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(54) Title: ANTI-INFECTIVE COMPOUNDS

(57) Abrégé/Abstract:
The present invention relates to small molecule compounds and their use in the treatment of bacterial infections, in particular Tuberculosis.

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(54) Title: PYRIDOPYRIMIDINE COMPOUNDS AS ANTI-TUBERCULAR AGENTS

(57) Abstract: The present invention relates to small molecule compounds and their use in the treatment of bacterial infections, in particular Tuberculosis.

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Anti-infective compounds

The present invention relates to small molecule compounds and their use in the treatment of bacterial infections, in particular Tuberculosis.

Background of the Invention

Tuberculosis (TB) as a disease continues to result in millions of deaths each year. Inadequate use of chemotherapy has led to an increasing number of drug resistant cases. This situation is likely to worsen with the emergence of extremely resistant strains to all currently known drugs (Van Rie and Enarson, 2006). The internationally recommended TB control strategy, also referred to as directly observed short-course chemotherapy (DOTS), relies on a combination of five antibacterial agents to be taken for a protracted period of more than six months (http://www.who.int/tb/dots/en/). With the use of a mathematical model, taking into consideration treatment duration and TB dynamics, benefits of reduced treatment length were predicted to be substantial and likely to greatly contribute to a reduced global TB burden (Salomon *et al.*, 2006).

Current chemotherapy consists of compounds that directly target *Mycobacterium tuberculosis* bacillus, either by neutralizing general information pathways and critical processes such as RNA polymerization and protein synthesis inhibition or by interfering with mycobacterial specific cell envelope synthesis. The most widely used dedicated anti-tubercular drugs isoniazid, ethionamide and pyrazinamide are pro-drugs that first require activation. As active forms, they demonstrate inhibitory activity on a wide range of mycobacterial targets, which have not yet been fully characterized. As for other chronic infectious diseases like human immunodeficiency virus, a multi-therapy approach, including drugs that target a wide range of critical features of *M. tuberculosis*, proved to be the most successful strategy to date. It is, thus, likely that a combination of current drug inhibitors, having different mechanisms of action against *M. tuberculosis*, will be the solution for the control of the disease.

The most challenging approaches for discovering new anti-TB drugs rely on screening for active compounds that target critical features essential for the survival of the bacillus. Although there is still a lack of understanding of the biological mechanisms behind tubercle

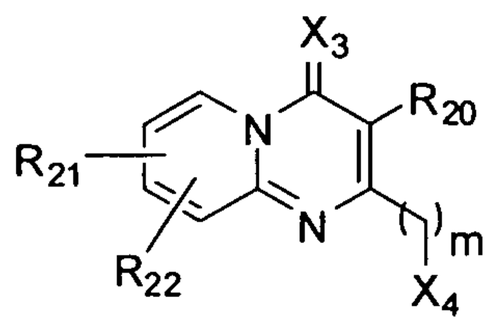
bacillus persistence, i.e. the location and state of latent bacteria, in humans, *M. tuberculosis* is thought to reside in primary granulomas under hypoxic conditions (Lenaerts *et al.*, 2007) as well as to hide within various types of cells (Houben *et al.*, 2006; Neyrolles et al., 2006). The bacillus mainly localizes inside phagocytic cells, such as macrophages and dendritic cells, and it has clearly been established that the tubercle bacillus adopts a different phenotype in the host macrophage's phagosome compared to growth in extracellular conditions (Rohde *et al.*, 2007; Schnappinger *et al.*, 2003). Upon infection, an inflammatory response is induced, thereby initiating recruitment of T lymphocytes that release interleukins and cytokines, which in turn activate the infected macrophages to enable the destruction of the pathogen. Upon the appropriate trigger, the host macrophage is, thus, able to eliminate the invading bacillus. This is further supported by the fact that of the people that inhale *M. tuberculosis*, more than 95% percent do not develop the disease, suggesting that the human host response is sufficient in most cases to thwart *M. tuberculosis* induced pathogenesis. This gives rise to the hypothesis that small molecular compounds could mimic the immune cell response signals and induce the host cells to clear the mycobacteria.

Accordingly, it was an object of the present invention to develop a phenotypic cell-based assay suitable for high throughput screening that allows for the search of compounds that would prevent *M. tuberculosis* multiplication inside the host macrophage.
Up to now, this type of investigation of the tubercle bacillus growth within host cells relied on colony forming units (CFUs) determination after host cell lysis followed by serial dilutions and a 3-week incubation period required for bacterial growth on agar plates. Luciferase-expressing mycobacteria have been shown to be efficient in reducing the experiment duration, although cell lysis and luciferin substrate addition steps are still required (Arain *et al.*, 1996). Also, these types of experiments are not easily amenable to large scale screening.

It was another object of the present invention to identify compounds effective against bacterial infections, in particular compounds that would prevent *M. tuberculosis* multiplication inside the host macrophage.

Description of the Invention

In one aspect, the present invention relates to compounds having the general formula VIII:



VIII

wherein

m is 0, 1, 2, or 3;

X_3 is selected from the group comprising CH_2, O, S and NH;

X_4 is selected from the group comprising halide, alkyl, OR_{23}, SR_{24} and $NR_{25}R_{26}$;

R_{20} is selected from the group comprising acyl, alkoxy, alkyl, alkylamino, alkylcarboxylic acid, arylcarboxylic acid, alkylcarboxylic alkylester, alkylene, alkylether, alkylhydroxy, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxylic acid, cyano, cycloalkyl, carboxylic acid, ester, halo, haloalkoxy, haloalkyl, haloalkylether, heteroaryl, heteroarylamino, heterocycloalkyl and hydrogen, any of which is optionally substituted;

R_{21} and R_{22} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylether, alkylthio, alkynyl, amido, amino, aryl, arylether, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted;

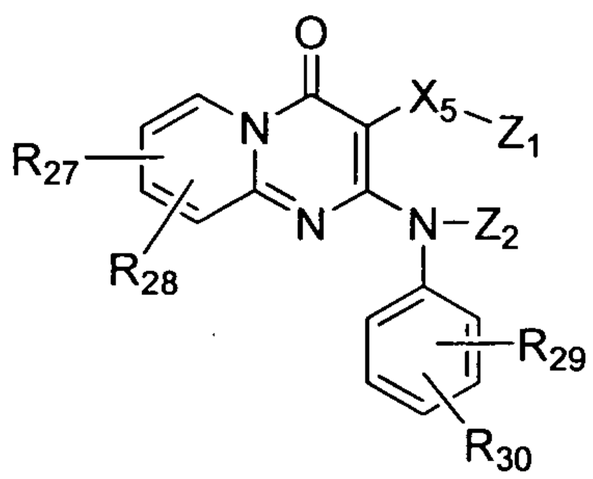
R_{23} is selected from the group comprising acyl, alkyl, alkylamino, alkylene, alkynyl, aryl, arylalkoxy, arylamino, arylthio, carboxy, cycloalkyl, ester, ether, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydrogen, thio, sulfonate, and sulfonylamino, any of which is optionally substituted;

R_{24} is selected from the group comprising alkyl, alkylaryl, alkylene, alkynyl, aryl, cycloalkyl, ester, halo, haloalkyl, heteroaryl, heterocycloalkyl, and hydrogen, any of which is optionally substituted; and

R_{25} and R_{26} are each independently selected from the group comprising acyl, alkyl, aminoalkyl, alkylene, alkylthio, alkynyl, aryl, arylalkoxy, arylamino, arylthio, carboxy, cycloalkyl, ester, ether, halo, haloalkoxy, haloalkyl, haloalkylether, heteroaryl, heteroarylamino, heterocycloalkyl and hydrogen, any of which is optionally substituted.

In general, the term "optionally substituted" as used herein is meant to indicate that a group, such as alkyl, alkylen, alkynyl, aryl, cycloalkyl, heterocycloalkyl, or heteroaryl, may be unsubstituted or substituted with one or more substituents. "Substituted" in reference to a group indicates that a hydrogen atom attached to a member atom within a group is replaced.

In another aspect, the present invention relates to compounds having the general formula VIIIa:



VIIIa

wherein

X_5 is selected from the group comprising CH_2, C=O and C=S;

Z_1 and Z_2 are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylether, alkylthio, alkynyl, amido, amino, aryl, arylether, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, and hydrogen, or two groups are connected each other to make five or six membered cyclic, heterocyclic and heteroaryl rings, any of which is optionally substituted;

R_{27} and R_{28} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylether, alkylthio, alkynyl, amido, amino, aryl, arylether, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted;

R_{29} and R_{30} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylether, alkylthio, alkynyl, amido, amino, aryl, arylether, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, or two groups are connected each other to make five or six membered cyclic, heterocyclic, aryl, and heteroaryl rings, any of which is optionally substituted.

The term "alkyl" as used herein is meant to indicate that a group, such as substituted or non-substituted C_1-C_{10} alkyl group which has the straight or branched chain.

The term "cycloalkyl" as used herein is meant to indicate that a group, such as substituted or non-substituted cyclic compound of C_3-C_8 ring structure.

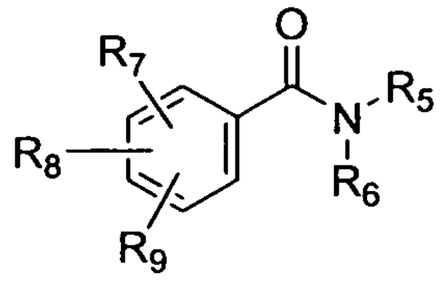
The term "heteroaryl" as used herein is meant to indicate that a group, such as substituted or non-substituted 5- to 9-membered aromatic compounds which have more than one heteroatom of N, O, and S in the ring structure itself.

The term "optionally substituted" as used herein is meant to indicates that a hydrogen atom attached to a member atom within a group is possibly replaced by group, such as C_1-C_{10} alkyl, halogen including fluorine, OH, NO_2, OR_{31}, CN, $NR_{31}R_{32}$, COR_{31}, SOR_{32}, SO_2R_{31}, SO_2NR_{31}, CR_{31}=$CR_{31}R_{32}$, CR_{31}=NR_{32}, aryl, aryloxy, C_4-C_{10} heteroaryl group, or –NR_{31}-COR_{32}, -O-COR_{31}.

R_{31} and R_{32} are each independently selected from the group comprising hydrogen, alkyl, alkyloxy, alkylamino, alkylcarbonyl, alkylcarbonylamino, alkylcarbonyloxy, alkylaminocarbonyl, alkyloxycarbonyl, cycloalkyl, cycloalkyloxy, cycloalkylamino, cycloalkylcarbonyl, cycloalkylcarbonylamino, cycloalkylcarbonyloxy, cycloalkylaminocarbonyl, cycloalkyloxycarbonyl, heteroaryl, heteroaryloxy, heteroaryl amino, heteroaryl carbonyl, heteroaryl carbonylamino, heteroaryl carbonyloxy, heteroaryl aminocarbonyl, heteroaryl oxycarbonyl, heteroaryl alkyl, heteroaryl alkyloxy, heteroaryl alkylamino, heteroaryl alkylcarbonyl, heteroaryl alkylcarbonylamino, heteroaryl alkylcarbonyloxy, heteroaryl alkylaminocarbonyl, heteroaryl alkyloxycarbonyl, phenyl, phenyloxy, phenylamino, phenylcarbonyl, phenylcarbonylamino, phenylcarbonyloxy, phenylaminocarbonyl, and phenyloxycarbonyl, any of which is optionally substituted.

In another aspect, the present invention relates to compounds having one of the formulas 125-301 as shown in Example 7, preferably 132-135, 137, 139-140, 147, 151-152, 160, 163, 173, 180, 184-185, 193, 195, 199-201, 204, 206-222, 224, 226, 229, 231-243, 245-278, 280-286 and 290-301 as shown in Table 4. Particularly preferred compounds are compounds having one of the formulas 133, 232 and 264 as shown in Table 4.

In one aspect, the present invention relates to compounds having the general formula II:



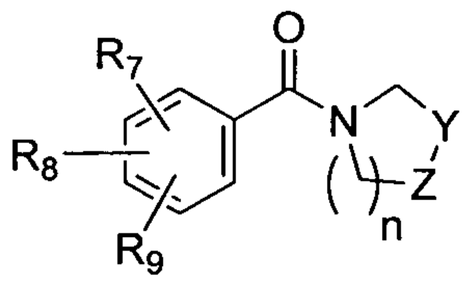
II

wherein

R_5 and R_6 are each independently selected from the group comprising acyl, alkyl, alkylamino, alkylene, alkylthio, alkynyl, aryl, arylalkoxy, arylamino, arylthio, carboxy, cycloalkyl, ester, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, sulfonate and sulfonyl , any of which is optionally substituted and

R_7, R_8 and R_9 are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted.

In another aspect, the present invention relates to compounds with the general formula II, wherein R_5 and R_6 are connected, having the general formula IIa:



IIa

wherein

n is 0, 1, 2, or 3;

Y and Z are each independently selected from the group comprising CH_2, $CHOR_{10}$, $CHNR_{10}R_{11}$, $CR_{10}R_{11}$ and NR_{10}; and

R_{10} and R_{11} are each independently selected from the group comprising acyl, alkyl, alkylamino, alkylene, alkylthio, alkynyl, aryl, arylalkoxy, arylamino, arylthio, carboxy, cycloalkyl, ester, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydrogen, sulfonate and sulfonyl , any of which is optionally substituted.

In another aspect, the present invention relates to compounds having one of the formulas with the general formula/scaffold II as shown in Table 2, as well as one of the formulas 1-123 as shown in Example 6, preferably 1-24, 26-34, 54, 56, 58-61, 63-64, 67, 90-101, 103-105, 107-

109, 112, 114-116 and 118-121 as shown in Table 4. Particularly preferred compounds are compounds having one of the formulas 4 and 24 as shown in Table 4.

Preferably, the compounds as defined above have an inhibitory activity, preferably an inhibitory activity above 65%, on bacterial growth, preferably on the growth of *M. tuberculosis*, inside a host cell, preferably a macrophage, at a concentration between 5-20 μM, preferably less than 5 μM.

In one aspect, the present invention relates to compounds as defined above for use in the treatment of bacterial infections.

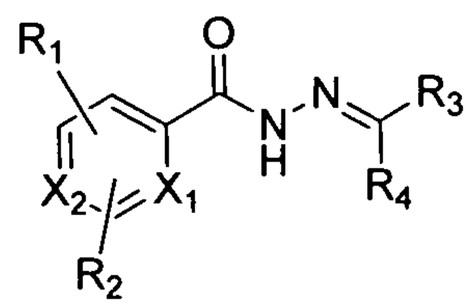
In one aspect, the present invention relates to compounds as defined above for use in the treatment of Tuberculosis.

In one aspect, the present invention relates to a pharmaceutical composition comprising a compound as defined above.

In one aspect, the present invention relates to a method of treatment of Tuberculosis, comprising the application of a suitable amount of a compound as defined above to a person in need thereof.

In another aspect, the present invention relates to compounds having one of the general formulas/scaffolds I, III-VII and IX-XX as shown in Table 3.

In one aspect, the present invention relates to compounds having the general formula I:



I

wherein

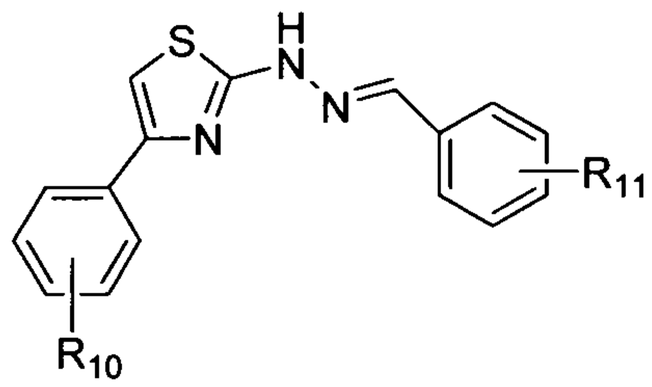
X_1 and X_2 are each independently selected from the group comprising CH and NH;

R_1 and R_2 are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino,

heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted; and

R_3 and R_4 are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, aryl, arylalkoxy, arylamino, arylthio, cyano, cycloalkyl, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl and hydrogen, any of which is optionally substituted.

In one aspect, the present invention relates to compounds having the general formula III:

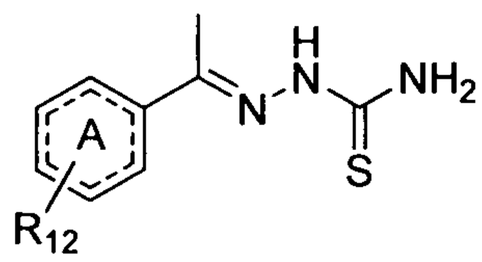


III

wherein

R_{10} and R_{11} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted.

In another aspect, the present invention relates to compounds having the general formula IV:



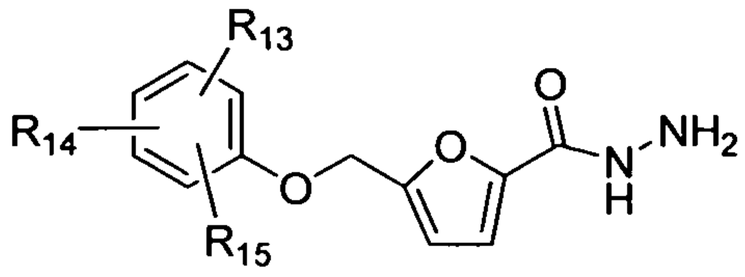
IV

wherein

A is an optionally substituted heteroaryl, naphthyl and phenyl and

R_{12} is selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted.

In one aspect, the present invention relates to compounds having the general formula V:

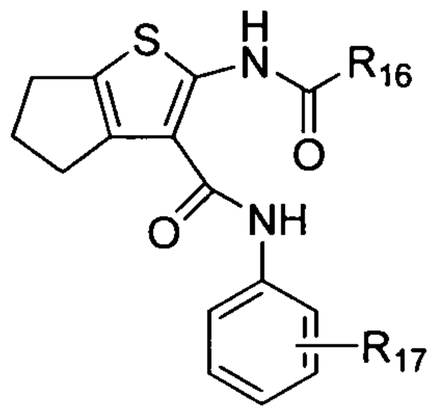


V

wherein

R_{13}, R_{14} and R_{15} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted.

In another aspect, the present invention relates to compounds having the general formula VI:



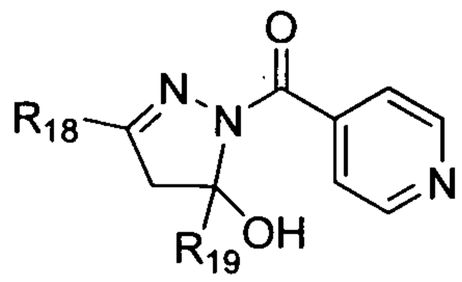
VI

wherein

R_{16} is selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, cycloalkyl, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl and hydrogen, any of which is optionally substituted and

R_{17} is selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thio, sulfonate, sulfonyl and sulfonylamino, any of which is optionally substituted.

In one aspect, the present invention relates to compounds having the general formula VII:



VII

wherein

R_{18} and R_{19} are each independently selected from the group comprising alkoxy, alkyl, alkylamino, alkylene, alkylthio, alkynyl, amido, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, ester, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl and hydrogen, any of which is optionally substituted.

In another aspect, the present invention relates to compounds having one of the formulas with the general formulas I, III-VII and IX-XX as shown in Table 2.

In one aspect, the present invention relates to a compound listed in Table 1.

In one aspect, the present invention relates to compounds as defined above for use in the treatment of bacterial infections.

In one aspect, the present invention relates to compounds as defined above for use in the treatment of Tuberculosis.

In one aspect, the present invention relates to a pharmaceutical composition comprising a compound as defined above.

In one aspect, the present invention relates to a method of treatment of Tuberculosis, comprising the application of a suitable amount of a compound as defined above to a person in need thereof.

In another aspect, the present invention relates to a screening method comprising the steps of

(a) batch infection of host cells with fluorescently labeled *M. tuberculosis* mycobacteria;

(b) removing free unbound mycobacteria;

(c) adding compounds that are to be tested to a multi-well plate;

(d) seeding said host cells infected with fluorescently labeled *M. tuberculosis* mycobacteria into said multi-well plate containing said compounds;

(e) incubating said multi-well plate containing host cells infected with fluorescently labeled *M. tuberculosis* mycobacteria and said compounds;

(f) fluorescently labeling said host cells;

(g) analyzing said multi-well plate using automated confocal microscopy.

The screening method according to the present invention represents a phenotypic cell-based assay enabling the search for drugs that interfere with the multiplication of *M. tuberculosis* within host macrophages. The assay makes use of fluorescently labeled living macrophages infected with fluorescently labeled mycobacteria and uses automated confocal fluorescence microscopy to measure intracellular mycobacterial growth. The assay has been set-up for the high throughput screening (HTS) of large scale chemical libraries.

Figures and Tables

Reference is now made to the figures and tables, wherein

Figure 1 shows the monitoring of tubercle bacillus intracellular growth inside macrophages by automated confocal microscopy: (**a**) Representative pictures of Raw264.7 cells infected with *M. tuberculosis* H37Rv-GFP at different time points after infection. (**b**) Image analysis: 1: Typical 2-color image; 2: Circled object corresponds to detected cells; 3: Circled object corresponds to bacterial aggregates; 4: Filled purple cells correspond to infected cells. **(c,d,e)** Image-based quantification of the percentage of infected cells and the mean number of cells from 2 hours to day 7 after infection with H37Rv-GFP at a multiplicity of infection of 0.5 (gray square), 1 (black circle) and 2 (dark gray triangle). Non-infected cells (black diamonds) were used as the negative control;

Figure 2 shows the pharmacological validation and MIC (minimal inhibitory concentration) comparison of the reference drugs in the *in vitro* growth fluorescence assay and the phenotypic cell-based assay: (**a**) Representative pictures of infected cells in presence of INH at 1 µg/mL or DMSO control. **(b,c,d)** Dose-response of INH, rifampin and ethionamide; black square and line corresponds to growth inhibition in cell-based assay; gray circle and line correspond to *in vitro* growth inhibition; shown is a representative data set;

Figure 3 shows assay automation validation of the phenotypic cell-based assay: **(a)** Percent of *M. tuberculosis* infected cells relative to 384-plate well-index. Black square, dark gray square, gray square and open square correspond to INH 1µg/mL, rifampin 5µg/mL, PBS and DMSO control respectively. (**b,c)** Percent of *M. tuberculosis* infected cells relative to INH and rifampin concentration. Experiments were performed on four different plates on two independent days;

Figure 4 shows primary screening results for the phenotypic cell-based assay and the *in vitro* growth assay for 26500 compounds: (**a**) Percent inhibition based on infection ratio relative to each compound and distribution. (**b**) Percent inhibition based on RFU relative to each compound and distribution. (**c**) Comparison of inhibition percentage for the phenotypic cell-based assay and the *in vitro* growth assay for each compound;

Figure 5 shows serial dilution results from the *in vitro* growth fluorescence assay and the phenotypic cell-based assay: Typical curves for compounds inhibiting (**a,b,c**) *in vitro* bacterial growth (**d,e,f**) both *in vitro* and intracellular growth and (**g,h,i**) intracellular growth only. (**a,d,g**) Infection ratio relative to compound concentration. (**b,e,h**) Cell number relative to compound concentration. (**c,f,i**) Relative fluorescence intensity relative to compound concentration. Compound concentration is given in M;

Figure 6 shows (**a**) a scheme of assay automation. (**b**) a 384-plate format description; (**c**) a 384-plate dose-response curve description, A to P and a to b correspond to 2-fold serial dilution of INH and Rifampin respectively with a starting concentration of 20 mg/mL in well A or a; RIF: Rifampin 5 μg/mL, Cpd: compound, INH100 1 μg/mL, INH50 0.05 μg/mL;

Figure 7 shows the anti-tuberculosis effect of compounds 4 and 24 (5 μM) on *M tuberculosis* H37Rv-GFP in (**a**) Raw267.4 (10^4 cells), (**b**) mouse bone marrow-derived macrophages and (**c**) human primary macrophages differentiated with 50 ng/mL rhM-CSF ($1.5*10^4$) after 7 days of infection with MOI 2.5:1 (control INH at 5 μM);

Figure 8 illustrates the colony forming units (CFUs) recovered from macrophages at different time points after infection with *M. tuberculosis* H37Rv. Either Raw264.7 cells (**a**) or murine BMDM (**b**) were infected at an MOI of 1:1 and treated with the indicated amount of pyridopyrimidione compound 232 (20 μM) with DMSO, INH (10 μM) and RIF (10 μM) as controls;

Table 1 lists 340 hits whose inhibitory activity was confirmed in an intracellular (QIM) assay or an *in vitro* (QUM) assay , wherein the abbreviation "QIM" stands for Quantification of Intracellular Mycobacteria, the abbreviation "QUM" stands for Quantification of *in vitro* grown Mycobacteria, and the abbreviation "CellNb" stands for cell number;

Table 2 lists 121 compounds which demonstrated an inhibitory activity above 65% at 2 μM without any apparent cell toxicity at 20 μM and consequently were selected for further confirmation by ten 3-fold serial dilutions;

Table 3 summarizes the independent/general molecular scaffolds/formulas of the 121 hits listed in **Table 2**;

Table 4 lists dinitrobenzamide and pyridopyrimidinone derivatives (general scaffold II and VIII, respectively, see **Table 3**) with their respective inhibitory activities, wherein the numbers in bold print refer to the compounds listed in Examples 6 and 7;

Table 5 shows the cytotoxicity and antibacterial spectrum of dinitrobenzamide compounds 4 and 24 (see **Table 4**);

Table 6 shows the cytotoxicity and antibacterial spectrum of pyridopyrimidinone compound 133 (see **Table 4**); and

Table 7 shows the frequency of spontaneous resistance for representative dinitrobenzamide and pyridopyrimidinone compounds.

Examples

The invention is now further described by reference to the following examples which are intended to illustrate, not to limit the scope of the invention.

Materials and Methods

Genetic constructs and mycobacterial strains

A recombinant strain of *M. tuberculosis* H37Rv expressing the green fluorescent protein (H37Rv-GFP) was obtained by transformation of an integrative plasmid (Abadie *et al.*, 2005; Cremer *et al.*, 2002). Within this plasmid, which is derived from the Ms6 mycobacteriophage, the *gfp* gene is cloned and constitutively expressed under the strong mycobacterial promoter *pBlaF*. Electrocompetent cells for *M. tuberculosis* H37Rv-GFP were prepared from 400 mL of a 15 days old Middlebrook 7H9 culture (Difco, Sparks MD, USA) supplemented with albumin-dextrose-catalase (ADC, Difco, Sparks MD, USA), glycerol and 0.05% Tween™ 80. Bacilli were harvested by centrifugation at 3000 g for 20 min, washed twice with H_2O at room temperature, and resuspended in 1-2 mL of 10% glycerol at room temperature after recentrifugation. 250 µl of bacilli were mixed with green fluorescent protein encoding plasmid and electroporated using a Biorad Gene Pulser (Biorad). After electroporation, bacilli were resuspended in medium and left one day at 37°C. Transformants were selected on Middlebrook 7H11 medium (Difco, Sparks MD, USA) supplemented with oleic acid-albumin-dextrose-catalase (OADC, Difco, Sparks MD, USA) and 50 µg/mL hygromycin (Invitrogen, Carlsbad, CA USA). The selected hygromycin-resistant and green fluorescent colonies appeared after 3 weeks. A 100 mL culture of the H37Rv-GFP strain was grown in Middlebrook 7H9-ADC medium supplemented with 0.05% Tween™ 80 and 50 µg/mL of hygromycin. Bacteria were harvested, washed twice and suspended in 50 mM sodium phosphate buffer (pH 7.5). The bacteria were then sonicated and allowed to stand for 1 hour to allow residual aggregates to settle. The bacterial suspensions were then aliquoted and frozen at -80°C. A single defrosted aliquot was used to quantify the CFUs (colony forming units) prior to inoculation and typical stock concentrations were about 2 to 5 x 10^8 CFU/mL.

Host cells

Mouse macrophage cell lines Raw 264.7 (ATCC # TIB-71), J774A.1 (ATCC # TIB-67) or human monocytes (ATCC # TIB-202) differentiated with 50 ng/mL PMA (Sigma) were grown in RPMI 1640 (Gibco) with 10% heat-inactivated fetal calf serum (Gibco).

Chemical compounds

The small synthetic molecules from the screening libraries were suspended in pure DMSO (Sigma, D5879-500 mL) at a concentration of 10 mM (Master plates) in Corning 96 well clear V-bottom polypropylene plates (Corning, #3956). The compounds were then reformatted in Greiner™ 384 well V-shape polypropylene plates (Greiner™, #781280) and diluted to a final concentration of 2 mM in pure DMSO. The compounds were kept frozen until use. For screening, compound plates were incubated at room temperature until thawed. The compounds were directly added into the assay plates from the DMSO stock using an EVObird liquid handler (Evotec Technologies), which transfers 250 nl of compound twice to reach a final dilution of 1:100. This one-step dilution reduces the risk of compound precipitation in intermediate plates and allows for a low final DMSO concentration (1%).

Positive control antibiotics (Isoniazid (Sigma, I3377-50G) and Rifampin (Euromedex, 1059-8, 5 g)) as well as negative controls (DMSO) were added manually in each plate in columns 1-2 and 23-24 (see **Figure 6 b** for plate description).

A total of 26500 compounds were tested. These compounds came from commercial libraries from Timtec (25000 from the ActiProbe diverse library, 1000 from the Kinase inhibitors ActiTargK library and 500 from the Protease inhibitors ActitargP library). The screened compounds were selected based on high diversity and drug-like properties (using Lipinski rule-of-five (Lipinski *et al.*, 2001)). They were first screened at one concentration (primary screen, concentration = 20 µM). The "positive" compounds selected from the primary screen were then confirmed by testing at 3 concentrations (20, 2 and 0.2 µM) to identify the most active and/or by ten 3-fold ten dilutions (from 20 µM to 0.5 nM).

Macrophage invasion assay set-up

Cells were first seeded in 50 µl at a density of 20,000 cells per well of a 384-well plate (Evotec technologies #781058) for 16 hours and then infected with bacterial suspensions at a multiplicity of infection (MOI) varying from 10:1 to 1:1 (bacteria:host cells). After 2 hours, cells were washed three times with phosphate buffered saline (PBS) and the compounds diluted in fresh culture medium were added. Cells were incubated at 37 °C, 5% CO_2 for up to seven days.

Macrophage batch infection assay scale-up

Cells (1.5×10^8 cells) were infected with H37Rv-GFP suspension at a MOI of 1:1 in 300 mL for 2 hours at 37 °C with shaking (100 rpm). After two washes by centrifugation at 1100 rpm (Beckman™ SX4250, 165 g) for 5 min., the remaining extracellular bacilli from the infected cells suspension were killed by a 1 hour amykacin (20 µM, Sigma, A2324-5G) treatment. After a final centrifugation step, cells were dispensed with the Wellmate (Matrix) into 384-well Evotec plates (#781058) preplated with 10 µl of the respective compound diluted in cell medium. Infected cells were then incubated in the presence of the compound for 5 days at 37 °C, 5% CO_2. After five days, macrophages were stained with SYTO 60 (Invitrogen, S11342) followed by plate sealing and image acquisition. During screening, staining of the live cells was carried out on a set of three plates every two hours to limit cell death due to prolonged incubation with cell chemical stain.

Image acquisition and data analysis

Confocal images were recorded on an automated fluorescent confocal microscope Opera™ (Evotec Technologies) using a 20X-water objective (NA 0.70), 488-nm and 635-nm lasers and a 488/635 primary dichroic mirror. Each image was then processed using dedicated in-house image analysis software (IM). Parameters determined were the total cell number and the number of infected cells. Briefly, the algorithm first segments the cells on the red channel using a sequence of processing steps as described elsewhere (Fenistein et al., manuscript in press). It is generally based on a succession of 1) thresholding the histogram of the original image (3 classes K-means) 2) gaussian filtering the original image with a standard deviation that is set equal to the cells' average radius, 3) searching for the local maxima of the filtered image that provides cell centers as seeds for 4) region growing that defines each cell's own surface and finally 5) removing extremely small cells as potential artifacts or noise. This step provides the total number of cells in the red channel. Infected cells are then defined as those having at least a given number of pixels (usually 3) whose intensity in the green channel is above a given intensity threshold. The ratio of infected cells to the total number of cells is the measure of interest (named infection ratio). For each well, 4 pictures were recorded and for each parameter, the mean of the four images was used.

Data obtained from either the intracellular assay image analysis or from the conventional antibacterial assay (see below) were then processed using ActivityBase (IDBS) to calculate the statistical data (% of inhibition, Z score for each compound, Z', CV etc. for the control

plates) and to store the data in an Oracle™ database. Additional analyses with regards to both quality control of the screens and hit identification were performed with various software packages including Spotfire (Tibco) and Pipelinepilot (Accelrys).

In vitro aerobic bacterial growth assay

A frozen aliquot of *M. tuberculosis* H37Rv-GFP was diluted at 1.5 x 10^6 CFU /mL in Middlebrook 7H9-ADC medium supplemented with 0.05% Tween™ 80. Greiner µclear-black 384-well plates (Greiner, #781091) were first preplated with 0.5 µl of compound dispensed by EVOBird (Evotec) in 10 µl of Middlebrook 7H9-ADC medium supplemented with 0.05% Tween™ 80. 40 µl of the diluted H37Rv-GFP bacterial suspension was then added on top of the diluted compound resulting in a final volume of 50 µl containing 1% DMSO. Plates were incubated at 37 °C, 5% CO_2 for 10 days after which GFP-fluorescence was recorded using a Victor 3 reader (Perkin-Elmer Life Sciences).

Macrophage infection assay and image analysis

Raw 264.7 (ATCC # TIB-71) ($1.5*10^8$ cells) were infected with H37Rv-GFP (Abadie *et al.*, 2005, Cremer *et al.*, 2002) in suspension at a MOI of 1:1 for 2 hours at 37 °C with shaking. After two washes by centrifugation, the remaining extracellular bacilli from the infected cell suspension were killed by a 1 hour Amikacin (20 µM, Sigma, A2324) treatment. After a final centrifugation step, cells were dispensed into 384-well Evotec plates (#781058) preplated with compounds and controls. Infected cells were then incubated for 5 days at 37°C, 5% CO_2. Murine Bone Marrow-Derived Macrophages (BMDM) were produced as described previously (Brodin *et al.*, 2006). Briefly, cells were extracted from the femurs and tibia of 6 weeks old female mice (C57BL/6, Orientbio) and cultivated in RPMI 1640 media containing 10% heat-inactivated fetal calf serum (FCS) (both from Gibco® at Invitrogen, Carlsbad, CA) and 10% L-929 cell conditioned medium. Peripheral Blood Mononuclear Cells (PBMC) were isolated from Buffy coat from healthy volunteers. Buffy coat diluted in PBS supplemented with 1% FCS was treated with 15 ml of Ficoll-Paque Plus (Amersham Biosciences, Sweden) and centrifuged at 2500 × *g* for 20 min. PBMC were obtained by $CD14^+$ bead separation (Miltenyi Biotec, Germany), washed 3-times with PBS (1% FCS) and transferred to 75 cm^2 culture flasks containing RPMI 1640 media, 10% FCS and 50 ng/ml of recombinant-human macrophage colony stimulating factor (R & D systems, Minneapolis). Six day old adherent murine BMDM and PBMC derived human macrophages were infected with H37Rv-GFP (Abadie *et al.*, 2005) in suspension at a MOI of 1:1 for 2 hours at 37°C and then extensively

washed and finally incubated with compounds or controls. After several days, macrophages were stained with SYTO 60 (Invitrogen, S11342) and image acquisition was performed on an EVOscreen-MarkIII fully automated platform (PerkinElmer) integrated with an Opera™ (20X-water objective, NA 0.70) and located in a BSL-3 safety laboratory. Mycobacteria-GFP were detected using a 488-nm laser coupled with a 535/50 nm detection filter and cells labeled with a 635-nm laser coupled with a 690/40 nm detection filter. Four fields were recorded for each plate well and each image was then processed using dedicated in-house image analysis software (IM) as described elsewhere (Fenistein *et al.*, in press).

Mycobacterial strains and *in vitro* bacterial growth assay

Mycobacterium tuberculosis H37Rv, H37Ra and BCG Pasteur were used as reference strains. All strains were diluted at 1.5 x 10^6 CFU /mL in Middlebrook 7H9-ADC medium supplemented with 0.05% Tween™ 80. 384-well plates (Greiner, #781091) were first preplated with 0.5 µl of compound dispensed by EVOBird (Evotec) in 10 µl of Middlebrook 7H9-ADC medium supplemented with 0.05% Tween™ 80. Forty microliters of the diluted H37Rv-GFP bacterial suspension was then added to the diluted compound resulting in a final volume of 50 µl containing 1% DMSO. Plates were incubated at 37°C, 5% CO_2 for 10 days. Mycobacterial growth was determined by measuring GFP-fluorescence using a Victor 3 reader (Perkin-Elmer Life Sciences) for H37Rv-GFP or with resazurin method. Isoniazid at 0.05 µg/mL and 1 µg/mL (Sigma, I3377), Rifampin at 1 µg/mL (Euromedex) and DMSO were used as controls.

Cytotoxicity Assay

In order to address compound toxicity, seven cell lines originating from different body tissues were cultivated in the presence of 3-fold dilutions of compounds starting from 100 µM. After 5 days of culture, cell viability was assessed by the resazurin test. Briefly, cells were incubated with 10 µg/mL of resazurin (Sigma-Aldrich St. Louis, MO) for 4 h at 37°C under 5% CO2. Resofurin fluorescence (RFU) was measured as indicated above. Percentage of toxicity on cells was calculated as follows: Cytotoxicity (%) = $(RFU_{DMSO}-RFU_{Blank}) - (RFU_{compound}-RFU_{blank}) / (RFU_{DMSO}-RFU_{Blank}) \times 100$. Percentage of cytotoxicity was plotted against compound concentration and the minimal toxic concentration (MTC_{50}) was determined by non-linear regression analysis as the lowest compound concentration where fifty percent toxicity was observed on the corresponding cell line.

Frequency of Spontaneous Resistance

The frequency of spontaneous mutations was determined on 7H10 plates containing increasing concentrations of dintirobenzamide (0.2, 0.8, 1.6 and 3.2 μg/ml) or pyridopyrimidinone (0.4, 0.8, 1.6 and 3.2 μg/ml) compounds. 10^6, 10^7 and 10^8 CFU containing bacterial suspensions were spread on compound containing agar plates. After 5-6 weeks at 37°C, colonies were counted and frequency of mutation was evaluated as the ratio of colonies relative to the original inoculum. DMSO and INH were used as negative and positive controls, respectively.

Example 1: Phenotypic macrophage-based assay set-up and automated image quantification

To set-up the optimal conditions of *M. tuberculosis* infection, Raw264.7 macrophages were first infected with mycobacteria that constitutively express green fluorescent protein (GFP) at different multiplicities of infection (MOI) followed by kinetic analysis. Up to 7 days post bacillus infection, the host live cells were daily labeled with the red chemical fluorescent dye Syto60, and confocal images of live samples were acquired using an automated confocal microscope. Typical images are displayed in **Figure 1a**. During the first 24 hours, a few discrete weakly fluorescent bacteria localized within the cells. By day 2, the average number of cells had increased and mycobacteria had started to spread into neighboring cells leading to zones of strongly fluorescent bacteria. The localization of the green signal is always within a distance of 5 μm to that of the red cell signal and in most cases actually overlaps with the cell signal. This confirms the intracellular nature of the mycobacteria growth. By day 4, the cell number has significantly diminished and the bacteria have formed large, highly fluorescent aggregates, which cover almost the entire image from day 5 onwards. As a control, non-infected cells grew up to confluence at day 2 and remained alive until day 7.

In order to automatically quantify the intracellular bacterial load, an in-house image analysis script was developed. This script enables the automated quantification of the number of cells and the percentage of infected cells, whereby an infected cell is a cell containing at least three green pixels with an intensity above a defined threshold (**Figure 1b**). 2 hours after infection, between 2 and 10% of Raw264.7 cells were found to harbor a low number of bacilli (**Figure 1c**). The percentage of infected cells, hereafter named infection ratio, continued to increase from 72 hours post-infection reaching up to 70% at seven days post infection. This increase in infection ratio correlated with an increase in cell mortality (**Figure 1d/e**).

Example 2: Comparative minimal inhibitory concentration of known anti-tubercular drugs

To validate the assay set-up, the effect of current anti-tuberculosis drugs on *M. tuberculosis* intracellular growth was investigated. 2-fold serial dilutions of isoniazid (INH), rifampin and ethionamide were performed, followed by testing on macrophages that had previously been infected with *M. tuberculosis* H37Rv-GFP. After 5 days of incubation, macrophages were stained, and images acquired on an automated confocal microscope as described above. A larger number of cells and a fewer number of bacteria are clearly seen on pictures corresponding to samples that were incubated with INH compared to the DMSO negative control. This shows that INH prevents both intracellular *M. tuberculosis* growth and bacillus mediated cytotoxicity (**Figure 2a**). A clear inhibition dose-response curve was obtained by image-extracted analysis (**Figure 2b**). In parallel, inhibition of *M. tuberculosis* H37Rv-GFP *in vitro* growth by INH was monitored by recording green fluorescence intensity under the same conditions. In both experiments, the minimal inhibitory concentration (MIC) for INH was 0.1 μg/mL, which is in accordance with the MIC reported in the literature for extracellular *M. tuberculosis* growth (Andries *et al.*, 2005). Similar results were obtained with the standard anti-tuberculosis drugs ethionamide (**Figure 2c**) and ethambutol (data not shown), whereas for rifampin, there was a log-fold decrease in the MIC in the cell-based assay compared to the *in vitro* assay (**Figure 2d**). The diminished efficacy of rifampin in the cell-based assay is likely due to impaired cell penetration and further demonstrates that it is the intracellular antibacterial activity that is being monitored in this assay. Thus, adaptation of both the intracellular and the *in vitro M. tuberculosis* growth assay for high throughput screening (HTS) was performed.

Example 3: Assay scale-up and validation

To simplify the protocol for HTS purposes, macrophages were infected in batch with *M. tuberculosis* before being dispensed onto the compounds. The batch infection was carried out with macrophages in suspension at 37°C under mild shaking. Free unbound mycobacteria were removed by washing three times with PBS and differential centrifugation, as well as by an additional one-hour incubation step with amykacin, an antibiotic known to selectively kill extracellular microbes (**Figure 6a**). *M. tuberculosis* infected macrophages were then seeded in plates that had been previously dispensed with the compounds, DMSO or antibiotic controls. The day-to-day as well as plate-to plate reproducibility was first tested. To this end, either

serial dilutions of INH or rifampin were dispensed into 8 plates along with the regular DMSO and positive control (INH at 1 μg/mL (MIC100) and at 0.05 μg/mL (MIC90) and rifampin at 1 μg/mL) wells that were subsequently seeded with infected cells. The same experiment was repeated over 2 consecutive days. After incubation for 5 days and macrophage staining, pictures from each plate were acquired. The mean infection ratio determined for the DMSO negative controls in each plate for the 2 days of experiments was between 50% and 70%, whereas for the INH and rifampin samples, the mean infection ratio fell to below 20% (**Figure 3a**). Despite some variation in the mean infection ratio between the two experiments, the difference between the INH-positive and DMSO-negative controls was above five-fold for both days. P values calculated for each plate using a paired t-student test also confirmed a significant difference between the positive and negative controls ($p < 0.000001$, data not shown). In addition, the inventors performed an experiment to determine if inhibitors of *M. tuberculosis* intracellular growth infection dispensed in any well on the plate could be detected by performing double-blind controls (spike of INH and rifampin at 3 different concentrations). Indeed, one hundred percent of the spikes were identified (data not shown). Taken together, these results prove that the assay is sensitive enough to be able to identify inhibitors under HTS conditions. Finally, the robustness of the assay was checked by monitoring the dose-response of reference compounds. Almost identical MICs for the antibiotic positive controls were determined independent of the plate or the day of the experiment (**Figure 3b/c**). Calculated MICs from the image based quantification of the infection ratio were 0.16 +/-0.05 μg/mL and 2.4 +/-1.3 μg/mL for INH and rifampin, respectively, and were confirmed by CFU plating (data not shown). In parallel, the extracellular growth assay was validated with a similar approach (data not shown).

Example 4: Primary screening of a large library of small synthetic compounds using the phenotypic cell-based assay

A 26500 small molecule compound library, that was selected for its high chemical diversity and drug-like properties according to the Lipinski rules (Lipinski *et al.*, 2001), was chosen as the first library to be screened using the validated phenotypic cell-based assay. The primary screen was carried out with compounds at 20 μM in singleton. The throughput was set to about 6000 compounds per working day encompassing 25 plates. The screening was performed with Raw264.7 cells that had been expanded from frozen stocks for ten days before infection with *M. tuberculosis* H37Rv-GFP. To accept the screening results, the MICs

obtained from 2 serial dilutions of INH and Rifampin processed at the beginning and at the end of the screening day should show similar results compared to the values obtained during the validation (see above). Each screened plate is then accepted by the quality control procedure if the window between DMSO and INH (1µg/ml) is higher than 3 and the CV calculated for the 320 compounds present in each plate is lower than 25. Such quality control criteria allow the identification of hits with an activity higher than 75%. Subsequently, the percent inhibition for each compound was determined relative to the corresponding mean infection ratio between 1 µg/mL INH (100%) and DMSO (0%) in the same 384-well plate. The percent inhibition distribution is centered around -20% of inhibition (**Figure 4a**). It was decided to select compounds that have an inhibitory effect greater than 65% which corresponds to a little less than 1.5 % of the total compounds.

In parallel, the same compounds were only tested for their inhibitory activity on the *M. tuberculosis* H37Rv-GFP bacterial growth. The results from this assay, which are based on classical fluorescence intensity, showed a higher degree of reproducibility and the criteria for plate validation was set to a Z' value (DMSO/INH) greater than 0.35. The throughput for this fluorescence based assay was approximately 20,000 compounds per day. Compounds that prevented *M. tuberculosis* growth *in vitro* with an inhibitory effect above 65% were then selected as hits (1.4%) as they belong to a clear independent population compared to the inactive population centered to 0% (**Figure 4b**).

The results gathered from the two different screenings were then compiled and compared (**Figure 4c**). Four different populations could be identified: compounds that are i) active only on extracellular bacteria, ii) active only on intracellular bacteria, iii) active in both settings or iv) not active. 657 compounds (2.5%) belonged to one of the first three categories and, thus, were selected for further investigation.

An important parameter that can be measured during image analysis is the total cell number, also referred to as cell cytotoxicity. A low cell number can be the result of two independent phenomena, the compound toxicity and *M. tuberculosis* growth mediated cell toxicity. Indeed, at day 5 after infection with *M. tuberculosis*, the cell number decreased to less than 100 cells per image compared to more than 500 cells per image for uninfected cells (**Figure 1e**). In contrast, a high cell number is obtained only when the compound is not toxic and prevents mycobacterial growth. This turns out to be a second relevant measurement of a compound's anti-mycobacterial activity. However, this criterion was not applied for the selection of hits from the primary screen as a low cell number was found for only a few compounds and the

inventors wanted to avoid failing to select highly active compounds that would later on prove to be active at much lower concentrations despite a cell toxicity at 20 μM. An additional validation criterion of a Z' (DMSO/INH) value of the total cell number greater than 0.2 was added for the following screening steps.

Example 5: Confirmation of screening results, dose-response analysis and hit classification

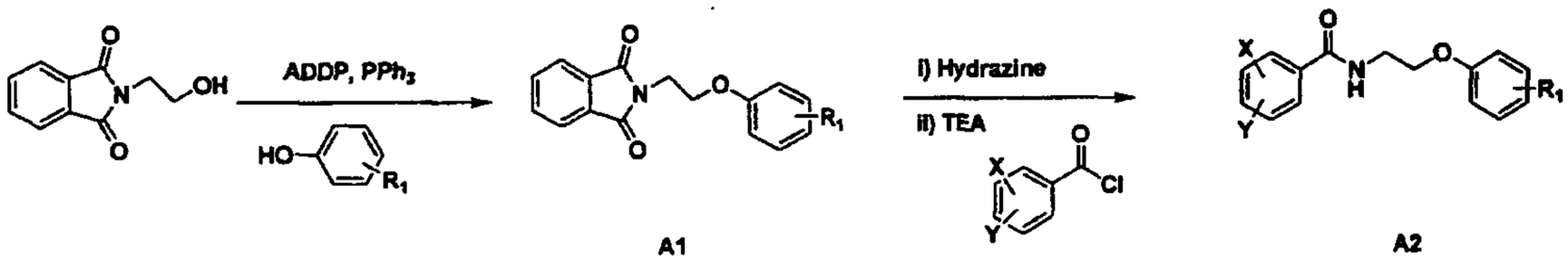
The 657 selected hits were first confirmed at 3 different concentrations, 20 μM, 2 μM and 0.2 μM. For 340 hits the activity was confirmed either at 20 μM or 2 μM, on the intracellular or the *in vitro* assay (see **Table 1**). From this latter list, 121 compounds demonstrated an inhibitory activity above 65% at 2 μM without any apparent cell toxicity at 20 μM and consequently were selected for further confirmation by ten 3-fold serial dilutions (see **Table 2**). All 121 compounds were confirmed by serial dilution with a MIC ranging between 250 nM and 20 μM. The results shown in **Figure 5** are representative of the three types of behavior observed: most of the compounds exhibited a clear dose response curve when activity was measured as infection ratio (**Figure 5b/e/h**). Compounds active on the bacilli level present a similar activity in the extracellular assay (**Figure 5c/f**) even if the MIC is different from one assay to the other. A few compounds don't present clear activity on the *in vitro* bacilli (**Figure 5i**) and may represent drugs acting through a cellular target or on a bacilli target involved only during the infection process. Furthermore, toxic compounds can be identified thanks to a dramatic decrease in the cell number when the compound concentration increases (**Figure 5d**) and activity of non-toxic compounds are validated by a dose response protective effect on the cell number (**Figure 5a**). Consequently cell number detection represents an independent secondary assay in the same well as the primary assay. The serial dilution results from all 121 compounds are presented in **Table 2**.

The 121 confirmed hits can be clustered as 20 independent/general scaffolds (**Table 3**). The number of compounds for each scaffold varied, ranging from 1 to 69 molecules. The molecules from the 69-compound scaffold share a common structure which is similar to INH thereby validating the screening results. One scaffold contains molecules that were only active in the intracellular assay and its mechanism of action will be the focus of further investigation.

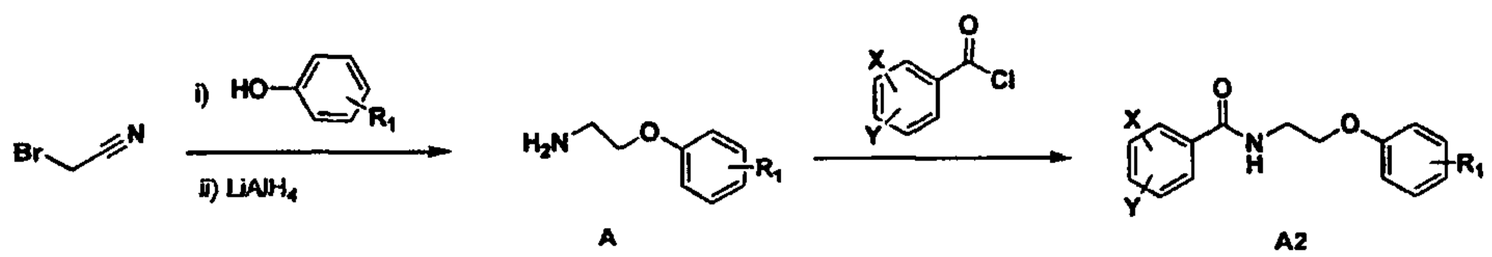
Example 6: Derivatization of the benzamide compounds

The benzamide compounds (scaffold II; see **Table 3**) underwent derivatization according to the methods outlined below (**Schemes 1-7**). Formation of the amide can be performed under general conditions using EDC or DCC coupling reagents with acids instead of acyl chloride. Resulting derivatives were examined for inhibitory activity using the assay described above and the results are summarized in **Table 4**.

Scheme 1



Scheme 2



<u>General procedure for the synthesis of 2-phenoxyethyl isoindoline-1,3-dione (A1)</u>

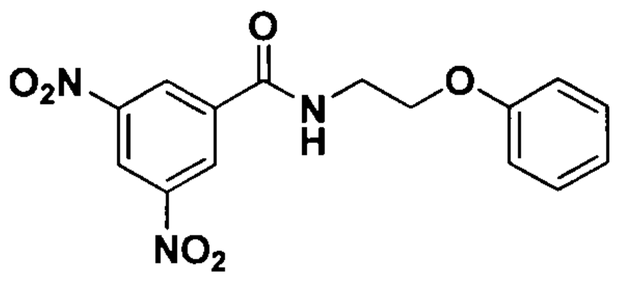
To a solution of 2-(2-hydroxyethyl)isoindoline-1,3-dione (1.68 mmol) in methylene chloride (10 mL) was added ADDP (1.68 mmol), triphenylphosphine (1.68 mmol) and phenol (3.18 mmol) and stirred at room temperature. After stirring overnight, the reaction mixture was diluted with methylene chloride (30 mL) and washed with 1 M NaOH aqueous solution (50 mL), and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (4:1 hexanes/ethyl acetate) and recrystallized from a mixture of hexanes and ethyl acetate to give A1.

<u>General procedure for the synthesis of *N*-(2-phenoxyethyl)-benzamide (A2)</u>

To a solution of A1 (1.14 mmol) in methanol (10 mL) was added hydrazine monohydrate (1.42 mmol) and the resulting mixture was refluxed under a nitrogen atmosphere. After 3 h, the reaction mixture was allowed to cool to room temperature and concentrated *in vacuo*. The residue was precipitated with methylene chloride (10 mL). The resulting precipitate was filtered through Celite™ and the filtrate was concentrated *in vacuo* to afford an amine intermediate. To a solution of the amine in methylene chloride (10 mL) was added

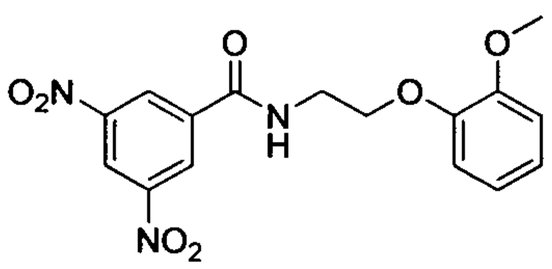
triethylamine (0.45 mmol) and a benzoylchloride (0.45 mmol) at 0 ℃ and the resulting mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with methylene chloride (10 mL) and washed with 1 M HCl aqueous solution (30 mL), saturated Na_2CO_3 aqueous solution (30 mL) and brine (30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (3:1 hexanes/ethyl acetate) and recrystallized from a mixture of hexanes and ethyl acetate to give A2.

3, 5-Dinitro-*N*-(2-phenoxyethyl)benzamide (1)



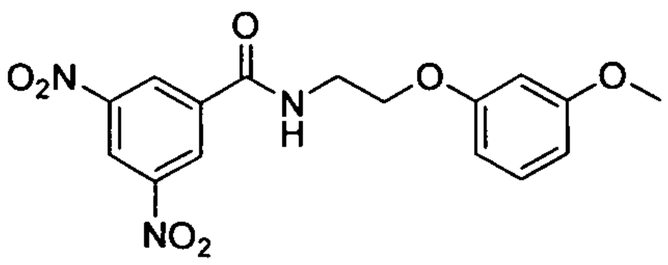
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.88 (t, J = 4.4 Hz, 2H), 4.21 (t, J = 5.2 Hz, 2H), 6.89 (d, J = 8.4 Hz, 3H), 7.24 (t, J = 8.0 Hz, 2H), 8.78 (brs, 1H), 9.02 (d, J = 2.0 Hz, 1H), 9.07 (d, J = 2.0 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 40.1, 66.0, 114.5, 120.8, 127.6, 129.6, 137.8, 148.8, 158.9, 163.0.

N-(2-(2-Methoxyphenoxy)ethyl)-3,5-dinitrobenzamide (2)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 3H), 3.92 (dd, J = 5.2, 10.4 Hz, 2H), 4.23 (t, J = 4.8 Hz, 2H), 6.91-7.02 (m, 4H), 7.63 (brs, 1H), 9.02 (d, J = 1.6 Hz, 2H), 9.14 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 56.1, 68.8, 112.2, 115.8, 121.0, 121.5, 122.9, 127.3, 137.8, 147.5, 148.6, 149.8, 162.6.

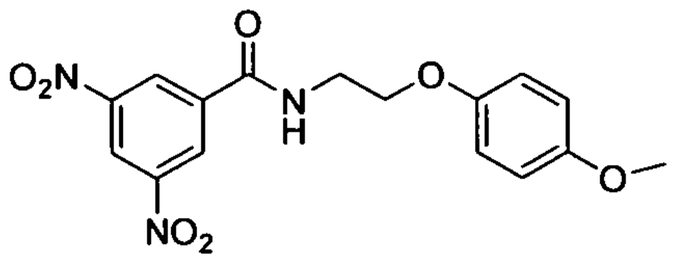
N-(2-(3-Methoxyphenoxy) ethyl)-3, 5-dinitrobenzamide (3)



^{1}H NMR (400 MHz, Acetone-d_6) δ 3.74 (s, 3H), 3.85 (dd, J = 5.6 Hz, 4.8 Hz, 2H), 4.21 (t, J = 5.2 Hz, 2H), 6.50 (m, 3H), 7.14 (t, J = 8.4 Hz, 1H), 8.75 (brs, 1H), 9.04 (s, 1H), 9.08 (s,

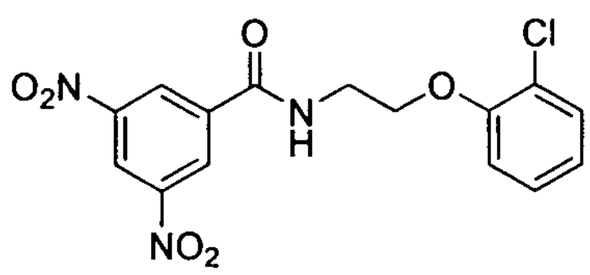
2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 40.1, 54.8, 66.1, 100.9, 106.5, 106.8, 120.9, 127.5, 130.0, 137.9, 148.8, 160.2, 161.2, 163.0.

N-(2-(4-Methoxyphenoxy)ethyl)-3,5-dinitrobenzamide (4)



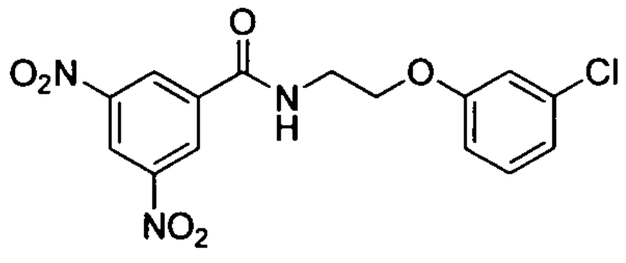
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (s, 3H), 3.91 (dd, J = 5.2, 10.8 Hz, 2H), 4.12 (t, J = 4.8 Hz, 2H), 6.74-6.80 (m, 4H), 7.21 (brs, 1H), 8.95 (d, J = 2.0 Hz, 2H), 9.07 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 55.6, 66.8, 114.7, 115.4, 121.0, 127.2, 137.6, 148.5, 152.2, 154.3, 163.1; LC-MS (ESI, *m/z*): 361 $[M+H]^+$.

N-(2-(2-Chlorophenoxy)ethyl)-3,5-dinitrobenzamide (5)



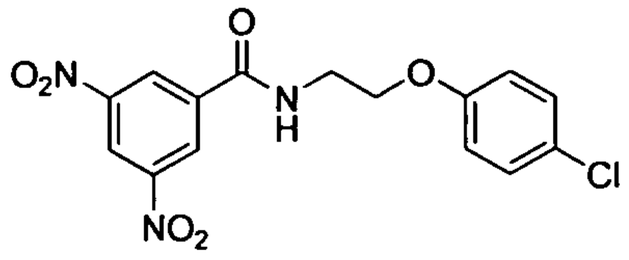
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, J = 5.2, 10.4 Hz, 2H), 4.25 (t, J = 5.2 Hz, 2H), 6.93-6.95 (m, 2H), 7.19-7.24 (m, 2H), 7.35 (dd, J = 1.2, 8.0 Hz, 1H), 8.98 (d, J = 2.0 Hz, 2H), 9.12 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.9, 63.0, 109.7, 116.2, 117.7, 118.2, 122.3, 123.1, 125.5, 132.6, 143.7, 148.7, 157.9.

N-(2-(3-Chlorophenoxy)ethyl)-3,5-dinitrobenzamide (6)



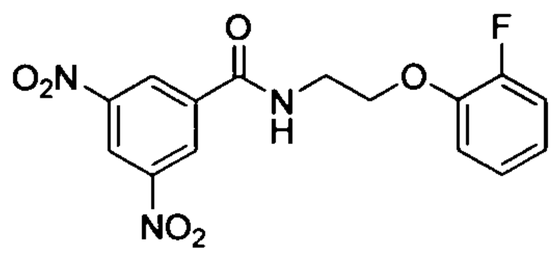
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, J = 5.6, 10.8 Hz, 2H), 4.19 (t, J = 4.8 Hz, 2H), 6.80-6.98 (m, 4H), 7.24 (t, J = 8.0 Hz, 1H), 8.96 (d, J = 2.0 Hz, 2H), 9.17 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 66.4, 110.7, 115.0, 121.2, 121.7, 127.2, 130.4, 135.1, 137.6, 148.7, 158.8, 163.0.

N-(2-(4-Chlorophenoxy)ethyl)-3,5-dinitrobenzamide (7)



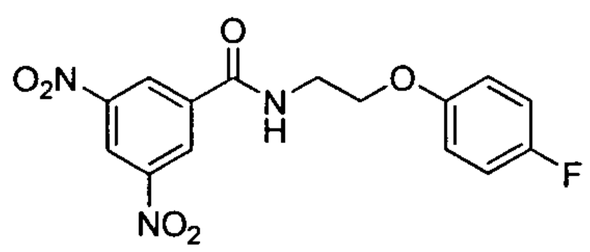
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (dd, *J* = 5.6, 10.4 Hz, 2H), 4.17 (t, *J* = 4.8 Hz, 2H), 6.78 (brs, 1H), 6.86 (dd, *J* = 2.4, 6.8 Hz, 2H), 7.23 (dd, *J* = 2.0, 6.8 Hz, 2H), 8.96 (d, *J* = 2.4 Hz, 2H), 9.17 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 66.5, 115.7, 121.2, 126.5, 127.2, 129.6, 137.6, 148.9, 156.8, 163.0.

N-(2-(2-Fluorophenoxy)ethyl)-3,5-dinitrobenzamide (8)



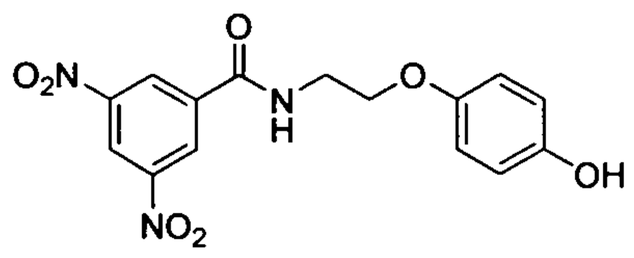
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, *J* = 5.2, 10.8 Hz, 2H), 4.25 (t, *J* = 5.2 Hz, 2H), 6.91-7.06 (m, 4H), 7.39 (brs, 1H), 8.97 (d, *J* = 2.0 Hz, 2H), 9.15 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 68.3, 115.7, 116.3 (d, *J* = 20 Hz, due to F), 121.1, 122.3 (d, *J* = 7 Hz, due to F), 124.6 (d, *J* = 5 Hz, due to F), 127.3, 137.6, 146.2, 148.6, 152.8 (d, *J* = 250 Hz, due to F), 163.1; LC-MS (ESI, *m/z*): 350 $[M+H]^+$.

N-(2-(4-Fluorophenoxy)ethyl)-3,5-dinitrobenzamide (9)



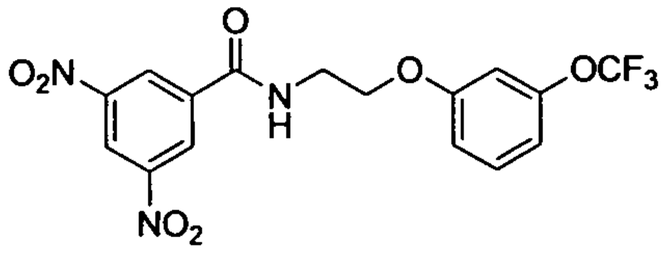
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.88 (dd, *J* = 5.2, 10.8 Hz, 2H), 4.23 (t, *J* = 5.2 Hz, 2H), 6.95-7.07 (m, 4H), 8.79 (brs, 1H), 9.07 (t, *J* = 2.4 Hz, 1H), 9.11 (d, *J* = 2.0 Hz, 2H).

N-(2-(4-Hydroxyphenoxy)ethyl)-3,5-dinitrobenzamide (10)



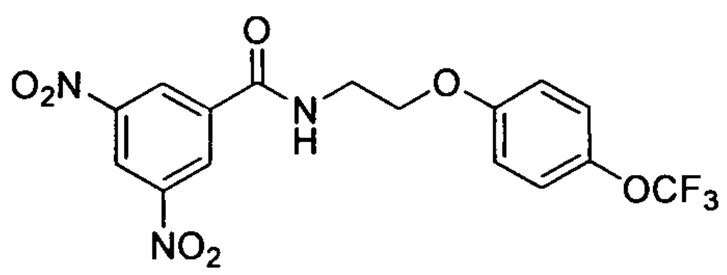
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.66 (dd, *J* = 5.6, 11.2 Hz, 2H), 4.06 (t, *J* = 5.2 Hz, 2H), 6.65-6.68 (m, 2H), 6.76-6.80 (m, 2H), 8.91 (brs, 1H), 8.98 (t, *J* = 2.0 Hz, 1H), 9.08 (d, *J* = 2.4 Hz, 2H), 9.42 (brs, 1H); ^{13}C NMR (100 MHz DMSO-d_6) δ 40.1, 66.9, 116.2, 116.4, 121.5, 128.2, 137.4, 148.8, 151.8, 152.0, 163.1.

N-(2-(3-(Trifluoromethoxy)phenoxy)ethyl)-3,5-dinitrobenzamide (11)



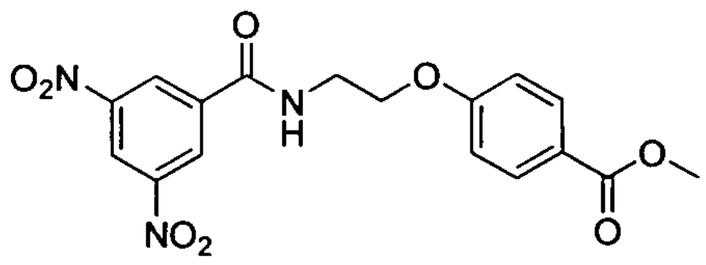
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.89 (dd, J = 5.6, 11.2 Hz, 2H), 4.29 (t, J = 5.6 Hz, 2H), 6.88 (d, J = 6.0 Hz, 2H), 6.99 (d, J = 8.0 Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 8.79 (brs, 1H), 9.05 (d, J = 1.2 Hz, 1H), 9.08 (d, J = 1.2 Hz, 2H) ; ^{13}C NMR (100 MHz, Acetone-d_6) δ 39.9, 66.7, 107.8, 113.1, 113.6, 120.9, 127.6, 130.9, 137.8, 148.9, 150.1, 160.2, 163.0.

N-(2-(4-(Trifluoromethoxy)phenoxy)ethyl)-3,5-dinitrobenzamide (12)



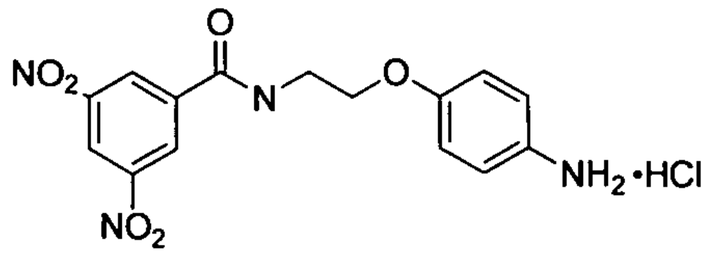
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.88 (dd, J = 10.8 Hz, 5.2 Hz, 2H), 4.27 (t, J = 5.6 Hz, 2H), 7.03 (dd, J = 7.2, 2.0 Hz, 2H), 7.23 (d, J = 8.8 Hz, 2H), 8.78 (brs, 1H), 9.04 (d, J = 2.0 Hz, 1H), 9.08 (d, J = 2.0 Hz, 2H) ; ^{13}C NMR (100 MHz, Acetone-d_6) δ 40.0, 66.8, 115.7, 120.9, 122.7, 127.6, 137.8, 142.7, 142.8, 148.9, 157.9, 163.1.

Methyl 4-(2-(3,5-dinitrobenzamido)ethoxy)benzoate (13)



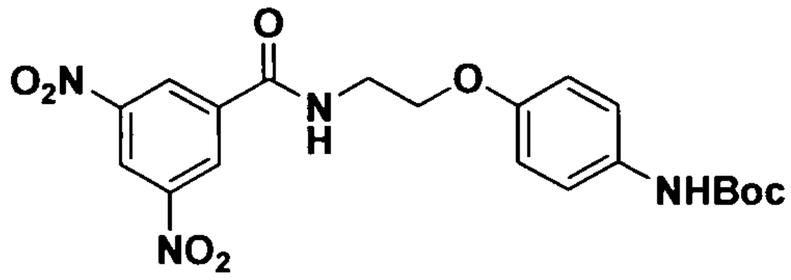
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.81 (s, 3H), 3.91 (t, J = 5.6 Hz, 2H), 4.33 (t, J = 5.6 Hz, 2H), 7.00 (t, J = 2.8 Hz, 1H), 7.03 (t, J = 2.8 Hz, 1H), 7.90 (t, J = 2.8 Hz, 1H), 7.92 (t, J = 2.8 Hz, 1H), 8.78 (brs, 1H), 9.03 (t, J = 2.4 Hz, 1H), 9.07 (d, J = 2.4 Hz, 2H) ; ^{13}C NMR (100 MHz, Acetone-d_6) δ 39.9, 51.3, 66.5, 114.4, 120.9, 123.0, 127.6, 131.5, 137.8, 148.9, 162.8, 163.0, 166.1.

N-(2-(4-Aminophenoxy)ethyl)-3,5-dinitrobenzamidehydrochloride (14)



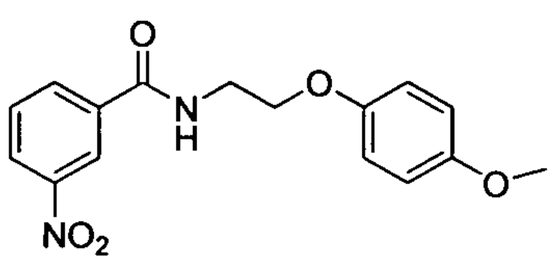
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.67 (d, J = 5.2 Hz, 2H), 4.15 (t, J = 5.2 Hz, 2H), 7.03 (d, J = 1.6 Hz, 2H), 7.29 (d, J = 1.6 Hz, 2H), 8.91 (d, J = 2.0 Hz, 1H), 9.04 (d, J = 2.0 Hz, 2H), 9.52 (brs, 1H), 10.28 (brs, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 40.1, 66.1, 115.4, 120.8, 124.3, 124.5, 127.5, 136.7, 148.1, 157.8, 162.4.

N-(2-(4-(*t*-Butoxycarbonylamino)phenoxy)ethyl)-3,5-dinitrobenzamide (15)



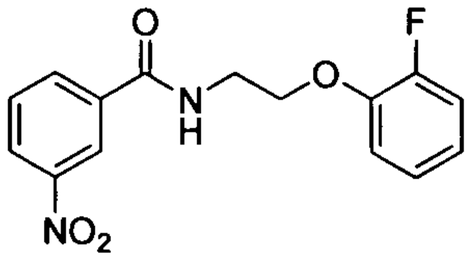
^{1}H NMR (400 MHz, Acetone-d_6) δ 1.44 (s, 9H), 3.83 (m, 2H), 4.18 (m, 2H), 6.84 (dd, J = 3.2, 9.2 Hz, 2H), 7.40 (d, J = 7.6 Hz, 2H), 8.15 (brs, 1H), 8.73 (brs, 1H), 9.03 (t, J = 2.0 Hz, 1H), 9.08 (d, J = 2.0 Hz, 2H) ; ^{13}C NMR (100 MHz, Acetone-d_6) δ 27.8, 40.1, 66.4, 78.9, 114.8, 119.9, 120.9, 127.6, 133.3, 137.9, 148.8, 153.2, 154.4, 163.0; LC-MS (ESI, *m/z*): 469 $[M+Na]^+$.

N-(2-(4-Methoxyphenoxy)ethyl)-3-nitrobenzamide (16)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3H), 3.81 (dd, J = 5.2, 10.4 Hz, 2H), 4.06 (t, J = 5.6 Hz, 2H), 6.73-6.78 (m, 4H), 7.48 (brs, 1H), 7.53 (t, J = 8.0 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 8.24 (d, J = 10.4 Hz, 1H), 8.56 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.8, 55.4, 66.7, 114.4, 115.2, 121.9, 125.8, 129.5, 133.1, 135.7, 147.8, 152.3, 153.9, 165.2.

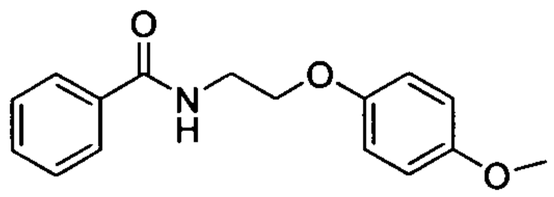
N-(2-(2-Fluorophenoxy)ethyl)-3-nitrobenzamide (17)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (dd, J = 5.6, 10.8 Hz, 2H), 4.23 (t, J = 4.8 Hz, 2H), 6.90-7.09 (m, 4H and brs, 1H), 7.62 (t, J = 8.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.63 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.8, 68.3, 115.6, 116.6 (d, J = 18.6 Hz, due to F), 122.3 (d, J = 5.3 Hz, due to F), 124.7 (d, J = 4.5 Hz, due to F), 126.0,

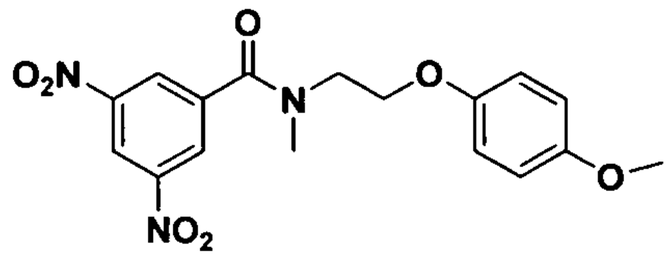
129.7, 133.0, 135.8, 146.3 (d, J = 10.4 Hz, due to F), 148.1, 152.6 (d, J = 245 Hz, due to F), 165.2.

N-(2-(4-Methoxyphenoxy)ethyl)benzamide (18)



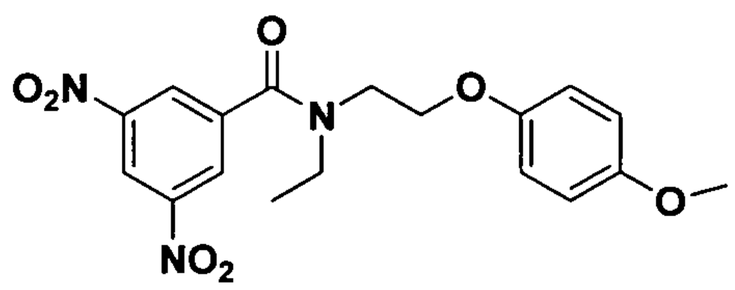
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (s, 3H), 3.80 (dd, J = 5.2, 10.8 Hz, 2H), 4.05 (t, J = 5.6 Hz, 2H), 6.78-6.83 (m, 4H), 7.03 (brs, 1H), 7.35-7.45 (m, 4H), 7.74 (d, J = 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.4, 55.4, 67.1, 114.5, 115.2, 126.8, 128.3, 131.3, 134.1, 152.4, 153.9, 167.6.

N-(2-(4-Methoxyphenoxy)ethyl)-*N*-methyl-3,5-dinitrobenzamide (19)



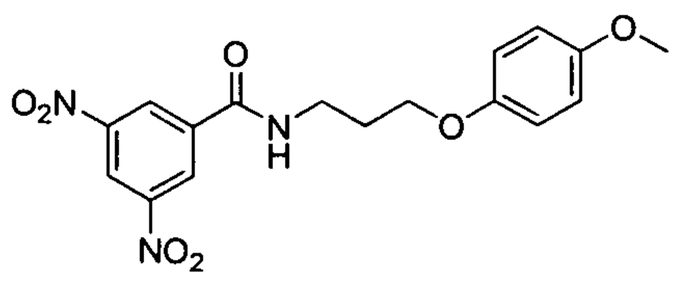
(Two rotamers, 1:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.18 (brs, 3H), 3.65 (brs, 1H), 3.75 (s, 3H), 3.94 (brs, 1H), 4.03 (brs, 1H),4.27 (brs, 1H), 6.79-6.84 (brd, 4H), 8.55 (brs, 1H), 8.72 (brs, 1H), 9.04 (br s, 1H).

N-Ethyl-*N*-(2-(4-methoxyphenoxy)ethyl)-3,5-dinitrobenzamide (20)



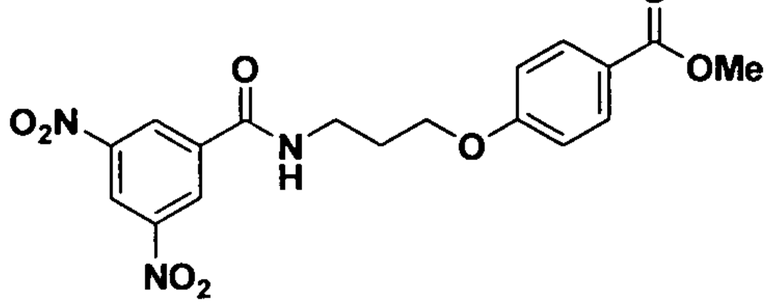
(Two rotamers, 1:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22-1.30 (m, 3H), 3.42 (brs, 1H), 3.63 (brs, 2H), 3.75 (s, 3H), 3.89 (brs, 1H), 4.01 (brs, 1H), 4.26 (brs, 1H), 6.80 (br, 4H), 8.53 (brs, 1H), 8.72 (brs, 1H), 9.04 (brs, 1H).

N-(3-(4-Methoxyphenoxy)propyl)-3,5-dinitrobenzamide (21)



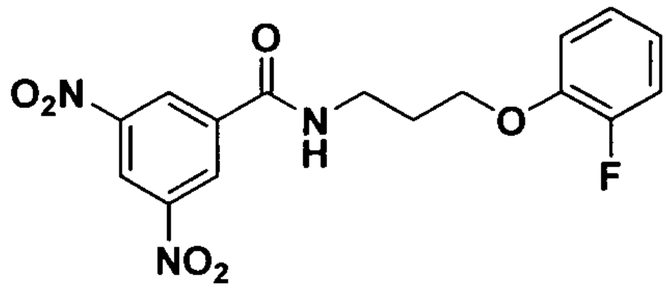
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04-2.20 (m, 2H), 3.76 (t, *J* = 6.0 Hz, 2H), 3.77 (s, 3H), 4.17 (t, *J* = 5.2 Hz, 2H), 6.85-6.91 (m, 4H), 7.24 (brs, 1H), 8.96 (d, *J* = 2.0 Hz, 2H), 9.16 (t, *J* = 2.0 Hz, 1H).

Methyl 4-(3-(3,5-dinitrobenzamido)propoxy)benzoate (22)



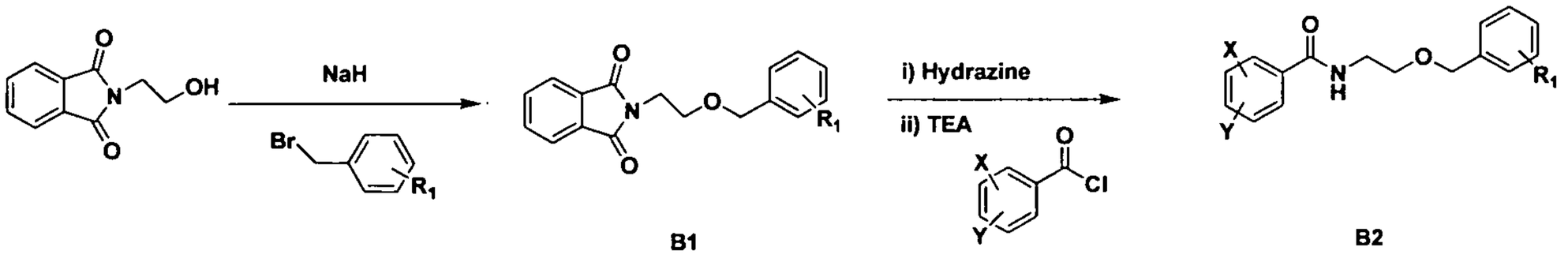
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21-2.24 (m, 2H), 3.77 (dd, *J* = 6.0, 12.0 Hz, 2H), 3.89 (s, 3H), 4.24 (t, *J* = 5.6 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 7.04 (brs, 1H), 8.00 (d, *J* = 8.8 Hz, 2H), 8.96 (d, *J* = 2.0 Hz, 2H), 9.16 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 39.3, 52.0, 67.2, 113.9, 121.1, 123.3, 127.0, 131.8, 137.8, 148.6, 161.9, 162.5, 166.6.

N-(3-(2-Fluorophenoxy)propyl)-3,5-dinitrobenzamide (23)



^{1}H NMR (400 MHz, $CDCl_3$) δ 2.19-2.25 (m, 2H), 3.83 (dd, *J* = 5.2, 11.2 Hz, 2H), 4.27 (t, *J* = 5.2 Hz, 2H), 6.90-7.11 (m, 4H), 7.50 (brs, 1H), 8.99 (d, *J* = 2.0 Hz, 2H), 9.16 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 40.0, 69.5, 114.0, 116.3 (d, *J* = 18 Hz, due to F), 120.9, 121.8 (d, *J* = 7.4 Hz, due to F), 124.7 (d, *J* = 3.7 Hz, due to F), 127.2, 127.3, 138.1, 147.3 (d, *J* = 245 Hz, due to F), 153.5, 162.7.

Scheme 3

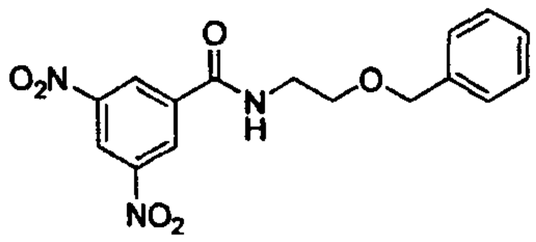


General procedure for the synthesis of of *N*-(2-(benzyloxy)ethyl)-dinitrobenzamide (B2)

To a solution of 2-(2-hydroxyethyl)isoindoline-1,3-dione (1.17 mmol) in dimethyl formamide (10 mL) was added sodium hydride (2.34 mmol) and a benzyl bromide (1.40 mmol) at 0°C

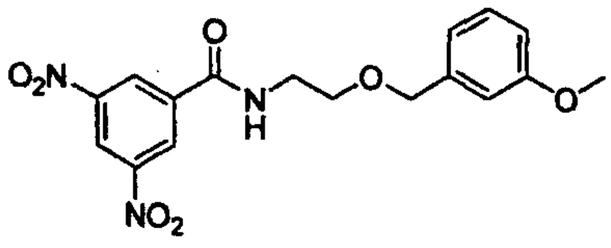
and the resulting mixture was stirred at room temperature. After stirring overnight, distilled water (50 mL) was added and the resulting precipitate was collected by filtration to afford B1. To a solution of B1 (0.85 mmol) in methanol (10 mL) was added hydrazine monohydrate (0.85 mmol) and the resulting mixture was refluxed under a nitrogen atmosphere. After 3 h, the reaction mixture was allowed to cool to room temperature and concentrated *in vacuo*. The residue was precipitated with methylene chloride (10 mL). The resulting precipitate was filtered off through Celite™, and the filtrate was concentrated *in vacuo* to afford an amine. To a solution of the amine in methylene chloride (10 mL) was added triethylamine (113 µl, 0.81 mmol) and a benzoylchloride (0.81 mmol) at 0 °C and the resulting mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with methylene chloride (30 mL) and washed with 1 M HCl aqueous solution (50 mL), saturated Na_2CO_3 aqueous solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (3:1 hexanes/ethyl acetate) and recrystallized from a mixture of hexanes and ethyl acetate to give B2.

<u>*N*-(2-(Benzyloxy)ethyl)-3,5-dinitrobenzamide (24)</u>



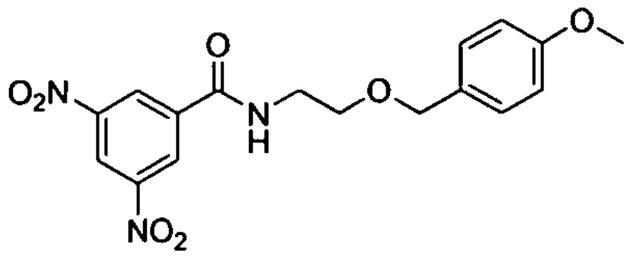
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68-3.72 (m, 4H), 4.55 (s, 2H), 6.75 (brs, 1H), 7.24-7.33 (m, 5H), 8.91 (d, J = 2.0 Hz, 2H), 9.13 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 68.1, 73.4, 121.0, 127.2, 128.0, 128.2, 128.7, 137.5, 138.0, 148.6, 162.7; LC-MS (ESI, *m/z*): 346 $[M+H]^+$.

<u>*N*-(2-(3-Methoxybenzyloxy)ethyl)-3,5-dinitrobenzamide (25)</u>



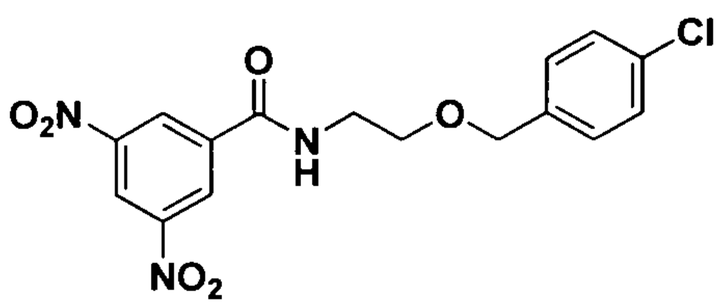
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71-3.74 (m, 4H), 3.76 (s, 3H), 4.52 (s, 2H), 6.77-6.90 (m, 3H), 6.97 (brs, 1H), 7.23 (t, J = 8.0 Hz, 1H), 8.91 (d, J = 2.0 Hz, 2H), 9.12 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.5, 55.2, 68.2, 73.1, 113.1, 113.6, 120.0, 120.9, 127.2, 129.6, 137.8, 139.1, 148.5, 159.7, 162.8.

N-(2-(4-Methoxybenzyloxy)ethyl)-3,5-dinitrobenzamide (26)



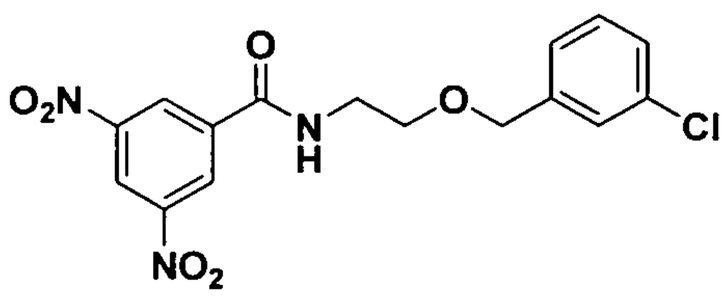
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.71 (m, 4H), 3.75 (s, 3H), 4.47 (s, 2H), 6.71 (brs, 1H), 6.84 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 8.87 (d, *J* = 2.4 Hz, 2H), 9.13 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.5, 55.3, 67.8, 73.1, 114.0, 121.0, 127.1, 129.6, 130.0, 137.9, 148.6, 159.5, 162.7.

N-(2-(4-Chlorobenzyloxy)ethyl)-3,5-dinitrobenzamide (27)



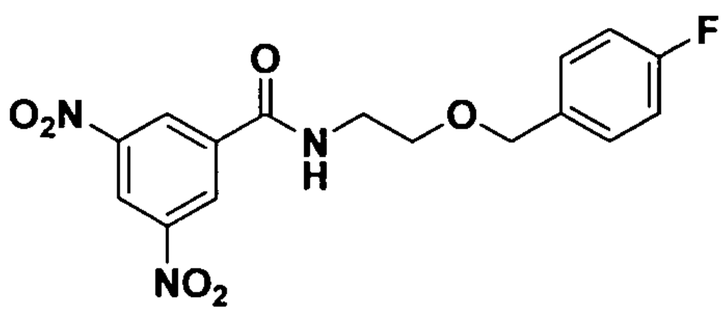
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68-3.76 (m, 4H), 4.53 (s, 2H), 6.77 (brs, 1H), 7.25-7.32 (m, 4H), 8.91 (d, *J* = 2.0 Hz, 2H), 9.15 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 68.3, 72.6, 121.1, 127.2, 128.8, 129.2, 134.0, 136.0, 137.8, 148.6, 162.7.

N-(2-(3-chlorobenzyloxy)ethyl)-3,5-dinitrobenzamide (28)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68-3.76 (m, 4H), 4.52 (s, 2H), 6.79 (brs, 1H), 7.17-7.29 (m, 4H), 8.91 (d, *J* = 2.0 Hz, 2H), 9.13 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 68.4, 72.5, 121.1, 125.8, 127.2, 127.8, 128.1, 129,2, 134.5, 137.8, 139.6, 148.6, 162.8.

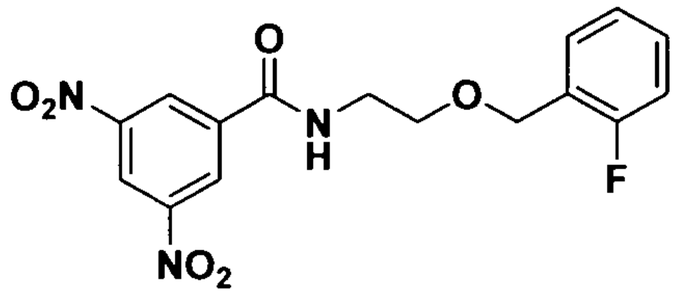
N-(2-(4-Fluorobenzyloxy)ethyl)-3,5-dinitrobenzamide (29)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68-3.76 (m, 4H), 4.53 (s, 2H), 6.74 (brs, 1H),7.02-7.06 (m, 2H), 7.30-7.33 (m, 2H), 8.92 (d, *J* = 2.0 Hz, 2H), 9.16 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100

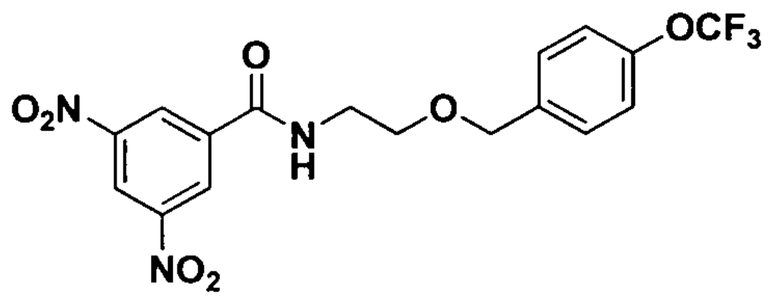
MHz, $CDCl_3$) δ 40.4, 68.1, 72.6, 115.5 (d, J = 22 Hz, due to F), 121.1, 127.1, 130.0 (d, J = 8.2 Hz, due to F), 133.5 (d, J = 3.0 Hz, due to F), 137.8, 148.6, 162.5 (d, J = 245 Hz, due to F), 162.7.

N-(2-(2-Fluorobenzyloxy)ethyl)-3,5-dinitrobenzamide (30)



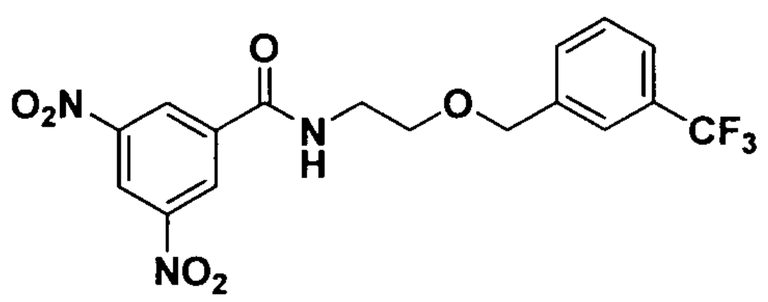
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (s, 4H), 4.64 (s, 2H), 7.07-7.17 (m, 3H), 7.29-7.39 (m, 1H and brs. 1H), 8.94 (d, J = 2.0 Hz, 2H), 9.17 (t, J = 2.0 Hz, 1H).

3,5-Dinitro-N-(2-(4-(trifluoromethoxy)benzyloxy)ethyl)benzamide (31)



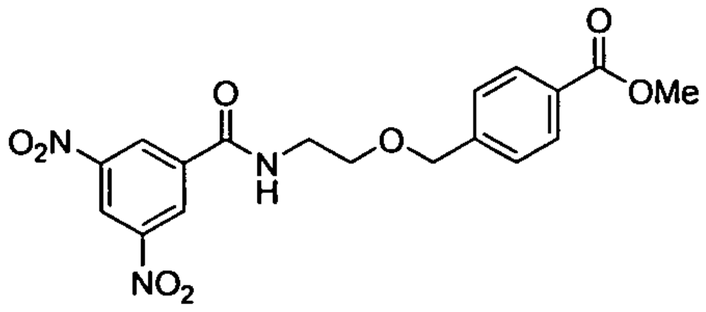
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72-3.76 (m, 4H), 4.54 (s, 2H), 7.13 (d, J = 8.0 Hz, 2H), 7.31-7.35 (m, 2H and brs, 1H), 8.94 (d, J = 2.0 Hz, 2H), 9.08 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 68.4, 72.2, 120.9, 121.0, 127.2, 129.1, 136.3, 137.7, 148.4, 148.7, 148.9, 162.9.

3,5-Dinitro-N-(2-(3-(trifluoromethyl)benzyloxy)ethyl)benzamide (32)



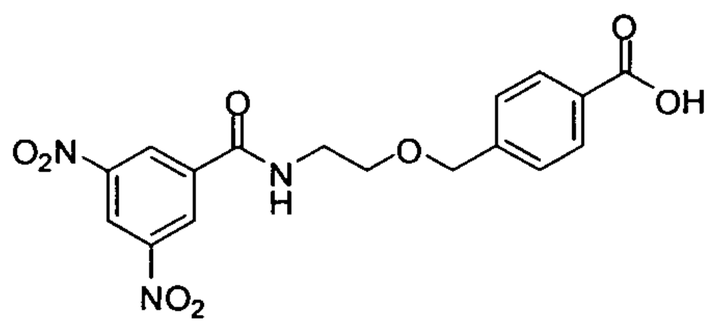
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72-3.79 (m, 4H), 4.61 (s, 2H), 7.06 (brs, 1H), 7.45-7.55 (m, 4H), 8.93 (d, J = 2.0 Hz, 2H), 9.10 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.4, 68.7, 72.4, 121.0, 124.1, 124.6, 124.7, 127.2, 129.0, 130.6 (q, J = 32 Hz, due to F), 130.8, 137.7, 138.6, 148.6, 162.9.

Methyl 4-((2-(3,5-dinitrobenzamido)ethoxy)methyl)benzoate (33)



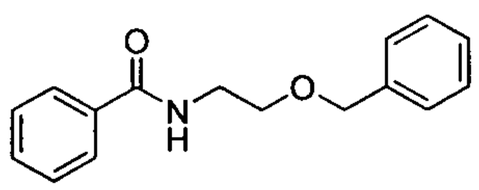
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71-3.74 (m, 4H), 3.84 (s, 3H), 4.55 (s, 2H), 7.29 (d, *J* = 8.0 Hz, 2H and brs, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 8.90 (d, *J* = 2.0 Hz, 2H), 9.01 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.6, 52.2, 68.8, 72.6, 120.9, 127.3, 129.5, 129.7, 137.8, 142.9, 148.5, 163.0, 166.8.

4-((2-(3,5-Dinitrobenzamido)ethoxy)methyl)benzoic acid (34)



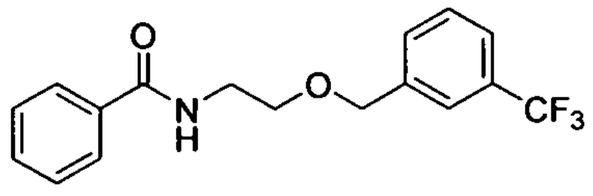
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.74 (t, *J* = 5.2 Hz, 2H), 3.81 (t, *J* = 5.2 Hz, 2H), 4.72 (s, 2H), 7.56 (d, *J* = 8.4 Hz, 2H) 7.72 (brs, 1H), 8.03 (d, *J* = 8.4 Hz, 2H), 9.02 (d, *J* = 2.0 Hz, 2H), 9.13 (t, *J* = 2.0 Hz, 1H).

N-(2-(Benzyloxy)ethyl)benzamide (35)



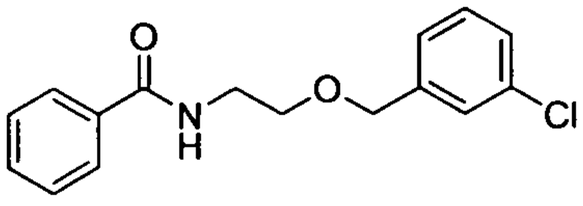
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62-3.68 (m, 4H), 4.52 (s, 2H), 6.71 (brs, 1H), 7.24-7.49 (m, 8H), 7.73-7.76 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.7, 68.8, 73.1, 126.9, 127.8, 128.4, 131.3, 134.5, 137.8, 167.5.

N-(2-(3-(Trifluoromethyl)benzyloxy)ethyl)benzamide (36)



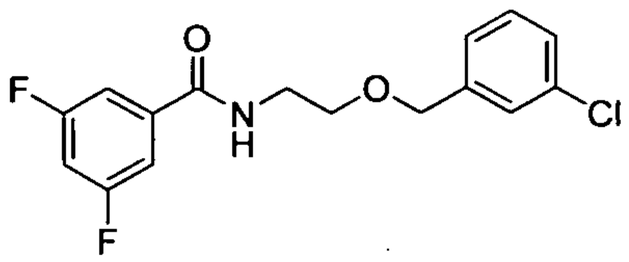
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63-3.70 (m, 4H), 4.56 (s, 2H), 6.72 (brs, 1H), 7.37-7.53 (m, 6H), 7.58 (s, 1H), 7.74-7.76 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.7, 69.3, 72.3, 124.2, 124.3, 124.6, 126.9, 128.5, 128.9, 130.8, 131.5, 134.4, 139.0, 148.6, 167.6.

N-(2-(3-Chlorobenzyloxy)ethyl)benzamide (37)



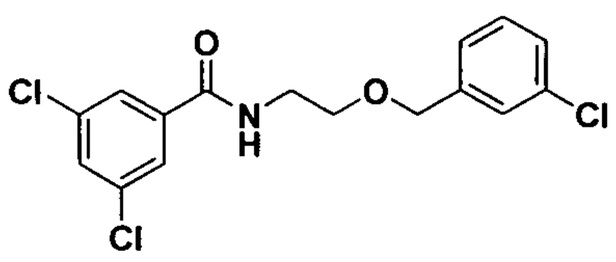
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62-3.69 (m, 4H), 4.49 (s, 2H), 6.71 (brs, 1H), 7.17-7.50 (m, 7H), 7.75-7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.7, 69.0, 72.2, 125.6, 126.8, 127.6, 127.8, 128.4, 129,7, 131.3, 134.3, 139.9, 167.4.

N-(2-(3- Chlorobenzyloxy)ethyl)-3,5-difluorobenzamide (38)



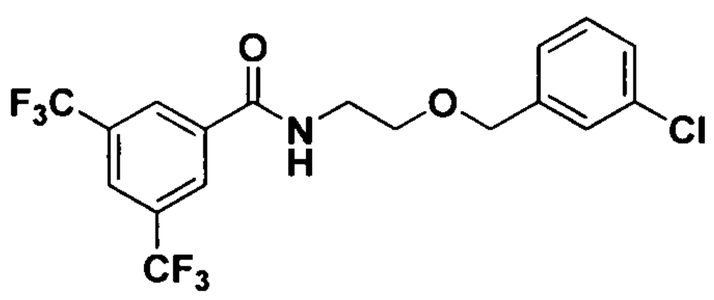
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64-3.69 (m, 4H), 4.52 (s, 2H), 6.54 (brs, 1H), 6.95 (tt, J = 2.4, 11.2 Hz, 1H), 7.19-7.33 (m, 6H).

N-(2-(3-Chlorobenzyloxy)ethyl)-3,5-dichlorobenzamide (39)



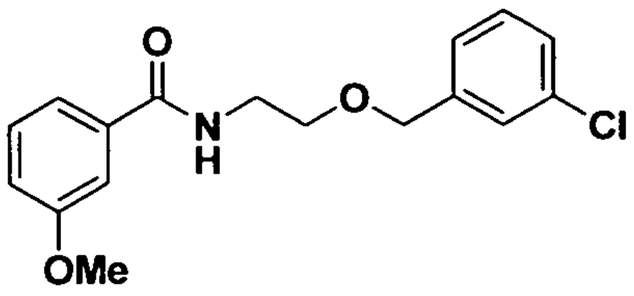
^{1}H NMR (400 MHz, CD_3OD) δ 3.13 (t, J = 5.2 Hz, 2H), 3.67 (t, J = 5.2 Hz, 2H), 4.55 (s, 2H), 7.27 – 7.29 (m, 3H), 7.42 (s, 1H), 7.46, (s, 1H), 7.81 (s, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.6, 67.2, 73.2, 127.0, 128.7, 128.8, 128.9, 130.7, 131.0, 135.4, 135.5, 141.4, 142.7, 171.5.

N-(2-(3-Chlorobenzyloxy)ethyl)-3,5-bis(trifluoromethyl)benzamide (40)



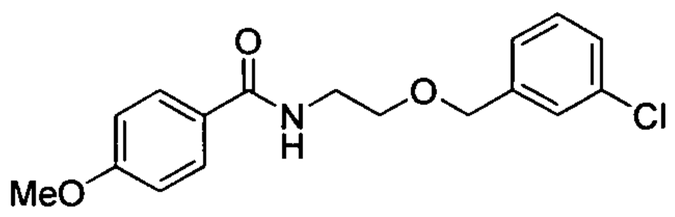
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 – 3.68 (m, 4H), 4.49 (s, 2H), 6.89 (brs, 1H), 7.15 (d, J = 3.6 Hz, 1H), 7.21 – 7.24 (m, 2H), 7.27 (s, 1H), 7.95 (s, 1H), 8.18 (s, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.3, 68.37, 72.5, 121.6, 125.0, 125.1, 125.7, 127.8, 128.1, 129.9, 132.0, 134.5, 136.6, 139.8, 164.8.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-methoxybenzamide (41)



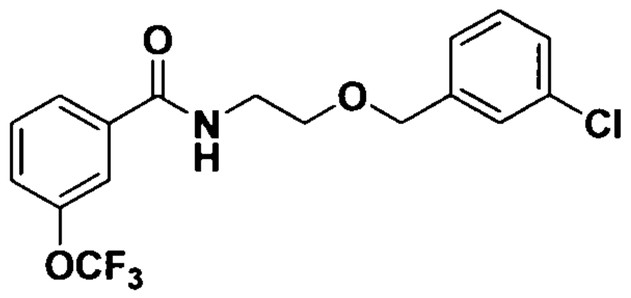
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (d, *J* = 3.6 Hz, 2H), 3.65 (d, *J* = 3.6 Hz, 2H), 3.81 (s, 3H), 4.49 (s, 2H), 6.51 (brs, 1H), 7.01 (dd , *J* = 8.0 Hz, 2.4 Hz, 1H), 7.16 (d, *J* = 4.4 Hz, 1H), 7.28 (m, 3H), 7.25 – 7.34 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.9, 55.5, 69.2, 72.5, 112.4, 117.8, 118.7, 125.8, 127.8, 128.0, 129.6, 129.9, 134.5, 136.0, 140.0, 159.9 ,167.5.

N-(2-(3-Chlorobenzyloxy)ethyl)-4-methoxybenzamide (42)



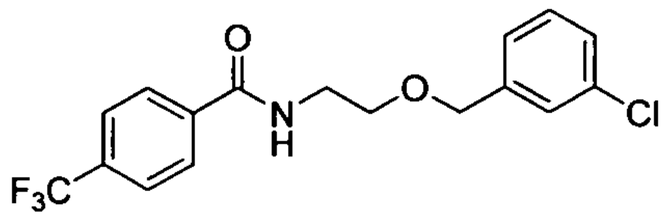
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 – 3.66 (m, 4H), 3.82 (s, 3H), 4.49 (S, 2H), 6.48 (brs, 1H), 6.89 (d, *J* = 8.8 hz, 2H), 7.17 (t, *J* = 4.4 Hz, 2H), 7.24 (m, 1H), 7.32 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.8, 55.5, 69.4, 72.4, 113.8, 125.7, 126.8, 127.8, 128.0, 128.8, 129.8, 134.5, 140.1, 162.2, 167.1.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-(trifluoromethoxy)benzamide (43)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 – 3.68 (m, 4H), 4.49 (s, 2H), 6.62 (brs, 1H), 7.15 (dd, *J* = 1.2, 8.8 Hz, 1H), 7.22 – 7.23 (m, 2H), 7.36 (t, *J* = 1.2 Hz, 2H), 7.43 (t, *J* = 8.4 Hz, 1H), 7.63 (dd, *J* = 1.2, 4.4 Hz, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 69.0, 72.4, 119.3, 120.1, 123.8, 125.1, 125.7, 127.8, 128.0, 129.9, 130.1, 134.5, 136.6, 140.0, 149.4, 166.1.

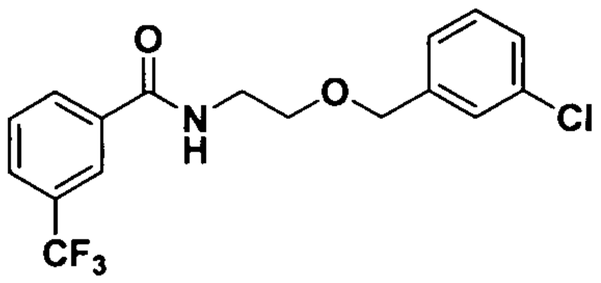
N-(2-(3-Chlorobenzyloxy)ethyl)-4-(trifluoromethyl)benzamide (44)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 – 3.68 (m, 4H), 4.49 (s, 2H), 6.71 (brs, 1H), 7.14 – 7.17 (m, 1H), 7.23 – 7.24 (m, 2H), 7.3 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H);

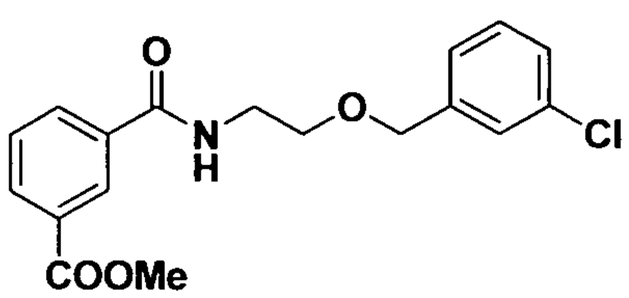
^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 68.9, 72.4, 125.6 (q, J = 3.7 Hz), 125.8, 127.5, 127.8, 128.1, 129.9, 138.1, 133.4, 134.5, 137.7, 140.0, 166.4.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-(trifluoromethyl)benzamide (45)



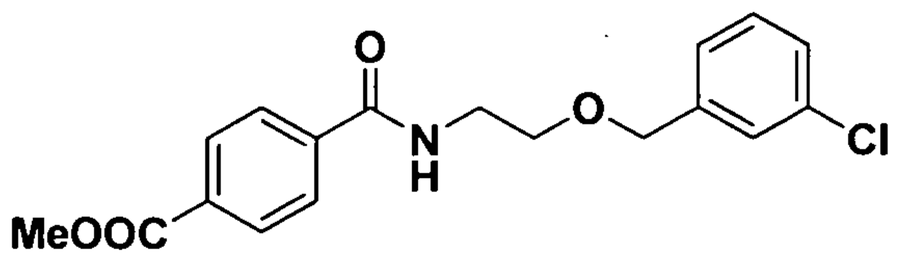
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (m, 4H), 4.46 (s, 2H), 6.96 (brs, 1H), 7.14 – 7.27 (m, 4H), 7.47 (t, J = 7.2 Hz, 1H), 7.68 (d, J = 3.2 Hz, 1H), 7.89 (d, J = 3.2 Hz, 1H), 8.01 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 68.9, 72.3, 122.4, 124.1, 125.7, 127.7, 127.9, 128.0, 129.1, 129.8, 130.3, 130.8, 134.4, 135.2, 140.0, 166.3.

Methyl 3-(2-(3-chlorobenzyloxy)ethylcarbamoyl)benzoate (46)



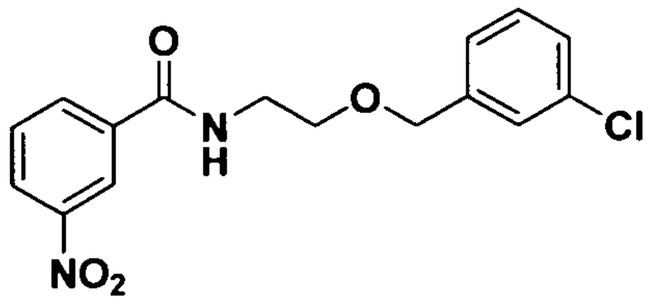
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 – 3.69 (m, 4H), 3.89 (s, 3H), 4.48 (s, 2H), 6.71 (brs, 1H), 7.15 – 7.16 (m, 1H), 7.21 – 7.24 (m, 2H), 7.28 (s, 1H), 7.47 (t, J = 4.0 Hz, 1H), 7.97 (d, J = 4.8 Hz, 1H), 8.11 (d, J = 4.8 Hz, 1H), 8.35 (t, J = 1.6 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 52.4, 69.0, 72.4, 125.7, 127.7, 127.8, 128.0, 128.9, 129.8, 130.5, 131.8, 132.4, 134.4, 134.8, 140.0, 166.3, 166.6.

Methyl 4-(2-(3-chlorobenzyloxy)ethylcarbamoyl)benzoate (47)



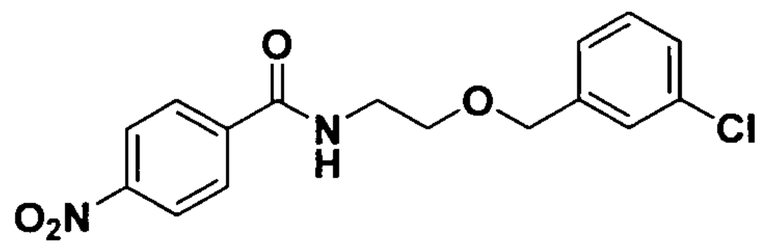
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 – 3.66 (m, 4H), 3.90 (s, 3H), 4.48 (s, 2H), 6.65 (brs, 1H), 7.14 – 7.17 (m, 1H), 7.22 (d, J = 5.2 Hz, 2H), 7.30 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 8.04 (d, J = 8.0 Hz, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 52.4, 69.0, 72.4, 125.7, 127.1, 127.8, 128.1, 129.9, 132.7, 134.5, 138.4, 140.0, 160.3, 166.7.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-nitrobenzamide (48)



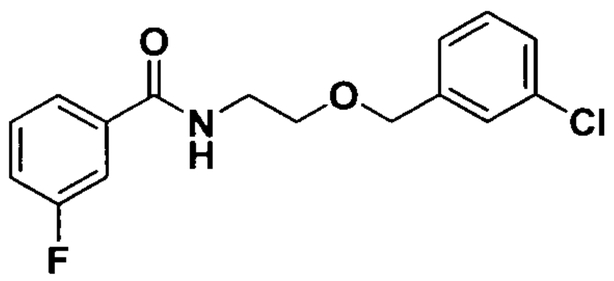
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (m, 4H), 4.45 (s, 2H), 7.13 – 7.23 (m, 5H), 7.53 (m, 1H), 8.08 (d, *J* = 6.8 Hz, 1H) 8.22 (d, *J* = 6.8 Hz, 1H), 8.54 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 68.7, 72.2, 122.0, 125.6, 125.9, 127.5, 127.8, 129.7, 129.8, 133.1, 134.2, 136.0, 139.9, 148.0, 165.3.

N-(2-(3-Chlorobenzyloxy)ethyl)-4-nitrobenzamide (49)



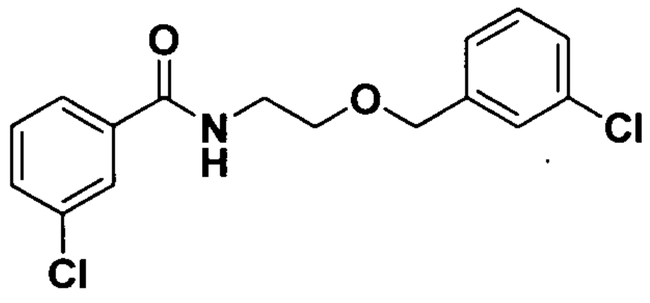
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (m, 4H), 4.45 (s, 2H), 6.97 (brs, 1H), 7.12 – 7.25 (m, 4H), 7.87 (d, *J* = 6.4 Hz, 2H), 8.15 (d, *J* = 6.4 Hz, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 68.7, 72.2, 123.6, 125.6, 127.5, 127.9, 128.2, 129.7, 134.3, 139.9, 140.0, 149.4, 165.6.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-fluorobenzamide (50)



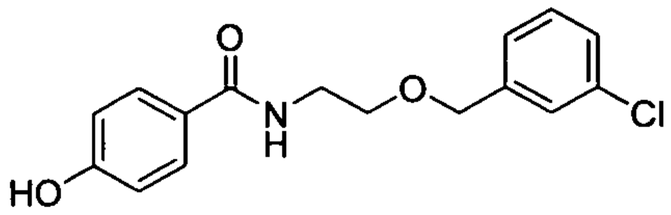
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 – 3.61 (m, 4H), 4.43 (s, 2H), 6.66 (brs, 1H), 7.10 – 7.12 (m, 2H), 7.18 – 7.19 (m, 2H), 7.25 (s, 1H), 7.30 – 7.31 (m, 1H), 7.41 – 7.45 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.9, 69.0, 72.4, 114.3 (d, *J* = 23.0 Hz, due to F), 118.4 (d, *J* = 20.8 Hz, due to F), 122.4 (d, *J* = 3.0 Hz, due to F), 125.7, 127.7, 128.0, 129.8, 130.2 (d, *J* = 8.2 Hz, due to F), 134.5, 136.7 (d, *J* = 6.7 Hz, due to F), 140.0, 163.0 (d, *J* = 245 Hz, due to F), 166.3 (d, *J* = 3.0 Hz, due to F).

N-(2-(3-Chlorobenzyloxy)ethyl)-3-chlorobenzamide (51)



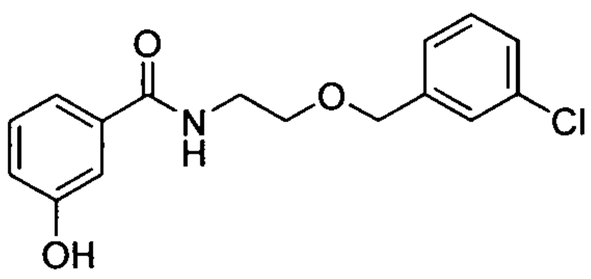
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (m, 4H), 4.49 (s, 2H), 6.52 (brs, 1H), 7.17 (d, *J* = 3.2 Hz, 1H), 7.24 (s, 2H), 7.31 – 7.36 (m, 2H), 7.44 (d, *J* = 3.6 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.73 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 69.1, 72.5, 125.1, 125.8, 127.5, 127.8, 128.1, 129.9, 130.0, 131.6, 134.6, 134.9, 136.3, 140.0, 166.3.

N-(2-(3-Chlorobenzyloxy)ethyl)-4-hydroxybenzamide (52)



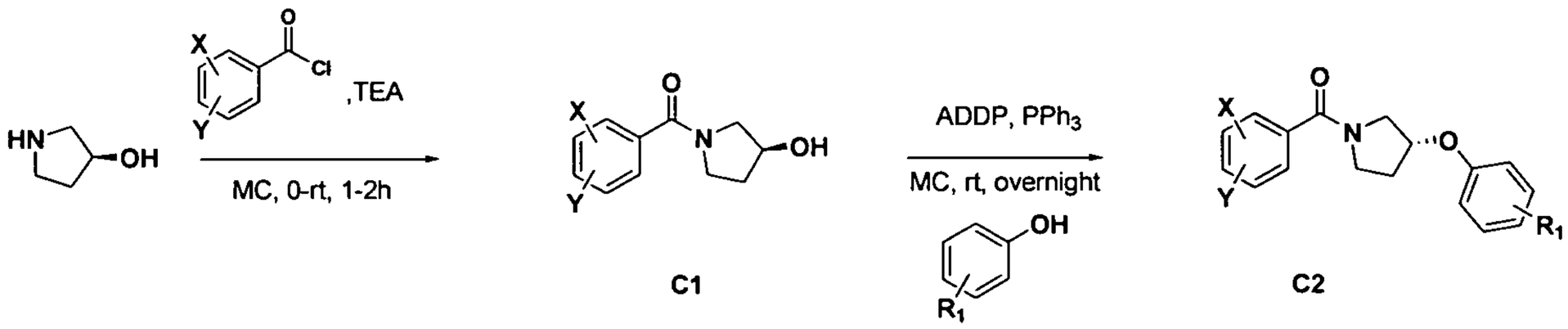
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 4H), 4.48 (s, 2H), 6.57 (brs, 1H), 6.84 (dd, *J* = 2.0, 8.8 Hz, 2H), 7.17 (d, *J* = 3.2 Hz, 1H), 7.23 (d, *J* = 3.2 Hz, 2H), 7.31 (s,1H), 7.60 (dd, *J* = 2.0, 8.8 Hz, 2H), 8.22 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 69.1, 72.5, 115.7, 125.4, 125.8, 127.8, 128.1, 129.0, 129.9, 134.5, 140.0, 160.2, 168.2.

N-(2-(3-Chlorobenzyloxy)ethyl)-3-hydroxybenzamide (53)



^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (m, 4H), 4.49 (s, 2H), 6.64 (brs, 1H), 6.98 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.17 – 7.26 (m, 5H), 7.30 (s, 1H), 7.50 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.0, 69.1, 72.5, 115.1, 117.8, 119.3, 125.9, 127.3, 128.1, 129.9, 130.0, 134.6, 135.4, 139.9, 157.2, 168.0.

Scheme 4



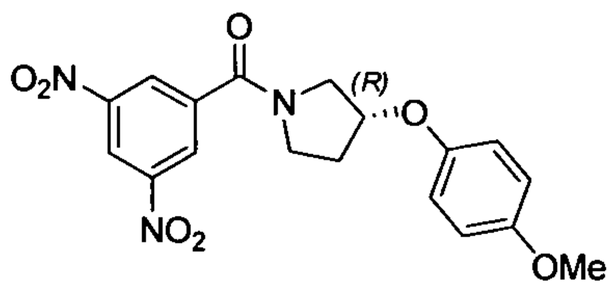
General procedure for the synthesis of phenoxy-pyrrolidin-1-yl-methanone (C2)

To a solution of (*S*)-3-pyrrolidinol (10 mmol) and triethylamine (11 mmol) in methylene chloride (50 mL) was added benzoyl chloride (8.67 mmol) at 0 °C. The reaction temperature was brought up to room temperature. After 2 h, the reaction mixture was diluted with

methylene chloride (50 mL) and then washed with 0.5 M HCl aqueous solution (100 mL) and brine (100 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (2:1 hexanes/ethyl acetate) to give C1.

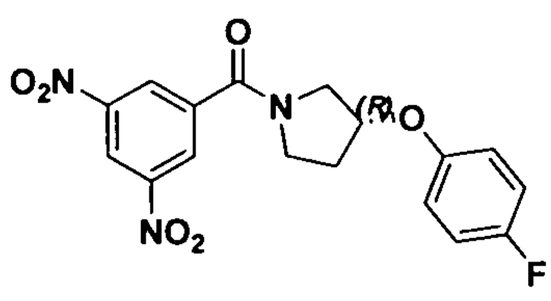
To a solution of C1 (1.07 mmol) in methylene chloride (10 mL) was added ADDP (1.28 mmol), triphenylphosphine (1.28 mmol) and a phenol (1.28 mmol) at room temperature. After stirring overnight, the reaction mixture was diluted with methylene chloride (30 mL) and washed with 1 M HCl aqueous solution (50 mL), saturated Na_2CO_3 aqueous solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude was purified by silica gel flash column chromatography (2:1 hexanes/ethyl acetate) and recrystallized from a mixture of hexanes and ethyl acetate to give C2.

(*R*)-(3,5-Dinitrophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (54)



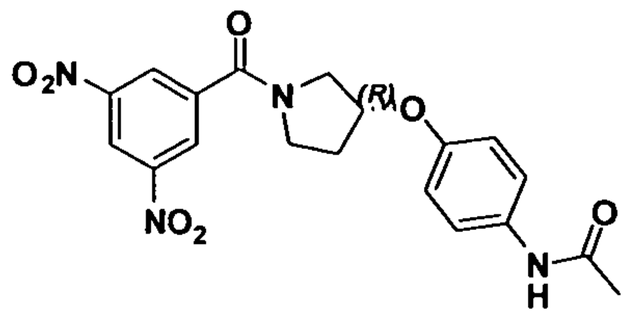
(Two rotamers, 1:1 ratio), m.p. 124 - 125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.11-2.19 (m, 1H), 2.30-2.34 (m, 1H), 3.54-3.64 (m, 1H), 3.72 & 3.76 (s, 3H), 3.81-3.99 (m, 3H), 4.86-4.94 (m, 1H), 6.74-6.84 (m, 4H), 8.68 & 8.75 (d, J = 1.6 Hz, 2H), 9.05 & 9.08 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.6, 32.4, 45.2, 47.7, 52.8, 54.8, 55.8, 55.9, 75.7, 115.0, 117.1, 117.3, 120.1, 120.2, 127.7, 127.9, 139.9, 140.0, 148.6, 150.4, 150.8, 154.8, 154.8, 164.7, 165.1; LC-MS (ESI, *m/z*): 388 $[M+H]^+$.

(*R*)-(3,5-Dinitrophenyl)(3-(4-fluorophenoxy)pyrrolidin-1-yl)methanone (55)



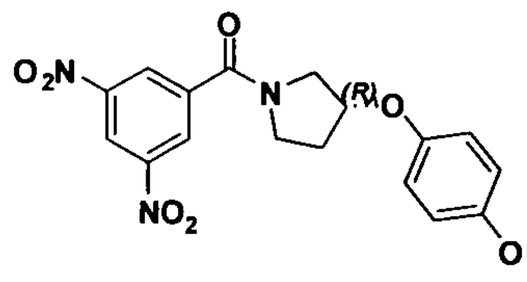
(Two rotamers, 1:1 ratio, 75 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.15-2.37 (m, 2H), 3.56-3.63 (m, 1H), 3.79-3.97 (m, 3H), 4.91-4.99 (m, 1H), 6.76-7.03 (m, 4H), 8.71 & 8.76 (d, J = 1.6 Hz, 2H), 9.08 & 9.10 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.3, 45.1, 47.7, 52.7, 54.8, 75.5, 77.0, 116.2, 116.5, 116.9, 117.0, 117.1, 120.1, 120.2, 127.7, 127.8, 139.8, 139.9, 148.6, 152.6, 152.9, 157.9 (d, J = 245 Hz, due to F), 164.7, 165.0.

(*R*)-*N*-(4-(1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yloxy)phenyl)acetamide (56)



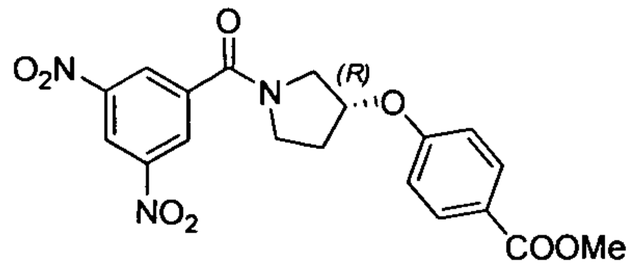
(Two rotamers, 1:1 ratio, 63 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.96 & 1.99 (s, 3H), 2.03-2.27 (m, 2H), 3.45-3.50 (m, 1H), 3.69-3.83 (m, 3H), 4.83-4.91 (m, 1H), 6.64 & 6.74 (d, *J* = 8.8 Hz, 2H), 7.26 & 7.33 (d, *J* = 8.8 Hz, 2H), 8.58 & 8.65 (d, *J* = 2.0 Hz, 2H), 8.95-8.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 23.3, 23.4, 29.7, 32.0, 45.0, 47.6, 52.6, 54.6, 75.0, 76.4, 115.8, 115.9, 120.0, 121.9, 127.4, 127.5, 127.6, 127.7, 132.4, 132.5, 139.4, 148.4, 152.8, 153.1, 165.0, 165.3, 169.7.

(*R*)-(3,5-Dinitrophenyl)(3-(4-(trifluoromethoxy)phenoxy)pyrrolidin-1-yl)methanone (57)



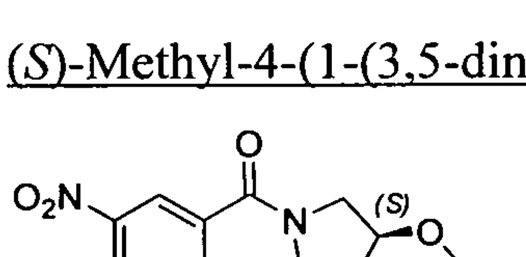
(Two rotamers, 6:4 ratio, 67 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20-2.40 (m, 2H), 3.59-3.66 (m, 1H), 3.84-4.00 (m, 3H), 4.97-5.05 (m, 1H), 6.83 & 6.92 (d, *J* = 8.8 Hz, 2H), 7.12 & 7.18 (d, *J* = 8.8 Hz, 2H), 8.73 & 8.77 (d, *J* = 2.0 Hz, 2H), 9.09 & 9.11 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.2, 45.1, 47.6, 52.6, 54.7, 75.2, 76.7, 116.4, 120.1, 122.8, 127.7, 127.8, 139.6, 139.7, 143.4, 148.5, 155.0, 155.2, 164.7, 164.9.

(*R*)-Methyl 4-(1-(3,5-dinitrobenzoyl)pyrrolidin-3-yloxy)benzoate (58)



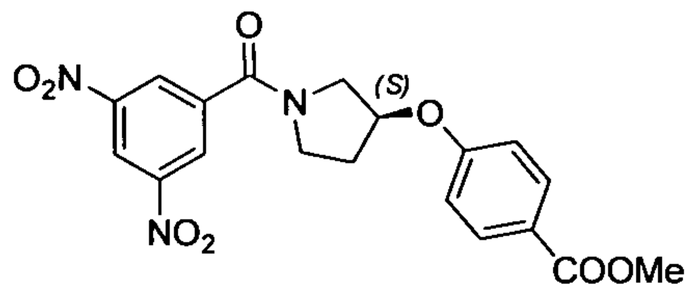
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 -2.37 (m, 2H), 3.57-3.65 (m, 1H), 3.85 & 3.87 (s, 3H), 3.89-3.99 (m, 3H), 5.03-5.11 (m, 1H), 6.82 & 6.91 (d, *J* = 7.2 Hz, 2H), 7.93 & 7.99 (d, *J* = 7.2 Hz, 2H), 8.70 & 8.75 (s, 2H), 9.07 & 9.09 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1, 30.0, 32.4, 45.2, 47.7, 52.2, 52.8, 54.8, 74.9, 76.3, 115.0, 120.36, 123.7, 123.8, 127.8, 127.9, 132.0, 139.7, 148.6, 160.2, 160.5, 164.7, 166.7.

(*R*)-(3,5-Dinitrophenyl)(3-(2-fluorophenoxy)pyrrolidin-1-yl)methanone (59)



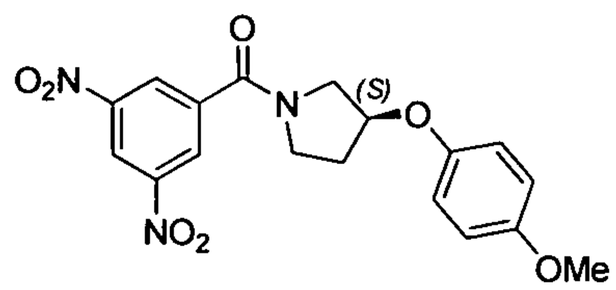
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, CD_3OD) δ 2.26-2.33 (m, 2H), 3.62-3.97 (m, 3H), 4.00 & 4.36 (s, 1H), 5.06 & 5.21 (s, 1H), 7.11 & 7.27 (m, 4H), 8.78 & 8.83 (d, *J* = 2.0 Hz, 2H), 9.01 & 9.04 (d, *J* = 2.0 Hz, 1H) ; ^{13}C NMR (100 MHz, CD_3OD) δ 29.9, 31.9, 44.9, 52.3, 54.4, 77.2, 78.7, 116.62, 116.67, 116.80, 116.85, 117.8 (d, *J* = 20 Hz, due to F), 120.04 (d, *J* = 3.7 Hz, due to F), 122.5, 122.6, 122.70, 122.77, 125.1, 125.15 (d, *J* = 3.7 Hz, due to F), 127.80 (d, *J* = 7.4 Hz due to F), 127.9, 139.8, 153.6 (d, *J* = 244 Hz, due to F), 165.4, 165.5.

(*S*)-Methyl-4-(1-(3,5-dinitrobenzoyl)pyrrolidin-3-yloxy)benzoate (60)



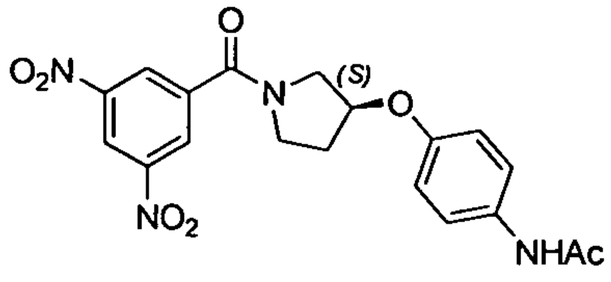
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, Acetone-d_6) δ 2.21-2.29 (m, 2H), 3.58 & 3.61 (s, 1H), 3.69 & 3.71 (s, 3H), 3.73-4.02 (m, 3H), 4.99 & 5.06 (s, 1H), 6.77-6.94 (m, 4H), 8.73 & 8.77 (s, 2H), 8.96 & 8.99 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 31.9, 44.1, 44.7, 52.2, 54.2, 55.1, 55.2, 76.0, 77.5, 114.82, 114.88, 117.2, 119.6, 127.7, 127.8, 140.5, 148.7, 151.1, 151.3, 154.7, 164.6, 164.7.

(*S*)-(3,5-dinitrophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (61)



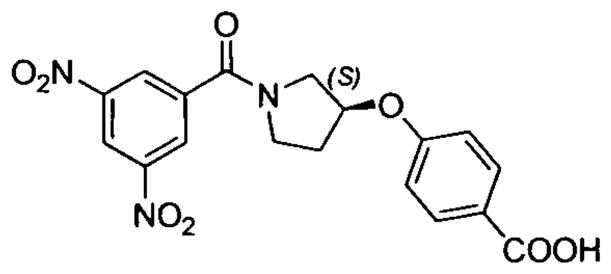
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, Acetone-d_6) δ 2.19-2.28 (m, 2H) 3.60-4.01 (m, 4H), 4.98 & 5.06 (s, 1H), 6.76-6.94 (m, 4H), 8.73 & 8.76 (s, 2H), 8.95 & 8.99 (s, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 31.9, 44.7, 52.2, 54.2, 55.0, 55.1, 65.8, 75.9, 77.5, 114.81, 114.87, 117.2, 119.6, 127.7, 127.8, 128.6, 129.8, 140.4, 148.7, 151.3, 154.7, 164.6, 164.7.

(*S*)-*N*-(4-(1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yloxy)phenyl)acetamide (62)



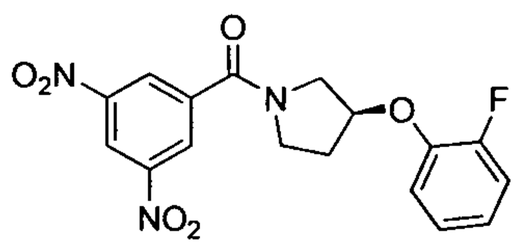
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, Acetone-d_6) δ 1.99 (s, 3H), 2.22-2.28 (m, 2H), 3.54-4.06 (m, 3H), 5.04 & 5.11 (s, 1H), 6.80 & 6.90 (d, J = 8.8 Hz, 1H), 7.46-7.70 (m, 4H, brs, 1H), 8.73 & 8.76 (s, 2H), 8.95 & 8.99 (s, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 24.1, 24.2, 30.0, 32.2, 45.2, 47.7, 52.7, 54.7, 75.1, 76.6, 115.9, 120.0, 120.1, 127.7, 127.8, 128.7, 128.8, 131.6, 132.0, 132.4, 132.6, 132.7, 132.8, 139.7, 148.4, 153.1, 165.0, 169.1.

(*S*)-4-(1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yloxy)benzoic acid (63)



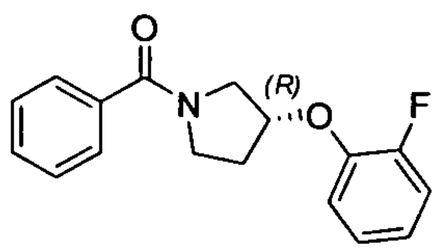
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, Acetone-d_6) δ 2.31-2.42 (m, 2H), 3.61-3.65 (m, 1H), 3.75-4.06 (m, 3H), 5.19 & 5.28 (s, 1H), 7.02 & 7.13 (d, J = 8.8 Hz, 2H), 7.98 & 8.06 (d, J = 8.8 Hz, 2H), 8.72 & 8.78 (d, J = 2.0 Hz, 2H), 9.02 & 9.05 (s, 1H).

(*S*)-(3,5-Dinitrophenyl)(3-(2-fluorophenoxy)pyrrolidin-1-yl)methanone (64)



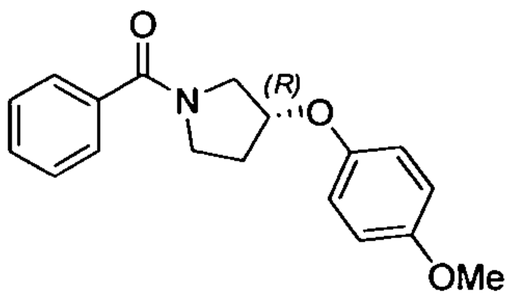
(Two rotamers, 1:1 ratio), ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.14-2.24 (m, 2H), 3.50-3.88 (m, 4H), 4.98 & 5.08 (s, 1H), 6.86-7.15 (m, 4H), 8.65 & 8.69 (s, 2H), 8.88 & 8.92 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.1, 31.1, 44.1, 51.5, 53.6, 76.4, 77.9, 115.7, 115.8, 115.9, 116.0, 117.1 (d, J = 22.3 Hz, due to F), 119.2 (d, J = 3.7 Hz, due to F), 121.7, 121.83, 121.88, 121.9, 124.2 (d, J = 3.7 Hz, due to F), 127.0, 139.0, 144.1, 144.4, 148.0, 152.8 (d, J = 242.6 Hz, due to F), 164.6, 164.7.

(*R*)-(3-(2-Fluorophenoxy)pyrrolidin-1-yl)(phenyl)methanone (65)



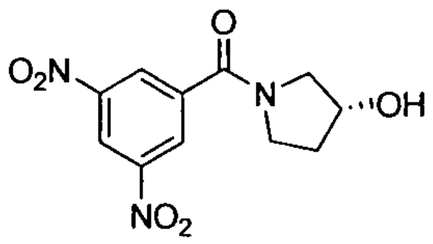
(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02-2.24 (m, 2H), 3.51-3.91 (m, 4H), 4.85 & 4.98 (s, 1H), 6.86-7.09 (m, 4H), 7.36-7.48 (m, 3H), 7.52 (d, *J* = 5.2, 1H), 7.53 (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.3, 32.4, 44.3, 47.5, 52.1, 54.8, 78.0, 79.0, 116.8, 117.0, 117.9, 118.6, 122.6, 122.7, 122.9, 123.0, 124.6 (d, *J* = 3.7 Hz due to F), 127.2, 127.4, 128.5, (d, *J* = 3.7 Hz, due to F), 130.1, 130.3, 136.7, 136.9, 144.7 (d, *J* = 20.1 Hz due to F), 153.8 (d, *J* = 245.6 Hz, due to F), 155.2, 170.0, 170.2.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(phenyl)methanone (66)



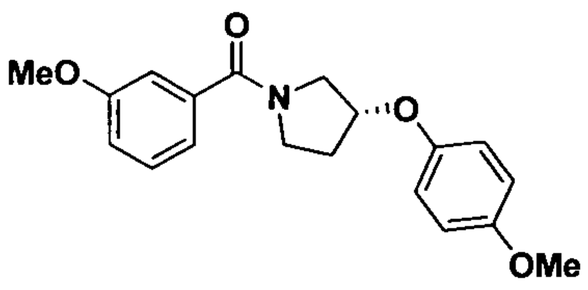
(Two rotamers, 1:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99-2.21 (m, 2H), 3.48-3.66 (m, 2H), 3.68 & 3.73 (s, 3H), 3.79-3.89 (m, 2H), 4.74 & 4.96 (s, 1H), 6.71 (s, 2H), 6.76 (s, 2H), 7.34 & 7.36 (d, *J* = 5.6 Hz, 3H), 7.46 & 7.52 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 32.3, 44.4, 47.6, 52.1, 54.8, 55.8, 55.9, 76.0, 114.9, 115.0, 117.1, 117.3, 127.3, 127.4, 128.50, 128.54, 130.1, 130.2, 136.8, 137.0, 150.9, 151.1, 154.5, 154.6, 169.9, 170.2; LC-MS (ESI, *m/z*): 298.1 $[M+H]^+$.

(*R*)-(3,5-Dinitrophenyl)(3-hydroxypyrrolidin-1-yl)methanone (67)



(Two rotamers, 1:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98-2.11 (m, 2H), 3.23 (brs, 1H), 3.37-3.48 (m, 1H), 3.61-3.79 (m, 3H), 4.47 & 4.56 (s, 1H), 8.62 & 8.67 (s, 2H), 8.99 - 9.00 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.0, 34.9, 45.1, 47.6, 55.5, 57.5, 69.4, 70.9, 120.1, 120.2, 127.8, 139.8, 139.9, 148.5, 165.1, 165.3.

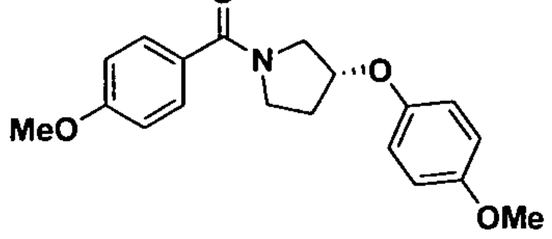
(*R*)-(3-(3-Methoxyphenoxy)pyrrolidin-1-yl)(3-methoxyphenyl)methanone (68)



(Two rotamers, 1:1 ratio, 85 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 – 2.22 (m, 2H), 3.48 – 3.65 (m, 2H), 3.68 & 3.71 (s, 3H), 3.73 & 3.76 (s, 3H), 3.79 – 3.89 (m,

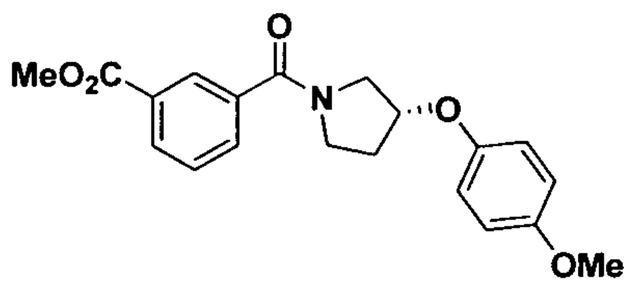
2H), 4.74 – 4.84 (m, 1H), 6.70 – 6.80 (m, 4H), 6.86 – 6.92 (m, 1H), 6.99 & 7.01 (s, 1H), 7.04 & 7.08 (s, 1H), 7.21 – 7.28 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 32.3, 44.5, 47.7, 52.2, 54.8, 55.6, 55.8, 76.0, 112.6, 112.8, 114.9, 115.0, 116.1, 116.6, 117.1, 117.2, 119.4, 119.6, 129.27, 129.32, 138.1, 150.9, 151.1, 154.5, 159.7, 169.8.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(3-methoxyphenyl)methanone (69)



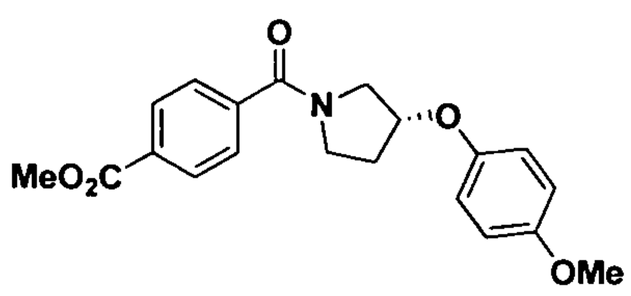
(Two rotamers, 1:1 ratio, 83 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 – 2.22 (m, 2H), 3.48 – 3.65 (m, 2H), 3.68 & 3.71 (s, 3H), 3.73 & 3.76 (s, 3H), 3.79 – 3.89 (m, 2H), 4.72 – 4.84 (m, 1H), 6.70 – 6.80 (m, 4H), 6.86 – 6.92 (m, 1H), 6.99 – 7.08 (m, 2H), 7.21 – 7.28 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.2, 32.1, 44.4, 47.6, 52.1, 54.4, 55.33, 55.62, 75.8, 113.4, 114.7, 116.9, 128.63, 128.75, 129.16, 129.32, 131.9, 150.9, 154.3, 160.9, 169.48, 169.79.

(*R*)-Methyl 3-(3-(4-methoxyphenoxy)pyrrolidine-1-carbonyl)benzoate (70)



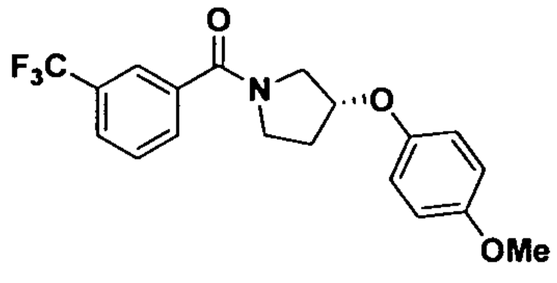
(Two rotamers, 1:1 ratio, 87 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 – 2.24 (m, 2H), 3.45 – 3.65 (m, 2H), 3.67 & 3.71 (s, 3H), 3.75 – 3.82 (m, 2H), 3.86 & 3.87 (s, 3H), 4.74 – 4.86 (m, 1H), 6.72 & 6.80 (m, 4H), 7.40 – 7.67 (m, 1H), 7.66 & 7.71 (d, J = 7.6 Hz, 1H), 8.04 (t , J = 9.0 Hz, 1H), 8.13 & 8.19 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.0, 44.3, 47.3, 52.2, 54.5, 55.5, 55.6, 75.7, 114.7, 114.8, 116.9, 117.0, 128.1, 128.2, 128.5, 128.6, 130.9, 134.0, 131.5, 131.6, 136.8, 136.9, 150.5, 150.7, 154.33, 154.38, 166.6, 168.6, 168.9.

(*R*)-Methyl 4-(3-(4-methoxyphenoxy)pyrrolidine-1-carbonyl)benzoate (71)



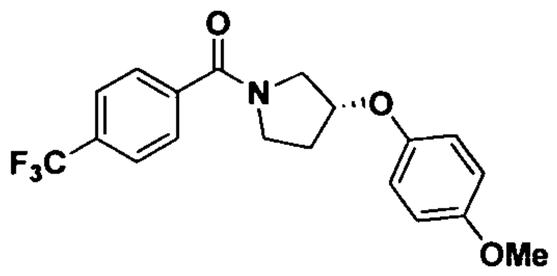
(Two rotamers, 1:1 ratio, 85 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 – 2.11 (m, 1H), 2.15 – 2.25 (m, 1H), 3.42 – 3.67 (m, 2H), 3.68 & 3.71 (s, 3H), 3.77 – 3.81 (m, 1H), 3.83 – 3.88 (m, 1H), 3.86 & 3.88 (s, 3H), 4.73 – 4.86 (m, 1H), 6.69 – 6.75 (m, 2H), 6.80 (s, 2H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.0, 44.2, 47.2, 51.9, 52.2, 54.3, 55.5, 55.6, 75.6, 114.7, 114.8, 116.8, 117.0, 127.0, 127.1, 129.5, 129.6, 131.2, 131.3, 140.7, 140.8, 150.5, 150.7, 154.3, 154.4, 168.7, 168.9.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(3-(trifluoromethyl)phenyl)methanone (72)



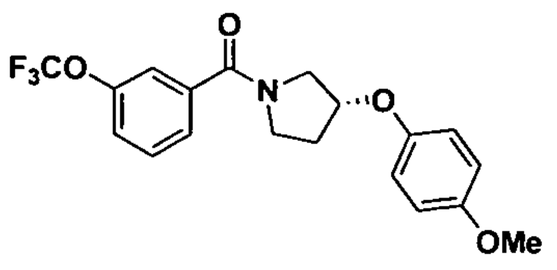
(Two rotamers, 1:1 ratio, 82 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 – 2.15 (m, 1H), 2.21 – 2.30 (m, 1H), 3.48 – 3.67 (m, 2H), 3.72 & 3.75 (s, 3H), 3.78 – 3.90 (m, 2H), 4.79 – 4.90 (m, 1H), 6.74 – 6.83 (m, 4H), 7.48 – 7.55 (m, 1H), 7.64 – 7.82 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.0, 32.1, 44.5, 47.5, 52.2, 54.6, 55.7, 55.8, 75.8, 114.8, 114.9, 117.0, 117.2, 124.2, 124.3, 129.0, 129.1, 130.4, 130.6, 137.3, 137.4. 150.6, 150.8, 154.5, 154.6, 168.3, 168.6.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(4-(trifluoromethyl)phenyl)methanone (73)



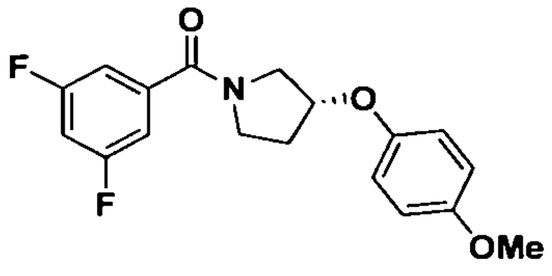
(Two rotamers, 1:1 ratio, 55 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 – 2.06 (m, 1H), 2.20–2.25 (m, 1H), 3.49–3.70 (m, 2H), 3.72 & 3.75 (s, 3H), 3.81–3.88 (m, 2H), 4.72 & 8.89 (m, 1H), 6.74–6.83 (m, 4H), 7.23–7.50 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.0, 32.2, 44.5, 47.5, 52.2, 54.6, 55.7, 55.8, 75.8, 76.8, 114.9, 117.0, 117.2, 119.8, 120.1, 122.5, 122.6, 125.6, 125.8, 130.0, 130.1, 138.5, 149.1, 150.6, 150.9, 154.5, 168.2.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(3-(trifluoromethoxy)phenyl)methanone (74)



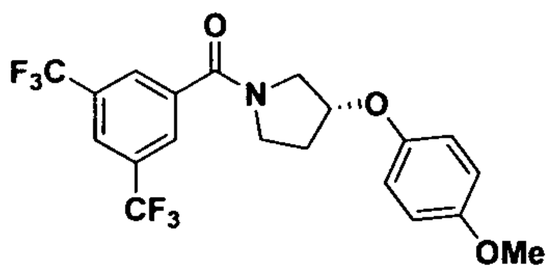
(Two rotamers, 1:1 ratio, 67 %), a yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.23 (m, 2H), 3.43 – 3.68 (m, 2H), 3.69 & 3.72 (s, 3H), 3.72 – 3.83 (m, 2H), 4.75 – 4.88 (m, 1H), 6.72 – 6.82 (m, 4H), 7.58 – 7.66 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 30.1, 32.2, 38.9, 44.6, 47.6, 52.2, 54.7, 55.8, 75.9, 114.9, 115.0, 117.1, 117.3, 125.5, 125.6, 127.7, 128.8, 150.8, 151.0, 154.6, 154.7, 168.5, 168.6.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(3-nitrophenyl)methanone (75)



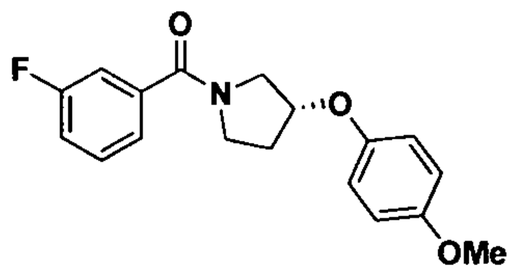
(Two rotamers, 1:1 ratio, 84 %), a yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 – 2.24 (m, 2H), 3.48 – 3.56 (m, 1H), 3.68 & 3.72 (s, 3H), 3.73 – 3.88 (m, 3H), 4.79 – 4.89 (m, 1H), 6.71 – 6.83 (m, 4H), 7.52 – 7.59 (m, 1H), 7.81 & 7.87 (d, *J* = 7.6 H, 1H), 8.22 (t, *J* = 9.8 Hz, 1H), 8.32 & 8.38 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.0, 44.5, 47.4, 52.2, 54.5, 55.5, 55.6, 75.6, 77.0, 114.7, 114.8, 116.9, 117.0, 122.2, 122.3, 124.6, 124.7, 129.6, 133.1, 133.2, 138.0, 138.1, 147.8, 150.4, 150.6, 154.3, 154.4, 166.9, 167.2.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(4-nitrophenyl)methanone (76)



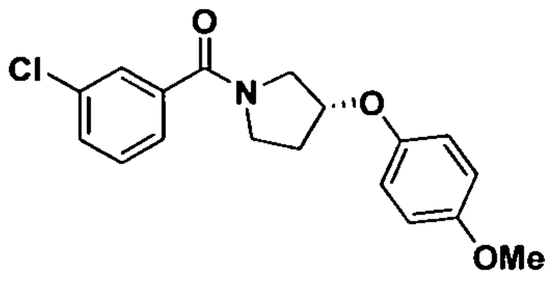
(Two rotamers, 1:1 ratio, 73 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.31 (m, 2H), 3.44 – 3.69 (m, 2H), 3.72 & 3.75 (s, 3H), 3.80 – 3.90 (m, 2H), 4.79 - 4.90 (m, 1H), 6.72 – 6.82 (m, 4H), 7.63 & 7.70 (d, *J* = 8.0 Hz, 2H), 8.22 & 8.24 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 31.9, 44.3, 47.2, 52.0, 55.5, 75.5, 76.7, 114.7, 114.8, 116.8, 116.9, 123.5, 128.0, 128.2, 128.6, 142.4, 142.5, 148.4, 150.3, 150.6, 154.3, 154.4, 167.3, 167.6.

(*R*)-(3-Fluorophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (77)



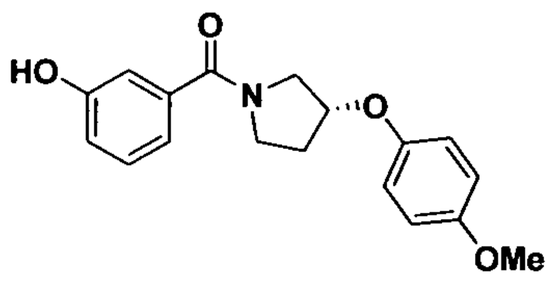
(Two rotamers, 1:1 ratio, 78 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.11 (m, 1H), 2.12 – 2.42 (m, 1H), 3.48 – 3.69 (m, 2H), 3.71 & 3.74 (s, 3H), 3.78 – 3.87 (m, 2H), 4.76 – 4.88 (m, 1H), 6.72 – 6.82 (m, 4H), 7.05 – 7.36 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.0, 44.3, 47.4, 52.0, 54.5, 55.6, 75.7, 114.4, 114.8, 116.9, 117.1, 122.8, 122.9, 130.1, 130.2, 138.6, 138.7, 150.6, 150.8, 154.4, 154.5, 162.4 (d, J = 245 Hz, due to F), 168.3, 168.5.

(*R*)-(3-Chlorophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (78)



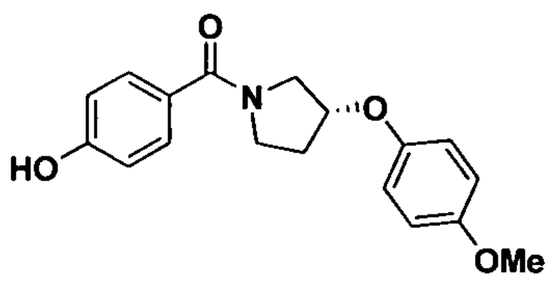
(Two rotamers, 1:1 ratio, 87 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.24 (m , 2H), 3.47 – 3.69 (m, 2H), 3.71 & 3.74 (s, 3H), 3.78 – 3.86 (m, 2H), 4.75 – 4.88 (m, 1H), 6.73 – 6.82 (m, 4H), 7.26 – 7.42 (m, 3H), 7.46 & 7.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.0, 44.3, 47.4, 52.0, 54.5, 55.6, 55.7, 75.7, 76.7, 114.7, 114.8, 116.6, 117.1, 125.1, 125.3, 127.3, 127.4, 129.7, 129.8, 130.0, 130.1, 134.3, 138.2, 138.3, 150.5, 150.7, 154.4, 168.1, 168.4

(*R*)-(3-Hydroxyphenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (79)



(Two rotamers, 1:1 ratio, 53 %), a white liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 – 2.25 (m, 2H), 3.53 – 3.74 (m, 2H), 3.77 & 3.81 (s, 3H), 3.83 – 3.94 (m, 2H), 4.73 & 4.87 (m, 1H), 6.72 – 6.82 (m, 4H), 6.85 – 6.98 (m, 2H), 7.08 – 7.20 (m, 2H), 8.21 (brs, 1H);

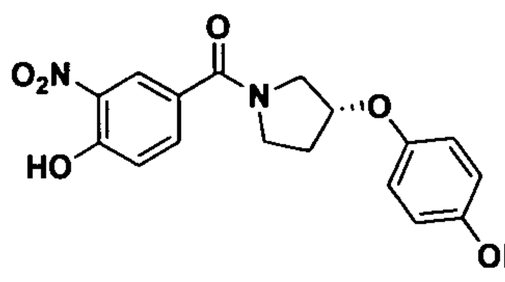
(*R*)-(4-Hydroxyphenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (80)



(Two rotamers, 1:1 ratio, 37 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 – 2.32 (m, 2H), 3.59 – 3.71 (m, 2H), 3.74 & 3.76 (s, 3H), 3.79 – 3.93 (m, 2H), 4.80 – 4.91 (m, 1H),

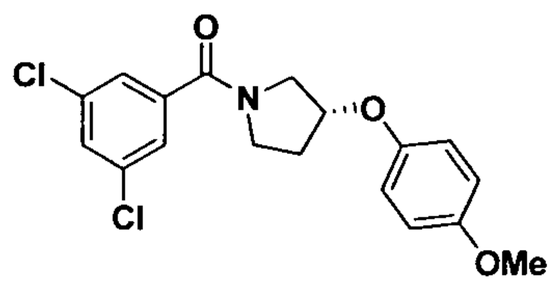
6.75 – 6.84 (m, 4H), 7.21 – 7.24 (m, 2H), 7.56 & 7.62 (d, *J* = 8.0 Hz, 2H), 8.01 & 8.03 (brs, 1H).

(*R*)-(4-Hydroxy-3-nitrophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (81)



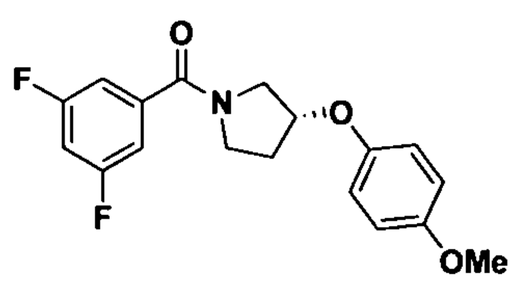
(Two rotamers, 1:1 ratio, 63 %), a yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.14 (m, 1H), 2.25 – 2.27 (m, 1H), 3.56 – 3.65 (m, 2H), 3.72 &3.74 (s, 3H), 3.81 – 3.91 (m, 2H), 4.81 - 4.89 (m, 1H), 6.76 (m, 4H), 7.16 (t, *J* = 9.4 Hz, 1H), 7.78 & 7.84 (d, *J* = 8.4 Hz, 1H), 8.29 & 8.37 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 31.8, 45.0, 47.6, 52.6, 54.9, 55.9, 115.1, 117.2, 117.3, 120.4, 124,7, 125.0, 128.8, 133.1, 136.9, 137.0, 151.0, 154.7, 156.4, 166.9, 167.3.

(*R*)-(3,5-Dichlorophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (82)



(Two rotamers, 1:1 ratio, 85 %), a pale yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 - 2.10 (m, 1H), 2.20 - 2.25 (m, 1H), 3.47 – 3.70 (m, 2H), 3.72 & 3.74 (s, 3H), 3.75 - 3.85 (m, 2H), 4.78 - 4.87 (m, 1H), 6.74 - 6.82 (m, 4H), 7.34 -7.41 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.0, 44.4, 47.4, 52.1, 54.4, 55.6, 55.7, 75.5, 114.8, 116.9, 125.6, 125.7, 130.0, 135.1, 139.2, 139.3, 150.4, 150.7, 154.4, 154.5, 166.7, 167.0.

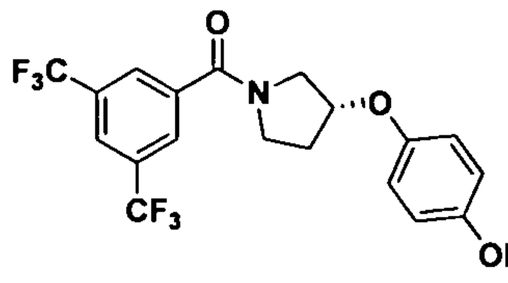
(*R*)-(3,5-Difluorophenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (83)



(Two rotamers, 1:1 ratio, 75 %), a yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 – 2.27 (m, 2H), 3.48 – 3.67 (m, 2H), 3.71 & 3.74 (s, 3H), 3.77 – 3.85 (m, 2H), 4.78 - 4.88 (m, 1H), 6.73 – 6.87 (m, 5H), 6.99 & 7.06 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.0, 44.4, 47.3, 52.1, 54.9, 55.6, 75.6, 105.3, 105.4, 110.3, 110.4, 110.5, 110.7, 114.8, 116.9,

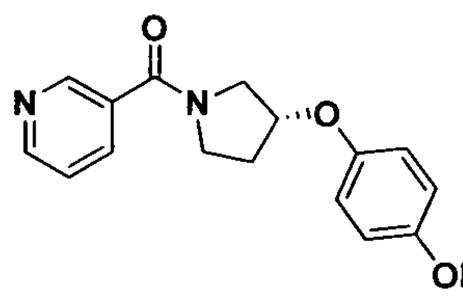
117.1, 150.2, 154.9, 162.4 (d, J = 250 Hz, due to F), 162.5 (d, J = 250 Hz, due to F), 167.0, 167.3.

(*R*)-(3,5-Bis(trifluoromethyl)phenyl)(3-(4-methoxyphenoxy)pyrrolidin-1-yl)methanone (84)



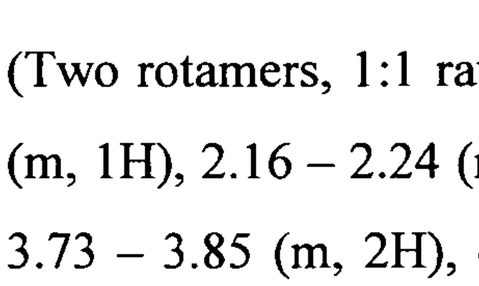
(Two rotamers, 1:1 ratio, 65 %), a yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08 – 2.14 (m, 1H), 2.24 – 2.29 (m, 1H), 3.47 – 3.67 (m, 2H), 3.71 & 3.74 (s, 3H), 3.76 – 3.91 (m, 2H), 4.81 – 4.91 (m, 1H), 6.74 – 6.83 (m, 4H), 7.90 – 8.12 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.1, 44.6, 47.4, 52.3, 54.5, 55.6, 75.6, 114.8, 114.9, 116, 9, 117.2, 123.7, 124.3, 127.5, 127.7, 131.1, 132.1, 138.5, 138.6, 150.4, 150.7, 154.5, 154.7, 166.5, 166.8.

(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(pyridin-3-yl)methanone (85)



(Two rotamers, 1:1 ratio, 82 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 – 2.10 (m, 1H), 2.16 – 2.24 (m, 1H), 3.48 – 3.58 (m, 1H), 3.64 – 3.73 (m, 1H), 3.67 & 3.69 (s, 3H), 3.73 – 3.85 (m, 2H), 4.75 – 4.85 (m, 1H), 6.69 – 6.78 (m, 4H), 7.25 – 7.31 (m, 1H), 7.78 &7.83 (d, J = 7.6 Hz, 1H), 8.57 – 8.61 (m, 1H), 8.71 & 8.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 31.9, 44.3, 47.2, 51.9, 54.4, 55.49, 55.53, 75.5, 114.66, 114.69, 116.8, 116.9, 123.1, 123.2, 132.3, 134.8, 134.9, 147.9, 148.1, 150.39, 150.63, 150.83, 150.89, 154.2, 154.3, 167.0, 167.3.

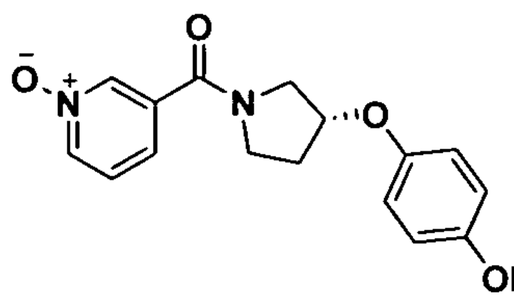
(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(pyridin-4-yl)methanone (86)



(Two rotamers, 1:1 ratio, 79 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 – 2.23 (m, 2H), 3.46 – 3.67 (m, 2H), 3.70 & 3.72 (s, 3H), 3.73 – 3.90 (m, 2H), 4.78 – 4.88 (m, 1H), 6.76 – 6.82 (m, 4H), 7.34 (s, 1H), 7.40 (s, 1H), 8.66 (d, J = 13.2 Hz, 2H); ^{13}C NMR (100

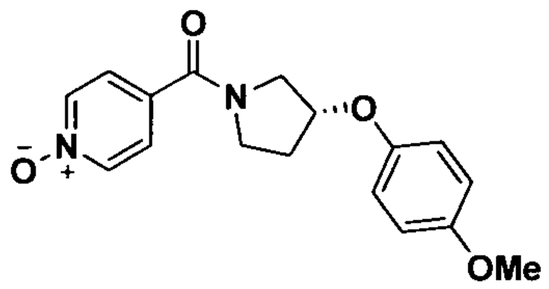
MHz, $CDCl_3$) δ 29.6, 31.7, 44.0, 46.8, 51.7 ,53.9, 55.3, 55.4, 75.3, 114.5, 114.6, 116.7, 116.8, 120.9, 121.0, 143.6, 143.7, 149.7, 150.2, 154.1, 154.2, 166.9, 167.1.

(*R*)-4-(3-(4-Methoxyphenoxy)pyrrolidine-1-carbonyl)pyridine 1-oxide (87)



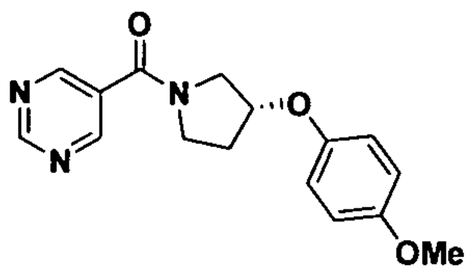
(Two rotamers, 1:1 ratio, 97 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 - 2.11 (m, 1H), 2.21 – 2.26 (m, 1H), 3.50 – 3.68 (m, 2H), 3.70 & 3.72 (s, 3H), 3.74 – 3.88 (m, 2H), 4.79 – 4.87 (m, 1H), 6.70 – 6.81 (m, 4H), 7.25 – 7.41 (m, 2H), 8.17 – 8.20 (m, 1H), 8.29 & 8.35 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.0, 44, 7, 47.3, 52.3, 54.4, 55.7, 75.4, 114.8, 116.9, 117.0, 124.5, 126.0, 126.1, 135.7, 135.8, 137.9, 138.1, 140.1, 150.3, 154.5, 154.5, 164.1, 164.3.

(*R*)-4-(3-(4-Methoxyphenoxy)pyrrolidine-1-carbonyl)pyridine-1-oxide (88)



(Two rotamers, 1:1 ratio, 95 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 - 2.10 (m, 1H), 2.22 – 2.27 (m, 1H), 3.52 – 3.68 (m, 2H), 3.70 & 3.72 (s, 3H), 3.76 – 3.83 (m, 2H), 4.80 – 4.87 (m, 1H), 6.70 – 6.79 (m, 4H), 7.40 (d, *J* = 6.4 Hz, 1H), 7.47 (d, *J* = 6.8 Hz, 1H), 8.13 – 8.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 32.1, 44.7, 47.3, 52.4, 54.4, 55.6, 75.4, 114.8, 116.9, 125.0, 125.1, 132.9, 133.0, 139.1, 150.3, 150.6, 154.4, 154.6, 165.3.

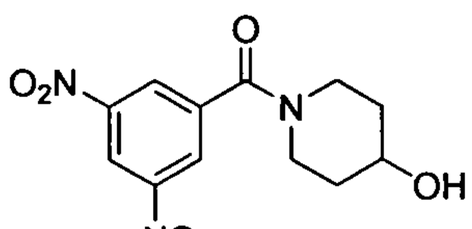
(*R*)-(3-(4-Methoxyphenoxy)pyrrolidin-1-yl)(pyrimidin-5-yl)methanone (89)



(Two rotamers, 1:1 ratio, 84 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 – 2.13 (m, 1H), 2.23 – 2.28 (m, 1H), 3.52 – 3.67 (m, 2H), 3.69 & 3.72 (s, 3H), 3.78 – 3.88 (m, 2H), 4.79 – 4.89 (m, 1H), 6.70 – 6.80 (m, 4H), 8.56 & 8.91 (s, 2H), 9.20 & 9.22 (s, 1H); ^{13}C

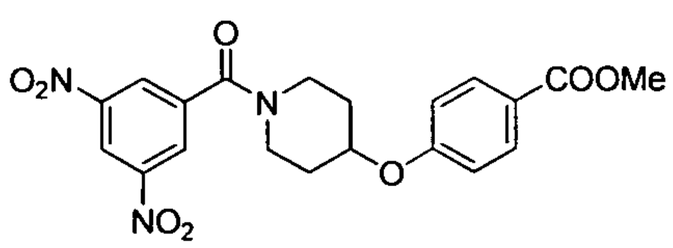
NMR (100 MHz, $CDCl_3$) δ 29.8, 32.1, 44.7, 52.3, 54.4, 55.6, 55.7, 75.5, 114.8, 116.9 ,117.0 130.2, 130.3, 150.3, 150.6, 154.5, 154.6, 155.5, 155.6, 159.4, 159.5, 164.5.

(3,5-Dinitrophenyl)(4-hydroxypiperidin-1-yl)methanone (90)



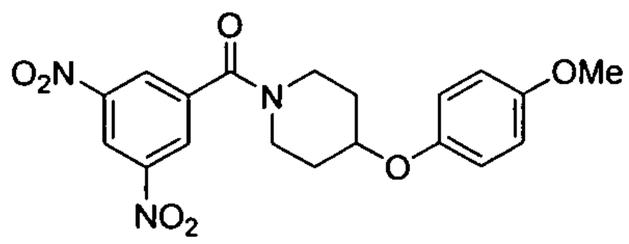
^{1}H NMR (400 MHz, Acetone-d_6) δ 1.50-1.56 (m, 2H), 1.80-1.90 (m, 2H), 3.30-3.42 (m, 2H), 3.63 (brs, 1H), 3.94-4.05 (m, 3H), 8.61 (d, J = 2.0 Hz, 2H), 8.95 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 33.7, 34.5, 39.5, 44.9, 66.0, 119.1, 127.4, 140.2, 148.8, 165.1.

Methyl 4-(1-(3,5-dinitrobenzoyl)piperidin-4-yloxy)benzoate (91)



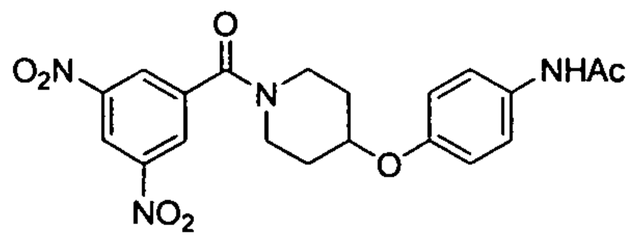
^{1}H NMR (400 MHz, Acetone-d_6) δ 1.84 (brs, 2H), 1.96 (brs, 2H), 3.31 (brs, 1H), 3.59-3.74 (m, 2H), 3.77 (s, 3H), 3.84-3.96 (m, 1H), 4.63-4.66 (m, 1H), 6.81-6.85 (m, 2H), 7.87-7.90 (m, 2H), 8.50 (d, J = 2.0 Hz, 2H), 8.97 (d, J = 2.0 Hz, 1H).

(3,5-Dinitrophenyl)(4-(4-methoxyphenoxy)piperidin-1-yl)methanone (92)



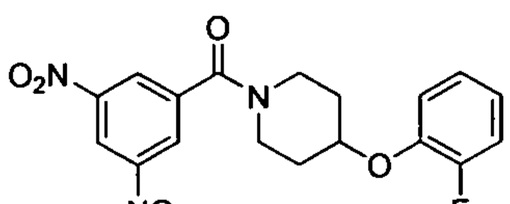
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85-1.98 (m, 4H), 3.35 (brs, 1H), 3.68-3.80 (m, 2H), 3.73 (s, 3H), 3.93 (brs, 1H), 4.49 (brs, 1H), 6.79 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 8.57 (s, 2H), 9.03 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.0, 31.2, 39.3, 44.6, 55.8, 71.9, 115.0, 117.9, 119.8, 127.5, 139.6, 148.7, 150.8, 154.6, 165.4.

N-(4-(1-(3,5-Dinitrobenzoyl)piperidin-4-yloxy)phenyl)acetamide (93)



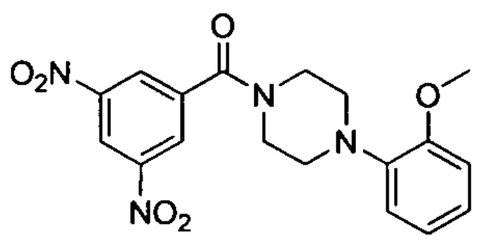
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62-1.96 (m, 4H), 1.97 (s, 3H), 3.48 (m, 3H), 3.93 (brs, 1H), 4.56 (s, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 8.64 (s, 2H), 8.33 (s, 1H), 9.74 (s, 1H) ;

(3,5-Dinitrophenyl)(4-(2-fluorophenoxy)piperidin-1-yl)methanone (94)



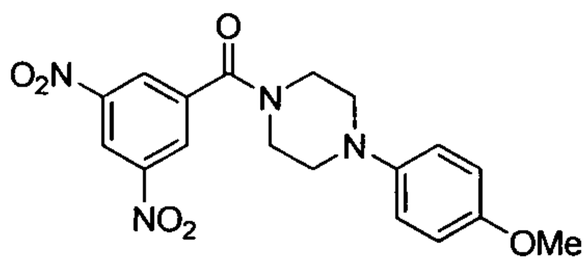
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.70-2.10 (m, 4H), 3.39-4.11 (m, 4H), 4.59 (m, 1H), 6.86-6.92 (m, 1H), 7.01-7.15 (m, 3H), 8.60 (d, J = 2.0 Hz, 2H), 8.89 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.1, 31.9, 45.5, 49.6, 75.0, 117.6 (d, J = 18.6 Hz, due to F), 119.5, 120.5, 123.3 (d, J = 6.7 Hz, due to F), 126.0 (d, J = 3.7 Hz, due to F), 128.6, 140.6, 146.1, 149.8, 154.8 (d, J = 242.6 Hz, due to F), 166.9.

(3,5-Dinitrophenyl)(4-(2-methoxyphenyl)piperazin-1-yl)methanone (95)



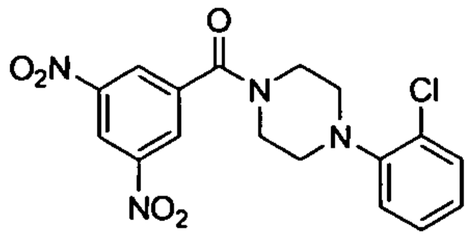
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.02-3.12 (m, 4H), 3.62 (brs, 2H), 3.82 (s, 3H), 3.87 (brs, 2H), 6.85-6.95 (m, 4H), 8.68 (d, J = 2.0 Hz, 2H), 8.96 (d, J = 2.4 Hz, 1H); LC-MS (ESI, *m/z*): 387 $[M+H]^+$.

(3,5-Dinitrophenyl)(4-(4-methoxyphenyl)piperazin-1-yl)methanone (96)



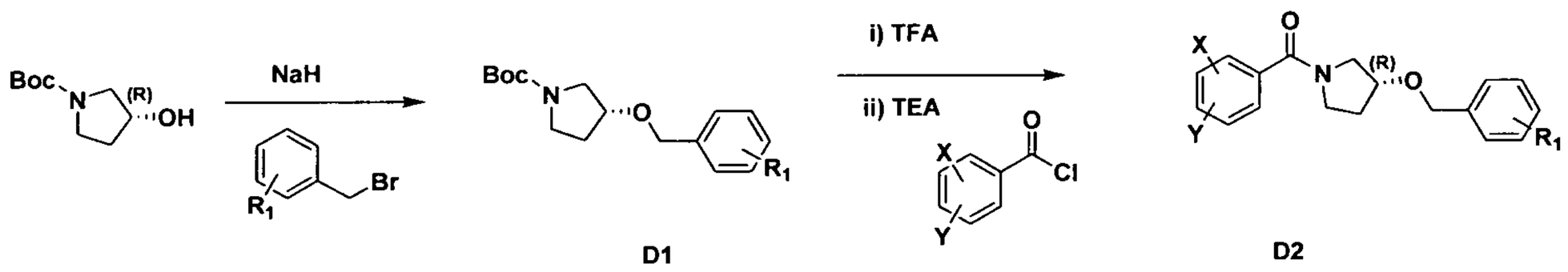
^{1}H NMR (400 MHz, Acetone-d_6) δ 3.08-3.17 (m, 4H), 3.68 (brs, 2H), 3.71 (s, 3H), 3.88 (brs, 2H), 6.82 (d, J = 8.8 Hz, 2H), 6.93, (d, J = 8.8 Hz, 2H), 8.69 (d, J = 2.0 Hz, 2H), 8.98 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 42.4, 47.7, 50.5, 50.9, 54.9, 114.4, 118.8, 119.3, 127.7, 139.9, 145.6, 148.8, 154.5, 165.2; LC-MS (ESI, *m/z*): 387 $[M+H]^+$.

(4-(2-chlorophenyl)piperazin-1-yl)(3,5-dinitrophenyl)methanone (97)



^{1}H NMR (400 MHz, Acetone-d_6) δ 3.09-3.17 (m, 4H), 3.70 (brs, 2H), 3.94 (brs, 2H), 7.07 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 8 Hz, 1H), 7.30 (t, J = 8 Hz, 1H), 7.41 (d, J = 8 Hz, 1H), 8.72 (s, 1H), 9.00 (s, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 43.3, 48.7, 51.6, 52.1, 120.0, 122.0, 125.3, 128.5, 128.9, 129.4, 131.4, 140.6, 149.6, 149.8, 166.1; LC-MS (ESI, *m/z*): 391 $[M+H]^+$.

Scheme 5



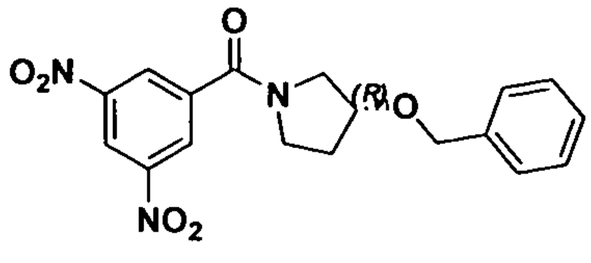
<u>General procedure for the synthesis of *t*-butyl-benzyloxypyrrolidine-1-carboxylate (D1)</u>

To a solution of (*R*)-*tert*-butyl 3-hydroxypyrrolidine-1-carboxylate (3.2 mmol) in dimethyl formamide (10 mL) was added sodium hydride (3.2 mmol) and benzyl bromide (3.2 mmol) at 0°C and the resulting mixture was stirred at room temperature. After stirring overnight, distilled water (50 mL) was added and the resulting precipitate was collected by filtration to afford D1.

<u>General procedure for the synthesis of benzyloxy-pyrrolidinyl-phenylmethanone (D2)</u>

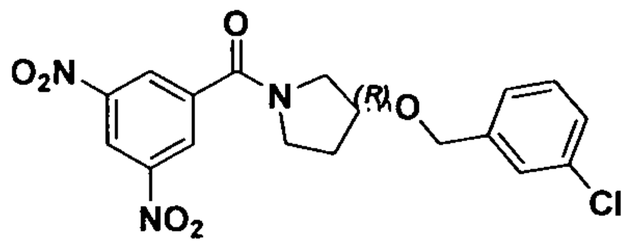
D1 (0.43 mmol) was dissolved in trifluoro acetic acid (5 mL) and stirred at room temperature. After 1 h, the reaction mixture was concentrated *in vacuo* to afford an amine. To a solution of the amine in methylene chloride (5 mL) was added triethylamine (0.51 mmol) and a benzoylchloride (0.51 mmol) at 0°C and the resulting mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with methylene chloride (30 mL) and washed with 1 M HCl aqueous solution (30 mL), saturated Na_2CO_3 aqueous solution (30 mL) and brine (30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (3:1 hexanes/ethyl acetate) and recrystallized from a mixture of hexanes and ethyl acetate to give D2.

(*R*)-(3-(Benzyloxy)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (98)



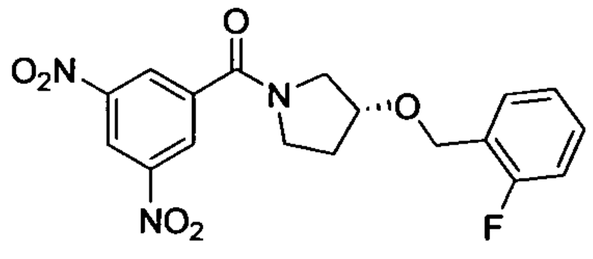
(Two rotamers, 1:1 ratio, 23 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 – 2.29 (m, 2H), 3.53 – 3.58 (m, 1H), 3.76 – 3.93 (m, 3H), 5.12 -5.37 (m, 3H), 7.34 – 7.44 (m, 5H), 8.67 & 8.73 (d, *J* = 1.6 Hz, 2H), 9.08 & 9.09 (d, *J* = 1.6 Hz, 1H).

((*R*)-3-(3-Chlorobenzyloxy)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (99)



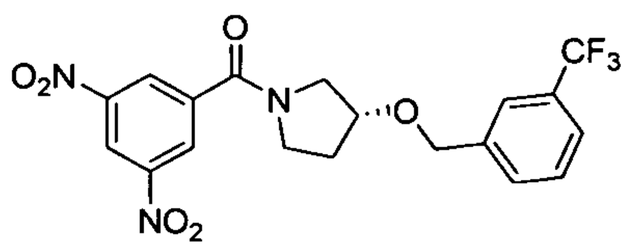
(Two rotamers 3:1 ratio, 75%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 – 2.21 (m, 2H), 3.38 – 3.83 (m, 4H), 4.13 – 4.47 (m, 1H), 4.99 & 5.07 (s, 1H), 5.17 & 5.29 (s, 1H), 7.07 – 7,29 (m, 4H), 8.64 & 8.69 (s, 2H), 8.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 32.2, 45.1, 47.6, 52.3, 54.8, 70.3, 70.4, 76.4, 78.0, 120.0, 120.1, 125.5, 125.6, 127.5, 127.7, 127.8, 127.9, 128.1, 129.9, 134.5, 134.6, 139.7, 139.8, 139.9, 148.5, 164.7, 164.8.

((*R*)-3-(2-Fluorobenzyloxy)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (100)



(Two rotamers 1:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 – 2.30 (m, 2H), 3.50 & 3.52 (s, 1H), 3.63 – 3.94 (m, 3H), 4.24 & 4.33 (s, 1H), 4.48 & 4.56 (d, *J* = 12.0 Hz, 1H), 4.65 (s, 1H), 6.99 – 7.44 (m, 4H), 8.69 & 8.75 (s, 2H), 9.10 (s, 1H).

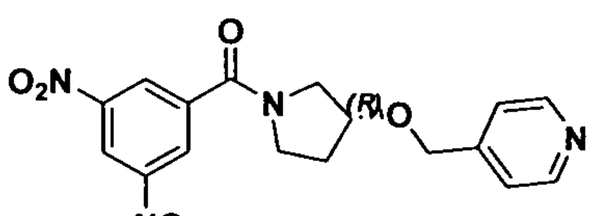
((*R*)-3-(3-(Trifluoromethyl)benzyloxy)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (101)



(Two rotamers 2:1 ratio), ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 – 2.29 (m, 2H), 3.53 & 3,55 (s, 1H), 3.78 – 3.96 (m, 3H), 4.27 & 4.35 (s, 1H), 4.51 & 4.62 (d, *J* = 12.4 Hz, 1H), 4.65 (s, 1H), 7.47 – 7.62 (m, 4H), 8.69 & 8.74 (s, 2H), 9.07 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7,

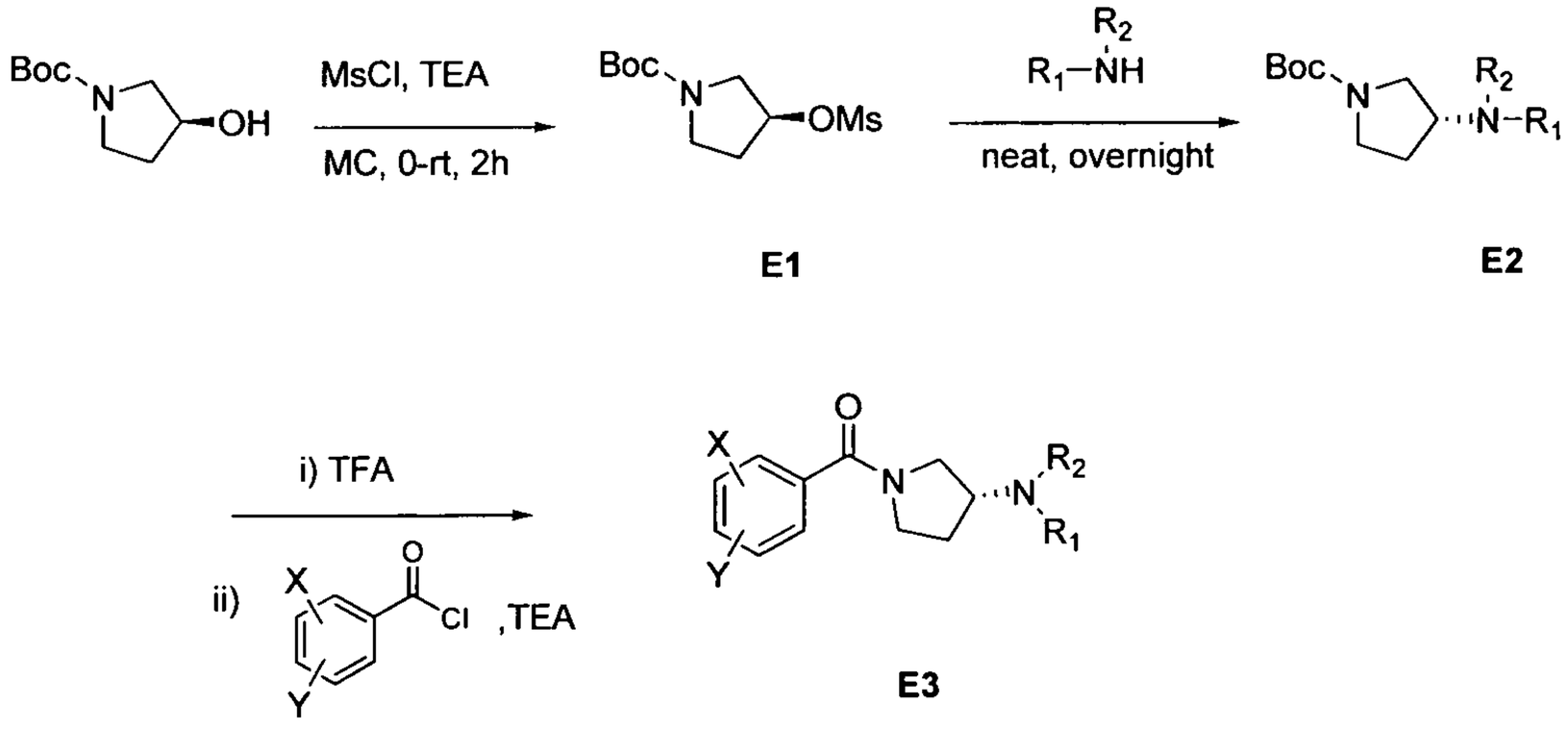
32.1, 45.1, 47.7, 52.4, 54.9, 70.4, 70.5, 76.7, 78.2, 120.1, 124.1, 124.3, 124.83, 124.87, 127.7, 127.8, 129.2, 130.8, 130.9, 138.7, 138.8, 139.7, 139.8, 148.5, 165.0.

(*R*)-(3,5-Dinitrophenyl)(3-(pyridin-4-ylmethoxy)pyrrolidin-1-yl)methanone (102)



(Two rotamers, 1:1 ratio, 75 %), a brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 – 2.24 (m, 2H), 3.49 – 3.92 (m, 4H), 4.20 – 4.28 (m, 1H), 4.41 – 4.61 (m, 2H), 7.14 – 7.24 (m, 2H), 8.49 – 8.56 (m, 2H), 8.67 & 8.70 (d, *J* = 1.6 Hz, 2H), 9.04 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 32.3, 45.1, 47.6, 52.3, 54.8, 69.4, 69.5, 76.9, 78.5, 120.1, 121.6, 121.7, 121.8, 127.7, 127.8, 139.8, 139.9, 146.6, 146.8, 148.5, 150.1, 150.2, 164.7.

Scheme 6



General procedure for the synthesis of aminopyrrolidinyl-phenyl-methanone (E3)

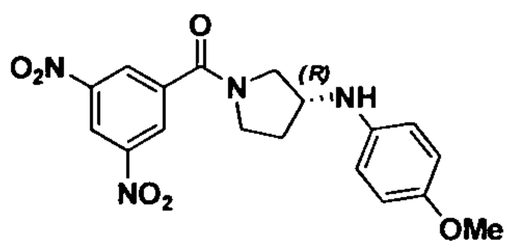
To a solution of (*S*)-(+)-*N*-Boc-3-pyrrolidinol (2.67 mmol) and triethylamine (4.01 mmol) in methylene chloride (50 mL) was added methansulfonyl chloride (4.01 mmol) under ice-bath and the resulting mixture was futher stirred at 4°C. After 2 h, the residue was diluted with methylene chloride (50 mL) and washed with water (100 mL) and brine (100 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (2:1 hexanes/ethyl acetate) to give E1.

A solution of E1 (0.75 mmol) and an amine (3.75 mmol) was stirred at 100°C. After stirring overnight, the residue was dissolved in methylene chloride (30 mL) and washed with water

(30 mL) and brine (30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (1:1 hexanes/ethyl acetate) to give E2.

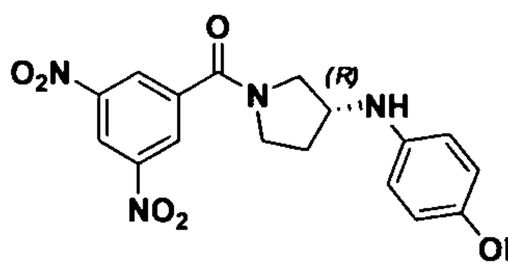
To a solution of E2 (0.96 mmol) in methylene chloride (20 mL) was added trifluoroacetic acid (0.5 mL). After 3 h, the solvent was removed *in vacuo*. The reaction mixture was dissolved in methylene chloride (20 mL) and cooled to 0°C. Triethylamine (4.83 mmol) and a benzoyl chloride (1.05 mmol) was added. After 2 h, the residue was diluted with methylene chloride (20 mL) and washed with water (40 mL) and brine (40 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude was purified by silica gel flash column chromatography (1:1 hexanes/ethyl acetate) to give E3.

(*R*)-(3,5-Dinitrophenyl)(3-(4-methoxyphenylamino)pyrrolidin-1-yl)methanone (103)



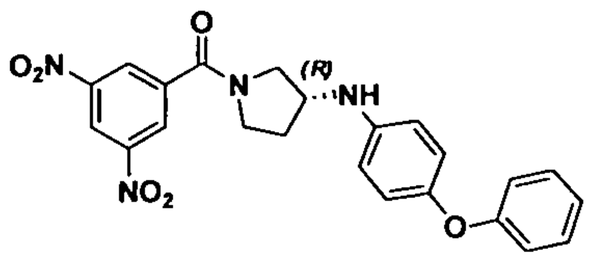
(Two rotamers, 1:1 ratio, 63 %), a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.93 – 2.01 (m, 1H), 2.14 – 2.30 (m, 1H), 3.26 – 3.30 & 3.44 – 3.50 (m, 1H), 3.54 – 3.72 (m, 2H), 3.61 & 3.68 (s, 3H), 3.80 – 3.91 (m, 1H), 3.95 – 4.05 (m, 1H), 6.43 & 6.55 (d, *J* = 8.8 Hz, 2H), 6.62 & 6.70 (d, *J* = 8.8 Hz, 2H), 8.58 & 8.67 (d, *J* = 2.4 Hz, 2H), 8.95 - 8.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 30.4, 32.4, 45.2, 47.9, 52.6, 53.0, 54.4, 55.0, 55.8, 55.9, 115.0, 115.1, 115.2, 115.3, 120.1, 127.6, 127.7, 139.6, 140.5, 140.7, 148.5, 148.6, 152.8, 152.9, 165.2, 165.4.

(*R*)-(3-(4-Butoxyphenylamino)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (104)



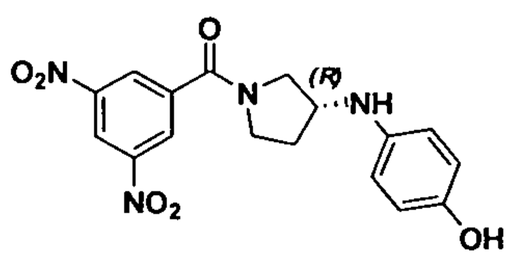
(Two rotamers, 1:1 ratio, 54 %), a brown solid; m.p. 118 – 120 □; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 – 0.98 (m, 3H), 1.39 – 1.52 (m, 2H), 1.61 – 1.76 (m, 2H), 2.02 – 2.05 (m, 1H), 2.24 – 2.41 (m, 1H), 3.33 – 3.37 & 3.50 – 3.63 (m, 2H), 3.66 – 4.13 (m, 6H), 6.47 & 6.60 (d, *J* = 8.4 Hz, 2H), 6.70 & 6.78 (d, *J* = 8.4 Hz, 2H), 8.66 & 8.74 (s, 2H), 9.05 & 9.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.1, 19.4, 19.5, 30.9, 31.6, 31.7, 32.9, 45.3, 47.9, 52.8, 53.4, 54.6, 55.2, 68.5, 68.6, 115.0, 115.2, 116.0, 116.2, 120.2, 127.8, 127.9, 139.9, 140.1, 140.4, 148.6, 152.7, 164.9, 165.1.

(*R*)-(3,5-Dinitrophenyl)(3-(4-phenoxyphenylamino)pyrrolidin-1-yl)methanone (105)



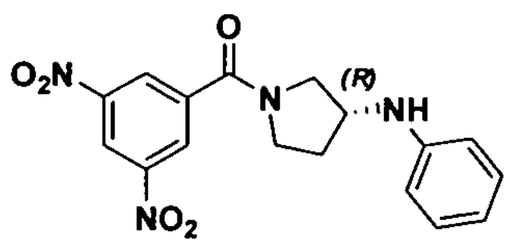
(Two rotamers, 1:1 ratio, 60 %), a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 2.00 – 2.06 (m, 1H), 2.18 – 2.35 (m, 1H), 3.32 – 3.35 & 3.48 – 3.54 (m, 1H), 3.61 – 3.78 (m, 2H), 3.82 – 4.12 (m, 2H), 6.47 & 6.60 (d, *J* = 8.8 Hz, 2H), 6.77 – 6.97 (m, 5H), 7.17, 7.24 (m, 2H), 8.63 & 8.69 (d, *J* = 1.6 Hz, 2H), 9.01 & 9.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 30.6, 32.5, 45.3, 47.9, 52.3, 53.0, 54.0, 55.1, 114.5, 114.8, 117.4, 117.5, 120.2, 121.3, 121.4, 122.4, 122.5, 127.7, 127.8, 129.7, 139.6, 142.8, 143.0, 148.6, 148.8, 165.2, 165.3.

(*R*)-(3,5-Dinitrophenyl)(3-(4-hydroxyphenylamino)pyrrolidin-1-yl)methanone (106)



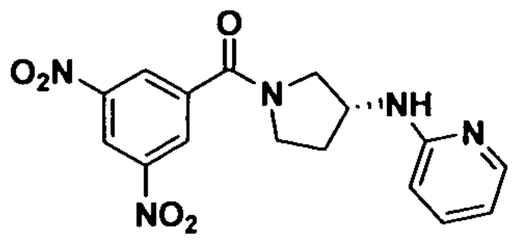
(Two rotamers, 1:1 ratio, 83 %), a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.78 – 1.89 (m, 1H), 2.03 – 2.15 (m, 1H), 3.12 – 3.17 (m, 1H), 3.37 – 3.45 (m, 1H), 3.52 – 3.95 (m, 3H), 5.15 – 5.23 (m, 1H), 6.36 – 6.56 (m, 4H), 8.38 & 8.44 (brs, 1H), 8.64 & 8.67 (s, 2H), 8.81 & 8.84 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.6, 31.3, 44.6, 46.9, 51.6, 51.9, 53.3, 54.1, 113.8, 114.2, 115.6, 115.7, 119.4, 127.4, 127.5, 139.6, 139.7, 140.3, 140.4, 148.0, 148.1, 148.5, 148.7, 164.2.

(*R*)-(3,5-Dinitrophenyl)(3-(phenylamino)pyrrolidin-1-yl)methanone (107)



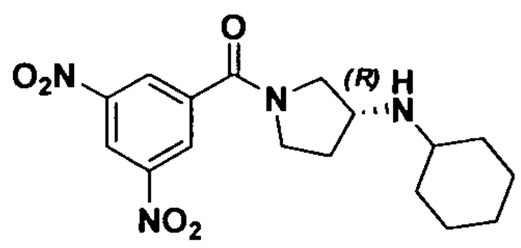
(Two rotamers, 1:1 ratio, 80 %), a red solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.99 – 2.04 (m, 1H), 2.17 – 2.33 (m, 1H), 3.28 – 3.31 & 3.57 -3.95(m, 4H), 4.04 – 4.11 (m, 1H), 6.46 – 6.48 (m, 1H), 6.59 – 6.70 (m, 2H), 7.02 – 7.14 (m, 2H), 8.60 & 8.67 (s, 2H), 8.98 & 9.01 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 30.3, 32.2, 45.1, 47.8, 51.7, 52.8, 53.3, 54.9, 113.2, 113.5, 118.3, 118.4, 120.0, 127.6, 127.7, 129.4, 139.5, 146.3, 146.4, 148.4, 148.5, 165.1, 165.3.

(*R*)-(3,5-Dinitrophenyl)(3-(pyridin-2-ylamino)pyrrolidin-1-yl)methanone (108)



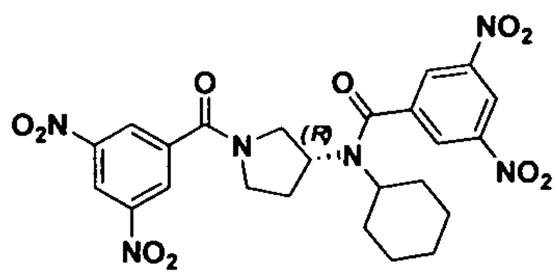
(Two rotamers, 1:1 ratio, 70 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 – 2.44 (m, 2H), 3.38 – 4.11 (m, 4H), 4.38 & 4.50 (m, 1H), 6.36 & 6.44 (d, *J* = 8.4 Hz, 1H), 6.57 & 6.64 (t, *J* = 6.0 Hz, 1H), 7.37 & 7.44 (t, *J* = 7.8 Hz, 1H), 7.98 & 8.11 (d, *J* = 5.2 Hz, 1H), 8.67 & 8.73 (s, 2H), 9.05 & 9.09 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.4, 32.6, 45.1, 47.7, 51.7, 52.9, 55.3, 76.7, 101.8, 108.3, 113.7, 119.9, 127.7, 137.5, 137.7, 147.8, 147.9, 148.3, 148.4, 157.2, 157.4, 164.8, 164.9.

(*R*)-(3-(Cyclohexylamino)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (109)



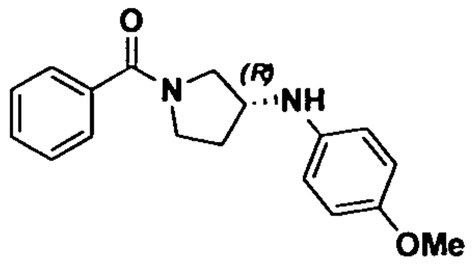
(Two rotamers, 1:1 ratio, 69 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 0.99 – 1.35 (m, 6H), 1.60 – 1.98 (m, 5H), 2.15 – 2.32 (m, 1H), 2.39 – 2.57 (m, 1H), 3.24 – 3.60 (m, 2H), 3.63 – 3.73 (m, 2H), 3.81 – 3.91 (m, 1H), 8.73 & 8.78 (s, 2H), 9.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 24.7, 24.8, 24.9, 25.6, 25.7, 30.6, 32.4, 33.3, 33.4, 45.2, 47.7, 52.6, 54.5, 54.8, 54.9, 55.1, 119.7, 127.4, 127.5, 139.5, 148.3, 164.9, 165.0.

(*R*)-*N*-Cyclohexyl-*N*-(1-(3,5-dinitrobenzoyl)pyrrolidin-3-yl)-3,5-dinitrobenzamide (110)



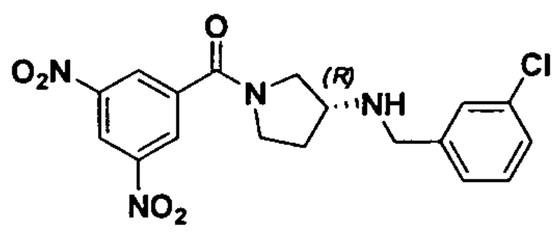
(Two rotamers, 1:1 ratio, 15 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 – 1.22 (m, 3H), 1.62 – 1.86 (m, 6H), 2.18 – 2.26 (m, 1H), 2.74 – 2.89 (m, 1H), 3.30 – 3.35 (m, 1H), 3.50 – 3.78 (m, 2H), 3.97 – 4.19 (m, 4H), 8.51 & 8.56 (s, 2H), 8.74 (s, 2H), 9.09 - 9.10 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 24.9, 25.3, 25.5, 27.3, 30.0, 31.8, 45.6, 48.7, 48.9, 50.0, 53.6, 54.8, 60.5, 119.8, 120.0, 126.7, 127.8, 139.8, 140.1, 140.2, 140.4, 148.6, 148.2, 164.4, 164.7, 166.6, 166.7.

(*R*)-(3-(4-Methoxyphenylamino)pyrrolidin-1-yl)(phenyl)methanone (111)



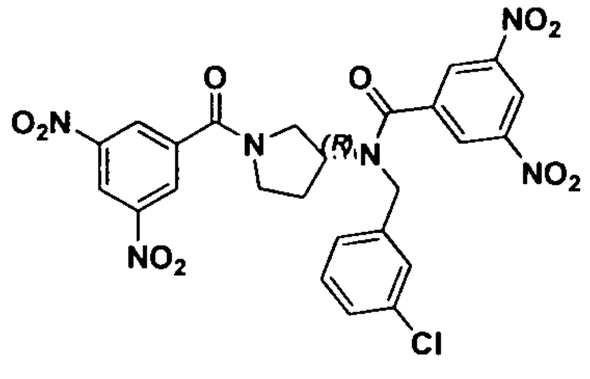
(Two rotamers, 1:1 ratio, 75 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84 – 1.88 (m, 1H), 2.08 – 2.32 (m, 1H), 3.26 – 3.34 & 3.49 – 4.03 (m, 5H), 3.69 & 3.72 (s, 3H), 6.48 & 6.50 (d, *J* = 6.4 Hz, 2H), 6.71 & 6.76 (d, *J* = 6.4 Hz, 2H), 7.36 – 7.51 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.7, 32.5, 44.5, 47.7, 52.6, 52.7, 54.2, 55.2, 55.8, 55.9, 114.7, 114.9, 115.0, 127.2, 128.3, 130.1, 136.7, 140.8, 141.0, 152.6, 170.0.

(*R*)-(3-(3-Chlorobenzylamino)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (112)



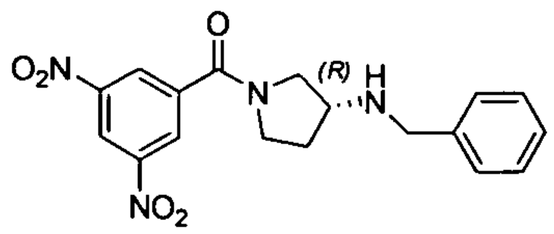
(Two rotamers, 1:1 ratio, 32 %) as a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.83 – 1.89 (m, 1H), 2.01 – 2.08 & 2.14 – 2.19 (m, 1H), 2.75 (brs, 1H), 3.15 – 3.19 & 3.35 – 3.83 (m, 7H), 7.05 – 7.23 (m, 4H), 8.58 & 8.67 (d, *J* = 2.0 Hz, 2H), 8.97 – 8.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 30.4, 32.1, 45.2, 47.7, 51.2, 51.4, 52.4, 54.9, 55.2, 57.5, 119.8, 126.0, 126.2, 127.2, 127.3, 127.5, 127.6, 127.8, 128.0, 129.7, 129.8, 134.1, 134.2, 139.5, 139.6, 141.3, 141.7, 148.2, 148.3, 164.7, 164.8.

(*R*)-*N*-(3-Chlorobenzyl)-*N*-(1-(3,5-dinitrobenzoyl)pyrrolidin-3-yl)-3,5-dinitrobenzamide (113)



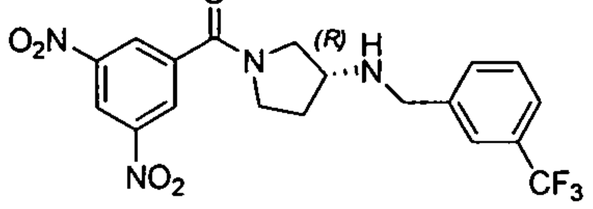
(Two rotamers, 1:1 ratio, 44 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26 – 2.35 (m, 2H), 3.56 – 4.05 (m, 4H), 4.57 – 4.65 (m, 3H), 7.06 – 7.15 (m, 2H), 7.24 – 7.35 (m, 2H), 8.50 – 8.62 (m, 4H), 8.97 – 9.02 (m, 2H); LC-MS (ESI, *m/z*): 599 $[M+H]^+$.

(*R*)-(3-(Benzylamino)pyrrolidin-1-yl)(3,5-dinitrophenyl)methanone (114)



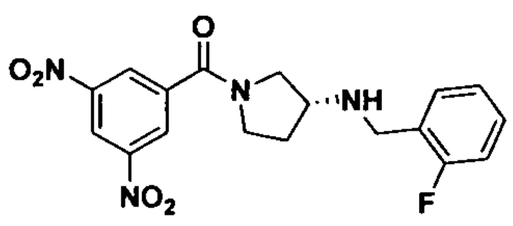
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (brs, 1H), 1.87-1.94 (m, 1H), 2.06-2.24 (m, 1H), 3.20 (dd, *J* = 4.8, 10.4 Hz, 0.5H), 3.46-3.89 (m, 6.5H), 7.15-7.36 (m, 5H), 8.63 (d, *J* = 2.0 Hz, 1H), 8.71 (d, *J* = 2.0 Hz, 1H), 9.03 (t, *J* = 2.0 Hz, 0.5H), 9.06 (t, *J* = 2.0 Hz, 0.5H).

(*R*)-(3,5-Dinitrophenyl)(3-(3-(trifluoromethyl)benzylamino)pyrrolidin-1-yl)methanone (115)



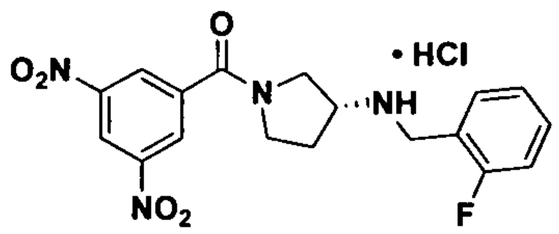
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (brs, 1H), 1.89-1.94 (m, 1H), 2.10-2.28 (m, 1H), 3.24 (dd, *J* = 5.2, 10.0 Hz, 0.5H), 3.45-3.92 (m, 6.5H), 7.40-7.61 (m, 4H), 8.65 (d, *J* = 2.0 Hz, 1H), 8.72 (d, *J* = 2.0 Hz, 1H), 9.06 (t, *J* = 2.0 Hz, 0.5H), 9.08 (t, *J* = 2.0 Hz, 0.5H).

(*R*)-(3,5-Dinitrophenyl)(3-(2-fluorobenzylamino)pyrrolidin-1-yl)methanone (116)



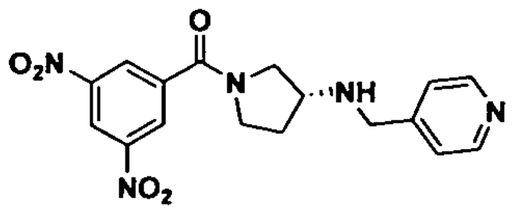
(Two rotamers, 1:1 ratio, 75 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 – 1.94 (m, 1H), 2.11 – 2.25 (m, 1H), 3.22 – 3.89 (m, 7H), 6.93 & 7.02 (t, *J* = 8.6 Hz, 2H), 7.20 & 7.33 (m, 2H), 8.66 & 8.72 (d, *J* = 2.0 Hz, 2H), 9.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.7, 32.5, 45.3, 47.8, 51.3, 51.5, 52.7, 55.1, 55.4, 57.7, 115.1, 115.3, 119.8, 119.9, 127.6, 127.7, 129.4, 129.6, 135.4, 135.5, 139.8, 148.3, 148.4, 162.0 (d, *J* = 245 Hz, due to F), 162.1 (d, *J* = 245 Hz, due to F), 164.5, 164.6.

(*R*)-(3,5-Dinitrophenyl)(3-(2-fluorobenzylamino)pyrrolidin-1-yl)methanone hydrochloride (117)



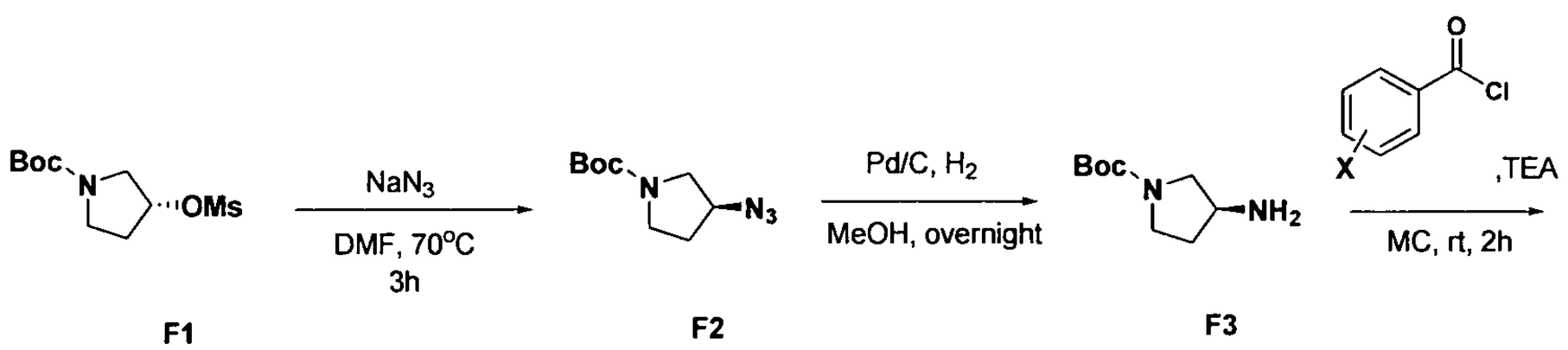
(Two rotamers, 1:1 ratio, 92 %), a white solid; ^{1}H NMR (400 MHz, $CD_3OD + D_2O$) δ 2.24 – 2.35 (m, 1H), 2.48 – 2.63 (m, 1H), 3.48 – 4.34 (m, 7H), 7.13 & 7.24 (t, *J* = 8.6 Hz, 2H), 7.47 & 7.58 (q, *J* = 7.0 Hz, 2H), 8.73 & 8.8 (d, *J* = 2.0 Hz, 2H), 9.16 (brs, 1H); ^{13}C NMR (100 MHz, $CD_3OD + D_2O$) δ 28.1, 29.7, 45.5, 50.6, 50.7, 51.9, 56.6, 57.7, 81.1, 117.0, 117.1, 127.7, 128.6, 128.7, 133.2, 133.3, 139.0, 147.1, 149.7, 167.5, 167.6.

(*R*)-(3,5-Dinitrophenyl)(3-(pyridin-4-ylmethylamino)pyrrolidin-1-yl)methanone (118)



(Two rotamers, 1:1 ratio, 69 %), a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (br, 1H), 1.88 – 2.23 (m, 2H), 3.23 – 3.89 (m, 7H), 7.17 & 7.26 (d, *J* = 5.2 Hz, 2H), 8.45 & 8.52 (d, *J* = 5.6 Hz, 2H), 8.65 & 8.69 (d, *J* = 2.0 Hz, 2H), 9.04 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2, 32.4, 44.7, 47.7, 52.4, 54.8, 55.9, 76.0, 115.0, 115.1, 117.2, 117.4, 124.4, 124.5, 127.0, 129.1, 130.6, 130.8, 137.5, 137.7, 150.8, 151.0, 154.7, 154.8, 168.5, 168.8.

Scheme 7



<u>General procedure for the synthesis of (*R*)-*N*-benzoylpyrrolidinyl-benzamide (F5)</u>

To a solution of F1 (3.77 mmol) in DMF (15 mL) was added sodium azide (11.00 mmol) and the resulting mixture was warmed to 70°C. After 3 h, the solvent was removed *in vacuo*, dissolved in ethylacetate (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silical gel flash column chromatography (1:1 hexanes/ethyl acetate) to give F2.

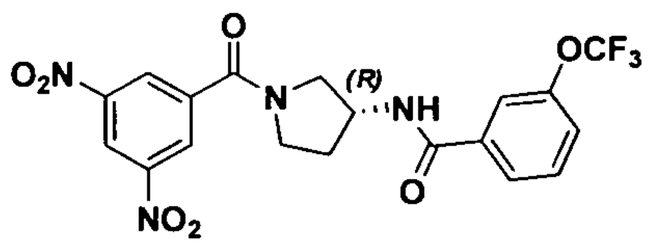
To a solution of F2 (2.68 mmol) was added 10% palladium on activated carbon and stirred overnight under hydrogen atmosphere. The reaction mixture was filtered using cellite 545 and the resulting filtrate was concentrated *in vacuo* to give F3.

To a solution of F3 (0.77 mmol) and triethylamine (1.16 mmol) in methylene chloride (10 mL) was added benzoyl chloride (1.00 mmol) under ice bath. The reaction mixture was brought up to room temperature. After 2 h, the raction mixture was diluted with methylene chloride (20 mL) and washed with water (30 mL) and brine (30 mL). The organic layer was

dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (2:1 hexanes/ethyl acetate) to give F4.

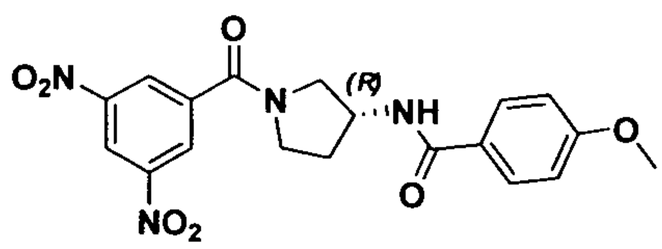
To a solution of F4 (0.59 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (0.5 mL) and stirred at room temperature. After 3 h, the solvent was removed *in vacuo*. The crude product was dissolved in methylene chloride (10 mL) and triethylamine (0.41 mL, 2.96 mmol) was added. The reaction mixture was cooled to 0°C and then 3,5-dichlorobenzoyl chloride (0.65 mmol) was added. The resulting mixture was brought up to room temperature. After 2 h, the solvent was removed *in vacuo* and the crude residue was purified by silica gel flash column chromatography (1:1 hexanes/ethyl acetate) to give F5.

(*R*)-*N*-(1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yl)-3-(trifluoromethoxy)benzamide (119)



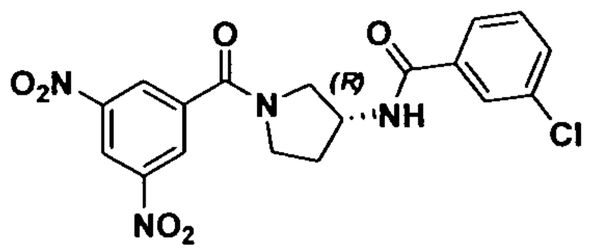
(Two rotamers, 1:1 ratio, 67 %), a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 – 2.18 (m, 1H), 2.29 – 2.40 (m, 1H), 3.49 – 3.60 (m, 1H), 3.68 – 3.76 (m, 1H), 3.87 – 3.98 (m, 2H), 4.60 – 4.74 (m, 1H), 7.19 – 7.60 (m, 5H), 8.51 & 8.59 (s, 2H), 8.91 & 8.96 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 32.5, 45.3, 48.0, 49.2, 50.8, 51.9, 54.8, 119.9, 120.0, 120.2, 124.3, 125.7, 127.6, 130.2, 135.7, 136.0, 139.4, 148.4, 148.5, 149.2, 164.9, 165.0, 166.5, 166.6.

(*R*)-*N*-(1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yl)-4-methoxybenzamide (120)



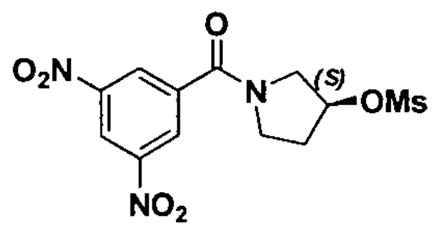
(Two rotamers, 1:1 ratio, 0.19 g, 76 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08 – 2.15 (m, 1H), 2.35 – 2.47 (m, 1H), 3.47 – 4.08 (m, 4H), 3.81 & 3.84 (s, 3H), 4.62 – 4.64 & 4.77 – 4.78 (m, 1H), 6.45 & 6.50 (brs, 1H), 6.82 & 6.88 (d, J = 8.4 Hz, 2H), 7.62 & 7.72 (d, J = 8.4 Hz, 2H), 8.62 & 8.71 (s, 2H), 9.04 & 9.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.0, 32.8, 45.2, 48.0, 48.9, 50.5, 52.2, 55.2, 55.6, 60.6, 113.9, 120.2, 125.6, 126.1, 127.7, 127.8, 129.0, 139.5, 148.5, 162.7, 164.9, 165.0, 167.4.

(*R*)-3-Chloro-*N*-(1-(3,5-dinitrobenzoyl)pyrrolidin-3-yl)benzamide (121)



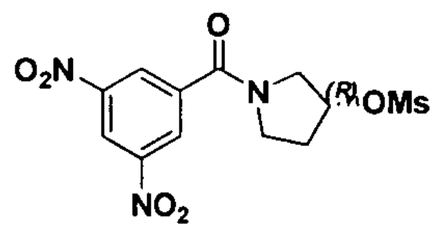
(Two rotamers, 1:1 ratio, 66 %), a pale yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.94 – 2.20 (m, 2H), 3.33 – 3.83 (m, 4H), 4.42 – 4.55 (m, 1H), 7.43 – 7.60 (m, 2H), 7.71 – 7.90 (m, 2H), 8.66 & 8.69 (d, J = 2.0 Hz, 2H, brs, 1H), 8.83 - 8.86 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.3, 31.5, 44.6, 47.0, 48.4, 49.9, 51.1, 53.3, 119.4, 119.5, 126.2, 126.3, 127.0, 127.1, 127.5, 130.2, 130.3, 131.1, 133.0, 133.1, 136.1, 136.3, 139.5, 139.6, 148.0, 164.0, 164.1, 165.0, 165.1.

(*S*)-1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yl methanesulfonate (122)



(Two rotamers, 1:1 ratio, 92 %), a white solid; m.p. 138 – 140 □; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 – 2.46 (m, 2H), 3.03 & 3.10 (s, 3H), 3.59 – 3.67 & 3.75 – 4.03 (m, 4H), 5.28 – 5.40 (m, 1H), 8.68 & 8.73 (s, 2H), 9.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.1, 33.6, 38.9, 39.0, 44.7, 47.2, 53.3, 55.3, 78.2, 78.6, 120.5, 127.8, 127.9, 139.3, 148.7, 164.8, 165.0; LC-MS (ESI, *m/z*): 360 $[M+H]^+$.

(*R*)-1-(3,5-Dinitrobenzoyl)pyrrolidin-3-yl methanesulfonate (123)



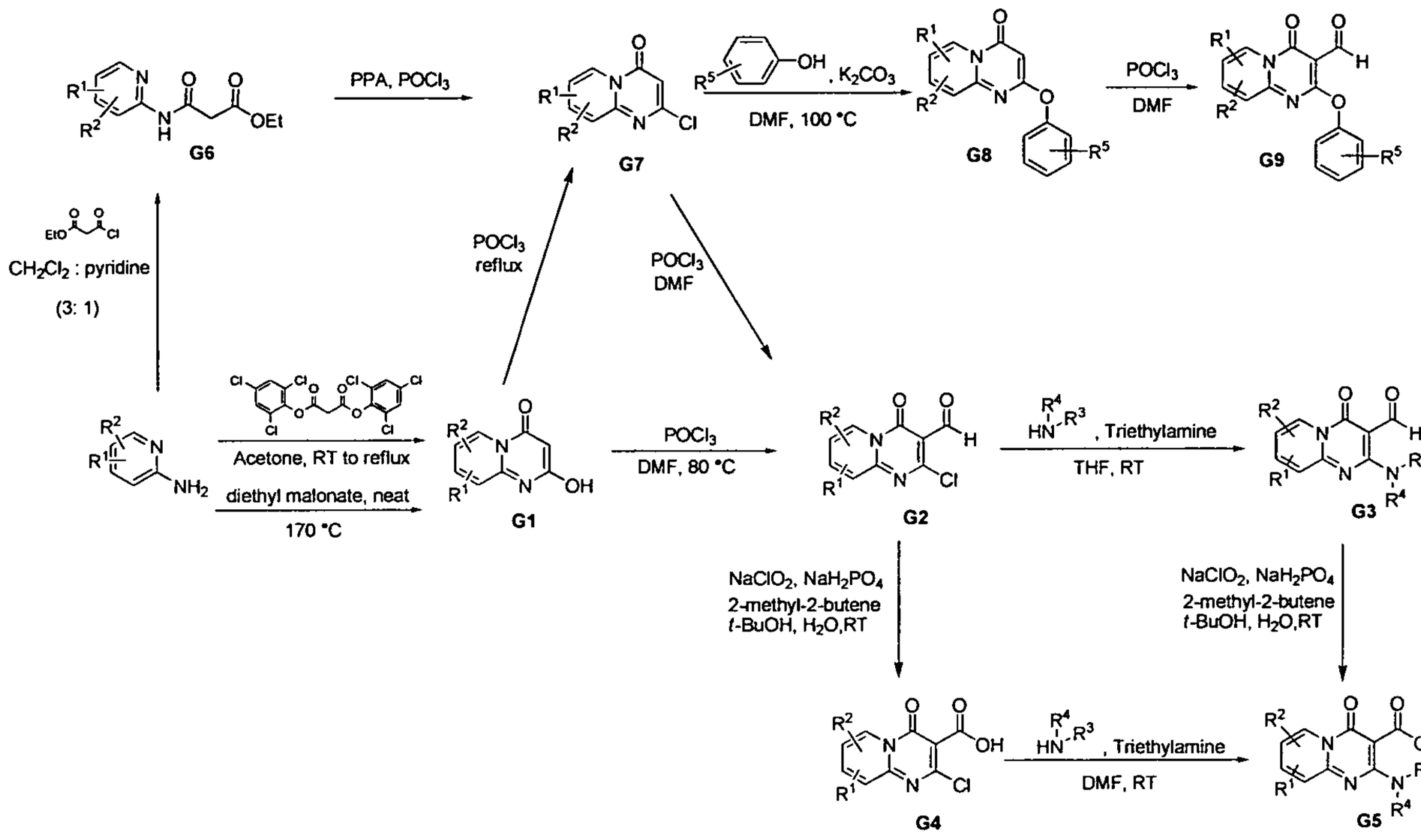
(Two rotamers, 1:1 ratio, 89 %), a white solid; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 2.16 – 2.32 (m, 2H), 2.94 & 3.02 (s, 3H), 3.50 – 3.91 (m, 4H), 5.19 – 5.30 (m, 1H), 8.58 & 8.63 (s, 2H), 8.97 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 30.7, 33.1, 38.3, 38.4, 44.5, 46.9, 53.0, 55.0, 78.5, 79.0, 120.1, 127.6, 139.0, 148.4, 164.9, 165.0.

Example 7: Derivatization of the pyridopyrimidinone compounds

The pyridopyrimidinone compounds (scaffold VIII; see **Table 3**) underwent derivatization according to the methods outlined below (**Schemes 8-10**). Resulting derivatives were

examined for inhibitory activity using the assay described above and the results are summarized in **Table 4**.

Scheme 8



General procedure for the synthesis of G1

2-Amino-3-picoline (1.0 mmol) was dissolved in diethyl malonate (1.0 mmol). The solution was heated to 170℃ for 12 h. After cooling, the dark residue was triturated with CH_2Cl_2 (10 mL). The residual pale solid was collected by filtration and washed with CH_2Cl_2 to give G1.

General procedure for the synthesis of G2

To a DMF (2.0 mL) was added $POCl_3$ (3.0 mmol) at 0℃. After the mixture was stirred at 0 ℃ for 40 min, a solution of G1 (1.0 mmol) in DMF (2.0 mL) was added and stirred at 80 ℃ for 1 h. The mixture was cooled and concentrated *in vacuo*. The residue was diluted with water and extracted with CH_2Cl_2 (10 mLx 3). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography to give G2.

General procedure for the synthesis of G3

To a stirred solution of G2 (1.0 mmol) in THF (2.0 mL) was added Et_3N (2.0 mmol). The mixture was cooled to 0°C. After 5 min, an amine (1.0 mmol) was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give G3.

General procedure for the synthesis of G4

G2 (0.5 mmol) was dissolved in 10.4 mL of *tert*-butyl alcohol and 2.5 mL of 2-methyl-2-butene. A solution of sodium chlorite (4.59 mmol) and sodium dihydrogenphosphate (3.46 mmol) in 4.2 mL of water was added dropwise. The reaction mixture was stirred at room temperature overnight. Volatile components were then removed under vacuum, and the residue was dissolved in 10 ml of water and extracted with two 10 ml portions of hexane. The aqueous layer was acidified to pH=3 with HCl(aq) and extracted with 10 mL portions of methylene chloride. The combined organic layers were washed with 20 mL of cold water, dried and concentrated to give G4.

General procedure for the synthesis of G5 from G3

G3 (36.6 μmol) was dissolved in 760 μl of *tert*-butyl alcohol and 180 μl of 2- methyl-2-butene. A solution of sodium chlorite (335 μmol) and sodium dihydrogenphosphate (253 μmol) in 300μl of water was added dropwise. The reaction mixture was stirred at room temperature overnight. Volatile components were then removed under vacuum and the residue was dissolved in 10 ml of water and extracted with two 10 ml portions of hexane. The aqueous layer was acidified to pH=3 with HCl(aq) and extracted with 10 ml portions of methylene chloride. The combined organic layers were washed with 20 ml of cold water, dried and concentrated to give G5.

General procedure for the synthesis of G5 from G4

To a stirred solution of G4 (1.0 mmol) in DMF (2.0 mL) was added Et_3N (2.0 mmol) and amine (1.5 mmol) and the mixture was stirred at 60°C overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with brine (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by recrystallization from a mixture of hexanes and methylene chloride to give G5.

General procedure for the synthesis of G6

The solution of 2-amino-3-picoline (4.0 mmol) in a solution of CH_2Cl_2 (3 mL) and dried pyridine (1 mL) was added dropwise at room temperature to a stirred solution of ethyl 3-chloro-3-oxo-propionate (5.3 mmol) in CH_2Cl_2 (3 mL) (an exothermic reaction with emission of white fume occurred during the addition). The resulting warm mixture was stirred at room temperature for 30 min and then poured into 30 mL of cold water; an excess of sodium carbonate was carefully added with stirring and the mixture was further stirred at room temperature for 1 h. The organic layer was then collected and the aqueous phase was extracted several times with CH_2Cl_2. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give G6.

General procedure for the synthesis of G7

A mixture of G6 (1.83 mmol), $POCl_3$ (0.5 mL) and polyphosphoric acid (137 mg) was heated with stirring at 130°C for 3 h. After cooling, anhydrous ethanol was added and the mixture was refluxed for 30 min, then allowed to cool. The mixture was treated with aqueous sodium carbonate and exhaustively extracted with CH_2Cl_2 (10 mLx 3). The combined layers were washed with water (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give G7.

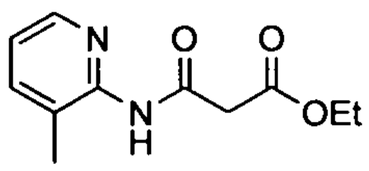
General procedure for the synthesis of G8

To a solution of G6 (1 mmol) in DMF (0.96 mL) was added potassium carbonate (5.0 mmol) followed by phenol (1.94 mmol). After 12 h at 100 °C, the solution was allowed to cool to 23 °C. The reaction mixture was washed with H_2O (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (20 mL x 3). The combined organic layers were washed with 1 N HCl (20 mL x 2), filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give G8.

General procedure for the synthesis of G9

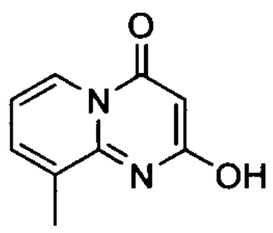
To DMF (2.0 mL) was added $POCl_3$ (3.0 mmol) at 0℃. After the mixture was stirred at 0 ℃ for 40 min, a solution of G8 (1.0 mmol) in DMF (2.0 mL) was added and stirred at 80 ℃ for 1 h. The mixture was cooled and concentrated *in vacuo*. The residue was diluted with water and extracted with CH_2Cl_2 (10 mL x 3). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography to give G9.

Ethyl 3-(3-methylpyridin-2-ylamino)-3-oxopropanoate (124)



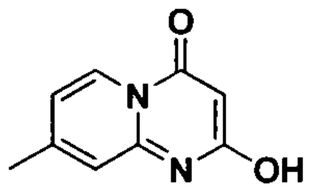
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.0 Hz, 3H), 2.25 (s, 3H), 3.45 (s, 2H), 4.20 (q, J = 7.2 Hz, 2H), 7.47 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 9.67 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 17.7, 42.6, 61.7, 113.8, 129.3, 138.8, 147.6, 148.8, 163.5, 168.4.

2-Hydroxy-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (125)



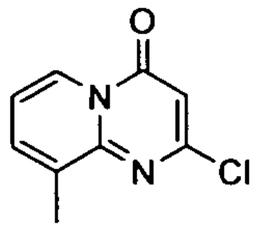
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.48 (s, 3H), 5.44 (s, 1H), 7.20 (t, J = 7.0 Hz, 1H), 7.87 (d, J = 6.8 Hz, 1H), 8.84 (d, J = 6.8 Hz, 1H), 11.52 (brs, 1H).

2-Hydroxy-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (126)



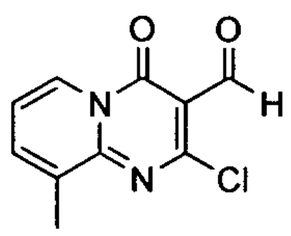
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (s, 3H), 4.88 (s, 1H), 7.20 – 7.24 (m, 2H), 8.85 (d, J = 6.8 Hz, 1H), 11.98 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.6, 80.3, 114.4, 117.1, 127.7, 146.7, 153.5, 155.3, 162.3.

2-Chloro-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (127)



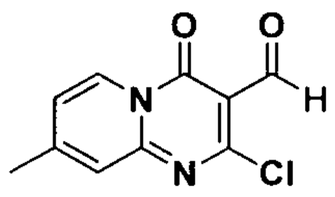
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H), 6.45 (s, 1H), 7.12 (t, J = 7.0 Hz, 1H), 7.68 (d, J = 6.8 Hz, 1H), 8.93 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 102.3, 115.8, 125.7, 134.7, 136.9, 150.0, 157.6, 157.9.

2-Chloro-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (128)



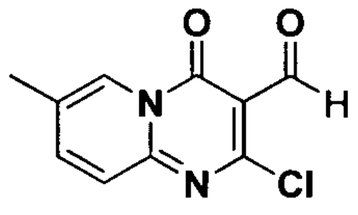
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.64 (s, 3H), 7.30 (t, J = 7.0 Hz, 1H), 7.92 (d, J = 7.2 Hz, 1H), 9.10 (d, J = 6.4 Hz, 1H), 10.42 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.7, 107.3, 117.7, 127.0, 135.6, 140.6, 150.0, 156.4, 160.2, 187.1.

2-Chloro-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (129)



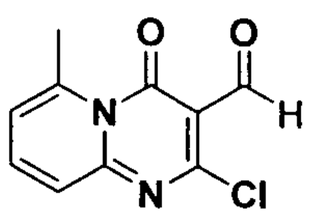
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (s, 3H), 7.24 (d, J = 7.2 Hz, 1H), 7.52 (s, 1H), 9.09 (d, J = 7.2 Hz, 1H), 10.40 (s, 1H).

2-Chloro-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (130)



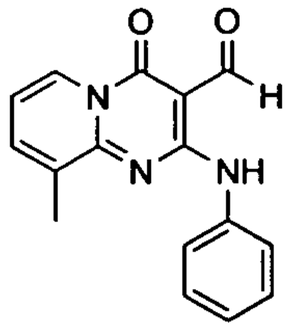
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H), 7.49 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 8.79 (s, 1H), 10.16 (s, 1H).

2-Chloro-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (131)



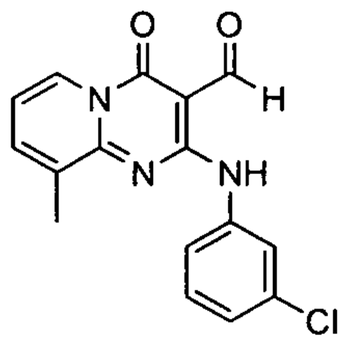
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.11 (s, 3H), 6.98 (d, J = 7.2 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.79 (t, J = 8.0 Hz, 1H), 10.29 (s, 1H).

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (132)



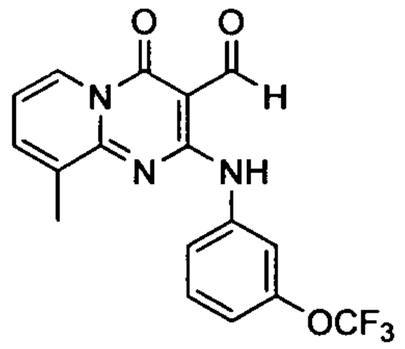
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 6.89 (t, *J* = 6.8 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 6.4 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 2H), 8.80 (d, *J* = 6.8 Hz, 1H), 10.27 (s, 1H), 11.67 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 94.6, 113.6, 121.8, 124.2, 125.9, 128.7, 133.6, 138.1, 138.9, 152.5, 153.8, 160.2, 190.2.

2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (133)



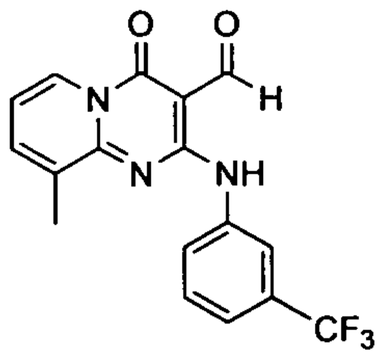
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.97 (t, *J* = 6.8 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 H, 1H), 7.69 (d, *J* = 6.8 Hz, 1H), 8.18 (s, 1H), 8.84 (d, *J* = 6.8 Hz, 1H), 10.27 (s, 1H), 11.72 (brs, 1H).

9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (134)



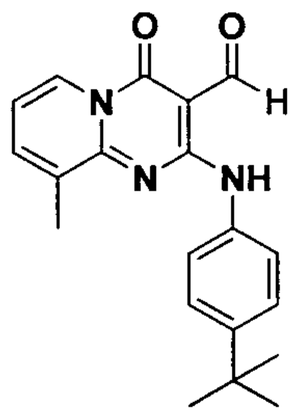
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.99 (t, *J* = 7.0 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 6.8 Hz, 1H), 8.16 (s, 1H), 8.88 (d, *J* = 8.0 Hz, 1H), 10.32 (s, 1H), 11.86 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 94.7, 114.2, 114.7, 116.5, 119.7, 126.1, 129.7, 133.8, 139.4, 139.7, 149.4, 152.6, 157.0, 160.1, 190.4.

9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (135)



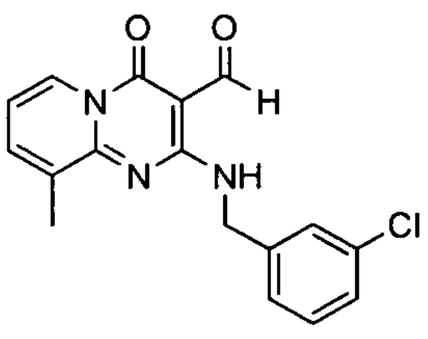
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 1H), 6.98 (t, *J* = 6.8 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 6.0 Hz, 1H), 8.61 (s, 1H), 8.87 (d, *J* = 6.8 Hz, 1H), 10.30 (s, 1H), 11.85 (brs, 1H).

2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (136)



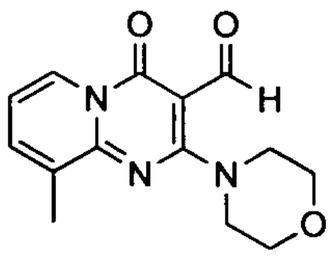
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 9H), 2.48 (s, 3H), 6.89 (t, *J* = 7.0 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 6.8 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 8.81 (d, *J* = 7.2 Hz, 1H), 10.30 (s, 1H), 11.68 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 31.3, 34.3, 94.6, 113.5, 121.4, 125.6, 125.9, 133.6, 135.6, 138.8, 147.2, 152.6, 156.7, 160.4, 190.2.

2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (137)



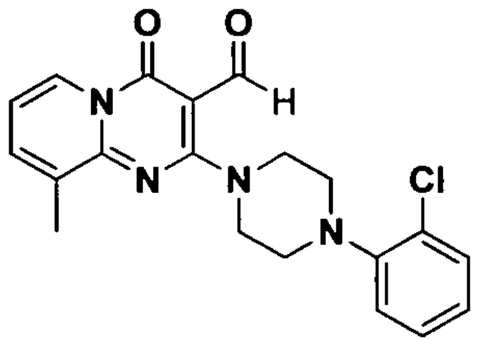
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 4.80 (d, *J* = 6.0 Hz, 2H), 6.87 (t, *J* = 7.0 Hz, 1H), 7.24-7.26 (m, 3H), 7.37 (s, 1H), 7.59 (d, *J* = 6.8 Hz, 1H), 8.79 (d, *J* = 7.2 Hz, 1H), 10.34 (brs, 1H), 10.30 (s, 1H).

9-Methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (138)



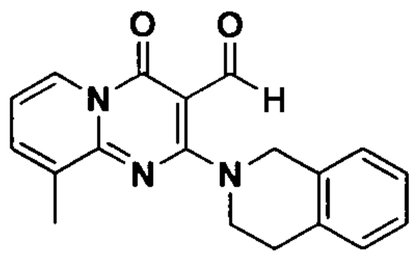
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H), 3.65 (d, J = 2.4 Hz, 4H), 3.72 (d, J = 3.2 Hz, 4H), 6.74 – 6.77 (m, 1H), 7.49 (d, J = 6.8 Hz, 1H), 8.62 (d, J = 7.2 Hz, 1H), 10.01 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 49.5, 67.0, 95.9, 112.9, 125.7, 133.0, 138.1, 150.5, 158.4, 162.3, 186.2

2-(4-(2-Chlorophenyl)piperazin-1-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (139)



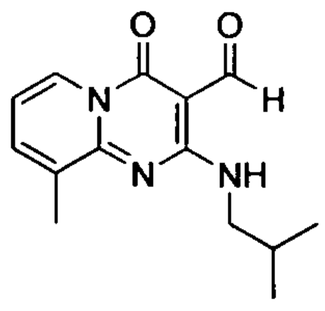
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H), 3.19 (t, J = 4.8 Hz, 4H), 3.92 (t, J = 4.6 Hz, 4H), 6.82 (t, J = 7.0 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 7.2 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 6.4 Hz, 1H), 8.73 (d, J = 6.8 Hz, 1H), 10.15 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 49.3, 51.4, 96.1, 112.7, 120.5 ,124.0, 125.8, 127.6, 128.8, 130.6, 133.0, 137.8, 148.7, 150.5, 158.6, 162.5, 186.4.

2-(3,4-Dihydroisoquinolin-2(1H)-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (140)



^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.05 (t, J = 5.8 Hz, 2H), 4.03 (t, J = 5.8 Hz, 2H), 4.73 (s, 2H), 6.78 (t, J = 7.0 Hz, 1H), 7.06 – 7.17 (m, 4H), 7.52 (d, J = 6.8 Hz, 1H), 8.70 (d, J = 7.6 Hz, 1H), 10.21 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 28.7, 46.3, 52.0, 96.1, 112.5, 125.8, 126.2, 126.6, 128.4, 133.0, 133.9, 134.6, 137.5, 150.3, 158.6, 162.3, 186.7.

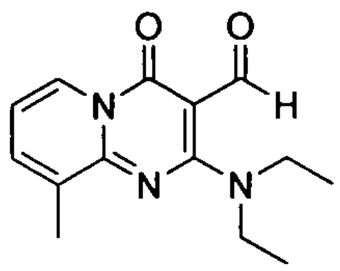
2-(Isobutylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (141)



^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (d, J = 4Hz, 6H), 1.90 (m, 1H), 2.37 (s, 3H), 3.41 (t, J = 6.8 Hz, 2H), 6.76 (t, J = 6.8 Hz, 1H), 7.24 – 7.52 (m, 1H), 8.69 (dd, J = 0.8, 7.2 Hz, 1H), 9.67

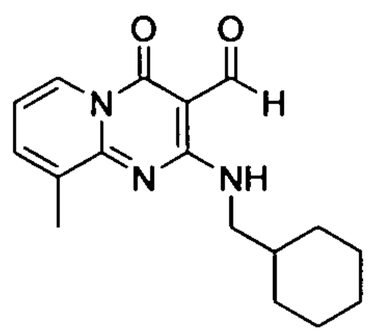
(brs, 1H), 10.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 20.4, 28.7, 48.1, 94.4, 112.5, 125.9, 133.2, 138.1, 152.8, 159.5, 160.7, 190.2.

2-(Diethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (142)



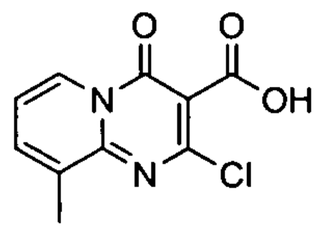
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, *J* = 6.8 Hz, 6H), 2.36 (s, 3H), 3.65 (q, *J* = 6.8 Hz, 4H), 6.72 (t, *J* = 6.8 Hz, 1H), 7.47 (d, *J* = 6.8 Hz, 1H), 8.65 (d, *J* = 6.4 Hz, 1H), 10.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.2, 17.7, 45.3, 96.2, 112.2, 125.8, 133.0, 137.3, 150.2, 158.5, 162.6, 186.9.

2-(Cyclohexylmethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (143)



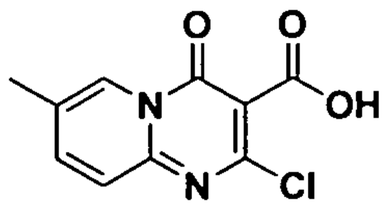
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 – 1.02 (m, 2H), 1.11 – 1.25 (m, 3H), 1.57 – 1.77 (m, 6H), 2.36 (s, 3H), 3.43 (t, *J* = 6.0 Hz, 2H), 6.75 (t, *J* = 7.2 Hz, 1H), 7.50 (d, *J*= 7.2 Hz, 1H), 8.67 (d, *J* = 6.8 Hz, 1H), 9.65 (brs, 1H), 10.21 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 26.0, 26.5, 31.1, 38.2, 47.0, 94.4, 112.5, 125.8, 133.2, 138.0, 152.8, 159.4, 160.6, 190.2

2-Chloro-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (144)



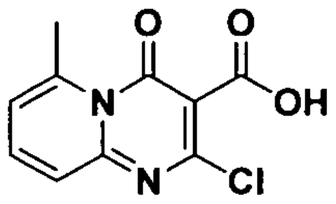
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (s, 3H), 7.53 (t, *J* = 7.0 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.97 (d. *J* = 6.8 Hz, 1H), 13.53 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.7, 108.1, 117.1, 125.6, 133.3, 138.7, 148.2, 152.0, 154.6, 163.9.

2-Chloro-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (145)



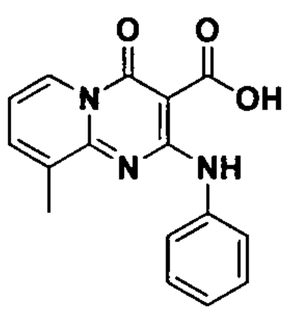
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H), 7.76 (d, J = 8.8 Hz, 1H), 8.11 (d, J = 8.8 Hz, 1H), 8.89 (s, 1H), 13.46 (br s, 1H).

2-Chloro-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (146)



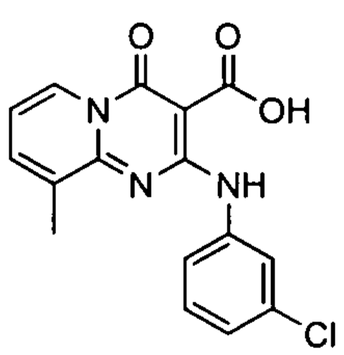
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.00 (s, 3H), 7.19 (d, J = 7.6 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.92 (t, J = 8.0 Hz, 1H), 13.35 (br s, 1H).

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (147)



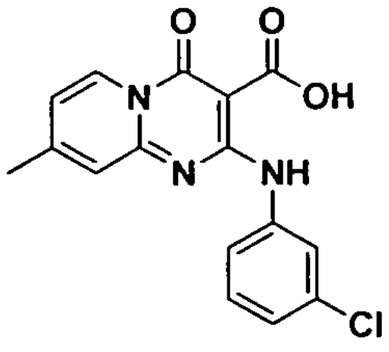
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.70 (dd, J = 6.8, 7.2 Hz, 1H), 7.15 (dd, J = 7.2, 7.2 Hz, 1H), 7.37 (dd, J = 7.2, 7.6 Hz, 2H), 7.65 (d, J = 6.8 Hz, 1H), 7.76 (d, J = 8.4 Hz, 2H), 8.76 (d, J = 7.2 Hz, 1H), 11.70 (brs, 1H), 14.31 (s, 1H).

2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (148)



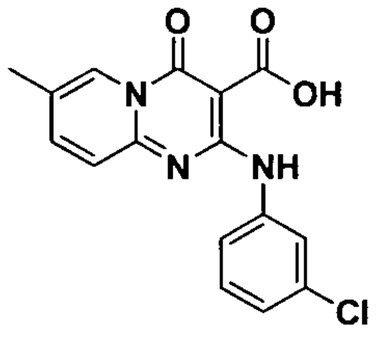
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 7.04 (t, J= 7.0 Hz, 1H), 7.12 (d, J= 8.0 Hz, 1H), 7.28 (J= 8.0 Hz, 1H), 7.71 (d, J= 8.0 Hz, 1H), 8.17 (s, 1H), 8.79 (d, J= 7.6 Hz, 1H), 11.78 (brs, 1H).

2-(3-Chlorophenylamino)-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (149)



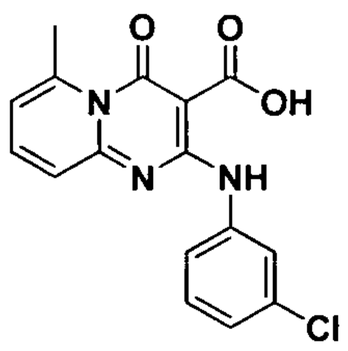
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 6.93 (d, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.25 – 7.29 (m, 2H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.96 (s, 1H), 8.76 (d, *J* = 7.2 Hz, 1H), 11.72 (br s, 1H), 14.19 (s, 1H).

2-(3-Chlorophenylamino)-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (150)



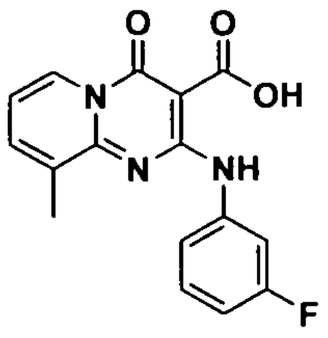
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.27 (t, *J* = 8.6 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.96 (s, 1H), 8.68 (s, 1H), 11.70 (br s, 1H), 14.28 (s, 1H).

2-(3-Chlorophenylamino)-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (151)



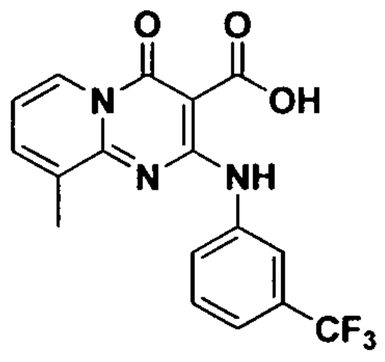
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (s, 3H), 6.70 (d, *J* = 6.8 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.23 – 7.27 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.91 (s, 1H), 11.76 (br s, 1H), 14.37 (s, 1H).

2-(3-Fluorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (152)



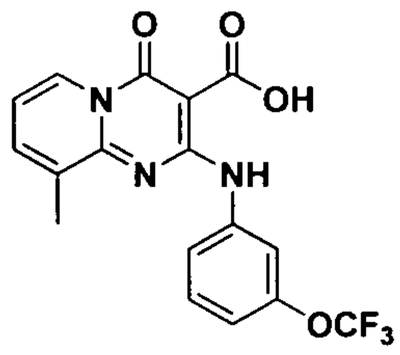
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (s, 3H), 6.81 – 6.87 (m, 1H), 7.03 (t, *J* = 7.2 Hz, 1H), 7.28 – 7.31 (m, 2H), 7.71 (d, *J* = 6.8 Hz, 1H), 7.89 (d, *J* = 10.4Hz, 1H), 8.79 (d, *J* = 7.2 Hz 1H), 11.83 (b s, 1H), 14.26 (br s, 1H).

9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (153)



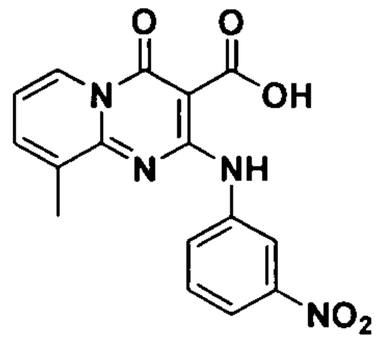
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (s, 3H), 7.05 (t, *J* = 7.0 Hz, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.73 (d, *J* = 6.8 Hz, 1H), 8.58 (s 1H), 8.81 (d, *J* = 6.8 Hz, 1H), 11.91 (br s, 1H).

9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (154)



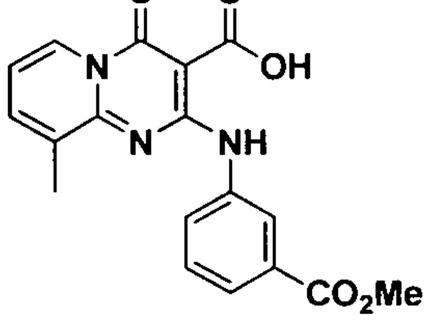
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (s, 3H), 7.00 (d, *J* = 8.0 Hz, 1H), 7.05 (t, *J* = 7.0 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 6.8 Hz, 1H), 8.09 (s, 1H), 8.81 (d, *J* = 7.2 Hz, 1H), 11.89 (br s, 1H), 14.26 (br s, 1H).

9-Methyl-2-(3-nitrophenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (155)



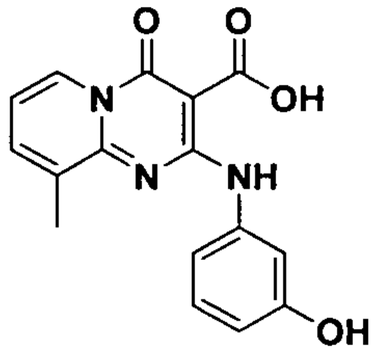
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (s, 3H), 7.40 (t, J = 7.0 Hz, 1H), 7.73 (t, J = 8.2 Hz, 1H), 7.96 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 8.13 (d, J = 6.8 Hz, 1H), 8.90 (d, J = 7.2 Hz, 1H), 9.33 (s, 1H), 11.84 (br s, 1H), 14.43 (br s, 1H).

2-(3-(Methoxycarbonyl)phenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (156)



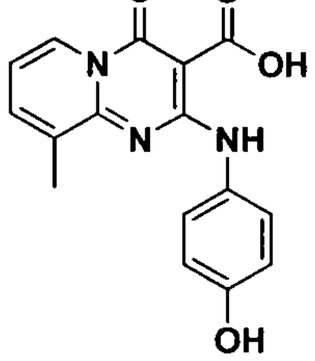
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H), 3.92 (s, 3H), 7.052 (t, J = 6.8 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.71 (t, J = 7.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 1H), 8.79 (d, J = 6.8 Hz, 1H), 8.83 (s, 1H), 11.83 (br s, 1H), 14.28 (br s, 1H).

2-(3-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (157)



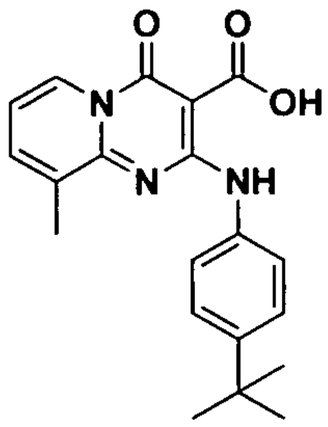
^{1}H NMR (400 MHz, CD_3OD) δ 2.55 (s, 3H), 6.61 (d, J = 8.0 Hz, 1H), 7.15 – 7.24 (m, 3H), 7.34 (s, 1H), 7.88 (d, J = 6.8 Hz, 1H), 8.82 (d, J = 7.2 Hz, 1H).

2-(4-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (158)



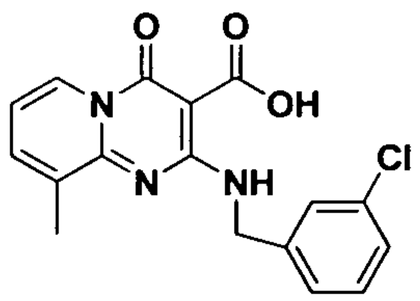
^{1}H NMR (400 MHz, CD_3OD) δ 2.45 (s, 3H), 6.81 (d, J = 8.8 Hz, 2H), 7.10 (t, J = 7.0 Hz, 1H), 7.57 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 6.8 Hz, 1H), 8.78 (d, J = 7.2 Hz, 1H), 11.26 (br s, 1H).

2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (159)



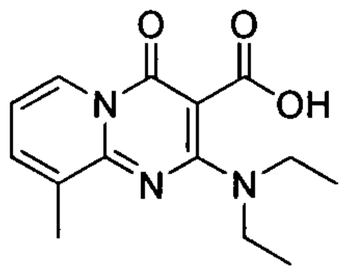
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 2.49 (s, 3H), 6.95 (t, *J* = 7.0 Hz, 1H), 7.37 (d, *J* = 7.2 Hz, 2H), 7.63 (d, *J* = 5.6 Hz, 1H), 7.69 (d, *J* = 6.8 Hz, 2H), 8.71 (d, *J* = 6.8 Hz, 1H), 11.64 (br s, 1H) 14.31 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 31.3, 34.4, 85.3, 114.1, 121.3, 125.5, 125.7, 133.6, 135.4, 138.2, 147.4, 150.2, 157.0, 161.8, 169.7.

2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (160)



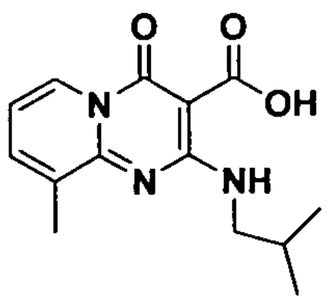
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H), 4.83 (d, *J* = 6.0 Hz, 2H), 7.17 (t, *J* = 7.0 Hz, 1H), 7.32 – 7.40 (m, 3H), 7.50 (s, 1H), 7.89 (d, *J* = 6.8 Hz, 1H), 8.68 (d, *J* = 7.2 Hz, 1H), 9.82 (d, *J* = 6.2 Hz, 1H), 14.25 (br s, 1H).

2-(Diethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (161)



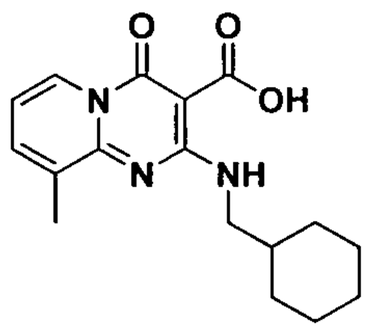
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, *J* = 6.8 Hz, 6H), 2.41 (s, 3H), 3.68 (q, *J* = 6.8 Hz, 4H), 6.67 (t, *J* = 7.2 Hz, 1H), 7.38 (d, *J* = 6.8 Hz, 1H), 8.71 (d, *J* = 7.2 Hz, 1H), 14.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 17.8, 45.4, 96.2, 112.2, 125.8, 133.0, 137.3, 150.2, 158.5, 162.6, 171.6.

2-(Isobutylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (162)



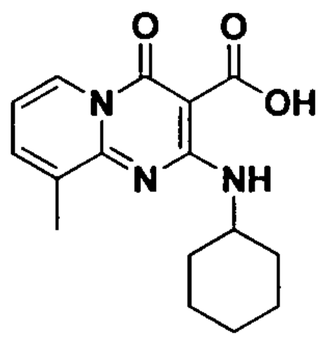
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (d, *J* = 6.8 Hz, 6H), 1.93 – 1.99 (m, 1H), 2.40 (s, 3H), 3.43 (t, *J* = 6.4 Hz, 2H), 6.84 (t, *J* = 7.2 Hz, 1H), 7.53 (d, *J* = 6.4 Hz, 1H), 8.62 (d, *J* = 7.6 Hz, 1H), 9.52 (brs, 1H), 14.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 20.4, 28.7, 48.6, 84.8, 113.2, 125.7, 133.2, 137.5, 150.5, 159.7, 162.0, 169.9.

2-(Cyclohexylmethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (163)



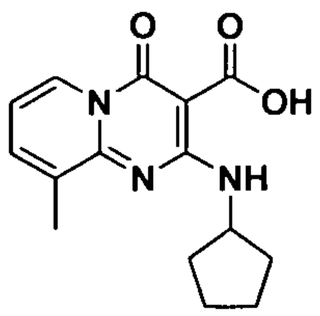
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 – 1.05 (m, 2H), 1.13 – 1.24 (m, 3H), 1.60 – 1.79 (m, 6H), 2.42 (s, 3H), 3.45 (t, *J* = 6.4 Hz, 2H), 6.83 (t, *J* = 7.2 Hz, 1H), 7.54 (d, *J* = 6.8 Hz, 1H), 8.62 (d, *J* = 7.2 Hz, 1H), 9.57 (brs, 1H), 14.13 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 26.0, 26.2, 31.2, 38.2, 47.4, 84.8, 113.2, 125.7, 133.2, 137.5, 150.5, 159.6, 162.0, 170.0.

2-(Cyclohexylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (164)



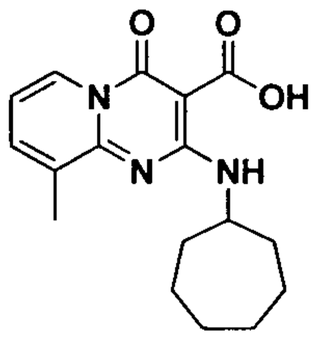
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 – 1.42 (m, 5H), 1.56 – 1.60 (m, 2H), 1.70 – 1.76 (m, 2H), 1.94 – 1.98 (m, 2H), 2.38 (s, 3H), 6.79 (t, *J* = 6.8 Hz, 1H), 7.51 (d, *J* = 6.8 Hz, 1H), 8.56 (d, *J* = 6.8 Hz, 1H), 9.42 (d, *J*= 6.8 Hz, 1H), 14.14 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 24.7, 25.7, 32.6, 50.0, 84.7, 113.1, 125.6, 133.1, 137.4, 150.5, 158.5, 162.0, 169.9.

2-(Cyclopentylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (165)



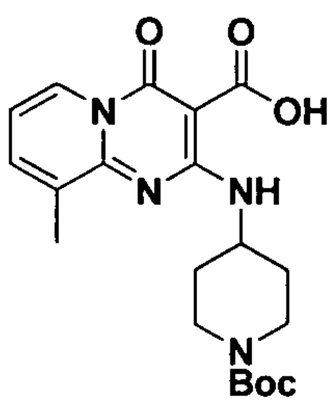
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 – 1.67 (m, 4H), 1.73 – 1.78 (m, 2H), 2.04 – 2.10 (m, 2H), 2.42 (s, 3H), 4.51 (q, *J* = 6.8 Hz, 1H), 6.83 (t, *J* = 6.8 Hz, 1H), 7.53 (d, *J* = 6.8 Hz, 1H), 8.59 (d, *J* = 6.8 Hz, 1H), 9.47 (d, *J*= 6.8 Hz, 1H), 14.15 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 24.1, 33.3, 53.0, 84.8, 113.3, 125.7, 133.3, 137.5, 150.5, 158.9, 162.0, 169.9.

2-(Cycloheptylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (166)



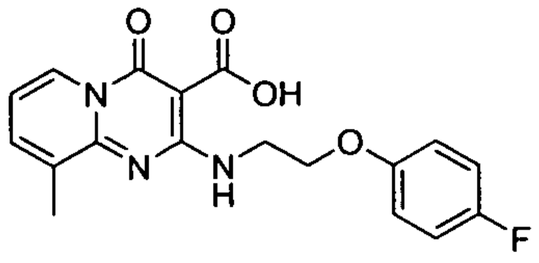
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 – 1.57 (m, 4H), 1.59 – 1.68 (m, 4H), 1.69 – 1.74 (m, 2H), 1.98 - 2.04 (m, 2H), 2.43 (s, 3H), 4.30 – 4.36 (m, 1H), 6.83 (t, *J* = 6.8 Hz, 1H), 7.53 (d, *J* = 6.8 Hz, 1H), 8.64 (d, *J* = 6.8 Hz, 1H), 9.53 (d, *J*= 6.8 Hz, 1H), 14.19 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 24.6, 28.1, 34.7, 52.3, 84.8, 113.1, 125.8, 133.2, 137.4, 150.4, 158.3, 162.1, 170.0.

2-(1-(tert-Butoxycarbonyl)piperidin-4-ylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (167)



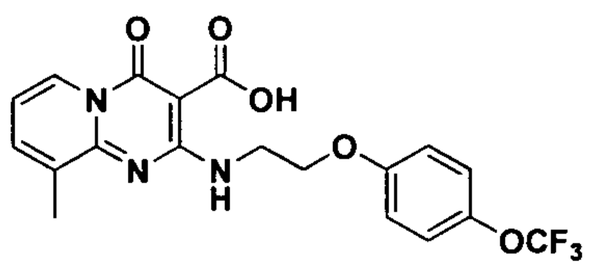
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H), 1.61 – 1.65 (m, 2H), 2.01 – 2.03 (m, 2H), 2.42 (s, 3H), 2.99 – 3.05 (m, 2H), 3.98 – 4.00 (m, 2H), 4.26 – 4.33 (m, 1H), 6.88 (t, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 6.8 Hz, 1H), 8.67 (d, *J* = 7.2 Hz, 1H), 9.56 (d, *J* = 6.8 Hz), 14.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 28.6, 31.6, 48.5, 66.4, 79.9, 85.0, 113.5, 125.9, 133.2, 137.8, 150.6, 154.9, 158.9, 162.0, 169.9.

2-(2-(4-Fluorophenoxy)ethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (168)



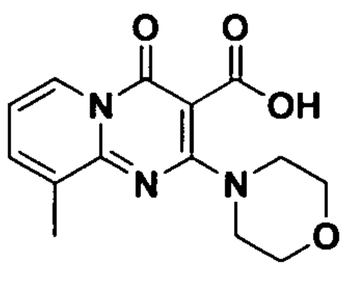
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 4.01 (t, J = 5.6 Hz, 2H), 4.15 (t, J = 5.6 Hz, 2H), 6.83 – 6.96 (m, 5H), 7.59 (d, J = 6.8 Hz, 1H), 8.68 (d, J = 7.2 Hz, 1H), 9.81 (brs, 1H), 14.01 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 40.5, 67.1, 85.3, 113.6, 115.8, 115.9, 116.0, 116.1, 125.9, 133.2, 137.9, 150.6, 154.8, 159.8, 161.9, 169.7.

9-Methyl-4-oxo-2-(2-(4-(trifluoromethoxy)phenoxy)ethylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (169)



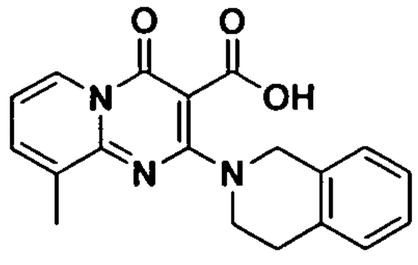
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 4.03 (t, *J* = 5.6 Hz, 2H),4.18 (t, *J* = 5.6 Hz, 2H), 6.90 (d, *J* = 9.2 Hz, 2H), 6.91 (t, *J* = 6.8 Hz, 1H), 7.11 (d, *J* = 9.2 Hz, 2H), 7.60 (d, *J* = 6.8 Hz, 1H), 9.70 (d, *J* = 7.2 Hz, 1H), 9.82 (brs, 1H), 14.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 40.5, 66.9, 77.4, 85.4, 113.7, 115.7, 122.6, 126.0, 133.2, 138.0, 155.8, 157.6, 159.9, 162.0, 169.0, 170.4 .

9-Methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (170)



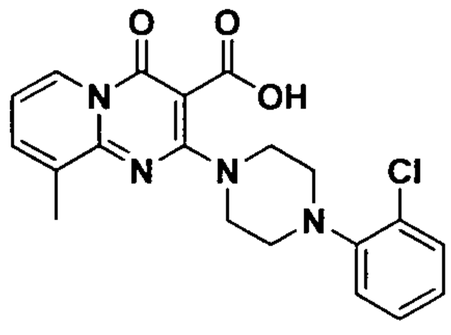
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H), 3.65 (t, *J* = 4.8 Hz, 4H), 3.74 (t, *J* = 4.8 Hz, 4H), 6.86 (t, *J* = 6.8 Hz, 1H), 7.51 (d, *J* = 6.8 Hz, 1H), 8.67 (d, *J* = 6.8 Hz, 1H), 13.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 58.4, 64.8, 97.5, 113.6, 124.6, 132.6, 136.0, 148.1, 160.5, 161.7, 171.3.

2-(3,4-Dihydroisoquinolin-2(1H)-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (171)



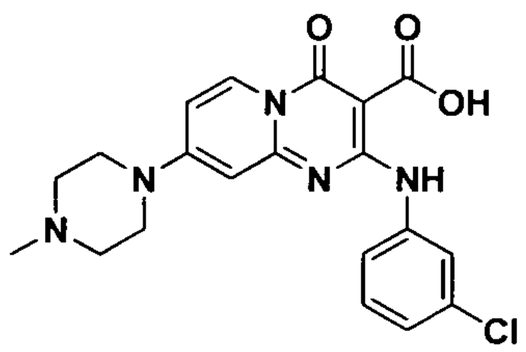
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (s, 3H), 3.03 (t, *J* = 5.8 Hz, 2H), 4.08 (m, 2H), 4.73 (m, 2H), 6.83 (t, *J* = 7.0 Hz, 1H), 7.06 – 7.18 (m, 4H), 7.52 (d, *J* = 6.8 Hz, 1H), 8.60 (d, *J* = 7.2 Hz, 1H), 13.73 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 28.5, 46.1, 52.4, 86.4, 113.0, 125.5, 126.1, 126.2, 126.6, 128.4, 132.9, 133.7, 134.4, 136.8, 148.1, 159.9, 163.2, 165.3.

2-(4-(2-Chlorophenyl)piperazin-1-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (172)



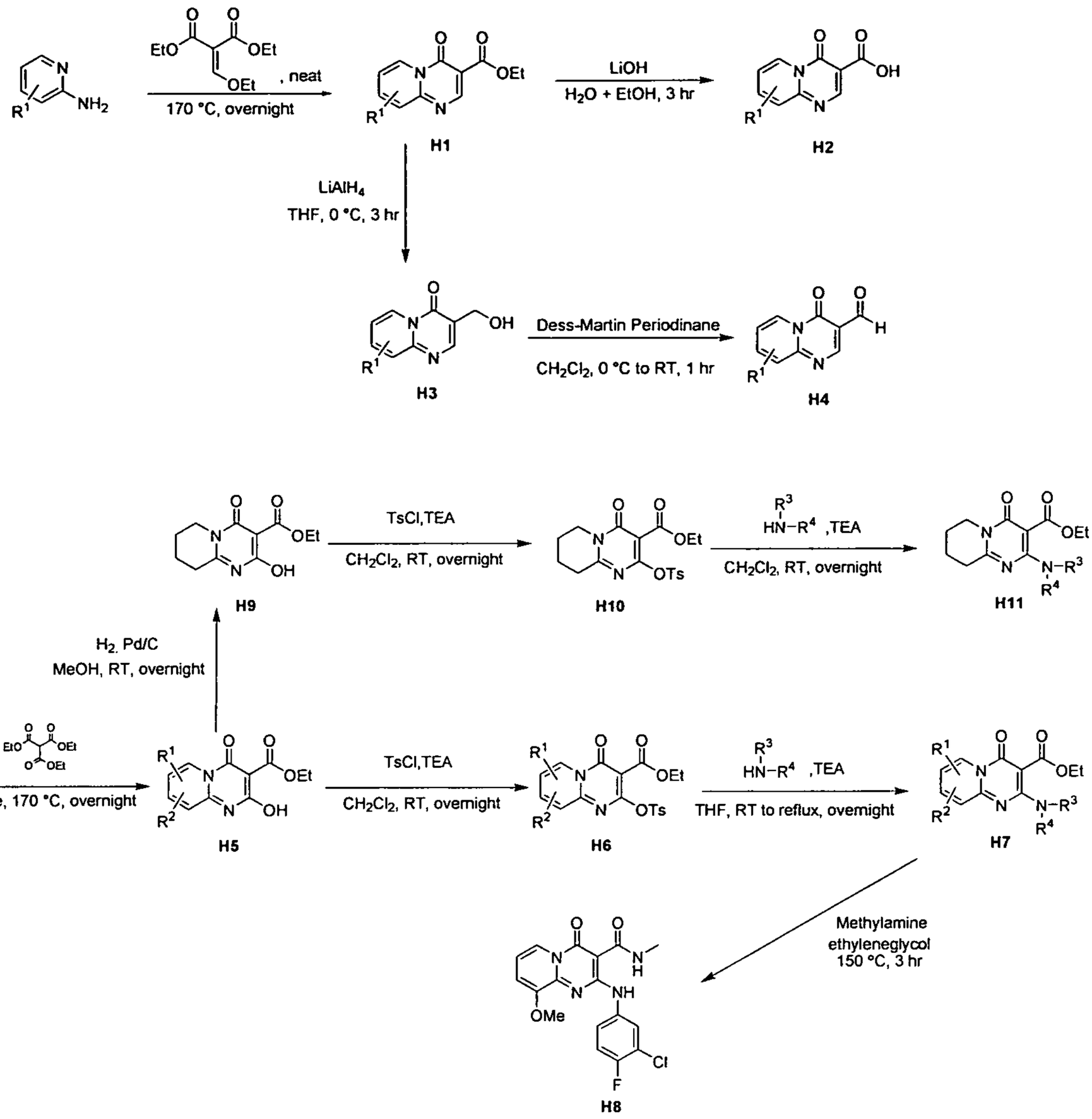
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 3.19 (t, *J* = 4.8 Hz, 4H), 3.96 (m, 4H), 6.87 (t, *J* = 7.0 Hz, 1H), 6.98 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 6.8 Hz, 1H), 8.66 (d, *J* = 7.2 Hz, 1H), 13.74 (br s, 1H).

2-(3-Chlorophenylamino)-8-(4-methylpiperazin-1-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (173)



^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.53 (t, *J* = 4.8 Hz, 4H), 3.54 (t, *J* = 4.8 Hz, 4H), 6.34 (d, *J* = 2.8 Hz, 1H), 6.55 (dd, *J* = 2.8, 8.4 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.49 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.86 (t, *J* = 2.0 Hz, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 11.5 (s, 1H), 14.18 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 46.1, 46.4, 54.4, 83.6, 98.8, 105.1, 120.0, 121.9, 124.0, 128.8, 129.9, 134.4, 139.9, 151.4, 155.6, 158.2, 161.8, 170.2.

Scheme 9



H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 G3 H13

General procedure for the synthesis of H1

2-Amino-3-picoline (1.0 mmol) was dissolved in diethyl ethoxymethylenemalonate (1.0 mmol). The solution was heated to 170 °C for 12 h. After cooling, the dark residue was triturated with EtOAc (10 mL). The residual pale solid was collected by filtration and washed with EtOAc to give H1.

General procedure for the synthesis of H2

To a stirred solution of H1 (0.43 mmol) in H_2O (3.0 mL) and EtOH (1.0 mL) was added LiOH (0.86 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with 1 N HCl (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H2.

General procedure for the synthesis of H3

To a stirred solution of H1 (0.38 mmol) in THF (2.0 mL) was added $LiAlH_4$ (0.57 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. After reaction was completed, 1N NaOH (2 mL) was added dropwise. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with H_2O (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H3.

General procedure for the synthesis of H4

To a stirred solution of H3 (95 μmol) in CH_2Cl_2 (1.0 mL) was added $NaHCO_3$ (285 μmol) and Dess-Martin Periodinane (114 μmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction mixture was filtered off and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H4.

General procedure for the synthesis of H5

To a stirred solution of 2-Amino-pyridine (10.6 mmol) in xylene (10.0 mL) was added diethyl ethoxymethylenemalonate (21.2 mmol). The mixture was stirred at 140 ℃ for 3 hr. After reaction was completed, the residual pale solid was collected by filtration and washed with diethyl ether to give H5.

General procedure for the synthesis of H6

To a stirred solution of H5 (0.42 mmol) in THF (5.0 mL) was added triethylamine (0.63 mmol) and *p*-toluenesulfonylchloride (0.46 mmol) at 0 °C. The reaction mixture was stirred at room temperature for overnight. After reaction was completed, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with 1N HCl (50 ml), saturated $NaHCO_3$ (50 ml) and brine (50 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H6.

General procedure for the synthesis of H7

To a stirred solution of H6 (0.25 mmol) in THF (1.2 mL) was added triethylamine (0.5 mmol) and an amine (0.26 mmol) at 0 °C. The reaction mixture was stirred at room temperature for overnight. After reaction was completed, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with 1N HCl (10 ml), saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H7.

General procedure for the synthesis of H8

To a stirred solution of H7 (0.27 mmol) in ethylene glycol (3.0 mL) was added methylamine (2 N solution in THF 1.3 mL). The mixture was stirred at 150 °C for 3 hr. The reaction mixture was added with ethylacetate (10 mL) and the residual pale solid was collected by filtration and washed with EtOAc. The crude product was purified by flash column chromatography to give H8.

General procedure for the synthesis of H9

To a stirred solution of H5 (2.13 mmol) in MeOH (8.0 mL) was added Pd/C (113 mg). The mixture was stirred at room temperature under H_2 for 3 h. After reaction was completed, the reaction mixture was filtered off and concentrated *in vacuo*. The crude product was recrystallized with EtOAc and hexane (1:4) to give H9.

General procedure for the synthesis of H10

To a stirred solution of H9 (0.42 mmol) in CH_2Cl_2 (5.0 mL) was added triethylamine (0.63 mmol) and *p*-toluenesulfonylchloride (0.46 mmol) at 0 °C. The reaction mixture was stirred at room temperature for overnight. After reaction was completed, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with 1N HCl (50 ml), saturated $NaHCO_3$ (50 ml) and brine (50 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (Hexane : EtOAc = 1: 2) to give H10.

General procedure for the synthesis of H11

To a stirred solution of H10 (0.25 mmol) in THF (2.0 mL) was added triethylamine (0.5 mmol) and an amine (0.37 mmol) at 0 °C. The reaction mixture was stirred at room

temperature for overnight. After reaction was completed, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with 1N HCl (10 ml), saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (Hexane : EtOAc = 1: 1) to give H11.

General procedure for the synthesis of H12

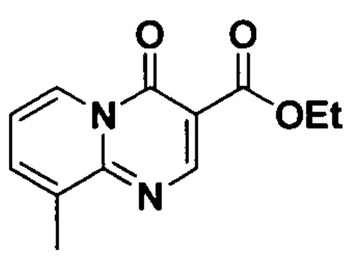
A solution of G3 (1.0 mmol), an amine (1.1 mmol) and triethylamine (2.0 mmol) in THF (2 mL) was refluxed for 1 h and cooled to room temperature. The solvent was evaporated to dryness, which was extracted with CH_2Cl_2 (20 mL x 3).

The reaction mixture was washed with 5% sodium bicarbonate. The organic layer was dried ($MgSO_4$) , filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H12.

General procedure for the synthesis of H13

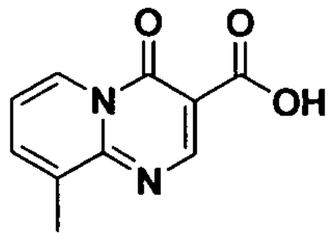
To a solution of G3 (1.1 mmol), an amine (1.0 mmol) in CH_2Cl_2 (5 mL) were added sodium triacetoxyborohydride (2.0 mmol) and glacial acetic acid (2.0 mmol) at room temperature for 20 h. The reaction mixture was added saturated ammonium chloride solution and stirred for 10 min. The reaction mixture was extracted with CH_2Cl_2 (20 mL). The organic layer was dried ($MgSO_4$) , filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give H13.

Ethyl 9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (174)



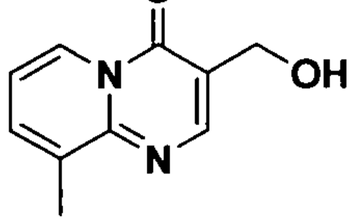
^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, J = 7.2 Hz, 3H), 2.62 (s, 3H), 4.39 (q, J = 7.2 Hz, 2H), 7.20 (t, J = 7.2 Hz, 1H), 7.77 (d, J = 7.2 Hz, 1H), 9.05 (s, 1H), 9.16 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 14.6, 18.2, 61.2, 105.3, 116.8, 127.0, 135.9, 138.2, 155.3, 158.4, 165.0, 189.1.

9-Methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (175)



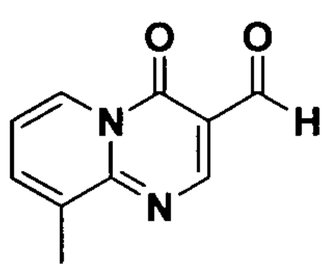
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3H), 7.12 (t, J = 6.8 Hz, 1H), 7.79 (d, J = 6.8 Hz, 1H), 8.87 (s, 1H), 9.21 (d, J = 7.2 Hz), 14.13 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ18.3, 110.9, 117.1, 128.1, 137.6, 141.1, 155.0, 157.1, 158.3, 171.3.

3-(Hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (176)



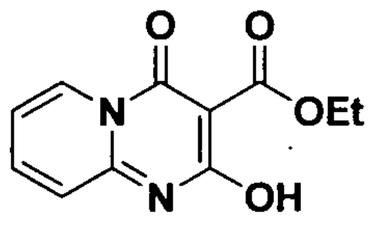
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 3.27 (brs, 1H), 4.66 (s, 2H), 7.01 (t, J = 6.8 Hz, 1H), 7.51 (d, J = 6.8 Hz, 1H), 8.32 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.2, 44.1, 111.2, 117.9, 127.1, 135.7, 139.8, 153.9, 155.6, 158.2.

9-Methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (177)



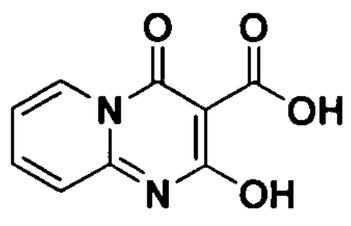
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 (s, 3H), 7.29 (t, J = 7.2 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 8.85 (s, 1H), 9.14 (d, J = 7.2 Hz, 1H), 10.33 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 110.9, 117.5, 126.7, 136.5, 139.5, 153.1, 155.6, 158.1, 188.5.

Ethyl 2-hydroxy-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (178)



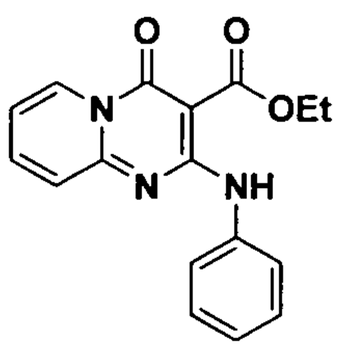
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, J = 7.2 Hz 3H), 4.45 (q, J = 7.2 Hz, 2H), 7.13 (ddd, J = 1.2, 6.8, 7.2 Hz, 1H), 7.49 (d, J = 8.8 Hz, 1H), 7.82 – 7.86 (m, 1H), 9.00 (d, J = 7.2 Hz, 1H), 13.64 (brs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 62.3, 87.1, 115.3, 125.1, 128.7, 140.3, 148.4, 152.6, 155.5, 171.7.

2-Hydroxy-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (179)



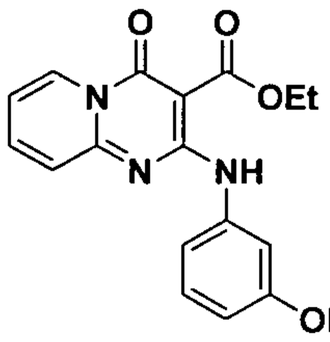
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.70 (dd, *J* = 6.8, 7.2 Hz, 1H), 7.15 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.37, (dd, *J* = 7.2, 7.6 Hz, 1H), 7.65 (d, *J* = 6.8 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 8.76 (d, *J* = 7.2 Hz, 1H), 11.70 (brs, 1H), 14.31 (s, 1H).

Ethyl 4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (180)



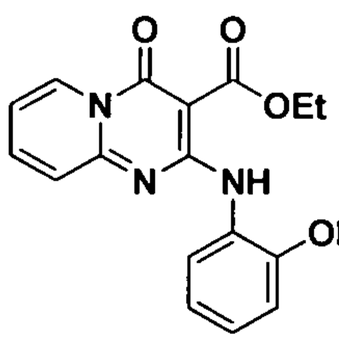
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (t, *J* = 7.2 Hz, 3H), 4.44 (q, *J* = 7.2 Hz, 2H), 6.93 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.29 – 7.36 (m, 3H), 7.65 – 7.68 (m, 3H), 8.97 (d, *J* = 7.2 Hz, 1H), 11.39 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 61.0, 85.5, 113.6, 122.5, 124.2, 124.5, 128.4, 128.6, 138.4, 139.0, 151.6, 155.9, 159.5, 169.6.

Ethyl 2-(3-hydroxyphenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (181)



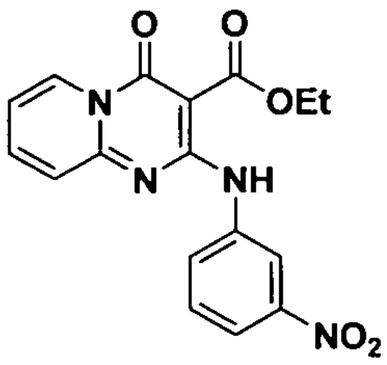
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 1.38 (t, *J* = 7.0 Hz, 3H), 4.37 (q, *J* = 7.2 Hz, 2H), 6.56 – 6.58 (m, 1H), 6.92 (dd, *J* = 6.8, 7.2 Hz, 1H0, 7.05 (d, *J* = 8.4 Hz, 1h0, 7.12 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.26 (m, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.66 (dd, *J* = 7.2, 7.6 Hz, 1H), 8.90 (d, *J* = 7.2 Hz, 1H), 11.22 (brs, 1H).

Ethyl 2-(2-hydroxyphenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (182)



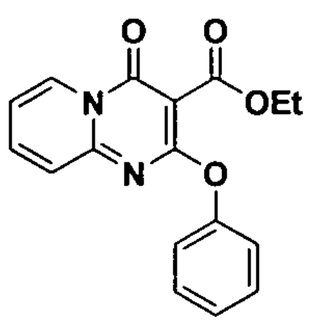
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (t, *J* = 7.2 Hz, 3H), 4.45 (q, *J* = 6.8 Hz, 2H), 6.90 (dd, *J* = 7.2, 8.0 Hz, 1H), 7.05 – 7.08 (m, 2H), 7.13 (dd, *J* = 7.6, 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.81 (dd, *J* = 7.6, 8.0 Hz, 1H), 9.03 (d, *J* = 6.8 Hz, 1H), 11.52 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 14.4, 61.3, 114.7, 120.1, 120.5, 122.9, 124.4, 127.0, 127.1, 129.0, 140.8, 149.3, 151.1, 158.6, 169.5.

Ethyl 2-(3-nitrophenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (183)



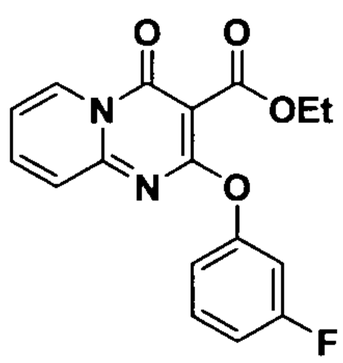
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, J = 6.4 Hz, 3H), 4.45 (q, J = 7.2 Hz, 2H), 7.05 (ddd, J = 1.2, 6.8, 6.8 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.47 (dd, J = 8.0, 8.4 Hz, 2H), 7.77 – 7.82 (m, 2H), 7.93 – 7.96 (m, 1H), 8.97 – 8.98 (m, 1H), 9.04 (dd, J = 0.8, 7.2 Hz, 1H), 11.74 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 14.4, 61.3, 86.1 ,114.5, 116.9, 118.4, 124.7, 127.4, 128.6, 129.2, 139.8, 148.5, 151.5, 155.7, 159.5, 169.6.

Ethyl 4-oxo-2-phenoxy-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (184)



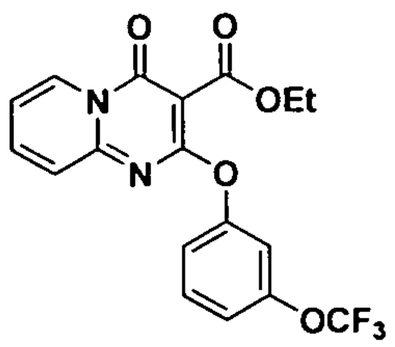
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 7.2 Hz, 3H), 4.42 (q, J = 7.2 Hz, 2H), 7.15 – 7.17 (m, 3H), 7.24 (d, J = 6.4 Hz, 1H), 7.36 - 7.41 (m, 3H), 7.77 (ddd, J = 1.6, 6.8, 6.8 Hz, 1H), 9.10 (dd, J = 0.8, 6.8 Hz, 1H);); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 61.3, 115.7, 121.8, 125.3, 128.5, 129.2, 128.7, 150.3, 152.5, 156.7, 164.1, 165.0.

Ethyl 2-(3-fluorophenoxy)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (185)



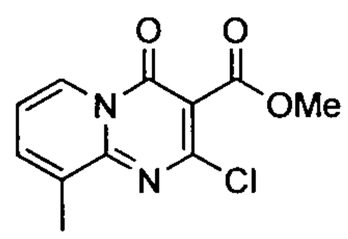
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, J = 7.0 Hz, 3H), 4.40 (q, J = 6.8 Hz, 2H), 6.91 – 6.98 m, 3H), 7.19 (ddd, J = 1.2, 7.2, 7.2 Hz, 1H), 7.32 – 7.36 (m, 1H), 7.39 (d, J = 8.8 Hz, 1H), 7.78 – 7.82 (m, 1H), 9.10 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 61.4, 94.6, 109.8, 110.0, 112.2, 112.4, 115.9, 117.5, 117.6, 125.3, 128.5, 129.8, 129.9, 139.9, 150.3, 153.3, 156.6, 161.6, 163.8, 164.0, 164.5.

Ethyl 4-oxo-2-(3-(trifluoromethyl)phenoxy)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (186)



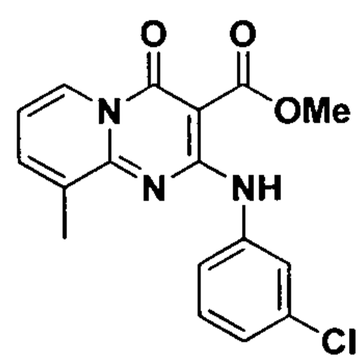
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.2 Hz, 3H), 4.43 (q, *J* = 7.0 Hz 2H), 7.21 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.47 – 7.52 (m, 2H), 7.81 (dd, *J* = 7.2, 8.4 Hz, 1H), 9.12 (d, *J* = 6.8 Hz, 1H).

Methyl 2-chloro-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (187)



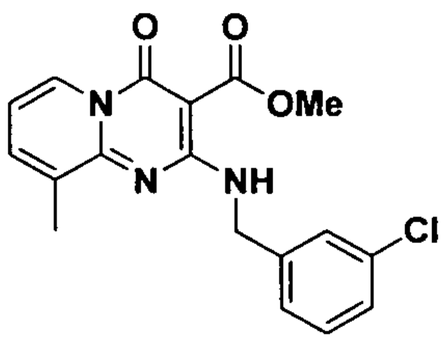
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3H), 3.93 (s, 3H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.75 (d, *J* = 6.8 Hz, 1H), 8.91 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 52.8, 108.0, 116.7, 126.1, 134.9, 138.3, 149.1, 155.1, 155.2, 164.2.

Methyl 2-(3-chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (188)



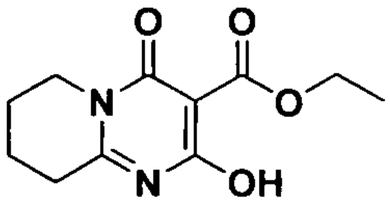
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 3.99 (s, 3H), 6.94 (t, *J* = 7.0 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 6.8 Hz, 1H), 8.18 (s, 1H), 8.91 (d, *J* = 7.2 Hz, 1H), 11.52 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 52.1, 85.3, 113.7, 119.6, 121.9, 123.5, 126.4, 129.4, 133.2, 134.1, 138.4, 139.9, 151.0, 156.2, 158.6, 170.1.

Methyl 2-(3-chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (189)



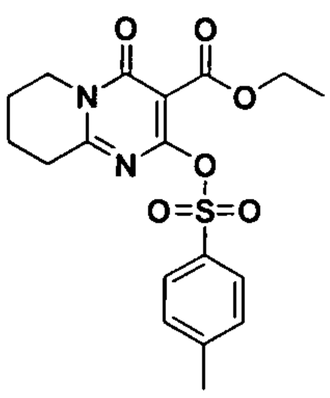
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.92 (s, 3H), 4.77 (d, *J* = 6.0 Hz, 2H), 6.80 (t, *J* = 6.8 Hz, 1H), 7.20-7.24 (m, 3H), 7.34 (s, 3H), 7.50 (d, *J* = 6.8 Hz, 1H), 8.82 (d, *J* = 7.2 Hz, 1H), 9.69 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 44.4, 51.8, 84.6, 112.6, 125.5, 126.4, 127.2, 127.7, 129.7, 132.7, 134.3 ,137.6, 141.1, 151.3, 156.4, 160.8, 170.1.

Ethyl 2-hydroxy-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (190)



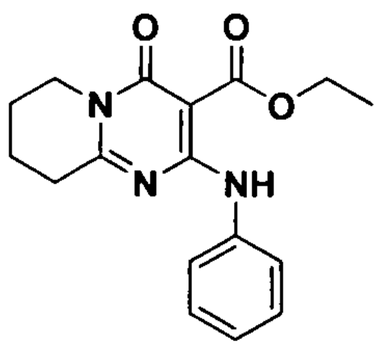
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, *J* = 7.2 Hz, 3H), 1.82 – 1.93 (m, 4H), 2.86 (t, *J* = 6.8 Hz, 2H), 3.84 (t, *J* = 6.0 Hz, 2H), 4.39 (q, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 18.9, 21.9, 32.2, 43.0, 62.4, 90.9, 159.8, 165.1, 171.7, 173.5 .

Ethyl 4-oxo-2-(tosyloxy)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (191)



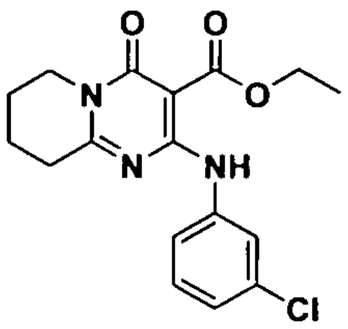
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, *J* = 7.2 Hz, 3H), 1.79 – 1.91 (m, 4H), 2.41 (s, 3H), 2.79 (t, *J* = 6.4 Hz, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 18.8, 21.6, 21.9, 31.8, 43.6, 61.9, 104.2, 129.1, 129.7, 134.2, 145.8, 159.4, 160.8, 162.0, 162.2.

Ethyl 4-oxo-2-(phenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (192)



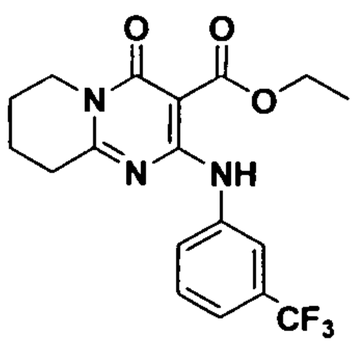
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 1.80 – 1.92 (m, 4H), 2.80 (t, *J* = 6.8 Hz, 2H), 3.87 (t, *J* = 6.0 Hz, 2H), 4.36 (q, *J* = 7.2 Hz, 2H), 7.08 (t, J = 7.2 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 2H), 7.53 (d, J = 7.6 Hz, 2H), 11.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 19.2, 22.2, 32.2, 42.4, 61.0, 88.4, 122.9, 124.4, 128.8, 138.4, 160.5, 160.8, 162.2, 169.8 .

Ethyl 2-(3-chlorophenylamino)-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (193)



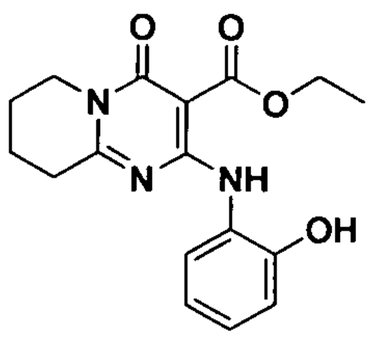
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (t, *J* = 7.2 Hz, 3H), 1.76 – 1.88 (m, 4H), 2.76 (t, *J* = 6.8 Hz, 2H), 3.78 (t, *J* = 6.0 Hz, 2H), 4.29 (q, *J* 7.06 (dd, *J* = 7.2 Hz, 2H), *J* = 1.2, 8.0 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.51 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.58 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 18.6, 22.1, 32.1, 42.6, 61.1, 81.4, 111.2, 111.7, 113.0, 128.4, 140 .4,149.6, 158.7, 161.12, 163.2, 170.4

Ethyl 4-oxo-2-(3-(trifluoromethyl)phenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (194)



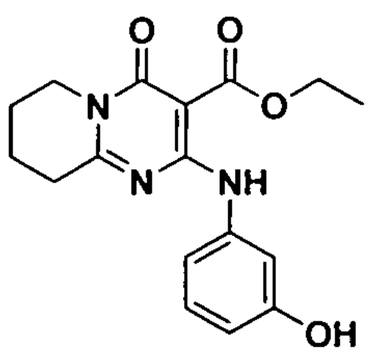
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (t, *J* = 7.2 Hz, 3H), 1.88 – 1.97 (m, 4H), 2.87 (t, *J* = 6.4 Hz, 2H), 3.93 (t, *J* = 5.6 Hz, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 8.05 (s, 1H), 11.2 (s, 1H);

Ethyl 2-(2-hydroxyphenylamino)-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (195)



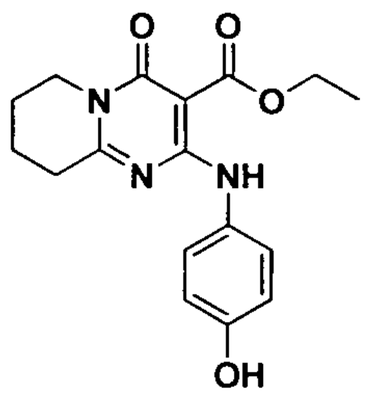
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 1.81 – 1.94 (m, 4H), 2.65 (t, *J* = 6.8 Hz, 2H), 3.65 (t, *J* = 6.0 Hz, 2H), 4.18 (q, *J* = 6.8 Hz, 2H), 6.85 (t, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 7.2 Hz, 1H), 7.06 – 7.12 (m, 2H), 9.98 (s, 1H), 11.3 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 18.8, 21.9, 31.6, 42.6, 61.3, 88.4, 120.2, 120.7, 124.5, 127.1, 127.2, 149.1, 159.4, 159.5, 163.0, 169.6 .

Ethyl 2-(3-hydroxyphenylamino)-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (196)



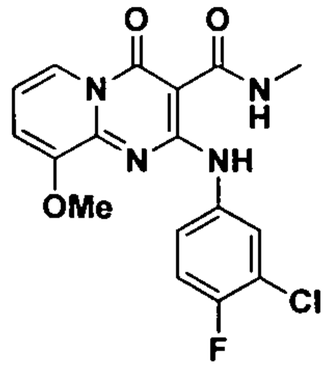
^{1}H NMR (400 MHz, $CDCl_3$+MeOD- d_4) δ 1.26 (t, J = 7.2 Hz, 3H), 1.71 – 1.81 (m, 4H), 2.72 (t, J = 6.4 Hz, 2H), 3.74 (t, J = 6.4 Hz, 2H), 4.23 (q, J = 7.2 Hz, 2H), 6.47 (d, J = 7.6 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 7.02 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + MeOD- d_4) δ 14.2, 18.8, 21.9, 31.8, 42.4, 60.9, 79.8, 109.8, 111.6, 114.0, 129.4, 139.4,149.7, 159.3, 160.2, 163.1, 169.6

Ethyl 2-(4-hydroxyphenylamino)-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (197)



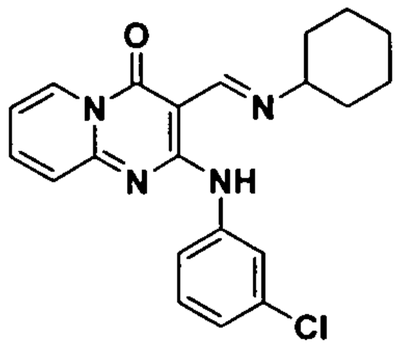
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (t, J = 7.2 Hz, 3H), 1.67 – 1.80 (m, 4H), 2.65 (t, J = 6.8 Hz, 2H), 3.65 (t, J = 6.0 Hz, 2H), 4.18 (q, J = 7.2 Hz, 2H), 6.68 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 8.8 Hz, 2H), 9.29 (s, 1H), 10.7 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 18.9, 21.9, 32.1, 42.3, 60.4, 87.2, 115.7, 125.0, 130.1, 154.9, 159.4, 160.6, 163.3, 169.6 .

2-(3-Chloro-4-fluorophenylamino)-9-methoxy-N-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (198)



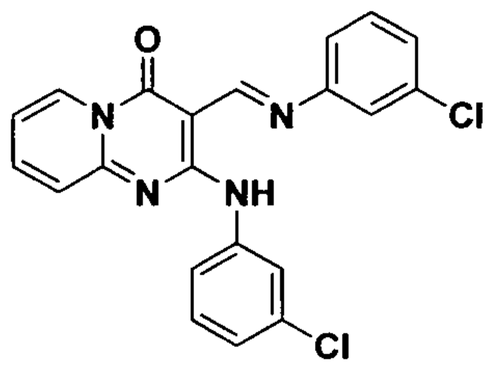
mp = 218 °C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.97 (d, J = 4.8 Hz, 3H), 4.41 (s, 3H), 6.89 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 6.97 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.05 (dd, J = 8.8 Hz, 8.8 Hz, 1H), 7.40 – 7.44 (m, 1H), 8.46 – 8.51 (m, 2H), 8.82 (d, J = 2.0 Hz, 1H), 12.98 (s, 1H);

(E)-2-(3-Chlorophenylamino)-3-((cyclohexylimino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (199)



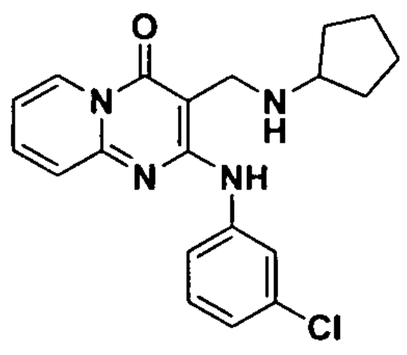
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 – 1.37 (m, 3H), 1.41 – 1.50 (m, 2H), 1.56 – 1.59 (m, 1H), 1.73 – 1.76 (m, 4H), 3.16 – 3.22 (m, 1H), 6.85 (ddd, *J* = 1.2, 6.8, 6.8 Hz, 1H), 6.94 (ddd, *J* = 0.8, 1.2, 8.0 Hz, 1H), 7.14 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.38 (ddd, *J* = 0.8, 1.2, 8.0 Hz, 1H), 7.54 – 7.58 (m, 1H), 7.90 – 7.91 (m, 1H), 8.83 (s, 1H), 8.85 (dd, *J* = 0.8, 1.2 Hz, 1H), 13.40 (brs, 1H);^{13}C NMR (100 MHz, $CDCl_3$) δ 24.4, 25.6, 34.9, 68.4, 91.6, 113.4, 119.2, 121.2, 123.0, 124.7, 127.6, 129.5, 134.2, 137.6, 140.8, 150.6, 156.3, 157.0, 158.3.

(E)-2-(3-Chlorophenylamino)-3-((3-chlorophenylimino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (200)



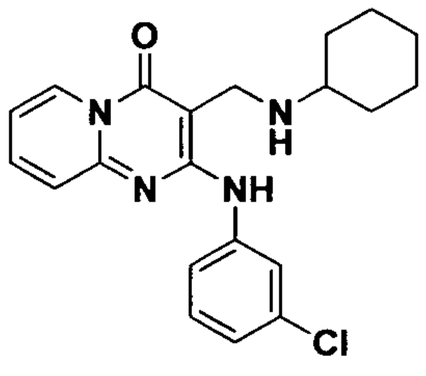
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (dd, *J* = 0.8, 1.2, 8.0 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 7.29 (dd, *J* = 2.0, 4.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.52 (ddd, *J* = 0.8, 1.2, 8.0 Hz, 1H), 7.17 – 7.76 (m, 1H), 8.02 – 8.04 (m, 1H), 8.98 (dd, *J* = 0.8, 6.8 Hz, 1H), 9.17 (s, 1H), 12.94 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 92.6, 114.0, 119.5, 119.8, 121.8, 123.9, 125.0, 125.7, 128.0, 129.7, 130.2, 134.4, 134.8, 138.7, 140.1, 151.3, 151.8, 157.0, 158.0, 158.9.

2-(3-Chlorophenylamino)-3-((cyclopentylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (201)



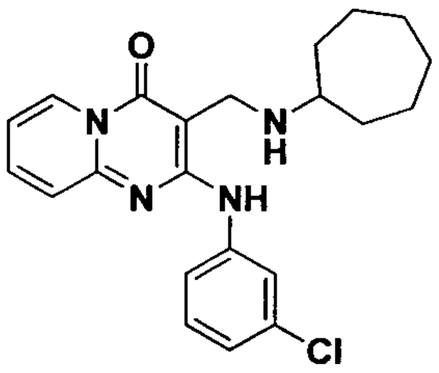
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 – 1.57 (m, 2H), 1.74 – 1.83 (m, 4H), 2.05 – 2.08 (m, 2H), 3.23 – 3.24 (m, 1H), 4.19 (s, 2H), 6.93 – 6.98 (m, 2H), 7.11 – 7.15 (m, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.51 (dd, J = 2.0, 8.4 Hz, 1H), 7.61 – 7.65 (m, 1H), 7.74 – 7.75 (m, 1H), 8.73 (d, J = 7.2 Hz, 1H).

2-(3-Chlorophenylamino)-3-((cyclohexylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (202)



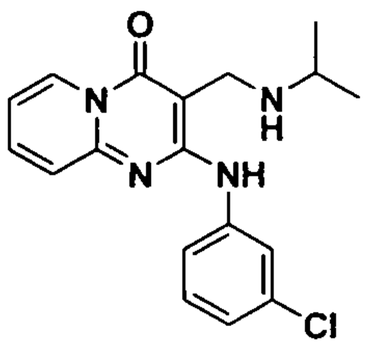
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 – 1.35 (m, 4H), 1.66 – 1.72 (m, 2H), 1.86 – 1.89 (m, 2H), 2.23 – 2.39 (m, 2H), 3.12 – 3.18 (m, 1H), 6.93 (ddd, J = 1.2, 6.8, 7.2 Hz, 1H), 6.99 (ddd, J = 0.8, 1.2, 7.6 Hz, 1H), 7.20 (dd, J = 8.0, 8.0 Hz, 1H), 7.25 (d, J = 8.8 Hz, 1H), 7.52 – 7.57 (m, 1H), 7.61 (dd, J = 1.2, 8.0 Hz, 1H), 7.84 – 7.85 (m, 1H), 8.76 (d, J = 6.4 Hz, 1H), 9.77 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6, 25.0, 41.2, 57.9, 88.9, 114.6, 119.2, 121.1, 122.8, 124.6, 127.3, 129.4, 133.7, 137.3, 140.8, 149.6, 157.2, 158.8.

2-(3-Chlorophenylamino)-3-((cycloheptylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (203)



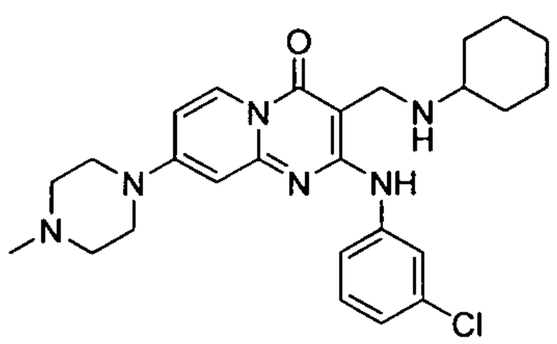
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 – 1.59 (m, 6H), 1.72 – 1.81 (m, 4H), 2.18 – 2.23 (m, 2H), 3.07 – 3.12 (m, 1H), 4.05 (m, 2H), 6.82 (ddd, J = 1.2, 6.8, 6.8 Hz, 1H), 6.91 (dd, J = 1.2, 8.0 Hz, 1H), 7.14 (dd, J = 8.0, 8.0 Hz, 1H), 7.44 – 7.49 (m, 2H), 7.78 – 7.80 (m, 1H), 8.70 (d, J = 6.8 Hz, 1H), 10.00 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.8, 32.3, 41.5, 59.7, 89.7, 114.2, 118.7, 120.6, 122.4, 124.4, 127.2, 129.3, 133.7, 136.8, 140.9, 149.4, 157.2, 158.2.

2-(3-Chlorophenylamino)-3-((isopropylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (204)



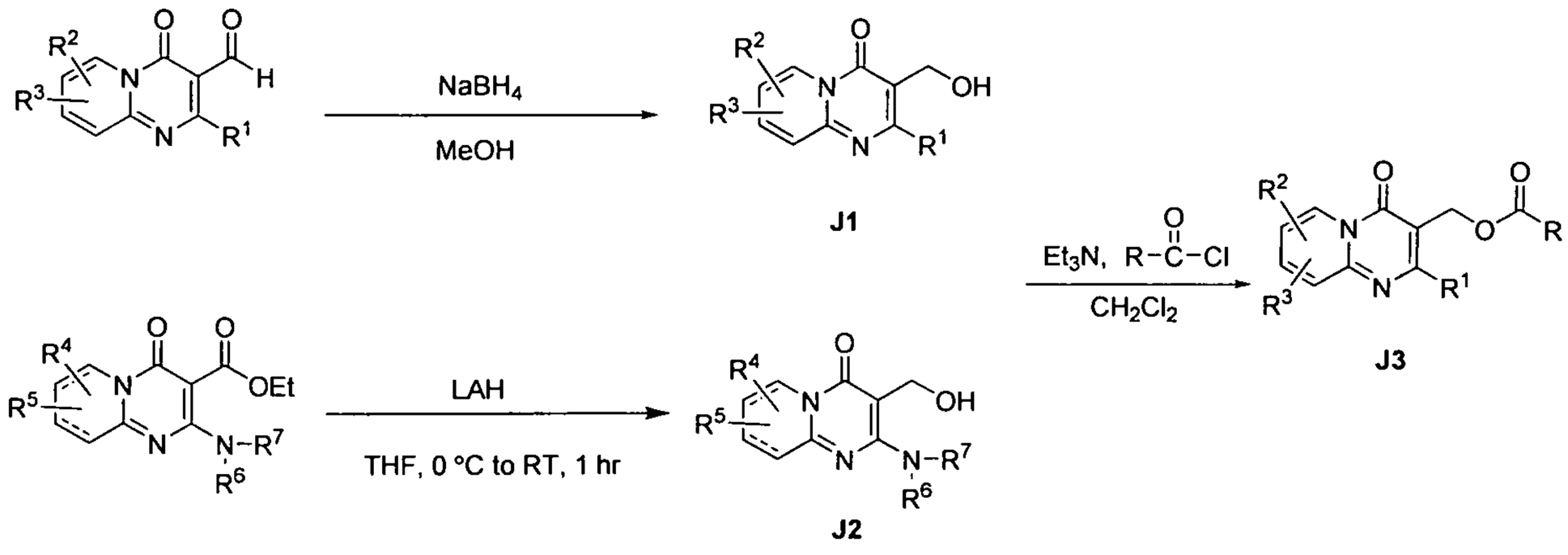
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.26 (s, 3H), 2.30 – 3.06 (m, 1H), 4.05 (s, 2H), 6.87 (dd, *J* = 6.4, 7.2 Hz, 1H), 6.95 (d, *J* = 7.2 Hz, 1H), 7.17 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.54 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.81 (s, 1H), 8.83 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1, 41.7, 48.9, 91.5, 113.7, 118.2, 120.1, 122.2, 124.6, 127.5, 129.5, 134.1, 136.2, 141.2, 149.5, 157.4, 157.8.

2-(3-Chlorophenylamino)-3-((cyclohexylamino)methyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (205)



^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 – 1.34 (m, 3H), 1.71 – 1.91 (m, 3H), 1.92 – 2.04 (m, 2H), 2.20 (s, 3H), 2.23 – 2.36 (m, 6H), 3.04 – 3.10 (m, 5H), 4.01 (s, 2H), 5.87 (s, 1H), 6.55 (s, *J* = 8.0 hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 7.6 Hz,1H), 7.84 (s, 1H), 8.46 (d, *J* = 7.6 Hz, 1H), 9.59 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 25.3, 30.2, 41.2, 46.1, 46.3, 54.2, 58.4, 86.2, 98.9, 106.5, 119.3, 121.0, 122.3, 128.3, 129.5, 133.9, 141.9, 150.8, 154.8, 157.7, 158.9.

Scheme 10



General procedure for the synthesis of J1

To a solution of an aldehyde (0.9 mmol) in methanol (0.5 mL) was added $NaBH_4$ (1.35 mmol) at room temperature. After stirring 1 h, the reaction mixture was diluted with methylene chloride (10 mL) and washed with brine (10 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by recrystallization from a mixture of hexanes and ethyl acetate to give J1.

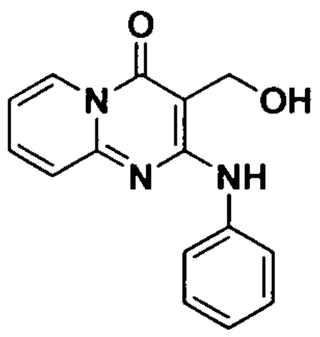
General procedure for the synthesis of J2

To a stirred solution of an ester (0.06 mmol) in THF (1.0 mL) was added $LiAlH_4$ (0.09 mmol). The reaction mixture was stirred at room temperature for 1 hr. After reaction was completed, H_2O (0.1 mL) was added dropwise. The reaction mixture was filtered off and concentrated *in vacuo*. The crude product was purified by flash column chromatography to give J2.

General procedure for the synthesis of J3

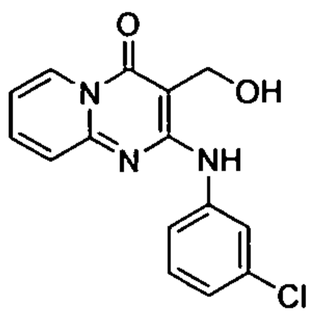
To a stirred solution of J1 or J2 (0.19 mmol) in CH_2Cl_2 (0.6 mL) was added triethylamine (0.38 mmol) and a benzoyl chloride (0.28 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After reaction was completed, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with brine (10 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (Hexane : EtOAc = 2: 1) to give J3.

3-(Hydroxymethyl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (206)



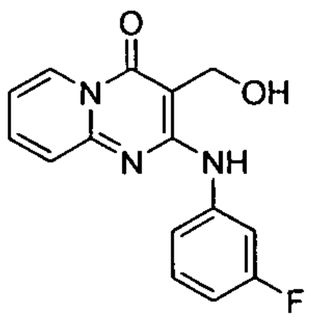
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 4.80 (s, 2H), 6.87 – 6.90 (m, 1H), 8.03 (dd, *J* = 7.2, 7.6 Hz, 1H), 7.27 (dd, *J* = 7.6, 8.0 Hz, 2H), 7.53 – 7.58 (m, 3H), 8.36 (brs, 1H), 8.82 (d, *J* = 6.8 Hz, 1H);^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 56.0, 94.80, 94.85, 113.8, 121.1, 121.2, 123.2, 123.3, 124.5, 127.5, 128.6, 136.4, 138.9, 139.0, 149.7, 157.1, 158.0, 158.1.

2-(3-Chlorophenylamino)-3-(hydroxy methyl)-4H-pyrido-[1,2-a] pyrimidin-4-one (207)



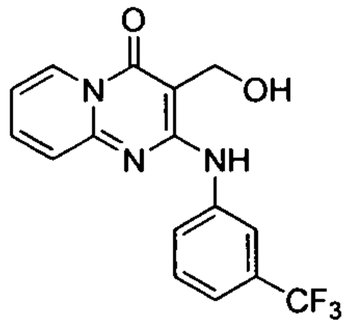
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (d, *J* =6.4 Hz, 2H), 6.93 (t, *J* =6.8 Hz, 1H), 7.05 (d, *J* =8.0 Hz, 1H), 7.38 (t, *J* =4.4 Hz, 2H), 7.42 (s, 1H), 7.63 (t, *J* =6.8 Hz, 1H), 7.81 (t, *J* =1.6 Hz, 1H), 8.20 (s, 1H), 8.92 (d, *J* =7.2 Hz, 1H),

2-(3-Fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido [1,2-a] pyrimidine-3-carbaldehyde (208)



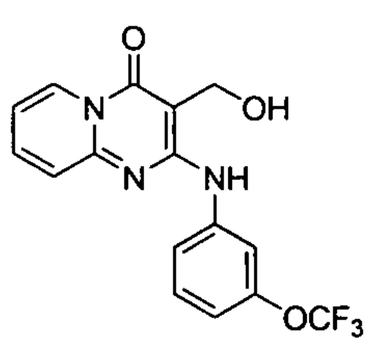
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (s, 2H), 6.94 (t, *J* =6.0 Hz, 2H), 7.17 (d, *J* =8.0 Hz, 1H), 7.43 (d, *J* =8.8 Hz, 2H), 7.63 (t, *J* =7.2 Hz, 2H), 7.70 (d, *J* =9.2 Hz, 1H), 8.26 (s, 1H), 8.93 (d, *J* =7.2 Hz, 1H).

3-(Hydroxymethyl)-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (209)



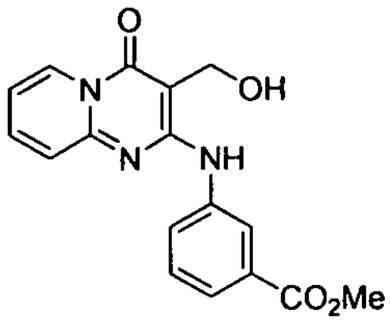
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.99 (s, 2H), 6.99 (d, *J* =6.0 Hz, 2H), 7.32 (d, *J* =8.0 Hz, 1H), 7.43 (d, *J* =7.6 Hz, 2H), 7.69 (brs, 2H), 8.06 (s, 1H), 8.27 (s, 1H), 8.96 (d, *J* =7.6 Hz, 1H).

3-(Hydroxymethyl)-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (210)



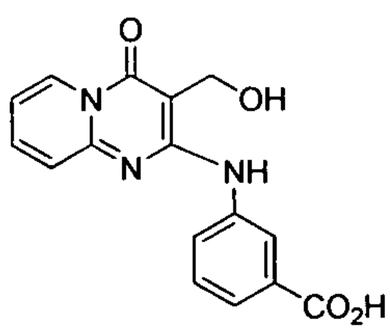
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (d, *J* =6.4 Hz, 2H), 6.84 (t, *J* =6.8 Hz, 1H), 6.92 (d, *J* =6.8 Hz, 1H), 7.30-7.34 (m, 3H), 7.59 (t, *J* =7.2 Hz, 1H), 7.86 (s, 1H), 8.36 (s, 1H), 8.87 (d, *J* =6.4 Hz, 1H),

Methyl 3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino)benzoate (211)



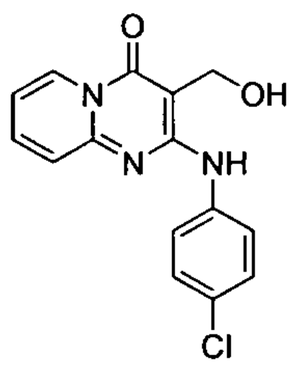
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 4.99 (d, *J* =6.4 Hz, 2H), 6.96 (t, *J* =7.2Hz, 1H), 7.38-7.42 (m, *2*H), 7.63 (t, *J* =7.8 Hz, 1H), 7.75 (d, *J* =7.6 Hz, 1H), 7.88 (d, *J* =8.0 Hz, 1H), 8.21(s, 1H), 8.25 (brs, 1H), 8.96 (d, *J* =7.6 Hz, 1H).

3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino) benzoic acid (212)



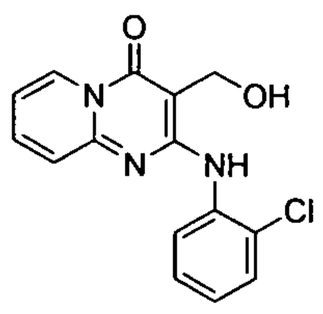
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.73 (s, 1H), 5.74 (s, 2H), 7.19 (t, *J* =7.2Hz, 1H), 7.38-7.42 (m, 2H), 7.45 (d, *J* =7.6 Hz, 1H), 7.86 (t, *J* =8.4 Hz, 1H), 8.00 (d, *J* =8.0 Hz, 1H), 8.19 (s, 1H), 8.82 (s, 1H), 8.89 (d, *J* =6.8 Hz, 1H).

2-(4-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (213)



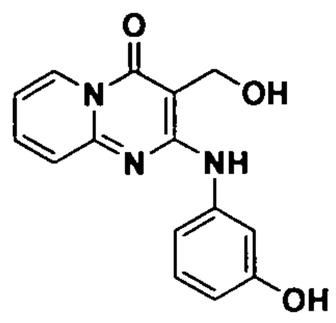
^{1}H NMR (400 MHz, DMSO) δ 4.05 (d, *J* =7.2 Hz, 2H), 7.37 (d, *J* =8.8Hz, 2H), 7.44 (d, *J* =8.8 Hz, 1H), 7.75 (d, *J* =6.8 Hz, 2H), 7.88 (t, *J* =8.8 Hz, 1H), 8.81 (s, 1H), 8.88 (d, *J* =6.4 Hz, 1H).

2-(2-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (214)



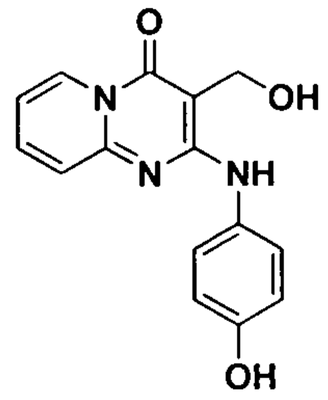
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.01 (d, *J* =5.6 Hz, 2H), 6.97-7.01 (m, 3H), 7.26-7.29 (m, 1H), 7.42 (t, *J* =8.8 Hz, 2H), 7.66 (t, *J* =7.2 Hz, 1H), 8.41 (t, *J* =5.2 Hz, 1H), 8.53(s, 1H), 8.99 (d, *J* =6.8 Hz, 1H).

3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (215)



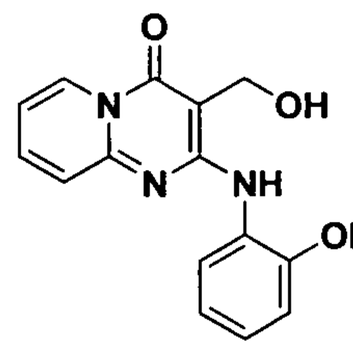
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 4.81 (s, 2H), 6.53 (d, *J* = 8.0 Hz, 1H), 6.99 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 7.12 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.18 (s, 1H), 7.42 (d, J = 9.6 Hz, 1H), 7.64 (dd, J = 6.8, 8.8 Hz, 1H), 8.88 (d, J = 7.2 Hz, 1H).

3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (216)



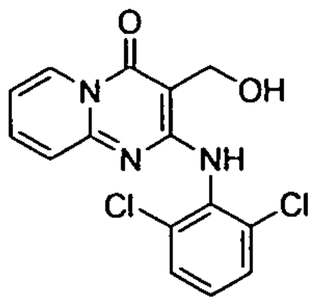
^{1}H NMR (400 MHz, CD_3OD) δ 4.83 (s, 2H), 6.77 (dd, *J* = 2.0 , 8.8 Hz, 2H), 7.04 (dd, *J* = 6.8, 6.8 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.34 – 7.67 (m, 2H), 7.67 – 7.73 (m, 1H), 8.84 (d, *J* = 6.8 Hz, 1H).

3-(Hydroxymethyl)-2-(2-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (217)



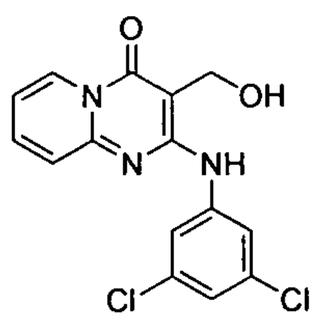
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 3.71 (s, 1H), 4.86 (s, 2H), 6.88 (ddd, *J* = 1.6, 7.6, 8.0 Hz, 1H), 6.93 (dd, *J* = 1.6, 8.0 Hz, 1H), 6.98 (ddd, *J* = 1.6, 7.2, 8.0 Hz, 1H(, 7.05 (ddd, *J* = 1.2, 6.8, 6.8 Hz,. 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.69 – 7.73 (m, 2H), 8.91 (dd, *J* = 0.8, 6.8 Hz, 1H).

2-(2,6-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (218)



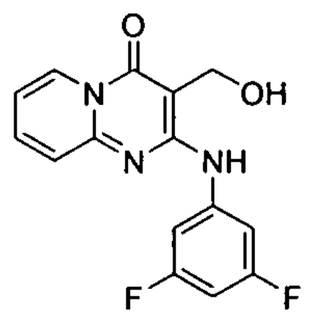
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.03 (d, *J* =6.0 Hz, 2H), 6.96 (t, *J* =7.2 Hz, 1H), 7.16 (t, *J* =7.6 Hz, 2H), 7.2 (s, 1H), 7.39 (d, *J* =8.0 Hz, 2H), 7.56 (t, *J* =7.6 Hz, 1H), 7.77 (s, 1H), 8.96 (d, *J* =7.2 Hz, 1H).

2-(3,5-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (219)



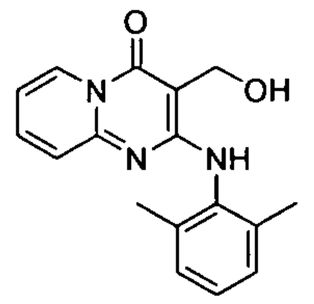
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97 (d, *J* =6.0 Hz, 2H), 7.01-7.04 (m, 2H), 7.50 (t, *J* =6.8 Hz, 1H), 7.60 (s, 2H), 7.71 (t, *J* =8.4 Hz, 2H), 8.24 (s, 1H), 8.98 (d, *J* =7.2 Hz, 1H).

2-(3,5-Difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (220)



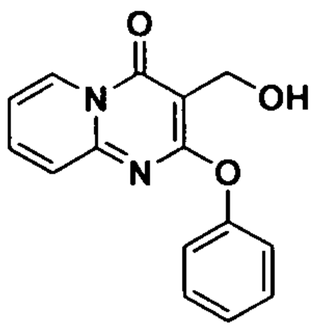
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.99 (d, *J* =6.0 Hz, 2H), 6.52 (t, *J* =8.8 Hz, 1H), 7.05 (t, *J* =5.6 Hz, 2H), 7.29 (d, *J* =2.0 Hz, 2H), 7.51 (s, 1H), 7.72 (t, *J* =7.6 Hz, 1H), 8.30 (s, 1H), 8.99 (d, *J* =6.4 Hz, 1H).

2-(2,6-Dimethylphenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (221)



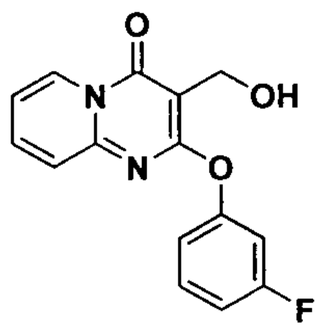
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 6H), 5.02 (d, *J* =6.4 Hz, 2H), 6.92 (t, *J* =6.8 Hz 1H), 7.12 (s, 3H), 7.20 (d, *J* =8.8 Hz, 1H), 7.33 (s, 1H), 7.53 (t, *J* =6.8 Hz, 1H), 8.94 (d, *J* =6.4 Hz, 1H).

3-(Hydroxymethyl)-2-phenoxy-4H-pyrido[1,2-a]pyrimidin-4-one (222)



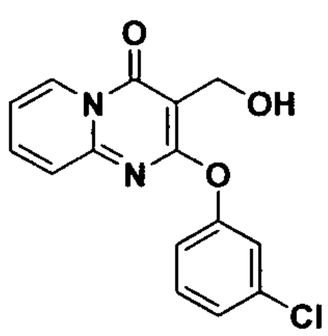
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.31 (brs, 1H), 4.86 (s, 2H), 7.03 – 7.09 (m, 3H), 7.13 – 7.18 (m, 1H), 7.28 – 7.34 (m, 3H), 7.58 – 7.62 (m, 1H), 8.94 – 8.96 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.0, 99.7, 115.2, 121.7, 125.1, 125.3, 127.4, 129.3, 136.8, 149.2, 152.8, 159.6, 164.0.

2-(3-Fluorophenoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (223)



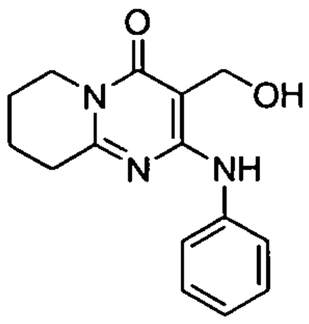
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (brs, 1H), 4.78 (s, 2H), 6.78 – 6.85 (m, 3H), 7.02 (ddd, *J* = 1.2, 6.8, 7.2 Hz, 1H), 7.18 – 7.23 (m, 1H), 7.25 (d, *J* = 9.2 Hz, 1H), 7.57 -7.62 (m, 1H), 8.89 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3, 99.7, 109.4, 109.6, 111.7, 111.9, 115.2, 117.2, 117.3, 125.0, 127.3, 129.7, 129.8, 137.0, 149.0, 153.5, 153.6, 159.4, 161.4, 163.6, 163.8.

2-(3-Chlorophenoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (224)



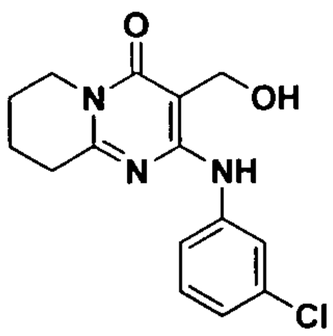
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (t, *J* = 6.4 Hz, 1H), 4.79 (d, *J* = 6.4 Hz, 2H), 6.95 – 6.98 (m, 1H), 7.04 (dd, *J* = 6.8, 7.2 Hz, 1H), 7.08 – 7.10 (m, 1H), 7.20 (dd, *J* = 8.4, 8.8 Hz, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.59 – 7.63 (m, 1H), 8.91 9dd, J = 0.4, 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.3, 55.4, 99.7, 115.3, 120.1, 122.2, 125.1, 127.4, 129.8, 134.3, 137.0, 153.2, 159.2, 163.6.

3-(Hydroxymethyl)-2-(phenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (225)



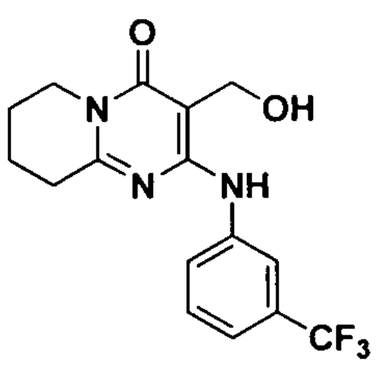
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 – 1.93 (m, 4H), 2.15 (s, 2H), 2.84 (t, *J* = 6.8 Hz, 2H), 3.87 (t, *J* = 6.2 Hz, 2H), 7.06 (t, *J* = 7.0 Hz, 1H), 7.26 (t, *J* = 7.0 Hz, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 11.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 19.2, 22.2, 32.2, 42.4, 88.4, 122.9, 124.4, 128.8, 138.4, 160.5, 160.8, 162.2.

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (226)



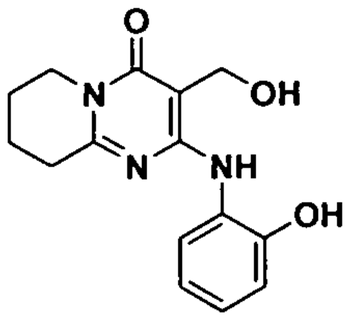
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 – 1.34 (m, 2H), 1.38 – 1.51 (m, 4H), 2.35 – 2.41 (m, 2H), 3.98 – 4.05 (m, 2H), 4.12 (s, 2H), 7.17 – 7.22 (m, 2H), 7.31 (t, *J* = 2.0 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.1, 23.1, 31.4, 42.4, 59.2, 61.4, 65.7, 122.8, 123.9, 125.6, 131.6, 134.3, 139.4, 157.9, 164.3

3-(Hydroxymethyl)-2-(3-(trifluoromethyl)phenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (227)



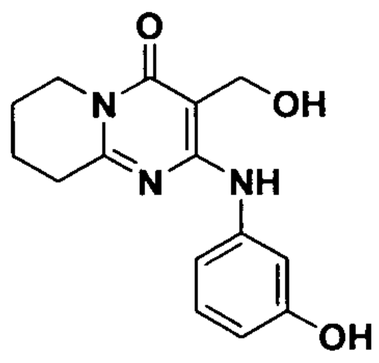
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 – 1.38 (m, 2H), 1.48 – 1.54 (m, 2H), 1.70 – 1.73 (m, 2H), 2.38 (t, *J* = 12.8 Hz, 1H), 3.98 – 4.06 (m, 2H), 4.13 (s, 2H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.55 – 7.59 (m, 2H), 7.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ14.3, 22.2, 30.5, 41.5, 58.4, 77.9, 119.8, 121.2, 127.0, 129.8, 130.1, (d, *J* = 26.8 due to CF_3), 138.2, 146.1, 157.1, 163.6, 169.1.

3-(Hydroxymethyl)-2-(2-hydroxyphenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (228)



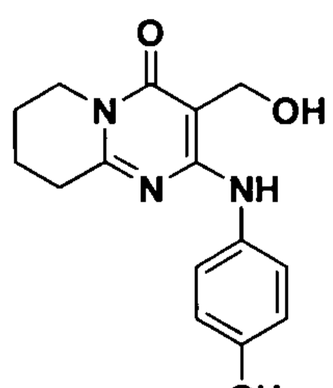
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78 – 1.94 (m, 4H), 2.13 – 2.23 (m,, 2H), 2.61 (t, *J* = 6.0 Hz, 1H), 3.98 – 4.05 (m, 2H), 4.12 (s, 2H), 6.81 (t, *J* = 7.2 Hz, 1H), 6.89 (d, *J* = 7.2 Hz, 1H), 6.98 – 7.12 (m, 2H), 10.11(s, 1H), 11.3 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 21.4, 31.3, 42.1, 61.1, 87.7, 121.2, 126.4, 128.3, 128.6, 151.1, 161.3, 162.5, 163.7, 169.4 .

3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (229)



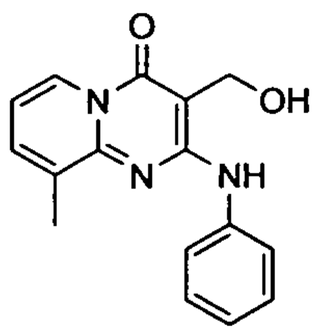
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 – 1.61 (m, 4H), 1.62 – 1.77 (m, 2H), 2.72 (t, *J* = 10.0 Hz, 1H), 3.78 – 3.95 (m, 2H), 4.17 (s,2H), 6.43 (d, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.98 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 21.8, 31.9, 42.4, 60.1, 79.8, 109.8, 111.6, 114.0, 129.4, 139.4,149.7, 159.3, 160.2, 163.1.

3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (230)



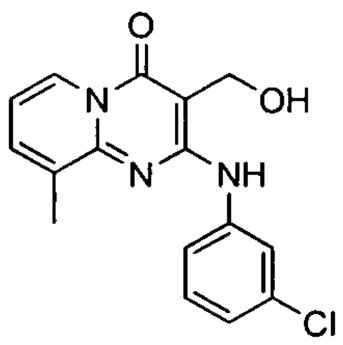
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 – 1.45 (m, 4H), 1.63 – 1.71 (m, 2H), 2.34 (t, *J* = 12.8 Hz, 1H), 3.98 – 4.05 (m, 2H), 4.19 (s, 2H), 6.75 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.9, 21.9, 32.1, 42.3, 60.4, 87.2, 115.7, 125.0, 130.1, 154.9, 159.4, 160.6, 163.3.

3-(Hydroxymethyl)-9-methyl-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (231)



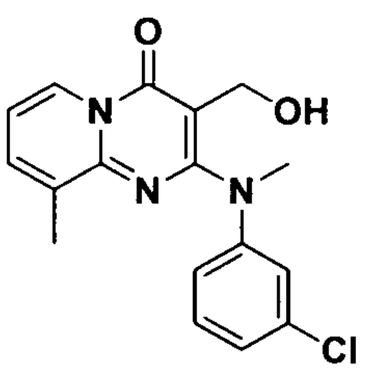
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 2.97 (brs, 1H), 4.93 (s, 2H), 6.89 (t, *J* = 6.8 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 6.4 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 2H), 8.73 (d, *J* = 6.8 Hz, 1H).

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (232)



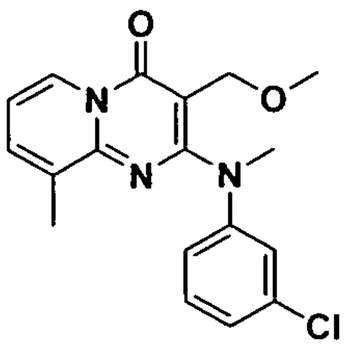
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.06 (t, *J*= 6.4 Hz, 1H), 4.92 (d, *J*= 6.4 Hz, 2H), 6.69 (d, *J* = 7.0 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 6.8 Hz, 1H), 8.03 (s, 1H), 8.38 (s, 1H), 8.71 (d, *J* = 7.2 Hz, 1H).

2-((3-Chlorophenyl)(methyl)amino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (233)



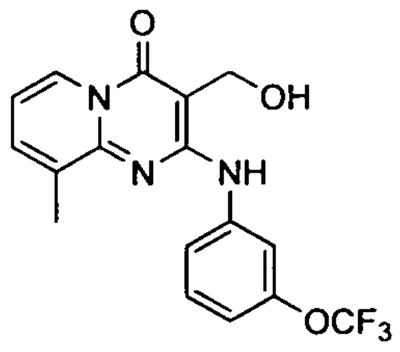
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 4.09 (t, *J* = 6.8 Hz, 1H), 4.12 (d, *J* = 7.2 Hz, 2H), 6.95 (t, *J* = 7.0 Hz, 1H), 7.04 – 7.06 (m, 2H), 7.20 (t, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 6.8 Hz, 1H), 8.84 (d, *J* = 7.2 Hz, 1H).

2-((3-Chlorophenyl)(methyl)amino)-3-(methoxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (234)



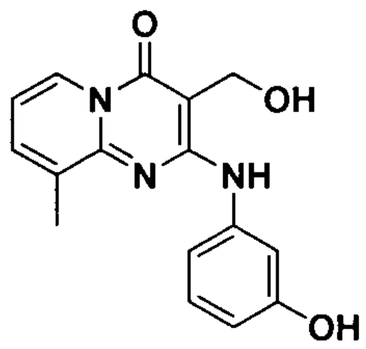
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 3.01 (s, 3H), 4.04 (s, 3H), 6.91 (t, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.20 (s, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 8.86 (d, *J* = 7.2 Hz, 1H).

3-(Hydroxymethyl)-9-methyl-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (235)



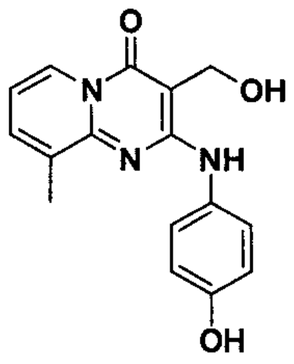
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 3.15 (t, *J* = 6.2 Hz, 1H), 4.93 (d, *J* = 6.4 Hz, 1H), 6.67 (t, *J* = 7.0 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 7.25-7.27 (m, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 6.8 Hz, 1H), 7.98 (s, 1H), 8.51 (s, 1H), 8.72 (d, *J* = 6.8 Hz, 1H).

3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (236)



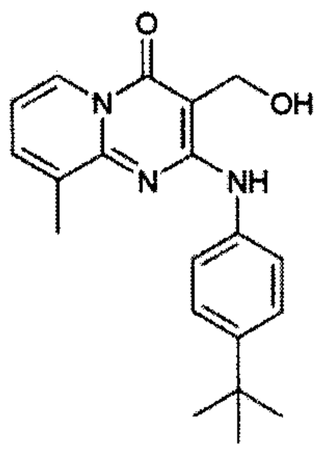
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 2.44 (s, 3H), 4.75 (s, 2H), 6.45 (dd, J = 2.4, 8.0 Hz, 1h), 6.84 (dd, J = 6.8, 6.8 Hz, 1H), 7.06 (dd, J = 8.0, 8.4 Hz, 1H), 7.11 (dd, J = 2.0, 2.4 Hz, 1H), 7.17 (dd, H = 2.0, 8.0 Hz, 1H), 7.45 (d, J = 6.8 Hzm 1H), 8.72 (d, J = 7.2 Hz, 1H).

3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (237)



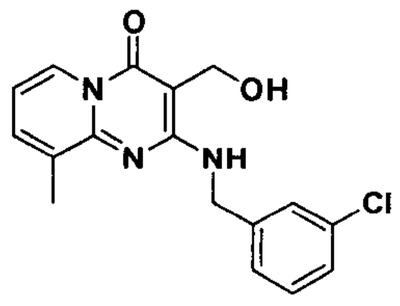
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 4.94 (d, J = 4.8 Hz, 1H), 6.81 -6.84 (m, 3H), 7.46 (d, J = 7.2 Hz, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.84 (s, 1H), 8.82 (d, J = 7.2 Hz, 1H).

2-(4-tert-Butylphenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (238)



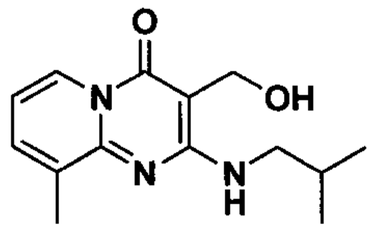
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.40 (s, 3H), 3.07 (t, *J* = 6.2 Hz, 1H), 4.91 (d, *J* = 6.4 Hz, 2H), 6.61 (t, *J* = 6.8 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 2H), 7.38 (d, *J* = 6.8 Hz, 1H), 8.21 (br s, 1H), 8.69 (d, *J* = 7.2 Hz, H).

2-(3-Chlorobenzylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (239)



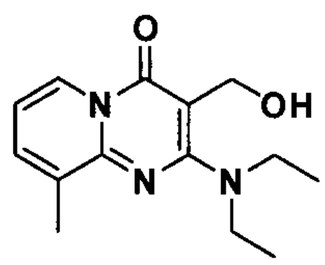
^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 2.31 (s, 3H), 3.02 (s, 1H), 4.68 (d, *J* = 5.6 Hz, 2H), 4.70 (s, 2H), 6.70 (dd, *J* = 5.6, 6.0 Hz, 1H), 6.74 (dd, *J* = 6.8, 7.2 Hz, 1H), 7.11 – 7.20 (m, 3H), 7.31 (s, 1H), 7.38 (d, *J* = 6.8 Hz, 1H), 8.66 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) δ 17.7, 44.2, 44.3, 55.8, 93.1, 93.2, 112.6, 125.4, 125.5, 126.9, 127.5, 129.5, 132.6, 134.0, 134.9, 141.7, 149.45, 149.47, 157.4, 159.10, 159.16.

3-(Hydroxymethyl)-2-(isobutylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (240)



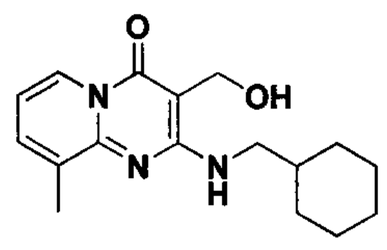
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (d, J = 6.8 Hz, 6H), 1.88 – 1.95 (m, 1H), 2.34 (s, 3H), 3.13 (brs, 1H), 3.32 (t, J = 6.0 Hz, 2H), 4.78 (d, J = 6.0 Hz, 2H), 6.08 (brs, 1H), 6.72 (t, J = 6.8 Hz, 1H), 7.37 (d, J = 6.8 Hz, 1H), 8.66 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 20.5, 28.9, 48.6, 57.1, 92.5, 112.1, 126.0, 132.5, 134.6, 149.6, 157.1, 159.5.

2-(Diethylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (241)



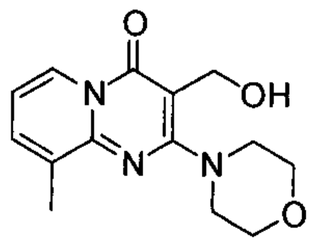
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 6.8 Hz, 6H), 2.35 (s, 3H), 3.41 (s, 1H), 3.63 (q, J = 6.8 Hz, 4H), 4.44 (s, 2H), 6.65 (t, J = 7.2 Hz, 1H), 7.31 (d, J = 6.8 Hz, 1H), 8.68 (d, J = 7.2 Hz, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 17.7, 44.0, 67.0, 92.2, 111.7, 125.8, 132.5, 134.4, 148.1, 160.7, 160.8.

2-(Cyclohexylmethylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (242)



^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 – 0.98 (m, 2H), 1.18 – 1.23 (m, 3H), 1.58 – 1.79 (m, 6H), 2.42 (s, 3H), 3.27 (t, J = 6.4 Hz, 2H), 3.85 (brs, 1H), 4.74 (m, 2H), 6.21 (t, J = 7.2 Hz, 1H), 6.68 (d, J = 6.8 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 8.57 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 26.2, 26.7, 31.3, 38.4, 47.5, 56.9, 92.8, 112.0, 126.0, 132.3, 134.5, 149.4, 156.9, 159.5.

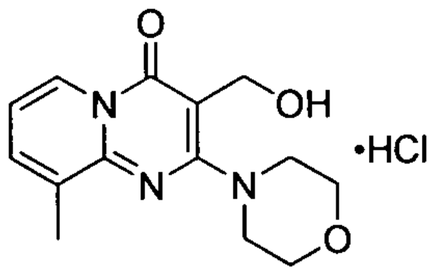
3-(Hydroxymethyl)-9-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (243)



^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (brs, 1H), 2.43 (s, 3H), 3.62 (t, *J* = 4.8 Hz, 4H), 3.78 (t, *J* = 4.8 Hz, 4H), 4.62 (s, 2H), 6.85 (t, *J* = 6.8 Hz, 1H), 7.46 (d, *J* = 6.8 Hz, 1H), 8.76 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 49.7, 58.9, 67.1, 95.5, 113.3, 125.2, 133.4, 135.0, 148.2, 160.6, 161.7.

3-(Hydroxymethyl)-9-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one hydrochloride

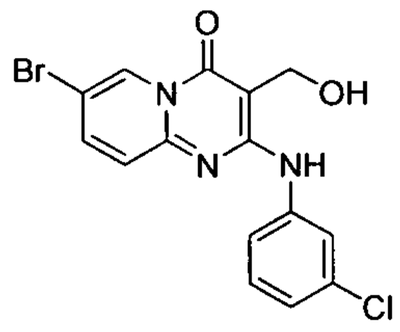
(244)



^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.42 (s, 1H), 3.62 (t, *J* = 4.8 Hz, 4H), 3.78 (t, *J* = 4.8 Hz, 4H), 4.62 (s, 2H), 6.85 (t, *J* = 6.8 Hz, 1H), 7.46 (d, *J* = 6.8 Hz, 1H), 8.76 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 49.7, 58.9, 67.1, 98.5, 113.3, 125.2, 133.4, 135.0, 148.2, 160.6, 161.7.

7-Bromo-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one

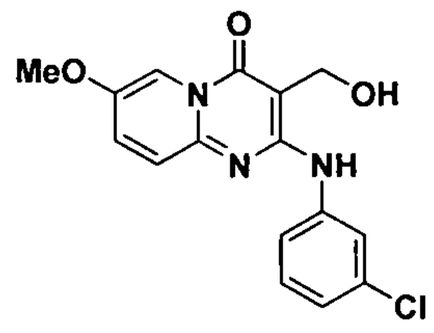
(245)



^{1}H NMR (400 MHz, DMSO-d_6) δ 4.78 (s, 2H), 5.37 (s, 1H), 7.12 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 7.32 (d, *J* = 8.0 Hz 1H), 7.42 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 7.54 (dd, *J* = 0.8 Hz, 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz 1H), 7.91 (d, *J* = 2.0 Hz, 1H), 8.47(s, 1H), 8.71 (s, 1H);

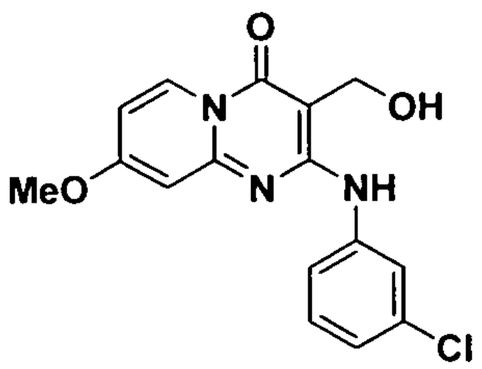
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-7-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one

(246)



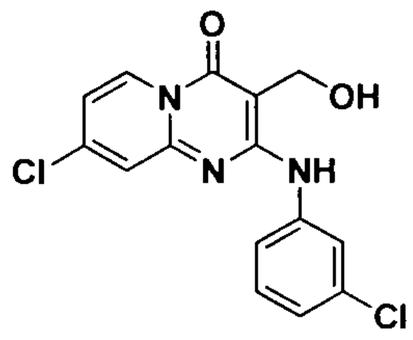
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (s, 3H), 4.70 (s, 2H), 5.22 (s, 1H), 7.02 (dd, *J* = 0.8 Hz, 8.0 Hz, 1H), 7.28 – 7.32 (m, 1H), 7.41 (dd, *J* = 1.2 Hz, 9.6 Hz, 1H), 7.58 (dd, *J* = 0.8 Hz, 8.0 Hz, 1H), 7.64 – 7.68 (m, 1H), 7.87 (d, *J* = 2.0 Hz, 1H), 8.36 (s, 1H), 8.69 (s, 1H)

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (247)



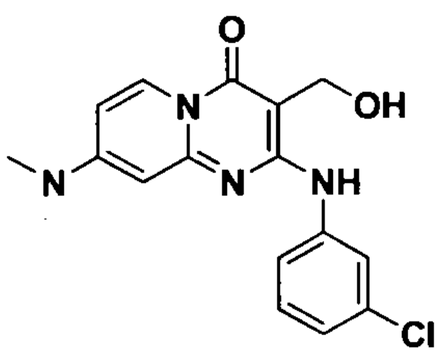
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.92 (s, 3H), 4.62 (s, 2H), 5.07 (s, 1H), 6.71 (d, J = 2.8 Hz, 1H), 6.83 (dd, J = 2.8 Hz, 8.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 1H), 7.28 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 2.0 Hz, 1H), 8.62 (s, 1H), 8.71 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 54.8, 57.3, 93.8, 101.5, 109.3, 120.0, 120.9, 122.5, 129.5, 130.7, 133.4, 142.2, 151.9, 156.9, 157.8, 166.2.

8-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (248)



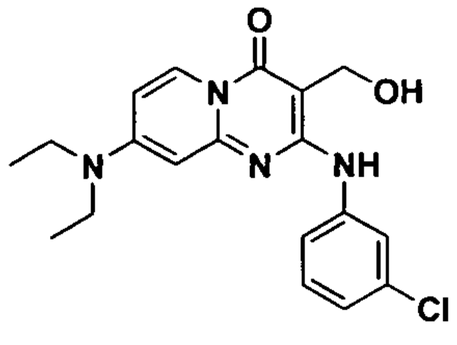
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 2H), 5.14 (brs, 1H), 7.03 (dd, J = 1.2, 8.0 Hz, 1H), 7.19 (dd, J = 2.4, 7.6 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 7.54, (d, J = 2.0 Hz, 1H), 7.58 (dd, J = 1.2, 8.4 Hz, 1H), 7.57 (t, J = 2.0 Hz, 1H), 8.78 (d, J = 8.0 Hz, 1H).

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(methylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (249)



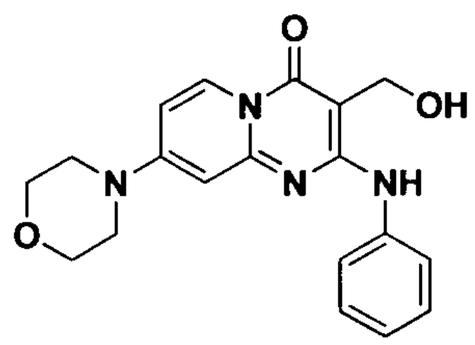
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (s, 3H), 3.85 (s, 2H), 6.02 (s, 1H), 6.32 (d, J = 7.6 Hz, 1H), 6.93 (d, J = 2 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.81 (s, 1H), 8.42 (s, 1H), 9.93 (s, 1H).

2-(3-Chlorophenylamino)-8-(diethylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (250)



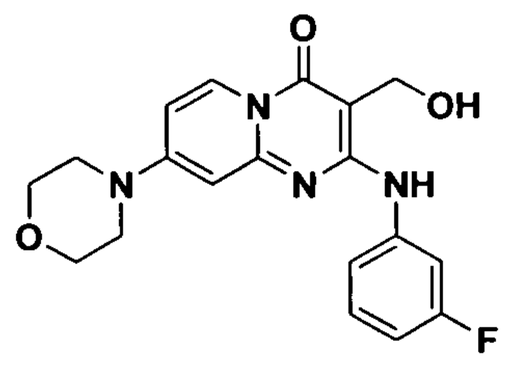
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 6.8 Hz, 6H), 3.44 (q, J = 6.8 Hz, 4H), 3.99 (s, 2H), 4.82 (t, J = 2.1 Hz, 1H), 6.29 (d, J = 2.1Hz, 1H), 6.54 (dd, J = 2.4, 8.4 Hz, 1H), 6.92 (d, J = 2 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 8.06 (t, J = 2.0 Hz, 1H), 8.85 (d, J = 8.4 Hz, 1H), 9.71 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7, 20.0, 44.7, 92.8, 97.1, 104.0, 118.9, 120.7, 121.9, 128.5, 129.5, 134.1, 142.8, 150.6, 151.9, 158.3, 159.2.

3-(Hydroxymethyl)-8-morpholino-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (251)



^{1}H NMR (400 MHz, DMSO-d_6) δ 3.43 (s, 4H), 3.67 (s, 4H), 4.59 (d, J = 5.2 Hz, 2H), 5.05, (t, J = 4.8 Hz, 1H), 6.41 (d, J = 2.0 Hz, 1H), 6.95 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 2.8, 8.4 Hz, 1H), 7.25 (t, J = 8.0 Hz, 2H), 7.64 (d, J = 7.6 Hz, 2H), 8.38 (s, 1H), 8.69 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.5, 55.1, 66.3, 91.5, 99.1, 105.4, 121.3, 122.6, 128.5, 129.1, 140.9, 151.4, 155.0, 156.7, 158.5.

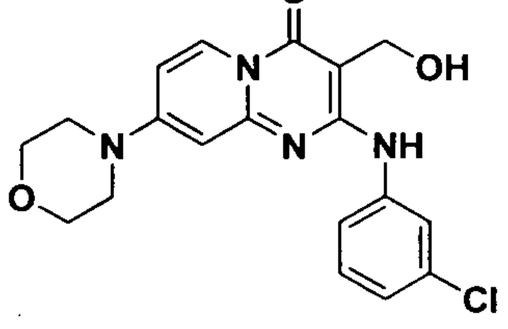
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (252)



^{1}H NMR (400 MHz, DMSO-d_6) δ 3.46 (s, 4H), 3.68 (s, 4H), 4.59 (d, J = 5.2 Hz, 2H), 5.06, (t, J = 5.2 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.74 (t, J = 7.2 Hz, 1H), 7.03 (dd, J = 2.8, 8.0 Hz, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 12.4 Hz, 1H), 8.52(s, 1H), 8.60 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.8, 54.2, 65.6, 91.3, 98.4,

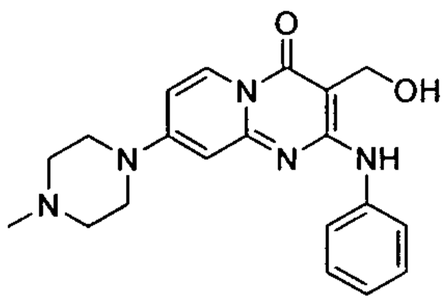
105.0, 108.0 (d, J = 20 Hz, due to F), 116.0, 128.0, 129.8 (d, J = 10 Hz, due to F), 142.1 (d, J = 11 Hz, due to F), 150.6, 154.4, 156.1, 157.4, 161.0, 163.3.

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (253)



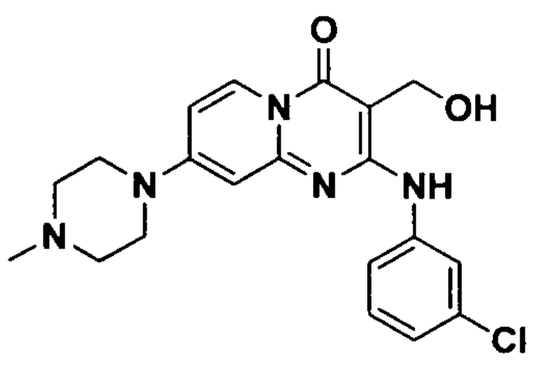
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.45 (t, J = 5.6 Hz, 4H), 3.69 (t, J = 5.6 Hz, 4H), 4.58 (d, J = 5.2 Hz, 2H), 5.01 (t, J = 5.2 Hz, 1H), 6.42 (d, J = 2.8 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.05 (dd, J = 2.8, 8.0 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.80 (t, J = 2.0 Hz, 1H), 8.48(s, 1H), 8.60 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.4, 53.6, 65.7, 84.7, 98.6, 105.3, 117.8, 118.7, 119.8, 127.1, 130.2, 129.2, 141.8, 149.7, 153.0, 155.3, 157.4; LC-MS (ESI, m/z): 386 $[M+H]^+$.

3-(Hydroxymethyl)-8-(4-methylpiperazin-1-yl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (254)



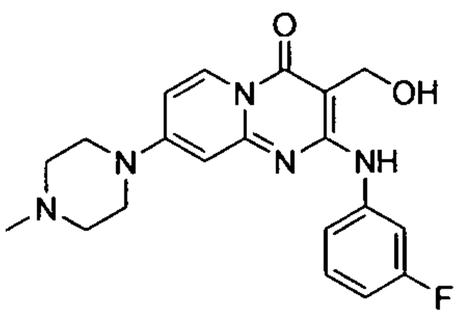
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.52 (t, J =5.2 Hz, 4H), 3.43 (t, J =5.2 Hz, 4H), 4.88 (s, 2H), 5.28 (s, 1H), 6.37 (s, 1H), 6.55 (d, J =8.0Hz, 1H), 7.05 (t, J =7.2Hz, 1H), 7.33 (t, J =7.6 Hz, 2H), 7.60 (d, J =7.6 Hz, 2H), 7.91 (s, 1H), 8.64 (d, J =8.0Hz, 1H).

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (255)



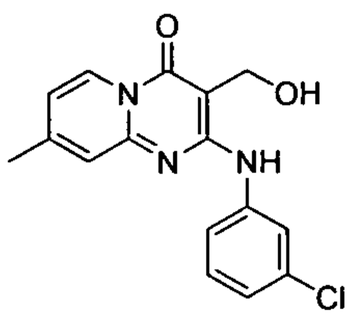
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.14 (s, 3H), 2.38 (t, *J* = 4.4 Hz, 4H), 3.45 (t, *J* = 4.4 Hz, 4H), 3.56 (s, 2H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.95 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.01 (dd, *J* = 2.4, 8.0 Hz, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 1.6 Hz, 1H), 8.0 (d, *J* = 8.0 Hz, 1H), 10.4 (s, 1H), 14.18 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.6, 51.6, 54.0, 55.0, 85.3, 98.3, 105.1, 117.7, 118.5, 121.0, 127.9, 130.3, 133.0, 142.1, 150.8, 154.1, 156.4, 157.8; LC-MS (ESI, *m/z*): 400 $[M+H]^+$.

2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a] pyrimidin-4-one (256)



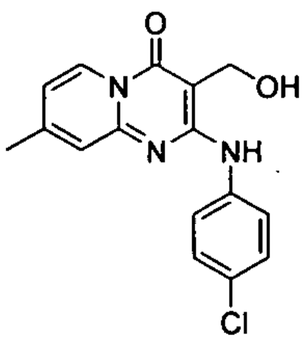
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 2.54 (t, *J* =4.4 Hz, 4H), 3.48 (t, *J* =4.8 Hz, 4H), 4.87 (s, 2H), 5.23 (s, 1H), 6.42 (s, 1H), 6.60 (d, *J* =8.4Hz, 1H), 6.73 (t, *J* =8.4Hz, 1H), 7.12 (d, *J* =8.4 Hz, 1H), 7.19 (d, *J* =8.4 Hz, 1H), 7.71-7.75 (m, 1H), 8.04 (s, 1H), 8.71 (d, *J* =8.0Hz, 1H).

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (257)



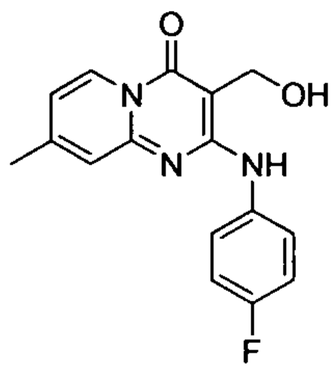
Colorless solid, mp 235°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H), 4.07 (q, *J* = 7.2 Hz, 2H), 7.03 (d, *J* = 8.8 Hz, 2H), 7.26 (t, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.84 (t, *J* = 2.0 Hz, 1H), 8.79 (d, *J* = 7.2 Hz, 2H).

2-(4-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (258)



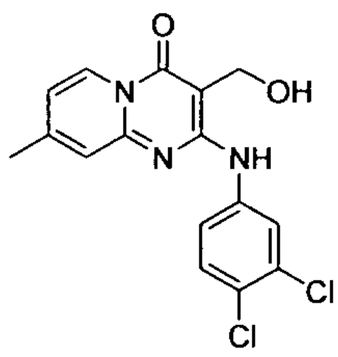
Colorless solid, mp 227°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H), 4.10 (s, 2H), 6.85 (d, *J* = 7.2Hz, 1H), 7.23-7.28 (m, 4H), 7.87 (d, *J* = 6.8Hz, 2H), 8.94 (d, *J* = 7.6 Hz, 1H).

2-(4-Fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (259)



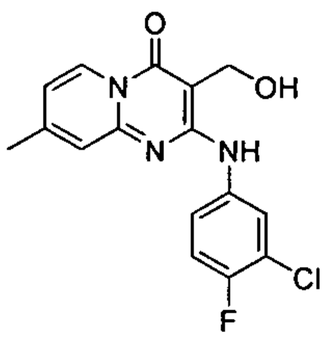
Colorless solid, mp 232°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42(s, 3H), 4.12 (s, 2H), 6.85 (d, *J* = 6.8 Hz, 1H), 7.05 (t, *J* = 8.4 Hz, 2H), 7.21 (s, 1H), 7.31-7.38 (m, 2H), 7.85 (q, *J* = 4.8 Hz, 2H), 8.94 (d, *J* = 7.2 Hz, 1H).

2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (260)



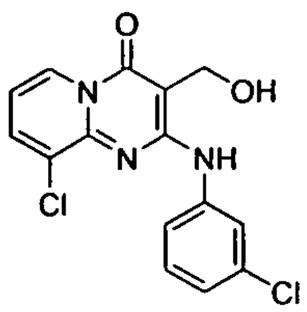
Colorless solid, mp 230°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 4.09 (s, 2H), 6.89 (d, *J* = 7.2 Hz, 1H), 7.26 (s, 1H), 7.36 (d, *J* =8.8 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* =2.4 Hz, 1H), 8.95 (d, *J* = 7.2 Hz, 1H), 9.71(s, 1H).

2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (261)



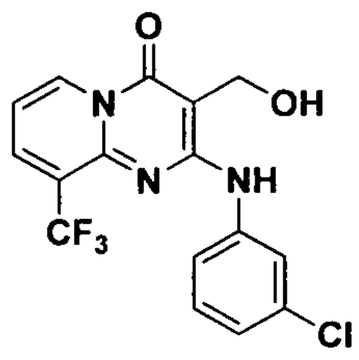
Colorless solid, mp 225°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 4.09 (s, 2H), 6.88 (d, *J* = 7.2 Hz, 1H), 7.11 (t, *J* = 8.8 Hz, 1H), 7.27 (s, 1H), 7.69-7.73 (m, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 8.95 (d, *J* = 7.2 Hz, 1H), 9.71 (s, 1H).

9-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (262)



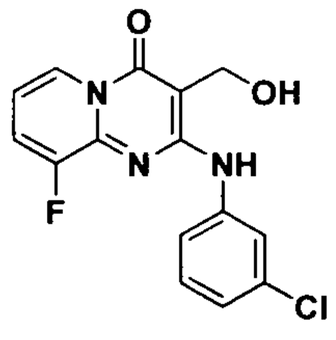
Colorless solid, mp 230°C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (d, J = 6.0 Hz, 2H), 6.80 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 7.2 Hz, 1H), 8.18 (t, J = 2.4 Hz, 1H), 8.43 (s, 1H), 8.81 (d, J = 7.2 Hz, 1H).

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (263)



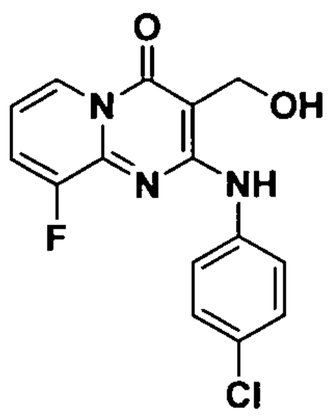
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.77 (s, 2H), 7.11 – 7.13 (m, 1H), 7.32 (dd, J = 7.2, 7.2 Hz, 1H), 7.35 (dd, J = 8.0, 8.0 Hz, 1H), 7.48 – 7.50 (m, 1H), 8.13 – 8.14 (m, 1H), 8.41 (d, J = 7.2 Hz, 1H), 9.12 (dd, J = 1.2, 7.2 Hz, 1H).

2-(3-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (264)



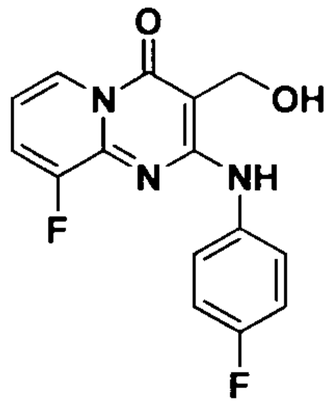
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.76 (s, 1H), 5.31 (brs, 1H), 7.11 – 7.13 (m, 1H), 7.18 - 7.23 (m, 1H), 7.38 (dd, J = 8.0, 8.0 Hz, 1H), 7.63 – 7.65 (m, 1H), 7.86 (dd, J = 8.4, 8.8 Hz, 1H), 8.12 - 8.13 (m, 1H), 8.73 (d, J = 7.2 Hz, 1H), 8.96 (brs, 1H).

2-(4-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (265)



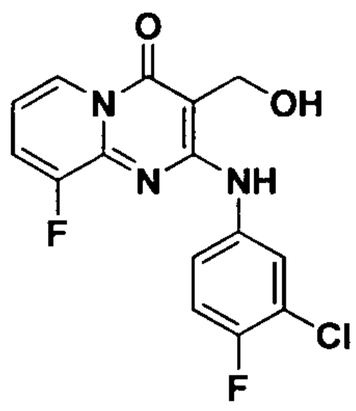
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.72 (s, 2H), 5.30 (brs, 1H), 7.15 – 7.20 (m, 1H), 7.41 – 7.44 (m, 2H), 7.79 – 7.82 (m, 2H), 7.84 – 7.86 (m, 1H), 8.72 (d, J = 7.2 Hz, 1H), 8.92 (brs, 1H).

9-Fluoro-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (266)



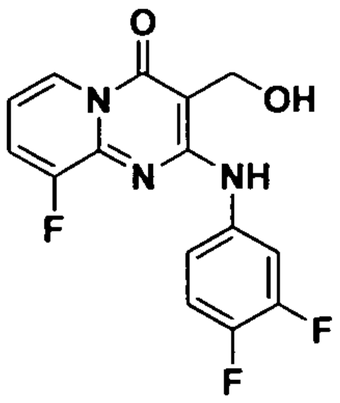
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.75 (s, 2H), 5.25 (brs, 1H), 7.13 – 7.25 (m, 3H), 7.73 – 7.77 (m, 2H), 7.80 – 7.85 (m, 1H), 8.72 (d, J = 7.2 Hz, 1H), 8.84 (brs, 1H).

2-(3-Chloro-4-fluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (267)



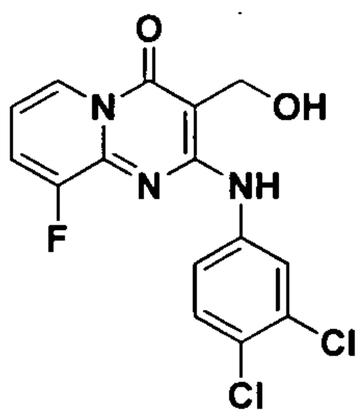
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.74 (s, 2H), 5.24 (brs, 1H), 7.18 – 7.22 (m, 1H), 7.39 – 7.44 (m, 1H), 7.65 – 7.69 (m, 1H), 7.83 – 7.87 (m, 1H), 8.20 – 8.22 (m, 1H), 8.72 (d, J = 7.2 Hz, 1H), 8.91 (brs, 1H).

2-(3,4-Difluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (268)



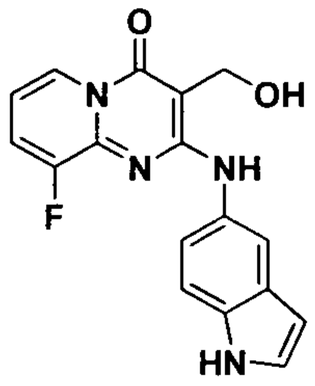
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.75 (s, 2H), 5.26 (brs, 1H), 7.17 – 7.22 (m, 1H), 7.39 – 7.49 (m, 1H), 7.84 – 7.88 (m, 1H), 8.08 – 8.14 (m, 1H), 8.73 (m, J = 7.2 Hz, 1H), 8.93 (brs, 1H).

2-(3,4-Dichlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (269)



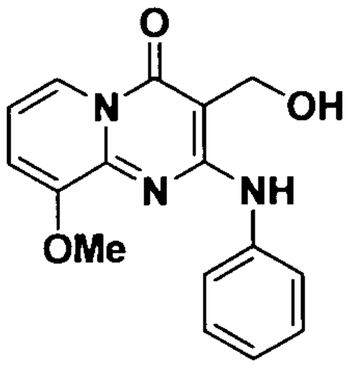
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.75 (s, 2H), 5.27 (brs, 1H), 7.19- 7.23 (m, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.7 (dd, J = 2.8, 8.8 Hz, 1H), 7.85 – 7.89 (m, 1H), 8.83 (d, J = 2.8 Hz, 1H), 8.73 (d, J = 8.8 Hz, 1H), 9.00 (brs, 1H).

2-(1H-Indol-5-ylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (270)



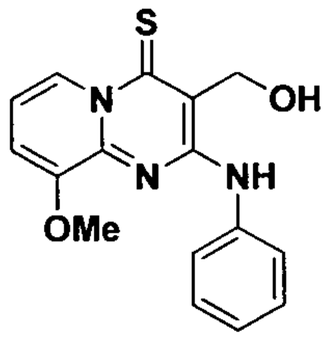
m.p=184 - 185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.70 (d, J = 5.2 Hz, 2H), 5.18 (t, J = 5.2 Hz, 1H), 6.35 (s, 1H), 7.00 – 7.04 (m, 1H), 7.23 (dd, J = 2 Hz, 8.8 Hz, 1H), 7.28 – 7.32 (m, 2H), 7.68 (dd, J = 8 Hz, J = 8 Hz, 1H), 7.82 (s, 1H), 8.61 (s, 1H), 8.64 (d, J = 6 Hz, 1H), 10.98 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 55.2, 94.6 ,101.7 (d, J = 5.2 Hz, due to F), 111.6, 112.1 (d, J = 7.4 Hz, due to F), 113.7, 118.0, 119.8 (d, J = 17.1 Hz, due to F), 124.2 (d, J = 4.4 Hz, due to F), 126.5, 128.2, 131.9, 133.5, 151.6, 154.1, 156.3, 157.6.

3-(Hydroxymethyl)-9-methoxy-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (271)



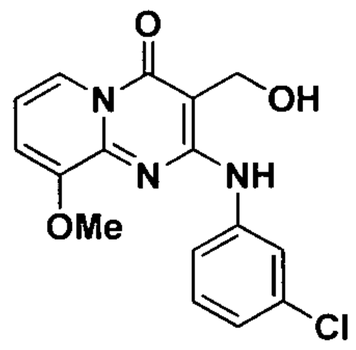
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.93 (s, 3H), 4.71 (d, J = 5.2 Hz, 2H), 5.29 (t, J = 5.2 Hz, 1H), 6.97 – 7.01 (m, 1H), 7.06 – 7.10 (m, 1H), 7.27 – 7.32 (m, 3H), 7.83 (d, J = 8.4 Hz, 2H), 8.47 (d, J = 7.2 Hz, 1H), 8.68 (s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-4-thione (272)



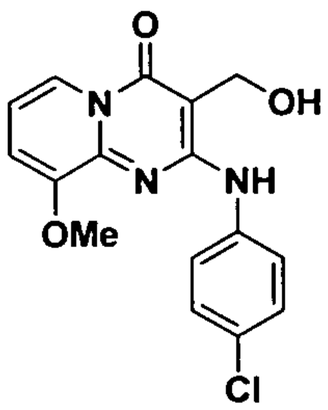
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (s, 3H), 4.11 (d, J = 7.2 Hz, 2H), 6.88 (t, J = 8.0 Hz, 2H), 7.04 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 2H), 7.82 (d, J = 7.6 Hz, 2H), 7.98 (s, 1H), 8.59 (d, J = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 26.9, 57.1, 94.2, 111.8, 112.7, 119.9, 121.1, 123.3, 128.9, 139.8, 143.7, 151.3, 155.6, 158.6.

2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (273)



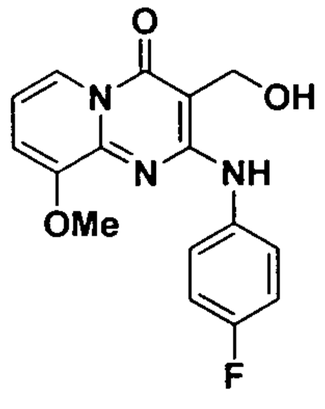
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (s, 3H), 4.68 (s, 2H), 6.99 (d, J = 7.6 Hz, 1H), 7.09 (dd, J = 7.2 Hz, J = 7.2 Hz, 1H), 7.25 – 7.29 (m, 2H), 7.56 (d, J = 8.0 Hz, 1H), 8.42 (s, 1H), 8.45 (d, J = 6.8 Hz, 1H), 8.77 (s, 1H).

2-(4-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (274)



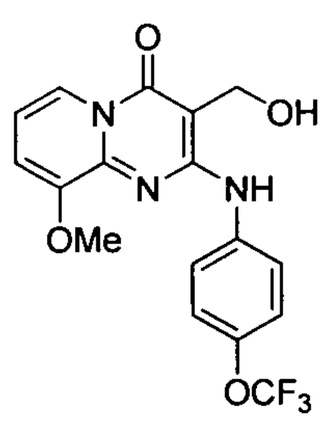
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.90 (s, 3H), 4.65 (d, J = 5.2 Hz, 2H), 5.19 (t, J = 5.2 Hz, 1H), 7.03 (dd, J = 7.2 Hz, 7.6 Hz, 1H), 7.23 (d, J = 7.6 Hz, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 9.2 Hz, 2H), 8.42 (d, J = 7.2 Hz, 1H), 8.72 (s, 1H).

2-(4-Fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (275)



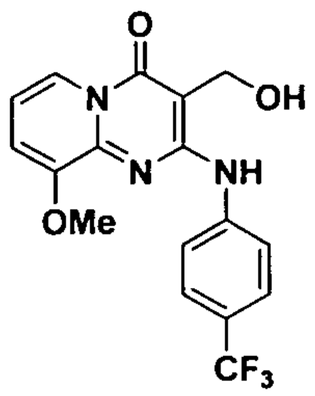
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 4.69 (d, J = 5.2 Hz, 2H), 5.19 (t, J = 5.2 Hz, 1H), 7.06 (t, J = 6.8 Hz, 1H), 7.13 (t, J = 8.8 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 7.83 – 7.86 (m, 1H), 8.45 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.66 (s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (276)



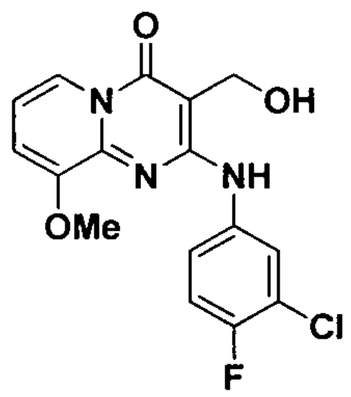
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (s, 3H), 4.67 (d, J = 4.0 Hz, 2H), 5.20 (s, 1H), 7.07 (dd, J = 7.2 Hz, J = 7.2 Hz, 1H), 7.23 (s, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.95 (dd, J = 8.8 Hz, J = 8.8 Hz, 2H), 8.45 (d, J = 7.6 Hz, 1H), 8.78 (s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (277)



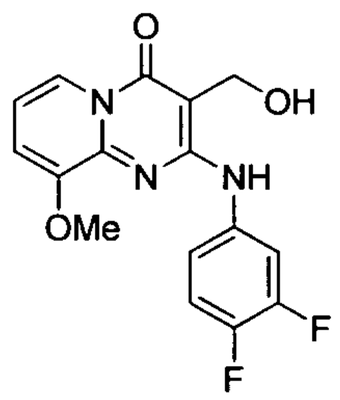
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.97 (s, 3H), 4.72 (s, 2H), 5.32 (s, 1H), 7.14, (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 8.8 Hz, 2H), 8.11 (d, J = 8.8 Hz, 2H), 8.49 (d, J = 7.2 Hz, 1H), 9.09 (s, 1H).

2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (278)



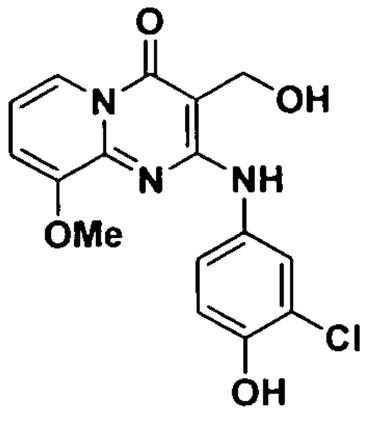
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.95 (s, 3H), 4.69 (d, J = 4.8 Hz, 2H), 5.16 (t, J = 4.8 Hz, 1H), 7.10 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.30 (dd, J = 0.8 Hz, 8.0 Hz, 1H), 7.32 (dd, J = 9.2 Hz, 9.2 Hz, 1H), 7.61 – 7.65 (m, 1H), 8.46 (dd, J = 0.8 Hz, 7.2 Hz, 1H), 8.59 (dd, J = 2.8 Hz, 7.2 Hz, 1H), 8.76 (s, 1H).

2-(3,4-Difluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (279)



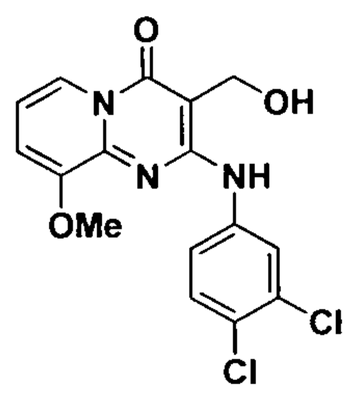
m.p=231 ℃ (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 4.66 (s, 2H), 5.17 (brs, 1H), 7.07 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.26 – 7.33 (m, 2H), 7.39 – 7.41 (m, 1H), 8.34 – 8.40 (m, 1H), 8.44 (d, J = 7.2 Hz, 1H), 8.74 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 54.1, 56.8, 95.2, 109.1, 113.4, 116.0 (d, J = 3.8 Hz, due to F), 116.8, 118.7, 137.5 (d, J = 9.7 Hz, due to F), 143.2 (d, J = 11.9 Hz, due to F), 145.6, 147.5 (d, J = 13.4 Hz, due to F), 149.9 (d, J = 13.4 Hz, due to F), 150.6, 155.5.

2-(3-Chloro-4-hydroxyphenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (280)



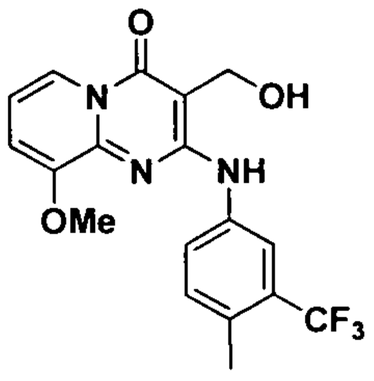
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.93 (s, 3H), 4.68 (s, 2H), 5.14 (s, 1H), 6.99 (d, J = 8.4 Hz, 1H), 7.06 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.26 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.38 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 8.25 (d, J = 2.8 Hz, 1H), 8.45 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.52 (s, 1H), 9.79 (s, 1H).

2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (281)



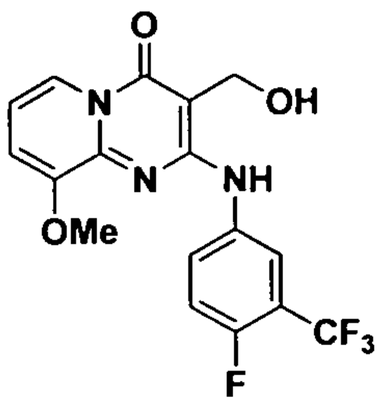
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.93 (s, 3H), 4.66 (d, J = 5.2 Hz, 2H), 5.16 (d, J = 5.2 Hz, 1H), 7.09 (t, J = 7.2 Hz, 1H), 7.29 (d, J = 6.8 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.64 (dd, J = 2.8 Hz, 8.8 Hz, 1H), 8.44 (d, J = 7.2 Hz, 1H), 8.67 (d, J = 2.8 Hz, 1H), 8.82 (s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(4-methyl-3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (282)



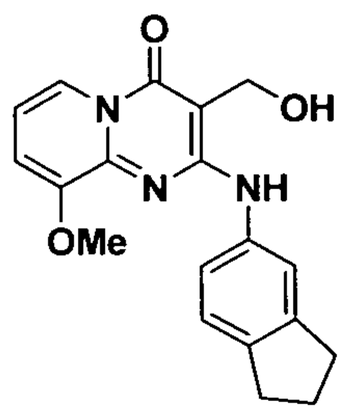
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (t, J = 2.0 Hz, 3H due to CF_3), 3.93 (s, 3H), 4.70 (d, J = 4.8 Hz, 2H), 5.19 (t, J = 4.8 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 7.29 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.74 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 8.46 (dd, J = 1.2 Hz, 6.8 Hz, 1H), 8.81 (s, 1H), 8.85 (d, J = 2.0 Hz, 1H).

2-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (283)



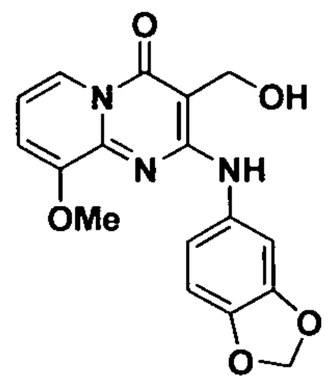
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.92 (s, 3H), 4.68 (d, J = 5.2 Hz, 2H), 5.12 (t, J = 5.2 Hz, 1H), 7.07 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.27 (d, J = 7.2 Hz, 1H), 7.37 – 7.42 (m, 1H), 7.86 – 7.88 (m, 1H), 8.43 (d, J = 7.2 Hz, 1H), 8.87 (s, 1H), 8.99 – 9.00 (m, 1H).

2-(2,3-Dihydro-1H-inden-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (284)



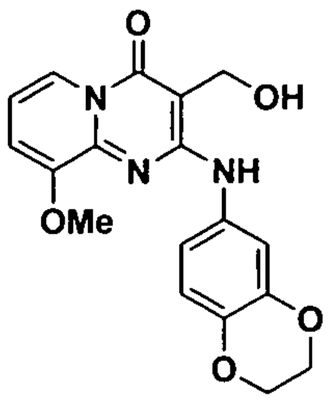
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.97 – 2.05 (m, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.85 (t, J = 7.6 Hz, 2H), 3.92 (s, 3H), 4.69 (d, J = 5.6 Hz, 2H), 5.26 (t, J = 5.6 Hz, 1H), 7.04 (dd, J = 7.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.24 (dd, J = 0.8 Hz, 7.6 Hz, 1H), 7.46 (dd, J = 2.0 Hz, 8.0 Hz, 1H), 7.82 (s, 1H), 8.45 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.59 (s, 1H).

2-(Benzo[d][1,3]dioxol-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (285)



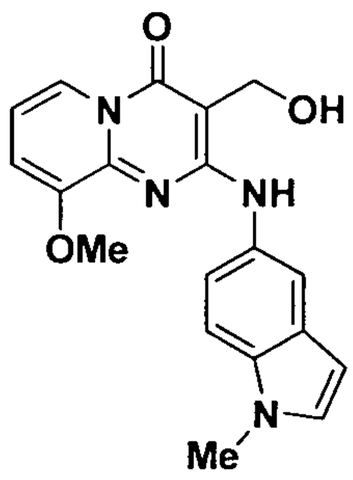
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 4.68 (d, J = 5.2 Hz, 2H), 5.21 (t, J = 5.2 Hz, 1H), 5.98 (s, 2H), 6.84 (d, J = 8.4 Hz, 1H), 7.05 – 7.07 (m, 1H), 7.26 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.82 (d, J = 2.0 Hz, 1H), 8.46 (d, J = 2.0 Hz, 1H), 8.45 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.56 (s, 1H).

2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (286)



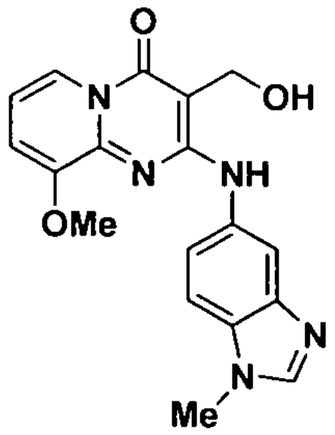
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.92 (s, 3H), 4.19 – 4.24 (m, 4H), 4.67 (d, J = 5.2 Hz, 2H), 5.19 (t, J = 5.2 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 7.05 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.12 (dd, J = 2.4 Hz, 8.4 Hz, 1H), 7.26 (d, J = 6.8 Hz, 1H), 7.64 (d, J = 2.4 Hz, 1H), 8.46 (dd, J = 2.0 Hz, 7.2 Hz, 1H), 8.47 (s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (287)



m.p=195-197 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H), 3.97 (s, 3H), 4.77 (d, J = 5.2 Hz, 2H), 5.28 (t, J = 5.2 Hz, 1H), 6.42 (d, J = 3.0 Hz, 1H), 7.09 (dd, J = 7.2, 7.6 Hz, 1H), 7.28 – 7.30 (m, 1H), 7.33 (d, J = 3.0 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 7.46 (dd, J = 2.0, 8.8 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 8.52 (dd, J = 1.2, 6.8 Hz, 1H), 8.62 (br s, 1H).

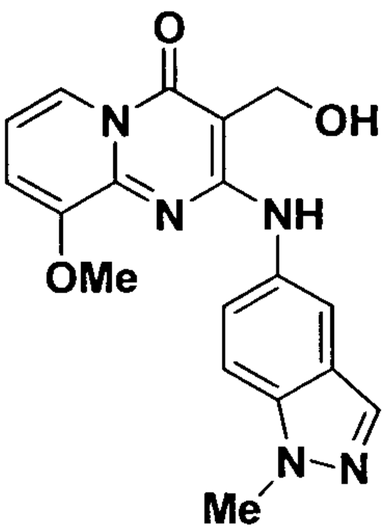
3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-benzo[d]imidazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (288)



m.p=186 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.87 (s, 3H), 3.98 (s, 3H), 4.79 (d, J = 5.6 Hz, 2H), 5.31 (t, J = 5.6 Hz, 1H), 7.08 (dd, J = 7.2, 7.2 Hz, 1H), 7.28 (dd, J = 0.8, 7.6 Hz, 1H), 7.50 (d, J = 8.8 Hz, 1H), 7.56 (dd, J = 2.0, 8.8 Hz, 1H), 8.13(s, 1H), 8.34 (d, J = 1.6

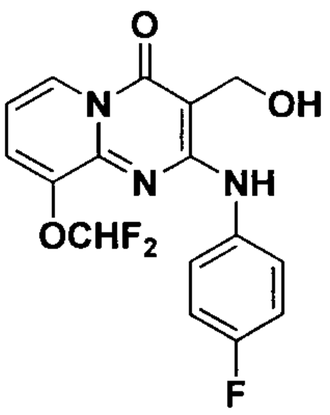
Hz, 1H), 8.53 (dd, J = 0.8, 7.2 Hz, 1H), 8.73 (br s, 1H).

3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (289)



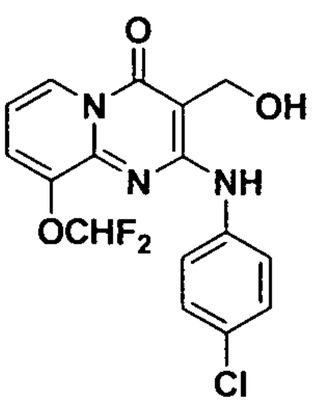
m.p=205 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.40 (s, 3H), 4.08 (s, 3H), 4.78 (d, J = 4.8 Hz, 2H), 5.28 (t, J = 5.0 Hz, 1H), 7.12 (dd, J = 7.2, 7.6 Hz, 1H), 7.32 (1H, J = 1.2, 7.6 Hz, 1H), 7.62 (d, J = 9.0 Hz, 1H), 7.68 (dd, J = 2.0, 9.0 Hz, 1H), 8.04 (m, 1H), 8.07(d, J = 1.2 Hz, 1H), 8.53 (dd, J = 1.2, 6.8 Hz, 1H), 8.75 (br s, 1H).

9-(Difluoromethoxy)-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (290)



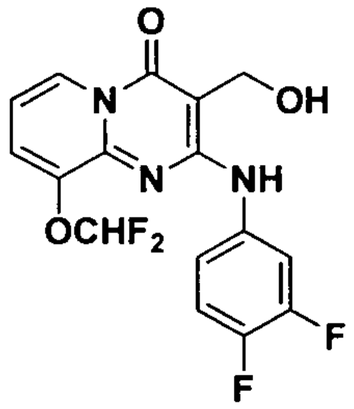
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.67 (d, J = 5.2 Hz, 2H), 5.14 (t, J = 5.2 Hz, 1H), 7.07 – 7.11 (m, 3H), 7.17 (t, J = 74 Hz due to F_2, 1H), 7.63 – 7.69 (m, 3H), 8.71 (d, J = 7.2 Hz, 1H), 8.75 (s, 1H).

2-(4-Chlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (291)



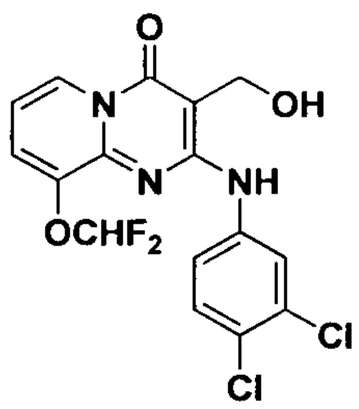
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.69 (d, J = 5.6 Hz, 2H), 5.23 (t, J = 5.2 Hz, 1H), 7.13 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.23 (t, J = 74 Hz, 1H, due to F_2), 7.30 – 7.33 (m, 2H), 7.72 – 7.75 (m, 3H), 8.75 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.86 (s, 1H);

9-(Difluoromethoxy)-2-(3,4-difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (292)



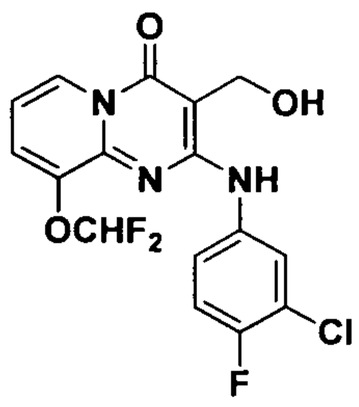
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.70 (d, J = 5.2 Hz, 2H), 5.22 (s, 1H), 7.16 (dd, J = 7.2 Hz, J = 7.2 Hz, 1H), 7.26 (t, J = 74 Hz, due to F2, 1H), 7.33 – 7.38 (m, 2H), 7.75 (d, J = 7.2 Hz, 1H), 8.12 (dd, J = 7.6 Hz, 12.8 Hz, 1H), 8.76 (d, J = 6.8 Hz, 1H), 8.90 (s, 1H); LC-MS (ESI, *m/z*): 370[M+H]$^+$.

2-(3,4-Dichlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (293)



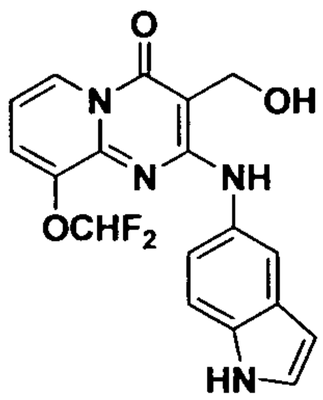
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.68 (s, 2H), 5.19 (s, 1H), 7.15 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 74 Hz, due to F_2, 1H), 7.47 – 7.57 (m, 2H), 7.72 (d, J = 7.2 Hz, 1H), 8.32 (d, J = 2.4 Hz, 1H), 8.73 (dd, J = 1.6 Hz, 7.2 Hz, 1H), 8.92 (s, 1H).

2-(3-Chloro-4-fluorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (294)



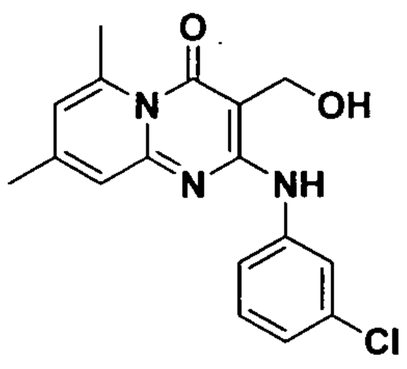
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.68 (d, J = 4.0 Hz, 2H), 5.18 (s, 1H), 7.15 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 7.24 (t, J = 74 Hz, 1H, due to F_2), 7.32 (dd, J = 9.2 Hz, 9.2 Hz, 1H), 7.50 – 7.54 (m, 1H), 7.73 (d, J = 7.6 Hz, 1H), 8.22 (dd, J = 2.8 Hz, 6.8 Hz, 1H), 8.74 (dd, J = 1.2 Hz, 7.2 Hz, 1H), 8.86 (s, 1H).

2-(1H-Indol-5-ylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (295)



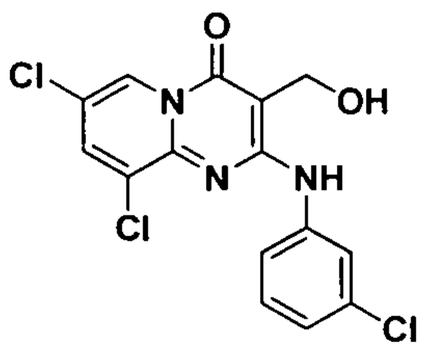
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.72 (d, J = 4.8 Hz, 2H), 5.23 (t, J = 4.8 Hz, 1H), 6.34 (s, 1H), 7.05 – 7.09 (m, 1H), 7.23 (dd, J = 8.8 Hz, 8.8 Hz, 1H), 7.25 (t, J = 74.4 Hz, 1H due to F_2), 7.31 – 7.33 (m, 2H), 7.68 (d, J = 7.2 Hz, 1H), 7.93 (s, 1H), 8.70 (s, 1H), 8.73 (d, J = 1.2 Hz, 1H), 10.99 (s, 1H).

2-(3-chlorophenylamino)-3-(hydroxymethyl)-6,8-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (296)



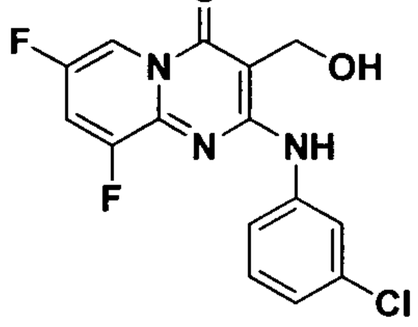
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H), 2.40 (s, 3H), 3.55 (s, 2H), 6.78 (s, 1H), 7.06 (d, J = 2.0 Hz, 1H), 7.21 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.71 (s, 1H), 9.60 (s, 1H); LC-MS (ESI, *m/z*): 330 $[M+H]^+$.

7,9-Dichloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (297)



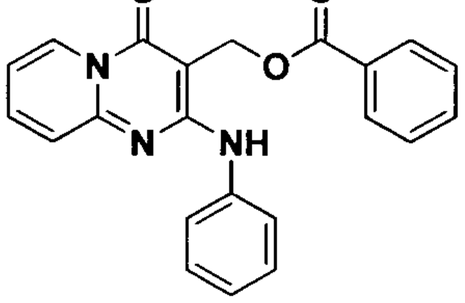
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.65 (s, 2H), 5.70 (d, J = 7.6 Hz, 1H), 7.29 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 7.57 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 8.25 (s, 1H), 8.32 (d, J = 2.0 Hz, 1H), 8.76 (d, J = 2.0 Hz, 1H).

2-(3-Chlorophenylamino)-7,9-difluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (298)



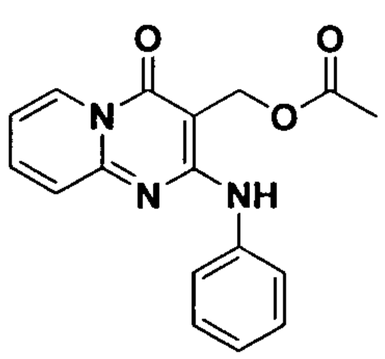
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.69 (d, J = 4.8 Hz, 2H), 5.31 (t, J = 4.8 Hz, 1H), 7.06 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.56 (dd, J = 1.2 Hz, 8.0 Hz, 1H), 8.02 (s, 1H), 8.18 – 8.23 (m, 1H), 8.68 (t, J = 2.0 Hz, 1H), 8.90 (s, 1H).

(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl benzoate (299)



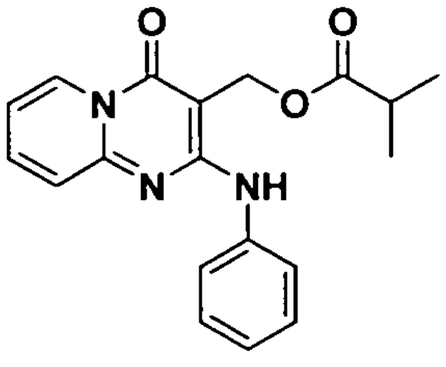
m.p=178 - 179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.66 (s, 2H), 6.96 (ddd, J = 1.2, 1.2, 6.8 Hz, 1H), 7.06 – 7.10 (m, 1H), 7.33 – 7.44 (m, 5H), 7.53 – 7.56 (m, 1H), 7.61 – 7.65 (m, 1H), 7.72 (m, 2H), 8.12 (dd, J = 1.2, 8.4 Hz, 1H), 9.14 (brs, 1H).

(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl acetate (300)



m.p=160 - 161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 6.92 (dd, J = 6.8, 7.2 Hz, 1H), 7.04 – 7.08 (m, 1H), 7.30 – 7.37 (m, 3H), 7.59 – 7.66 (m, 3H), 8.91 (brs, 1H), 8.94 (d, J = 7.2 Hz, 1H).

(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl isobutyrate (301)



m.p=161 - 163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (d, J = 7.2 Hz, 6H), 2.62 – 2.65 (m, 1H), 6.94 (dd, J = 6.8, 7.2 Hz, 1H), 7.04 – 7.08 (m, 1H), 7.31 – 7.38 (m, 3H), 7.60 – 7.67 (m, 3H), 8.95 (brs, 1H), 8.95 (d, J = 6.8 Hz, 1H).

Example 8: Additional studies on dinitrobenzamide compounds

Two representative molecules, compounds 4 and 24, were re-synthesized in-house and subjected to conventional CFU-based activity testing in primary macrophages (**Figure 7**). A ten-fold decrease in the number of CFUs, similar to that seen with INH, was observed for both compounds five days after infection on three different cell lines. This confirms the potency of this series of compounds.

To address the issue of toxicity, compounds 4 and 24 were tested on a panel of five cell lines derived from different body tissue. Cells were incubated with increasing amounts of compound and cell viability was assessed with resazurin after 5 days of co-incubation. Percentage cytotoxicity was determined by taking as a reference the resofurin fluorescence measured by DMSO containing wells. The concentration where fifty percent of the cells died was defined as the Minimal Toxic Concentration (MTC_{50}). Both compounds 4 and 24 showed no cytotoxicity against the panel of cell lines suggesting this series of compounds to be promising new anti-tuberculosis drugs (**Table 5**).

To gain insight into the possible specificity of acitivity of compounds 4 and 24, analysis of the broad antimicrobial spectrum was undertaken and showed that the effect of these dinitrobenzamide derivatives was mainly restricted to actinomycetes with the most potent activity observed against *Mycobacterium* (**Table 5**). Of particular importance, the tested DNB were also highly active against multidrug-resistant (MDR) and extensively drug-resistant (XDR) clinical isolates, suggesting that they might act on different targets than current antituberculosis compounds.

Mutation frequency of *M. tuberculosis* H37Rv was determined for compounds 4 and 24. Increasing numbers of bacteria grew on 7H10 agar medium supplemented with different concentrations of compounds. After a 6-week growth, colonies were counted in order to

evaluate the proportion of spontaneous mutational frequency (**Table 7**). For compound 4, 1×10^{-6} and 1×10^{-8} frequencies of resistance were found at 0.2 μg/ml and 3.2 μg/ml, respectively. Spontaneous mutational rate was therefore calculated to be 1×10^{-7}. For compound 24, at 0.2 μg/ml and 3.2 μg/ml, frequency of mutation was 7×10^{-7} and 1×10^{-8}, respectively which corresponds to a mean frequency of 3.5×10^{-7}. Overall, these values were superior to frequency of mutation observed for INH-resistant mutants (3×10^{-6}). These results, thus, demonstrate that this class of compounds result in a low frequency of mutation.

Example 9: Additional studies on pyridopyrimidinone compounds

Table 6 shows the minimal inhibitory concentration (MIC) of one representative compound, 133, on different Mycobacterial species. While it has no effect on the fast growing *Mycobacterium smegmatis* mc^2, it was able to inhibit typical laboratory strains such as H37Rv, H37Ra and BCG Pasteur with an MIC of 2 μM. More importantly, the antimicrobial activity of 133 was also tested against clinical isolates strains of mycobacteria. The MIC values for multi-drug-resistant (MDR-TB) and extensive-drug-resistant (XDR-TB) isolates strains were within the micromolar range.

To address the issue of toxicity, compound 133 was tested on a panel of seven cell lines derived from different body tissue. Cells were incubated with increasing amounts of compound and cell viability was assessed with resazurin after 5 days of co-incubation. Percentage of cytotoxicity was determined by taking as a reference the resofurin fluorescence measured by DMSO containing wells. The concentration where fifty percent of the cells died was defined as the Minimal Toxic Concentration (MTC_{50}). Compound 133 showed no cytotoxicity for all tested cell lines up to 100 μM (**Table 6**). The selectivity index, which consists of the ratio between antitubercular activitiy and cytotoxicity was therefore above 50 for both extracellular and intracellular mycobacteria suggesting this series of compounds to be promising new anti-tuberculosis drugs.

The effect of this series of compounds on primary macrophages was further determined. Host cells that had priory been incubated with compound 232 harbored fewer bacteria compared to DMSO control and were more abundant at day 5 after infection as shown in **Figure 8**. Similar data were obtained for compound 133 (data not shown). Conventional CFU determination was then performed seven days after infection to quantify the remaining bacterial load. A ten-

fold decrease in the number of CFUs, similar to that seen with INH, was observed for both compounds on both human and mouse cells (**Figure 8**). This confirms the potency of this series of compounds.

Mutation frequency of *M. tuberculosis* H37Rv was determined for compound 264. Increasing numbers of bacteria grew on 7H10 agar medium supplemented with different concentrations of compound. After a 6-week growth, colonies were counted in order to evaluate the proportion of spontaneous mutational frequency (**Table 7**). Compound 264 gave frequencies of resistance of 3.4×10^{-6} and 8×10^{-6} at 0.4 and 0.8 µg/ml, respectively, and 2×10^{-8} at both 1.6 µg/ml and 3.2 µg/ml. Accordingly, spontaneous mutational rate was calculated to be 7×10^{-7}. Overall, these values are better than the frequency of mutation observed for INH (2.9×10^{-6}). These results, therefore, demonstrate that this class of compounds result in a low frequency of mutation.

One of the current challenges for TB drug discovery is the identification of compounds that are active against persistent bacteria. Although the location and state of latent bacteria remains a matter of debate, one commonly shared hypothesis for mycobacterial persistence is that *M. tuberculosis* bacilli are able to survive in macrophages for prolonged periods of time and, unlike other bacteria, are able to actively replicate. The intraphagosomal profile of *M. tuberculosis* is complex; a large variety of genes are over-expressed and timely regulated and are also dependent on environmental factors. Altogether, this makes the identification of one specific tubercle factor that could be selected as the ideal target difficult. Consequently, non-target cell-based assays are a critical tool in the search of intracellular *M. tuberculosis* inhibitors.

Investigation of bacillus growth inhibitors within macrophages has long been limited due to cumbersome CFU plating, slow bacillus growth, safety requirements and difficulties in setting-up appropriate infection conditions. As a consequence, this approach was always used as a secondary assay after the initial selection of compounds that are active on *in vitro* extracellular growth. With the advent of automated confocal microscopy, the above mentioned limitations could be readdressed and the inventors show the feasibility of large scale compound screening. It was decided to perform suspension macrophage batch infection in order to minimize the steps and to meet safety requirements. To this end, careful attention was paid to the removal of the extracellular non-phagocytosed mycobacteria. The

centrifugation conditions used during the wash steps were set up in order to recover only the infected cells and discard most of the extracellular bacteria. By microscopy the inventors confirmed that unbound mycobacteria represented less than 10% of the total bacterial load (data not shown). Mycobacteria are able to grow independently of host cells and consequently any remaining extracellular bacilli would greatly compromise the validity of the inventors' model. To this end, an additional amikacin treatment step was added to the protocol to further eliminate any remaining mycobacteria. Thus with the optimized protocol, there is almost no non-phagocytosed mycobacteria left by the time compound is added. The obtained results also demonstrate that it is specifically the effect on the intracellular mycobacteria that is being measured with compound treatment. Indeed, the inventors observed a weak inhibition with rifampin, an antibiotic that is known to poorly penetrate cells. The 50-fold reproducible decrease in MIC for rifampin in the intracellular assay compared to the *in vitro* growth assay proved that the targeted bacteria are not extracellular. Otherwise no difference would have been seen in MIC between the two assays. Similarly, compounds able to inhibit mycobacterial growth in the phenotypic cell-based assay, but not the *in vitro* growth assay were also identified. In addition, the fact that the compounds are mixed with previously infected cells should decrease the chance for the identification of primary infection inhibitors. However, such compounds may still be identified as blockers of neighboring cell infection.

Compared to a conventional CFU-plating method, the microscopy based detection of fluorescent bacteria is not sufficiently sensitive to distinguish between dead and live bacilli as the GFP signal is stable for several days. Indeed, at a high concentration of INH, rifampin or active compound, there is always 10% of the cells that appear to be infected, which is similar to the initial infection ratio. Surprisingly, no CFU could be recovered after plating such samples. Owing to the fact that latent bacilli are able to recover growth (Cho *et al.*, 2007), the microscopy-detected bacilli must be dead bacilli rather than latent bacilli. Thus, the inventors' assay detects compounds that interfere with bacilli growth within macrophages.

As it is well established and confirmed (**Figure 1a**), macrophages are able to support high bacterial loads which end up encompassing a large part of the cell cytoplasm and eventually lead to macrophage cell death. It is obvious when *M. tuberculosis* is the infectious agent compared to BCG (Bacille Calmette-Guerin), which even at high MOI fails to induce much cytotoxicity (data not shown). Taking this into account, it was decided to set the data acquisition at day 5 post-infection when the cell number in the DMSO samples had significantly decreased relative to the antibiotically protected controls. Thus, monitoring cell

number was an additional parameter enabling the inventors to confirm the compound's antibacterial activity.

Unlike direct fluorescence based assays, analysis for image-based assays proved to be much more variable. Several parameters that are inherent to the biology of the assay partially explain the lower Z'-values that are usually accepted for HTS validation. The remaining fluorescent dead bacilli do not have much of an impact on the Z'-value, rather the variability in the infection ratio for the DMSO controls seems to account for the discrepancy. Also of importance is the fact that, upon infection, the macrophages had a tendency to migrate which in turn led to a heterogeneous set of images (**Figure 2a**). However, the aim of the primary screen was to identify compounds fully active at a concentration of 20 μM. Thus, for this purpose, a positive Z' for the infection ratio (INH/DMSO) was considered an acceptable value. The best proof of the validity of the hit selection according to the present invention comes from the subsequent serial dilution analysis, whereby almost 100% of the hits were confirmed. For each of the hits, a nicely fitted dose-response curve for the infection ratio was obtained as well as for the non-toxic compound in terms of cell number. Again, cell number brought an additional confirmation of the results that is totally independent of green fluorescence emission and GFP expression.

Obviously compounds found to be active against both intracellular and *in vitro M. tuberculosis* growth are the most promising. The best inhibitors isolated from this library have an inhibitory activity within the same range as INH. Further structure activity relationship studies will contribute to determine if their activity could be improved. In the course of another study using this phenotypic cell-based model, MIC down to the ng/mL scale was obtained for compounds with known *in vitro* antibacterial efficacy showing that compounds with a lower MIC than INH can be identified by the assay according to the present invention (data not shown). Of utmost interest are the compounds that are active only in the intracellular bacteria assay as they are likely to have a new mechanism of action independent of the infecting strain suggesting that they may also be active on the non-curable multi-drug-resistant (MDR)-strains.

Taken together, the above results show that monitoring *M. tuberculosis* growth with automated fluorescence microscopy is highly robust and reliable and that this method enables fast selection of potent anti-TB compounds.

References

Abadie, V., Badell, E., Douillard, P., Ensergueix, D., Leenen, P. J., Tanguy, M., Fiette, L., Saeland, S., Gicquel, B., and Winter, N. (2005). Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. Blood *106*, 1843-1850.

Andries, K., Verhasselt, P., Guillemont, J., Gohlmann, H. W., Neefs, J. M., Winkler, H., Van Gestel, J., Timmerman, P., Zhu, M., Lee, E., *et al.* (2005). A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science *307*, 223-227.

Arain, T. M., Resconi, A. E., Singh, D. C., and Stover, C. K. (1996). Reporter gene technology to assess activity of antimycobacterial agents in macrophages. Antimicrob Agents Chemother *40*, 1542-1544.

Brodin, P., Majlessi, L., Marsollier, L., de Jonge, M.I., Bottai, D., Demangel, Cl., Hinds, J., Neyrolles, O., Butcher, P.D., Leclerc, C., Coles, S.T., Brosch, R., (2006). Dissection of ESAT-6 system 1 of Mycobacterium tuberculosis and impact on immunogenicity and virulence. Infect Immun *74*, 88-98.

Cho, S. H., Warit, S., Wan, B., Hwang, C. H., Pauli, G. F., and Franzblau, S. G. (2007). Low-oxygen-recovery assay for high-throughput screening of compounds against nonreplicating Mycobacterium tuberculosis. Antimicrob Agents Chemother *51*, 1380-1385.

Cremer, I., Dieu-Nosjean, M. C., Marechal, S., Dezutter-Dambuyant, C., Goddard, S., Adams, D., Winter, N., Menetrier-Caux, C., Sautes-Fridman, C., Fridman, W. H., and Mueller, C. G. (2002). Long-lived immature dendritic cells mediated by TRANCE-RANK interaction. Blood *100*, 3646-3655.

Fenistein, D., Lenseigne, B., Christophe, T., Brodin, P., and Genovesio, A. (2008). A fast fully automated cell segmentation algorithm for high throughput and high content screening. Cytometry part A, in press.

Houben, E. N., Nguyen, L., and Pieters, J. (2006). Interaction of pathogenic mycobacteria with the host immune system. Curr Opin Microbiol *9*, 76-85.

Lenaerts, A. J., Hoff, D., Aly, S., Ehlers, S., Andries, K., Cantarero, L., Orme, I. M., and Basaraba, R. J. (2007). Location of persisting mycobacteria in a Guinea pig model of tuberculosis revealed by r207910. Antimicrob Agents Chemother *51*, 3338-3345.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews *46*, 3-26.

Neyrolles, O., Hernandez-Pando, R., Pietri-Rouxel, F., Fornes, P., Tailleux, L., Barrios Payan, J. A., Pivert, E., Bordat, Y., Aguilar, D., Prevost, M. C., *et al.* (2006). Is adipose tissue a place for Mycobacterium tuberculosis persistence? PLoS ONE *1*, e43.

Rohde, K. H., Abramovitch, R. B., and Russell, D. G. (2007). Mycobacterium tuberculosis invasion of macrophages: linking bacterial gene expression to environmental cues. Cell Host Microbe *2*, 352-364.

Salomon, J. A., Lloyd-Smith, J. O., Getz, W. M., Resch, S., Sanchez, M. S., Porco, T. C., and Borgdorff, M. W. (2006). Prospects for advancing tuberculosis control efforts through novel therapies. PLoS Med *3*, e273.

Schnappinger, D., Ehrt, S., Voskuil, M. I., Liu, Y., Mangan, J. A., Monahan, I. M., Dolganov, G., Efron, B., Butcher, P. D., Nathan, C., and Schoolnik, G. K. (2003). Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment. J Exp Med *198*, 693-704.

Van Rie, A., and Enarson, D. (2006). XDR tuberculosis: an indicator of public-health negligence. Lancet *368*, 1554-1556.

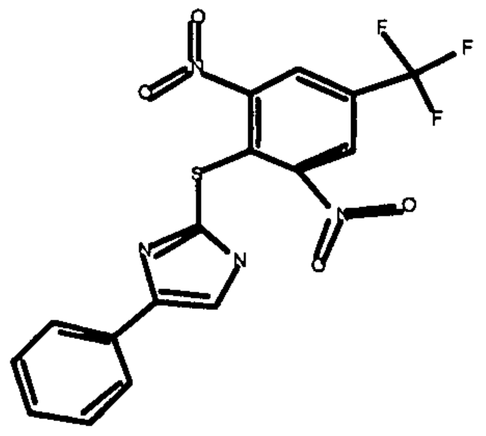
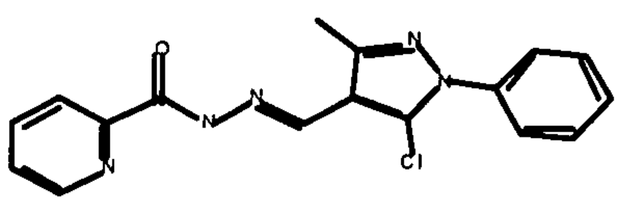
ID	Structure	Primary QIM CellNb	QIM Confirm CellNb 20uM	QIM Confirm CellNb 2uM	QIM Confirm CellNb 0.2uM	Primary QIM % Inhibition	QIM Confirm % Inhibition 20uM	QIM Confirm % Inhibition 2uM	QIM Confirm % Inhibition 0.2uM	Primary QUM % Inhibition	QUM Confirm % Inhibition 20uM	QUM Confirm % Inhibitio n 2uM	QUM Confirm % Inhibition 0.2uM
IPK 000 001 32		88.3	113.8	208.5	241.4	25.4	50.7	0.2	10.3	99.9	89.1	41.2	43.9
IPK 000 001 90		435.5	317.6	173.6	190.0	91.9	96.5	13.2	12.7	2.9	42.3	29.4	34.6

Table 1

Table 1

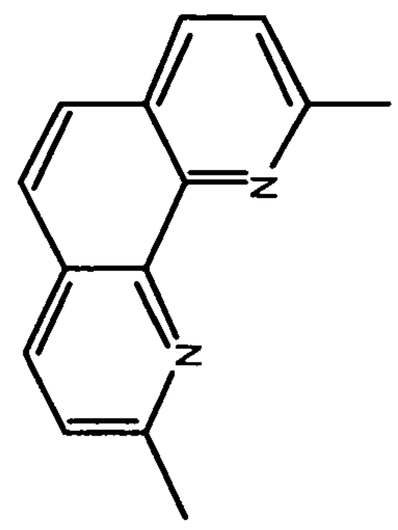
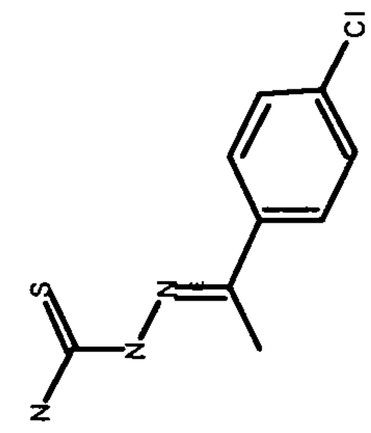
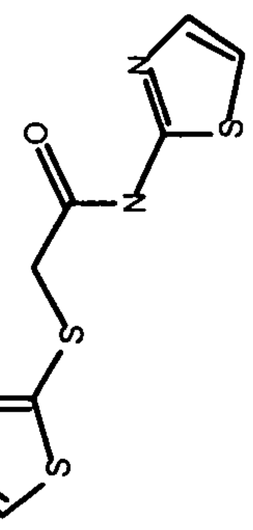
32.5	56.9	45.9
52.4	67.4	36.1
69.7	49.7	36.4
99.7	98.9	-13.8
-2.0	54.5	11.4
7.7	70.4	-1.3
-12.3	24.5	66.0
-28.5	26.6	65.9
241.4	472.3	315.9
92.0	541.6	246.1
148.0	249.8	412.9
77.0	235.5	350.3
		
IPK 000 002 03	IPK 000 002 17	IPK 000 002 87

Table 1

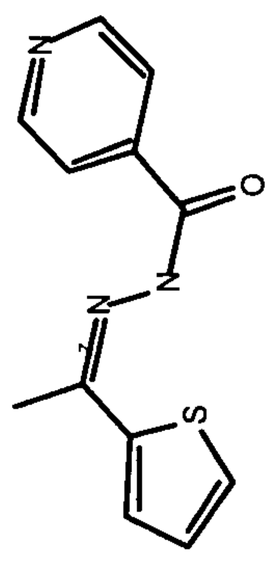
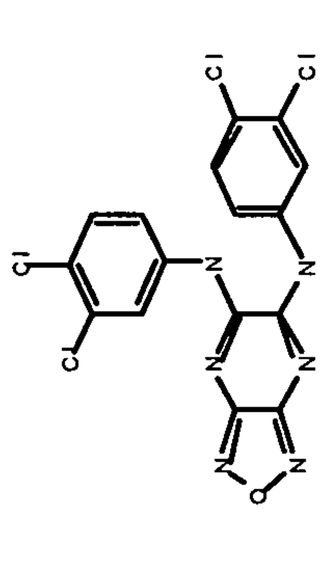
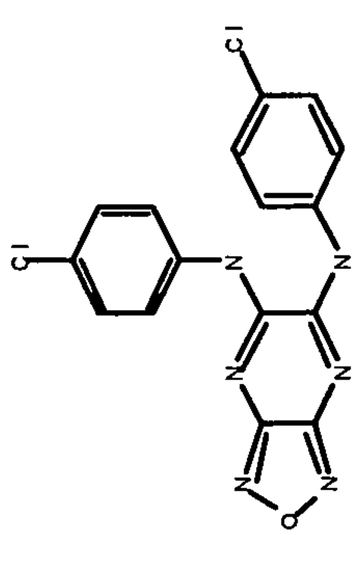
IPK 000 003 01		373.5	248.3	457.3	232.6	88.2	40.4	77.1	4.7	98.7	43.0	82.8	46.4
IPK 000 003 89		72.5	103.0	200.6	265.3	27.7	84.6	86.6	20.1	100.3	67.0	76.5	44.0
IPK 000 003 90		78.0	133.4	75.6	142.3	15.7	67.9	43.2	2.2	99.7	72.7	68.8	44.0

Table 1

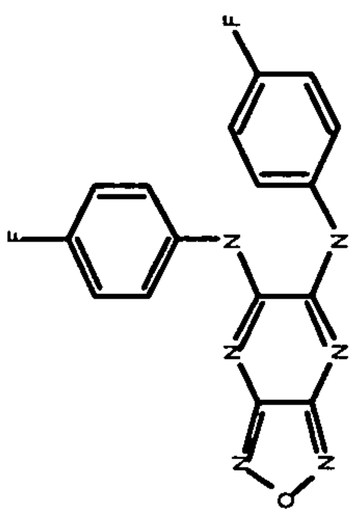
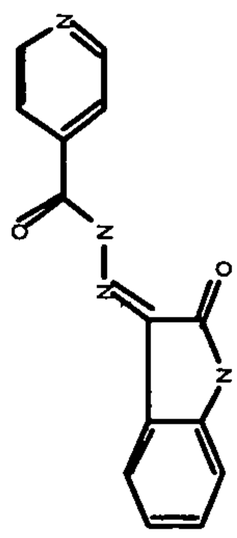
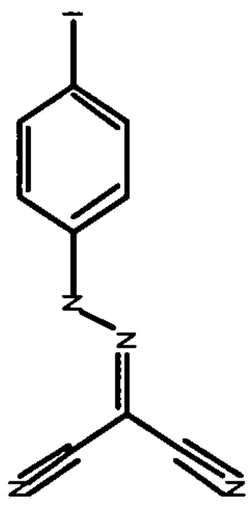
IPK 000 003 91		63.0 128.8 148.9 220.9 31.6 76.4 36.8 2.9 99.6 76.7 41.4 46.6	424.3 328.8 320.9 262.8 97.6 65.2 22.8 17.7 42.0 43.4 41.9 23.7
IPK 000 006 35		61.3 166.5 308.8 393.1 -28.2 25.8 14.7 45.7 76.6 80.8 33.0 41.0	IPK 000 007 31
			

Table 1

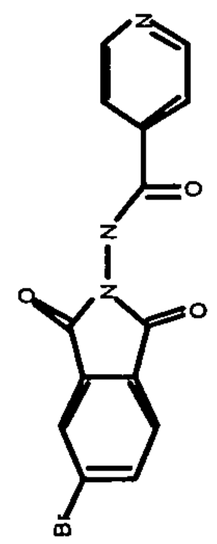
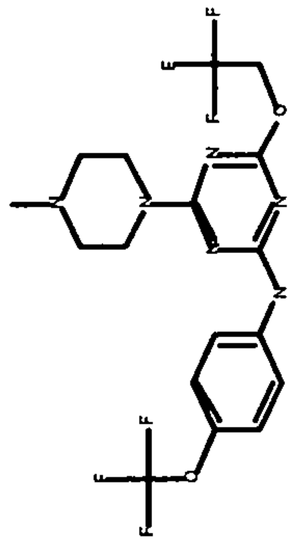
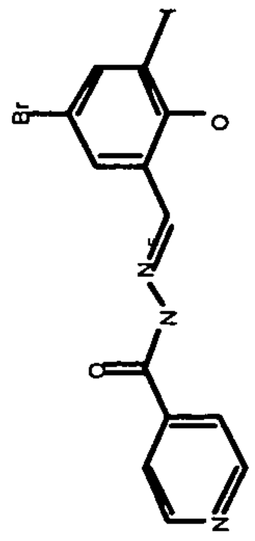
IPK 000 008 02		305.8	484.5	218.8	306.6	83.2	98.0	9.3	0.7	34.8	98.2	31.9	36.4
IPK 000 008 12		396.3	248.0	225.6	292.9	64.1	78.4	18.9	2.7	97.2	48.0	39.7	36.5
IPK 000 009 33		314.5	333.6	475.9	264.8	79.9	56.9	92.5	8.5	30.3	69.8	44.5	31.8

Table 1

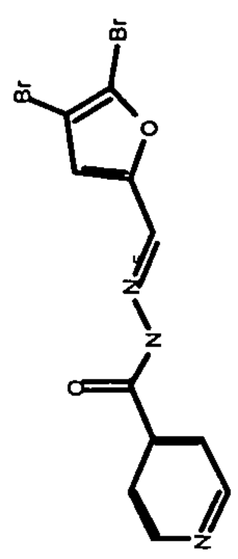
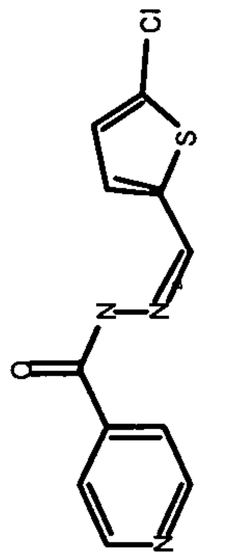
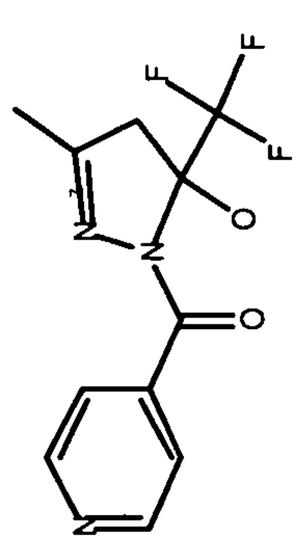
IPK 000 009 41		345.8	28.3
		446.5	59.6
		488.3	99.4
		257.8	97.7
		92.9	19.6
		99.6	92.8
		92.8	99.6
		19.6	92.9
		97.7	257.8
		99.4	488.3
		59.6	446.5
		28.3	345.8
IPK 000 009 42		376.5	28.8
		255.0	50.3
		473.5	100.0
		326.3	92.6
		93.1	21.3
		97.9	92.3
		92.3	97.9
		21.3	93.1
		92.6	326.3
		100.0	473.5
		50.3	255.0
		28.8	376.5
IPK 000 009 78		454.5	30.0
		376.1	61.1
		414.4	100.1
		325.5	39.5
		102.6	17.0
		88.6	50.2
		30.0	88.6
		61.1	102.6
		100.1	325.5
		39.5	414.4
		17.0	376.1
		50.2	454.5

Table 1

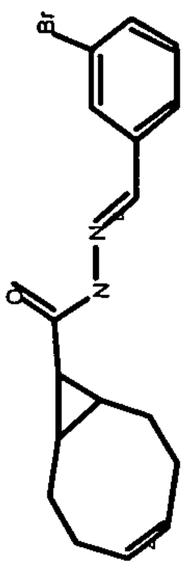
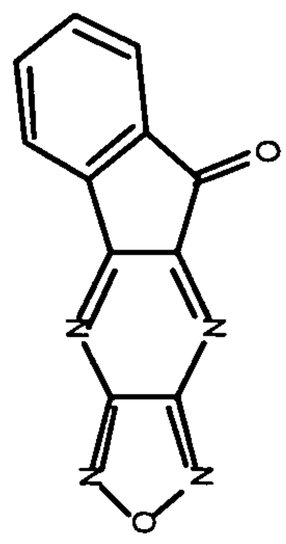
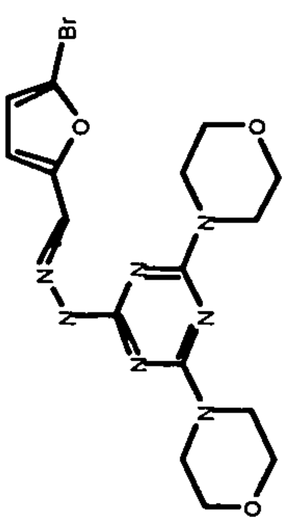
39.5	67.2	39.8
43.1	93.2	40.6
39.2	43.1	96.3
98.4	90.5	100.0
17.1	9.7	4.7
18.0	31.6	23.4
81.8	47.6	41.9
81.0	60.0	6.5
412.0	248.8	259.6
344.9	80.0	336.5
380.9	279.1	201.1
322.8	190.8	145.5
		
IPK 000 010 06	IPK 000 011 19	IPK 000 011 65

Table 1

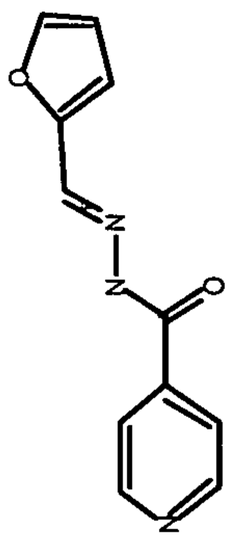
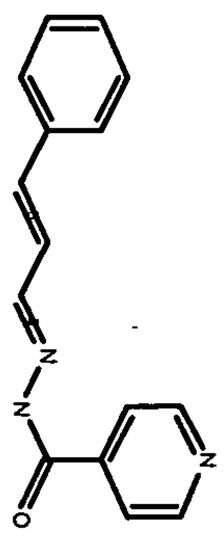
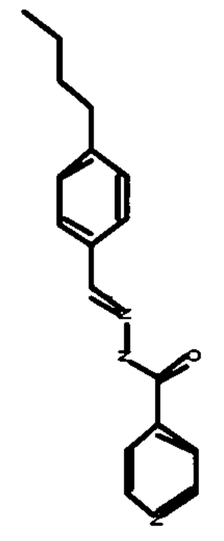
IPK 000 013 67		358.0	457.3	545.1	452.5	98.7	66.7	102.1	84.1	98.7	69.7	90.2	49.4
IPK 000 013 68		276.3	438.8	528.8	400.1	65.0	67.2	101.8	50.9	98.9	77.6	77.1	50.4
IPK 000 013 69		327.5	443.3	532.1	405.8	91.3	58.6	104.9	68.9	99.2	62.4	79.1	51.2

Table 1

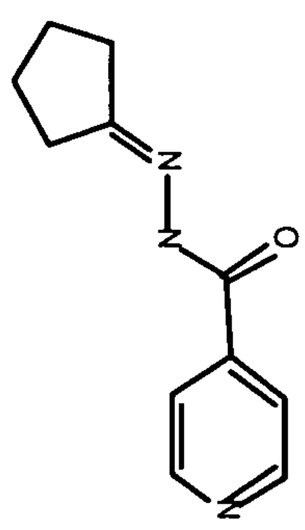
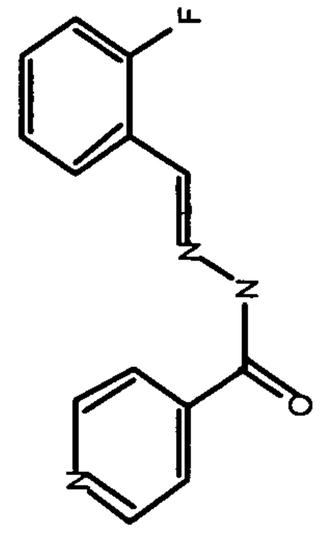
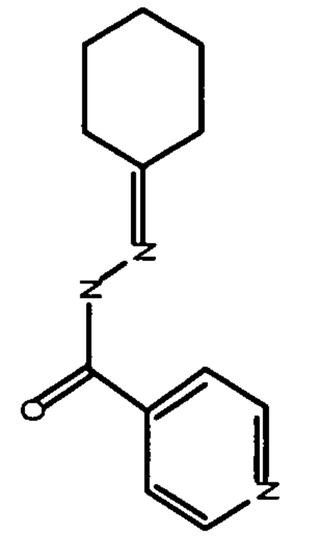
IPK 000 013 70		309.3	518.5	510.9	412.6	88.3	103.0	98.1	50.5	98.4	67.6	79.4	46.3
IPK 000 013 71		358.8	377.4	544.3	476.5	102.9	97.5	105.2	86.3	99.4	68.4	92.2	51.6
IPK 000 013 72		355.5	457.3	541.6	448.5	82.2	100.6	103.7	63.2	100.0	98.0	73.9	57.7

Table 1

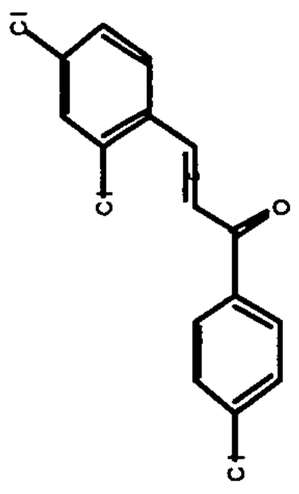
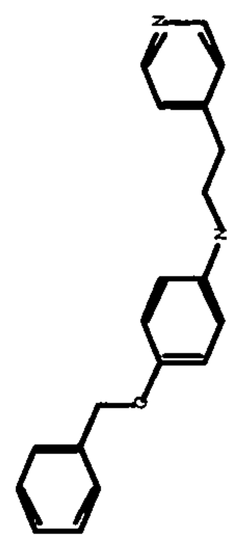
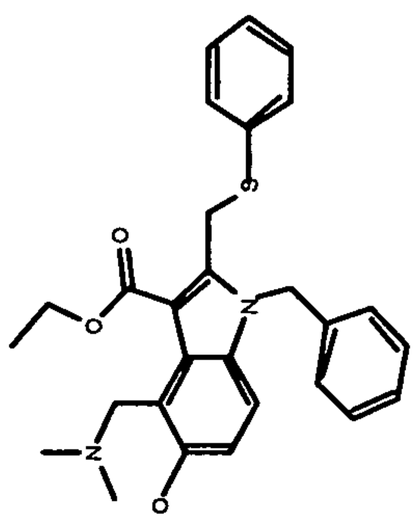
IPK 000 015 36		146.5	168.0	286.0	331.0	38.1	-4.8	36.1	15.8	100.3	79.5	59.4	41.9
IPK 000 016 00		93.0	300.3	265.4	278.8	-19.0	19.4	37.9	3.2	90.5	68.0	48.6	47.1
IPK 000 016 05		192.0	282.0	148.9	206.8	67.9	0.8	43.4	-2.2	98.7	70.0	42.5	36.6

Table 1

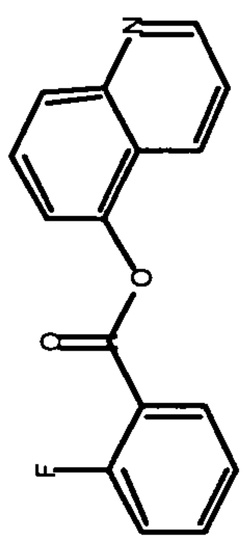
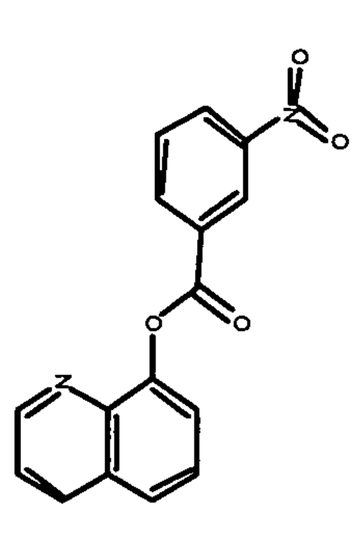
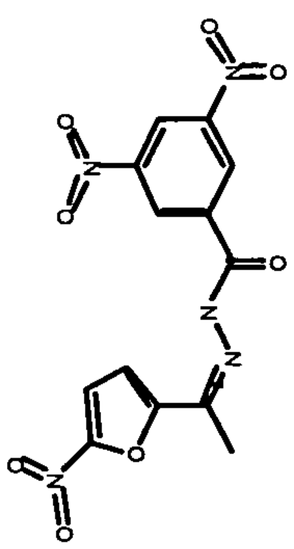
IPK 000 018 65		218.0	256.4	218.3	256.6	23.1	39.2	14.0	28.0	99.9	99.0	54.0	36.4	
IPK 000 018 66		63.3	130.4	296.6	258.1	-26.8	16.7	0.4	8.6	100.6	99.6	72.0	39.3	
IPK 000 018 82		106.8	184.1	209.0	443.0	-57.3	4.1	17.6	42.7	99.3	67.6	41.9	41.1	

Table 1

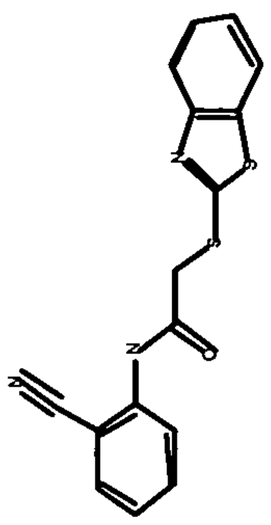
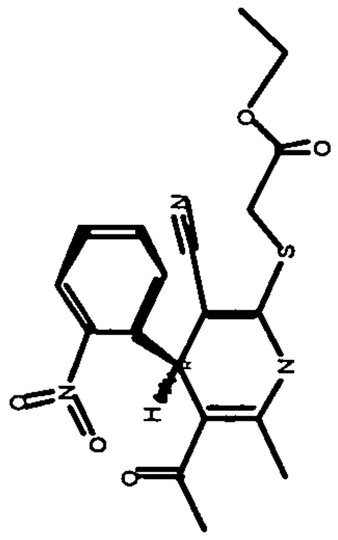
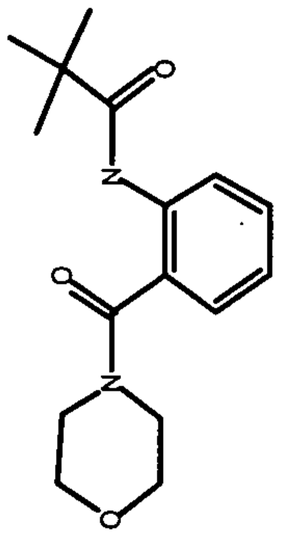
IPK 000 018 97		314.0	553.9	299.8	288.5	76.7	83.8	20.0	7.3	10.3	36.1	33.9	40.5	
IPK 000 019 84		402.0	610.5	329.0	287.9	88.8	94.6	2.2	7.8	-10.5	43.7	36.8	46.7	
IPK 000 021 87		405.0	609.0	403.4	305.3	90.2	96.5	29.8	15.4	2.7	41.9	42.9	49.1	

Table 1

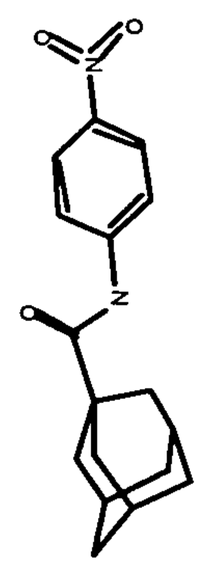
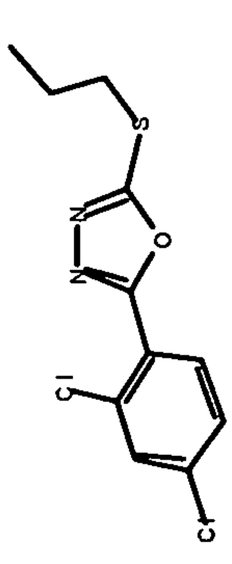
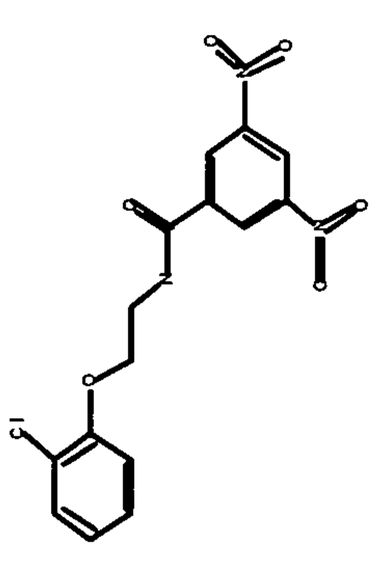
IPK 000 022 33		372.0	517.1	472.3	315.6	66.0	90.0	33.5	30.3	96.0	74.2	45.4	35.7
IPK 000 024 43		203.8	205.0	349.0	352.1	5.8	59.1	58.3	50.4	75.8	67.7	40.1	34.7
IPK 000 027 72		333.3	238.1	440.4	267.0	82.6	100.0	77.7	29.3	100.0	100.9	97.6	35.1

Table 1

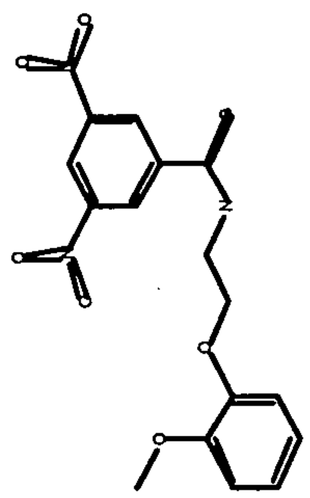
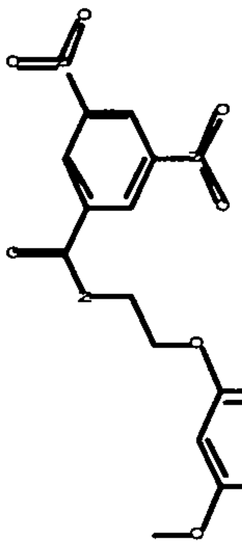
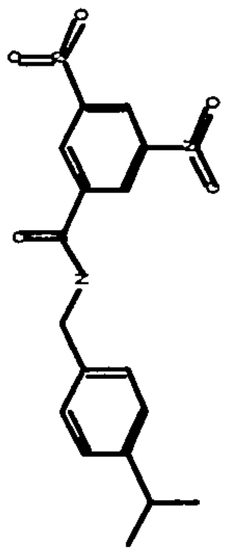
IPK 000 027 74	IPK 000 027 77	IPK 000 027 78
		
366.0	378.0	332.0
435.8	322.3	499.4
478.8	315.3	543.5
268.5	218.1	366.0
87.2	89.1	79.6
96.9	86.3	96.3
83.3	47.7	99.3
-2.7	-12.6	33.0
98.8	99.6	98.8
100.4	100.4	78.2
97.5	96.8	97.5
43.5	40.2	71.7

Table 1

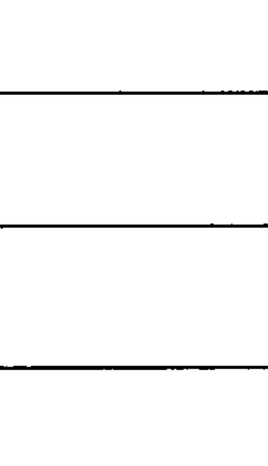
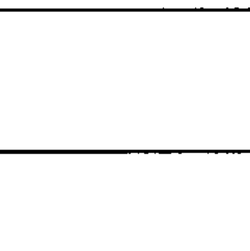
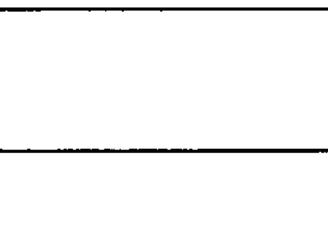
IPK 000 027 85		315.0 224.8 487.4 429.3 100.3 89.0 40.1 99.4 83.0 99.6 71.7 97.9 97.2	410.8 325.6 434.1 295.3 89.0 46.1 98.5 14.2 99.3 75.0 97.6 65.8
IPK 000 027 91		315.3 308.9 478.6 489.8 94.5 17.8 102.4 64.5 99.8 48.3 98.1 94.0	
IPK 000 028 35			

Table 1

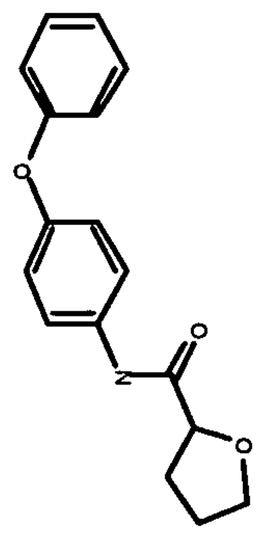
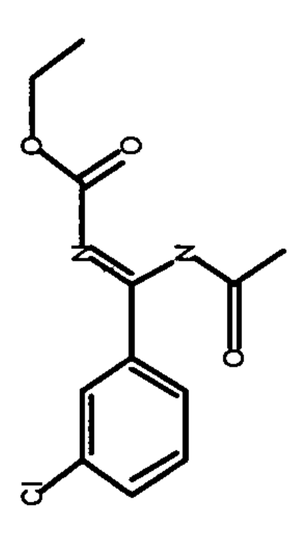
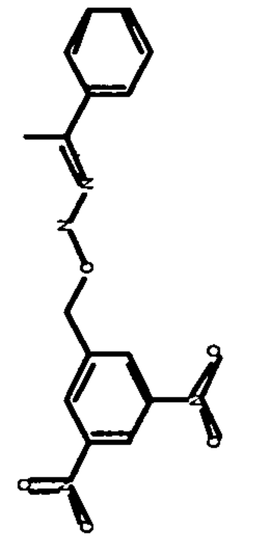
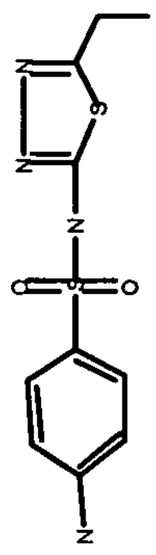
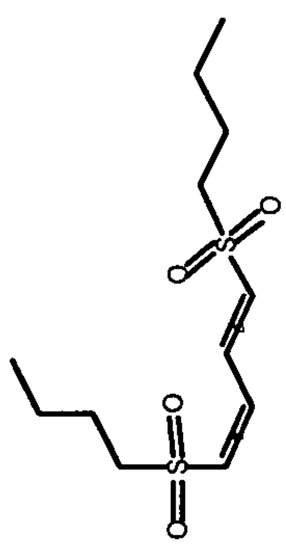
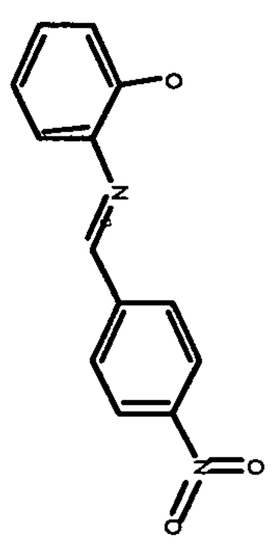
IPK 000 033 16		283.0	296.1	81.9	92.3	69.6	5.0	43.4	61.3	42.0	34.1	303.9	573.9	
IPK 000 033 61		188.3	210.8	31.6	62.9	13.8	16.2	94.9	67.1	37.6	51.6	111.8	434.1	
IPK 000 035 56		226.0	247.1	83.3	89.0	18.8	9.1	71.9	65.6	39.6	41.5	524.3	313.1	

Table 1

IPK 000 035 58		104.0	279.9	330.0	292.3	-51.3	27.7	2.8	59.5	34.4	32.0	5.1	17.2	87.2	96.4	65.3	70.6	46.4	47.9	45.1	42.3	38.7
IPK 000 036 07		142.3	164.4	293.9	267.4	27.7	59.5	32.0	17.2	96.4	70.6	47.9	42.3	38.7	46.8	68.4	97.7	20.4	15.6	18.1	-38.6	321.4	262.3	330.0
IPK 000 040 14		95.5	330.0	262.3	321.4	-38.6	18.1	15.6	20.4	97.7	68.4	46.8	38.7	42.3	47.9	70.6	96.4	17.2	32.0	59.5	27.7	267.4	293.9	164.4

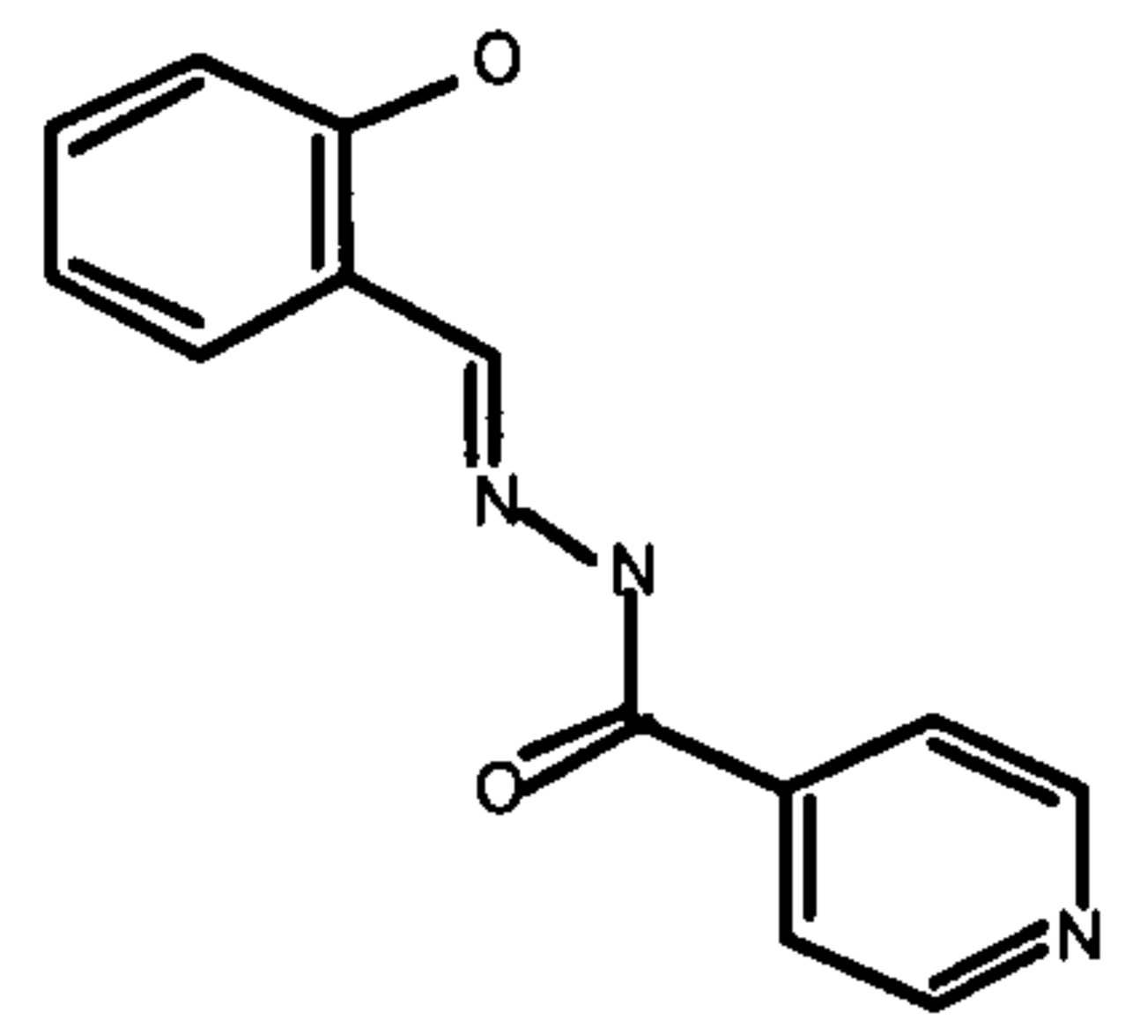
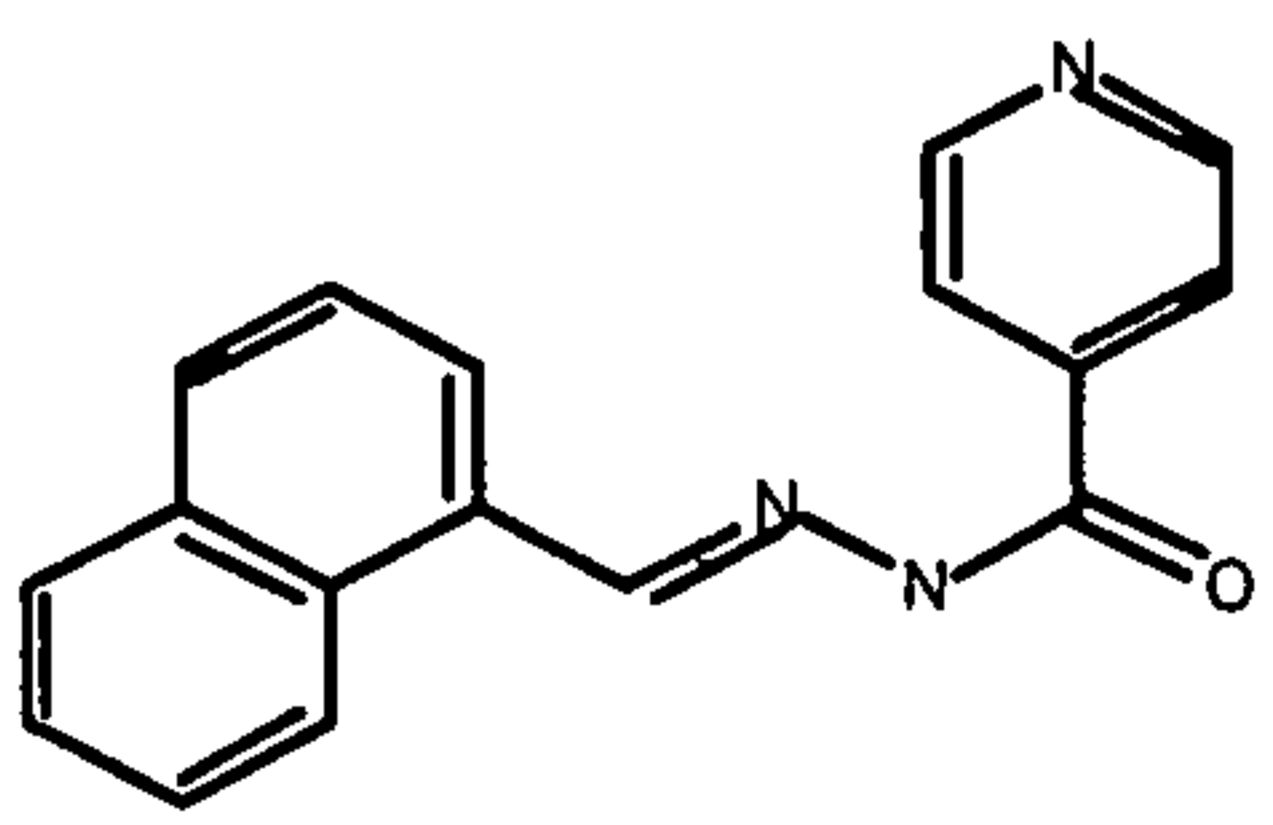
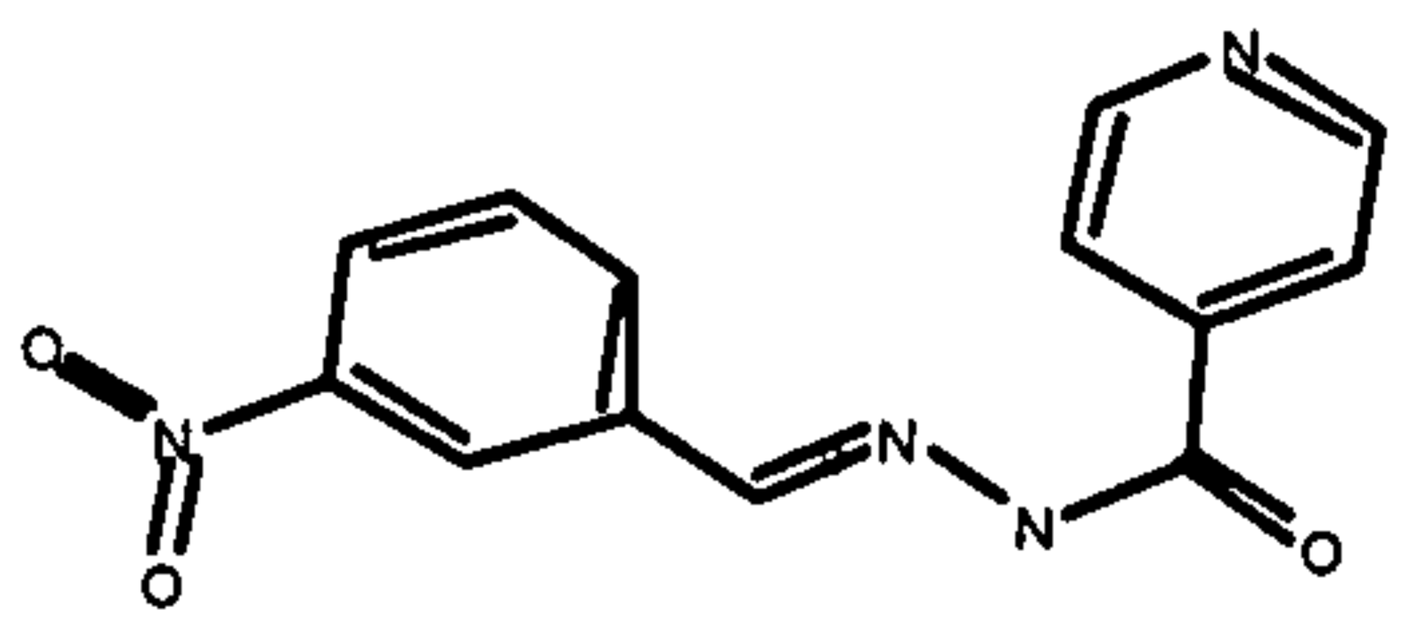
IPK 000 041 45		324.5	243.8	527.9	437.4	88.6	87.2	103.2	56.7	44.7	100.5	47.4	38.4
IPK 000 041 46		320.0	347.1	542.6	386.9	81.2	93.3	101.2	41.8	98.3	57.9	81.4	41.1
IPK 000 041 47		362.8	345.8	516.1	479.8	102.4	95.5	101.8	74.5	91.8	99.5	82.3	43.8

Table 1

Table 1

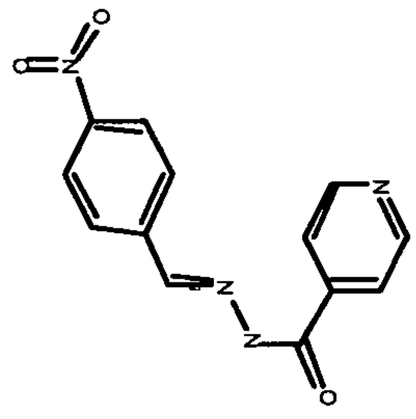
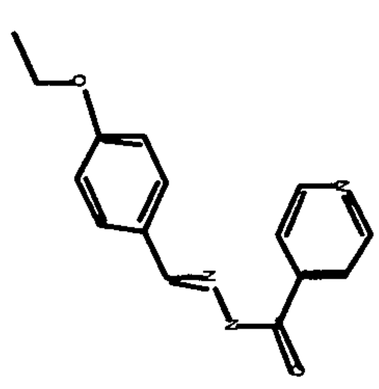
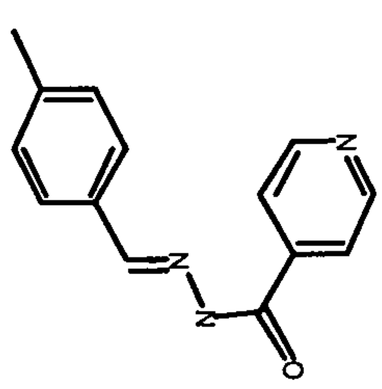
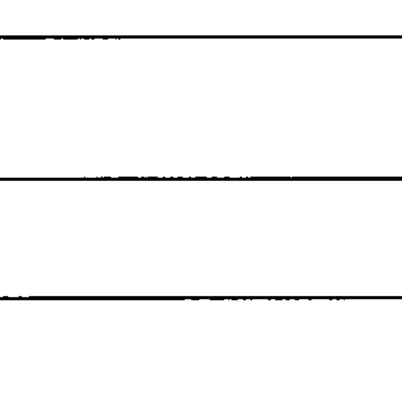
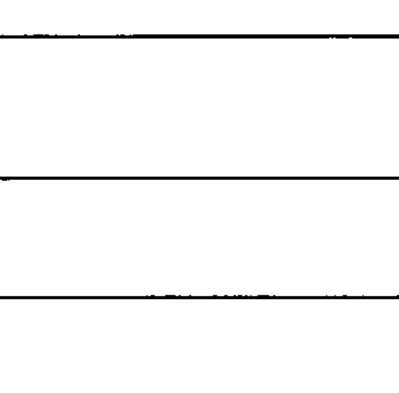
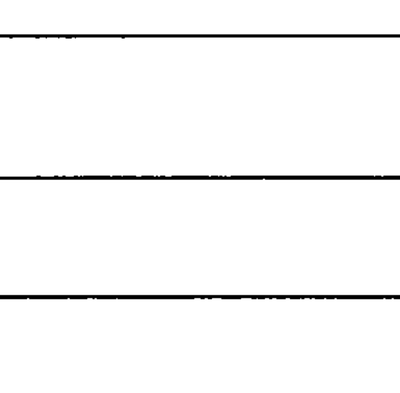
IPK 000 041 48		315.3	347.6	508.4	414.6	84.5	88.4	97.5	55.9	96.4	38.6	84.5	43.7	336.5	338.1	535.4	394.6	83.8	94.5	102.3	56.4	98.9	39.4	85.5	44.4
IPK 000 041 49		296.3	315.9	515.5	396.3	81.6	90.8	102.2	66.3	99.2	77.7	94.3	52.0												
IPK 000 041 50																							

Table 1

IPK 00041 51		351.3	350.6	505.6	368.0	89.0	75.6	94.0	102.9	55.1	98.4	70.9	85.2	40.8
IPK 00041 52		262.5	362.8	523.5	451.8	100.2	93.7	103.2	89.7	99.0	56.0	93.0	53.1
IPK 00041 53		426.8	431.1	523.3	197.8	97.0	98.1	18.8	96.9	44.4	82.2	15.1

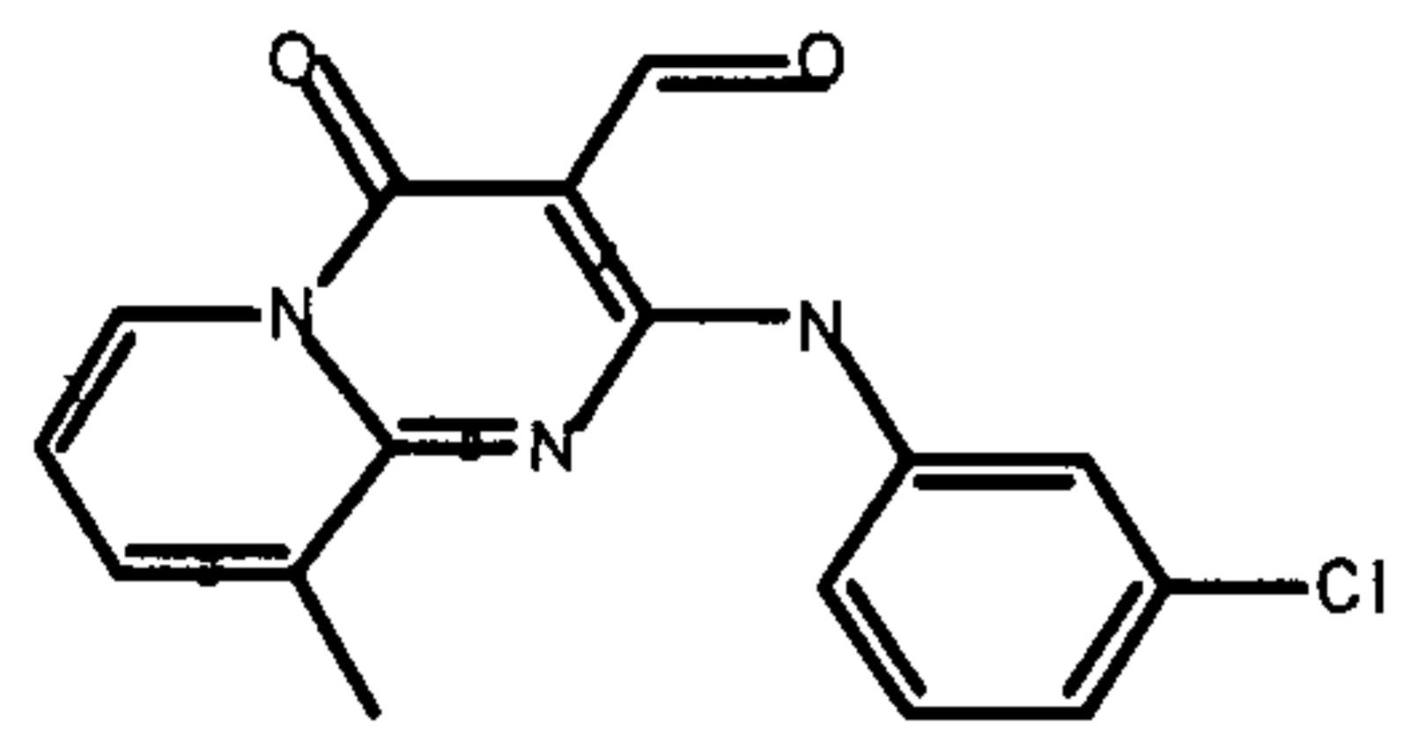
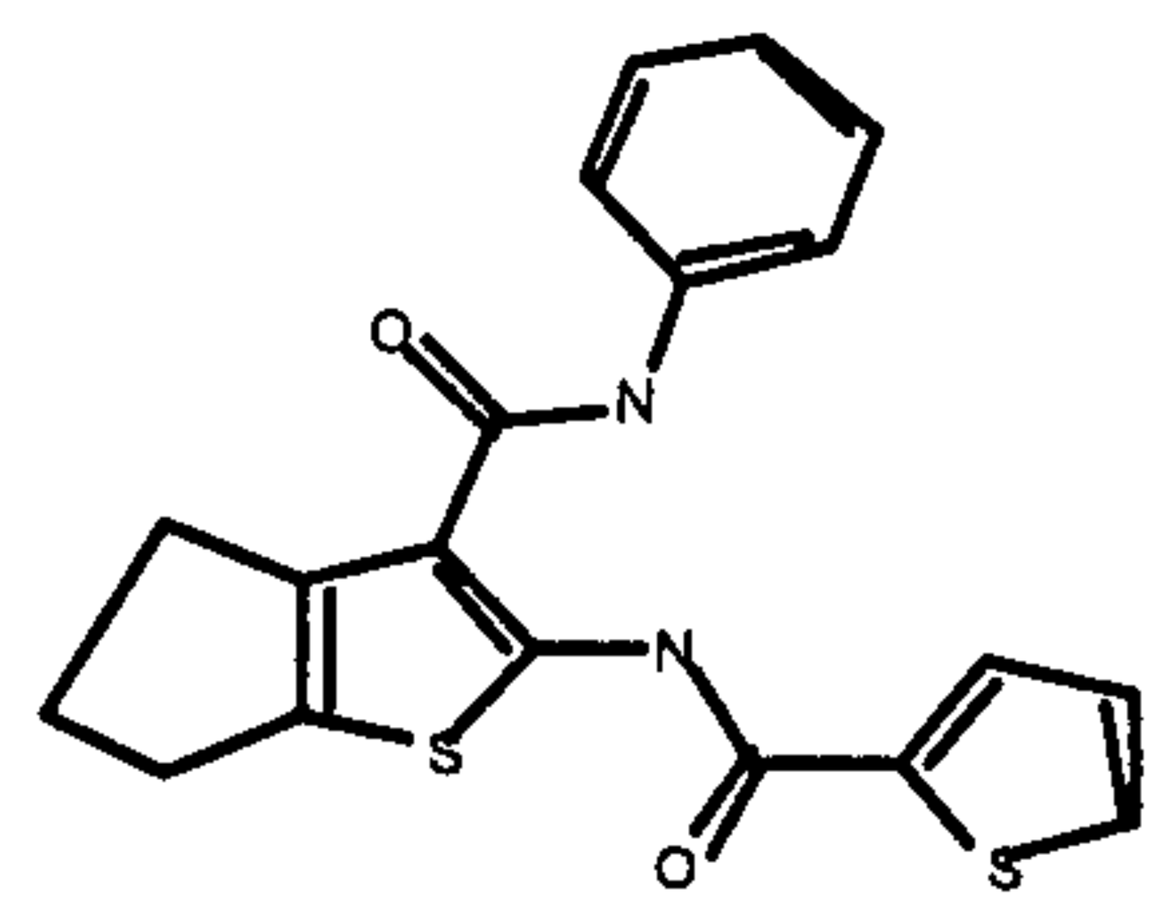
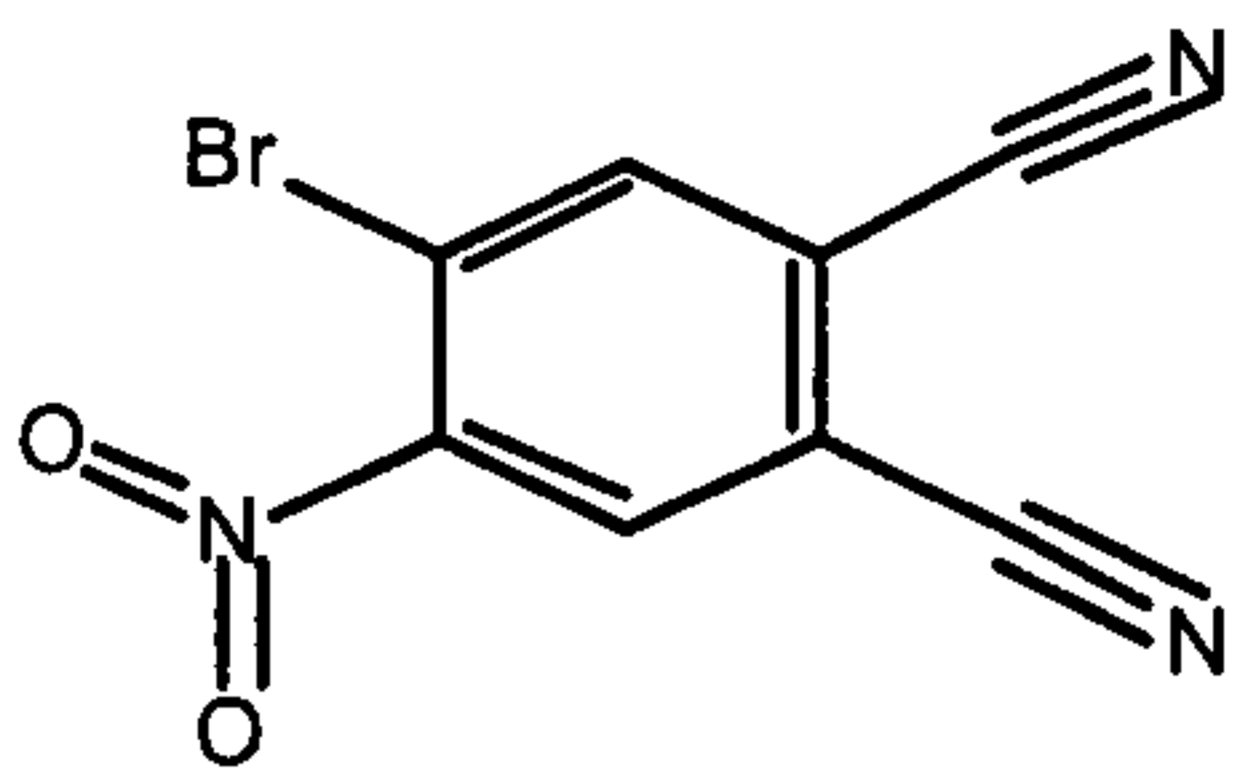
IPK 000 042 07		198.0	596.4	616.9	259.4	34.7	107.1	104.0	12.4	93.4	98.0	96.5	70.8
IPK 000 042 30		193.8	376.8	658.0	384.8	73.7	95.9	75.2	11.0	85.2	95.8	62.9	39.1
IPK 000 042 72		152.0	199.9	96.1	227.8	11.9	57.6	-1.9	2.5	82.5	67.7	43.7	39.6

Table 1

Table 1

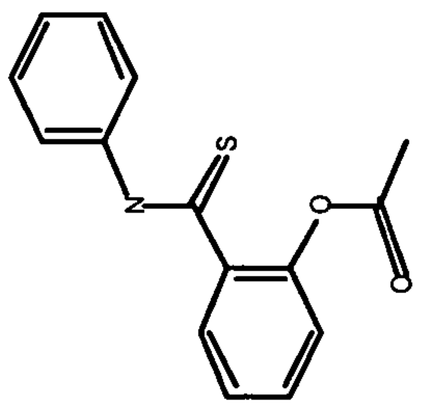
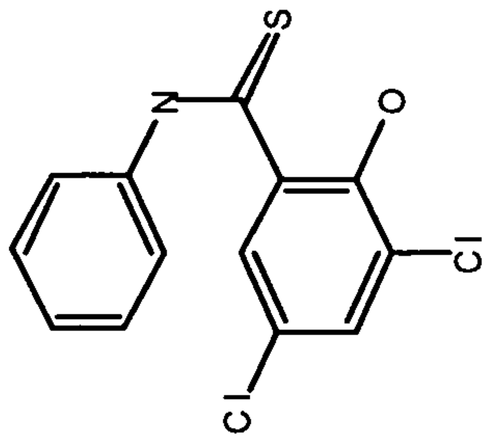
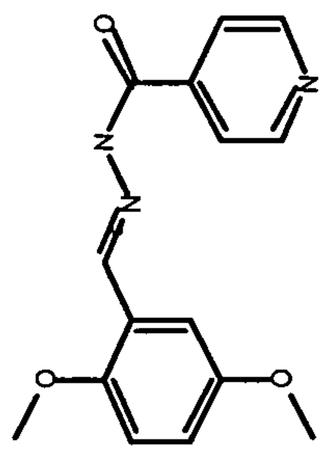
IPK 000 042 77		367.0	425.5	255.3	352.6	104.9	73.9	48.9	91.7	7.7	33.0	22.4	25.8	99.2	98.7	67.1	67.1	51.3	56.4	33.5	42.9
IPK 000 042 78		194.5	341.9	311.5	322.6	73.9	91.7	105.5	33.0	105.1	25.8	49.3	98.7	96.6	67.1	98.5	56.4	76.0	42.9	44.3	
IPK 000 042 93		321.8	451.8	532.9	387.9	108.6	73.9	105.5	33.0	105.1	25.8	49.3	98.7	96.6	67.1	98.5	56.4	76.0	42.9	44.3	

Table 1

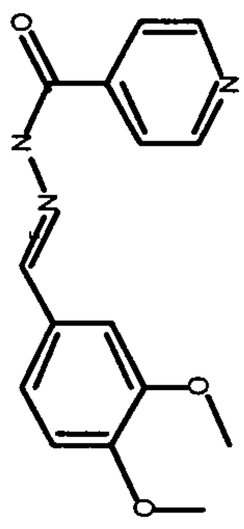
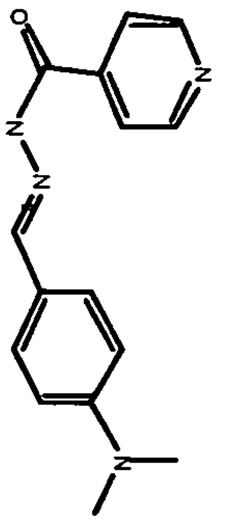
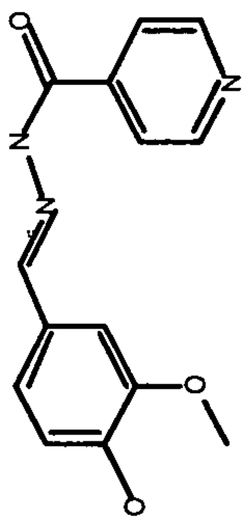
IPK 000 042 95		143.5	433.9	494.6	493.0	64.7	98.8	106.0	70.4	96.6	53.1	82.0	42.2
IPK 000 042 96		216.3	477.0	472.5	491.4	84.3	105.4	101.1	77.4	94.3	97.2	83.3	43.3
IPK 000 042 97		307.8	483.0	502.4	312.9	99.7	103.4	99.2	19.2	97.0	98.8	73.9	40.1

Table 1

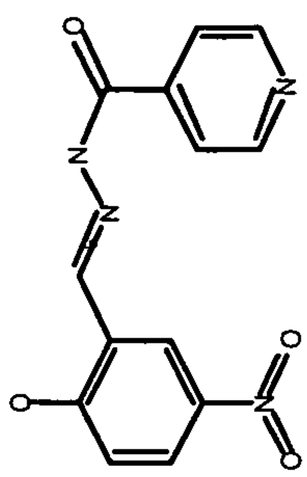
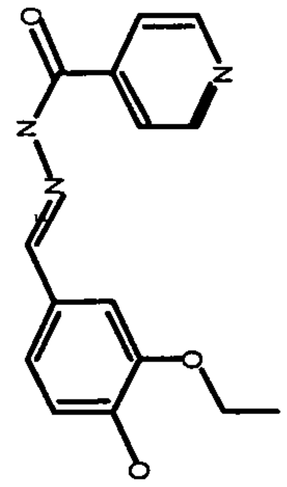
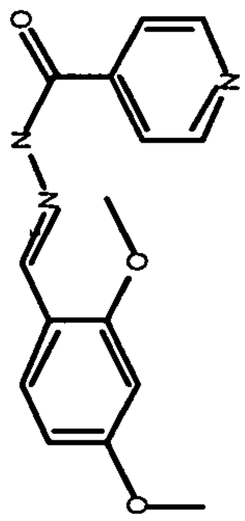
IPK 000 042 98		350.0	554.9	494.5	279.6	102.3	105.4	95.3	19.0	30.3	82.9	48.5	31.8
IPK 000 042 99		364.0	488.3	567.3	378.4	102.7	106.3	104.1	43.4	97.9	97.1	79.3	51.8
IPK 000 043 00		333.3	413.1	537.9	371.0	104.4	101.8	101.4	37.5	98.3	98.6	70.6	51.0

Table 1

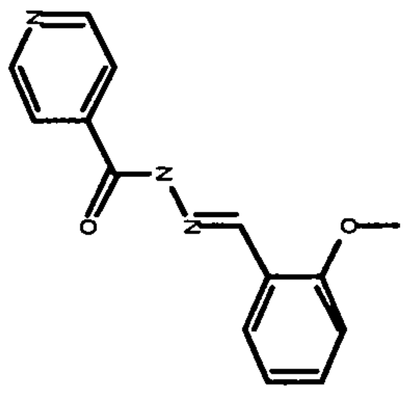
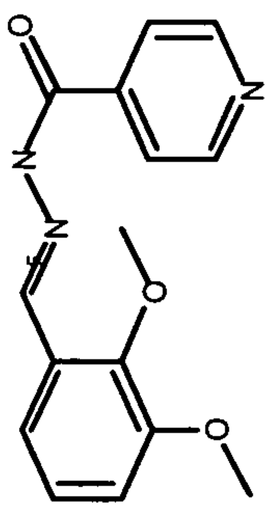
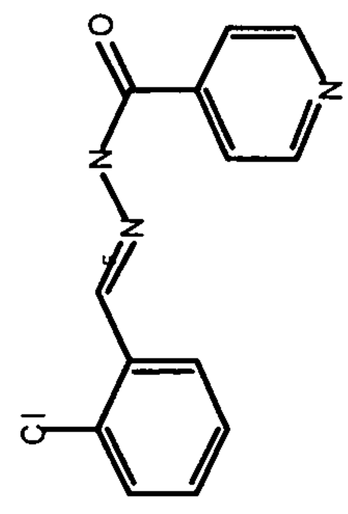
IPK 000 043 01		287.8	448.5	584.3	385.1	86.6	102.6	103.6	56.3	98.6	98.8	74.2	47.4
IPK 000 043 02		229.5	422.1	483.0	476.3	81.1	99.8	98.7	71.3	98.4	98.2	82.6	48.4
IPK 000 043 05		228.8	494.4	502.8	469.0	97.1	103.8	101.6	80.2	99.4	98.8	94.4	51.4

Table 1

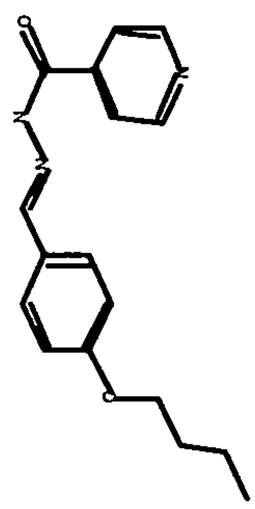
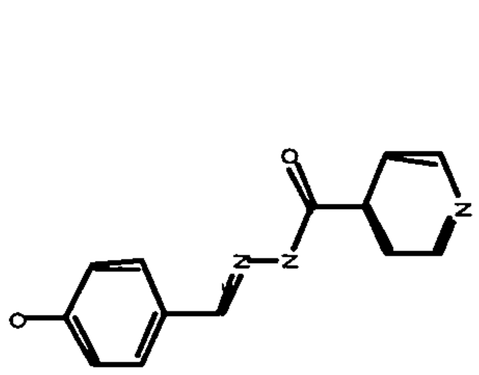
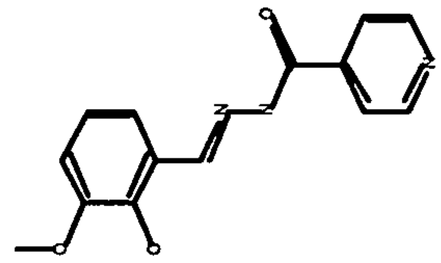
IPK 000 043 06		200.3	436.0	520.1	213.5	81.4	104.3	303.5	416.6	541.6	392.0	255.5	367.6	486.8	397.6	87.3	104.3	38.5	75.2	98.5	99.8	-0.4	98.1	98.0	81.4	213.5	520.1	436.0	200.3
IPK 000 043 07		303.5	416.6	541.6	392.0	104.3	81.4	255.5	367.6	486.8	397.6	87.3	104.3	44.8	79.2	98.9	99.7	68.4	102.5	98.6	104.3	541.6	392.0	416.6	303.5	200.3	436.0	520.1	213.5
IPK 000 043 08		255.5	367.6	486.8	397.6	87.3	104.3	39.8	63.5	63.9	77.2	72.9	102.7	95.1	87.3	104.3	392.0	367.6	486.8	397.6	87.3	104.3	81.4	95.1	102.7	72.9	77.2	63.9	63.5	39.8

Table 1

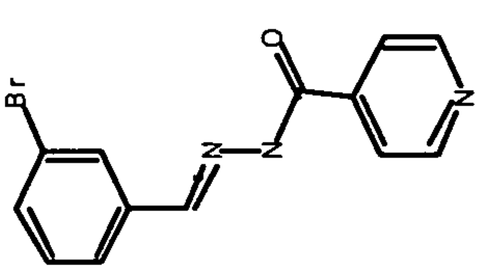
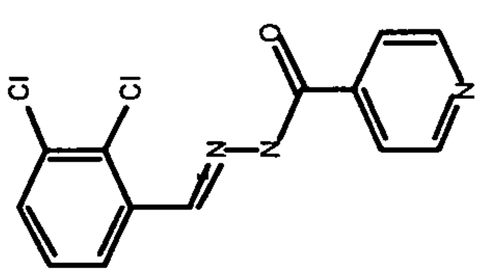
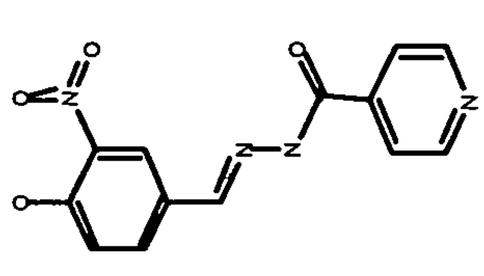
IPK 000 043 09		258.5	459.3	516.8	420.0	95.8	102.4	100.5	59.3	99.5	98.4	80.1	49.1
IPK 000 043 10		176.8	441.6	509.8	367.0	14.3	98.3	92.7	28.5	98.7	66.9	73.1	46.5
IPK 000 043 11		366.0	514.6	532.3	350.4	104.4	104.5	99.5	40.3	97.5	70.9	76.7	43.5

Table 1

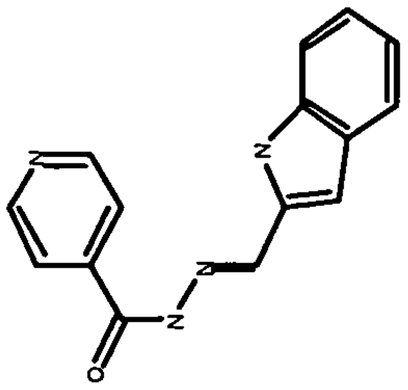
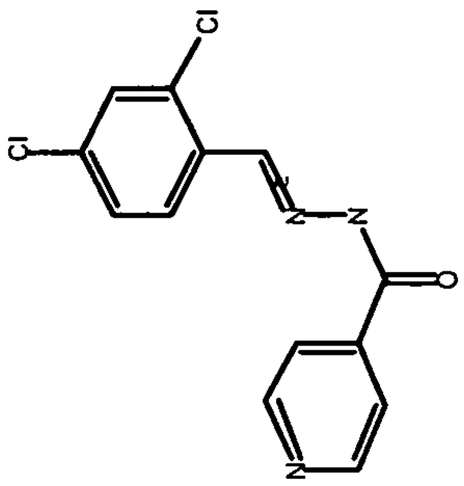
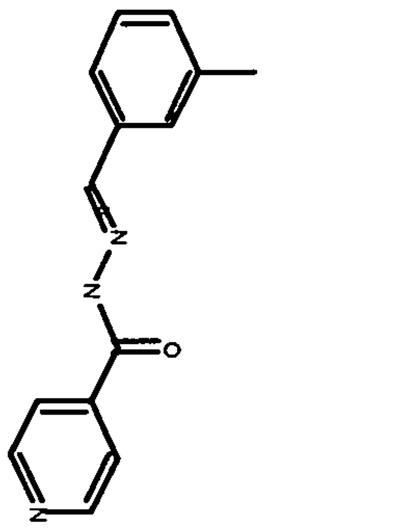
IPK 000 043 12		195.0 177.8 347.3	
		406.3 468.8 430.3	
		500.3 469.0 523.3	
		432.0 269.9 413.6	
		69.9 49.2 109.4	
		100.0 104.2 102.1	
		104.6 101.6 102.2	
		56.5 17.8 72.4	
		98.5 98.7 99.5	
		69.8 98.5 98.6	
		84.5 75.0 89.6	
		48.9 48.9 52.7	
IPK 000 043 13			
IPK 000 043 26			

Table 1

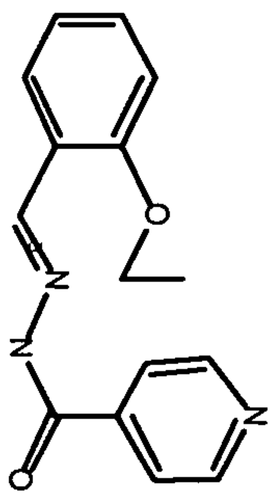
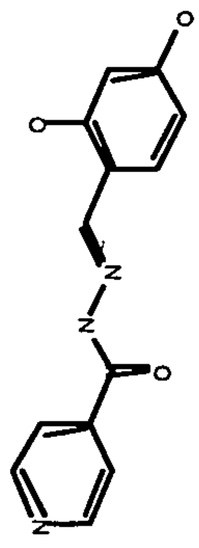
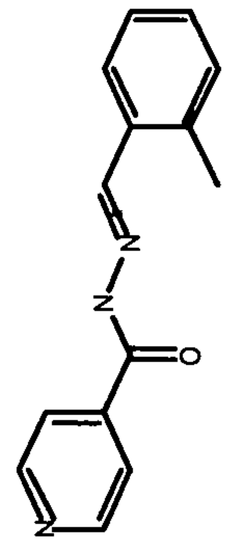
IPK 000 043 27		125.8	438.6	549.8	459.1	47.6	102.0	105.2	87.8	99.2	98.8	86.3	67.0
IPK 000 043 28		326.5	408.3	482.8	332.6	94.9	95.9	87.1	11.2	12.2	69.7	38.8	43.0
IPK 000 043 29		415.5	454.4	567.1	489.3	111.0	100.2	105.2	71.9	98.3	98.6	76.8	51.3

Table 1

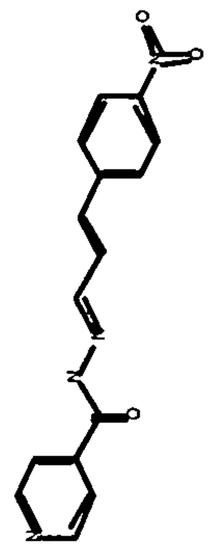
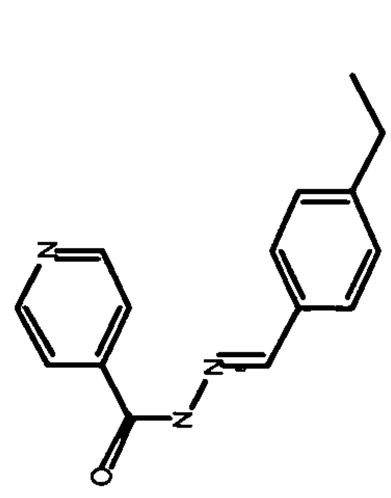
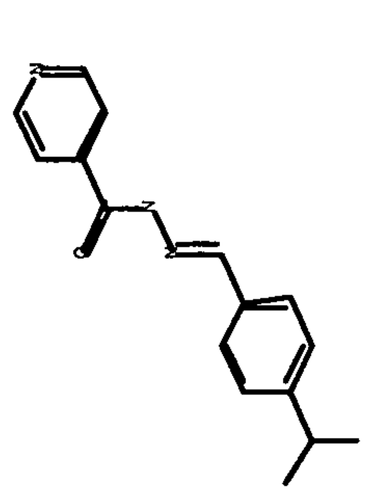
IPK 00043 30		305.0	503.3	575.8	251.1	107.2	102.5	100.8	-5.6	98.0	97.9	69.7	32.8
IPK 00043 31		334.0	442.5	526.9	321.1	94.6	100.4	101.5	43.8	98.7	52.7	69.8	24.0
IPK 00043 32		164.0	452.4	425.4	481.6	60.5	102.5	94.5	72.0	98.9	99.1	78.1	37.0

Table 1

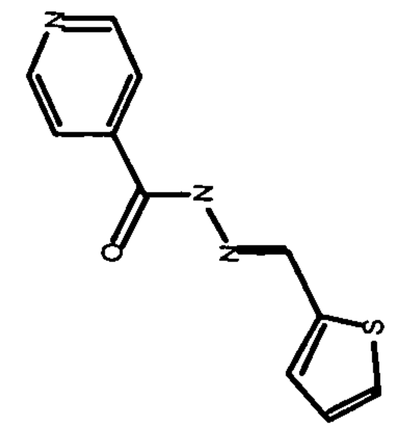
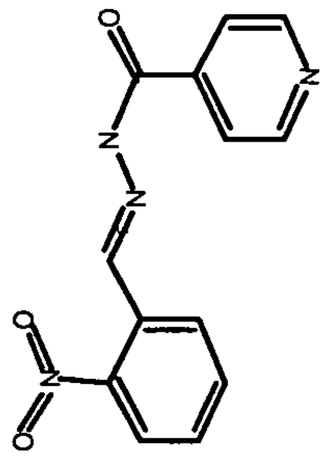
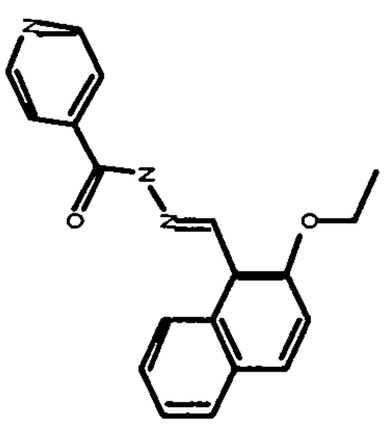
IPK 000 043 33		270.8	522.8	515.1	362.8	83.0	104.4	94.5	31.3	98.4	98.8	59.3	34.8						
IPK 000 043 35		245.5	461.5	484.3	335.4	104.0	100.9	94.4	31.6	96.1	67.1	75.7	39.9						
IPK 000 043 62		393.8	523.9	561.9	502.1	98.2	104.5	98.8	63.7	99.4	98.3	71.0	42.9						

Table 1

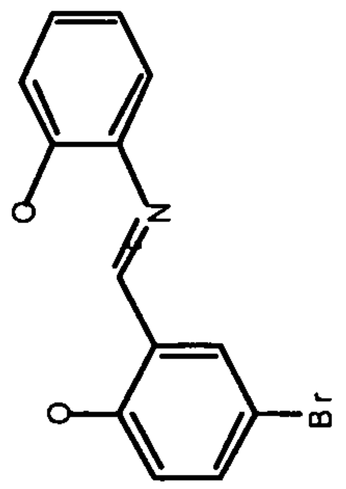
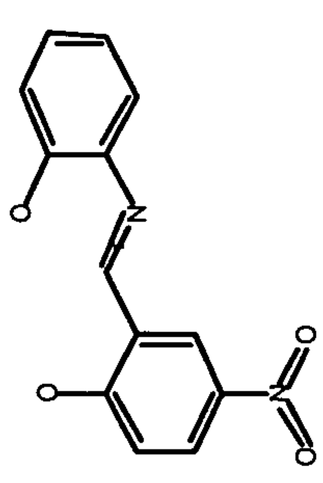
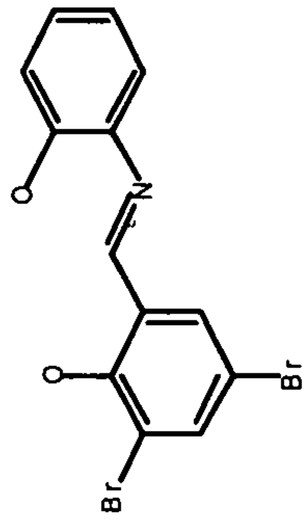
IPK 000 043 83		89.0	252.3	303.8	296.4	-80.9	31.3	17.1	22.6	99.7	99.2	50.6	34.2	1.1	1.1
IPK 000 044 20		135.0	219.0	278.5	303.0	-67.5	30.7	6.3	24.5	99.6	98.5	52.4	40.3	1.1	1.1
IPK 000 044 41		126.8	307.0	377.5	260.0	-120.3	29.9	34.0	18.0	98.6	84.0	41.4	37.1	1.1	1.1

Table 1

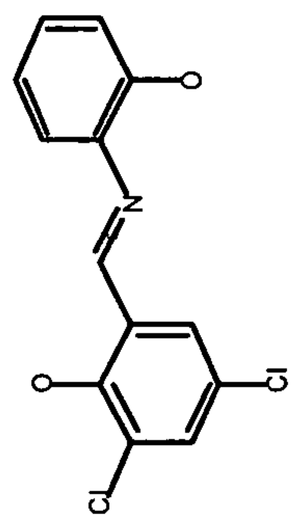
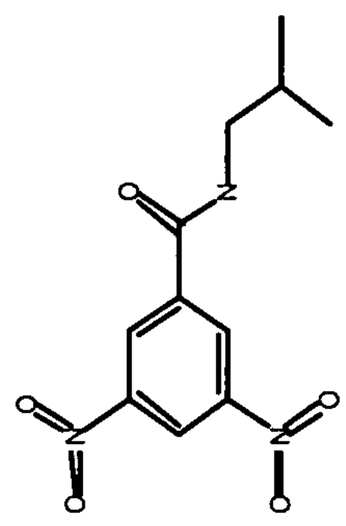
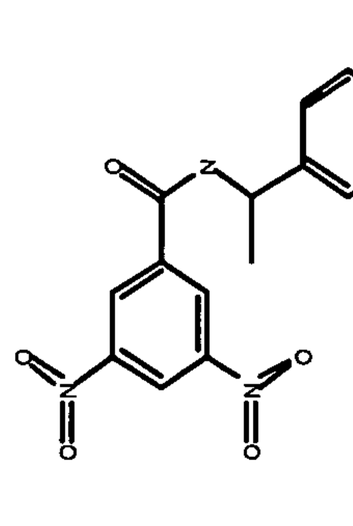
IPK 000 045 01		145.0 273.8 328.5 305.8 -9.3 57.6 66.8 99.3 19.3 24.3 30.6	193.3 284.4 388.9 335.3 73.3 65.2 99.1 100.8 13.9 36.5 88.9
IPK 000 046 78		238.8 389.1 277.8 353.3 76.9 41.8 73.5 99.2 38.6 3.1 64.6	36.5 44.4
IPK 000 046 80			

Table 1

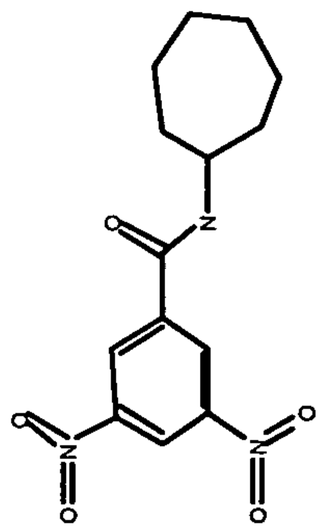
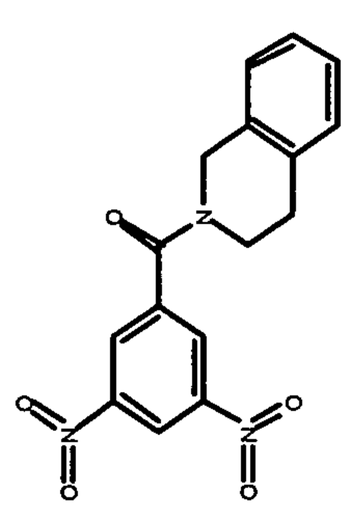
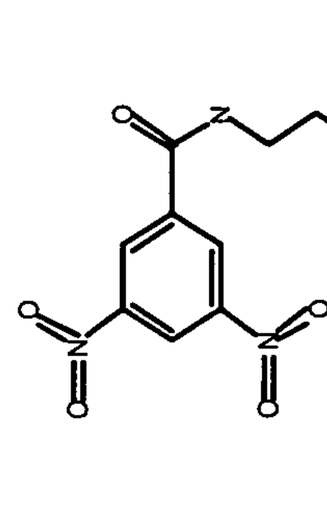
IPK 00046 83		204.0	379.6	437.5	355.0	75.8	61.7	91.7	37.2	100.1	69.6	97.1	44.3
IPK 00046 86		129.3	285.6	305.0	300.1	26.6	40.8	15.0	17.3	100.2	70.3	41.2	38.3
IPK 00046 87		239.0	343.3	411.1	328.6	74.4	56.3	61.3	14.4	99.2	62.5	70.4	44.2

Table 1

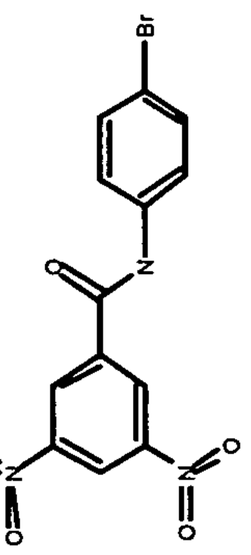
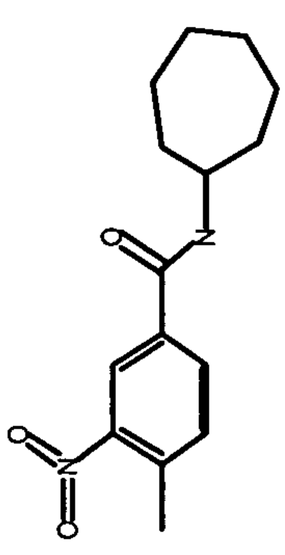
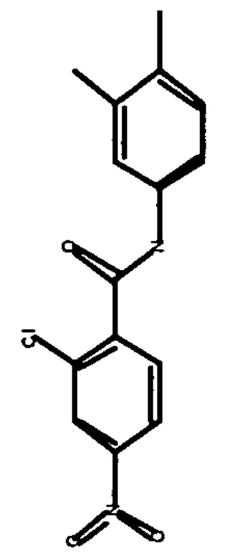
IPK 000 046 92		303.5	309.6	325.4	304.1	90.2	93.4	8.1	31.2	17.0	53.1	38.4	42.2
IPK 000 047 06		290.8	440.0	365.8	300.0	57.3	69.9	17.6	35.0	97.4	76.4	41.9	41.1
IPK 000 047 15		281.8	432.3	482.3	298.0	69.7	85.8	61.1	13.3	99.3	97.7	36.4	36.8

Table 1

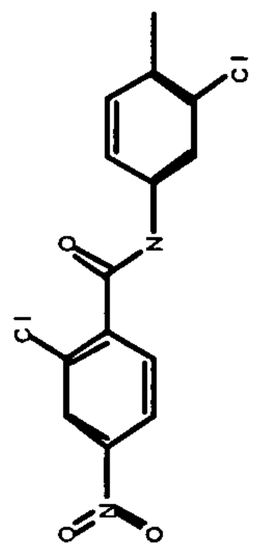
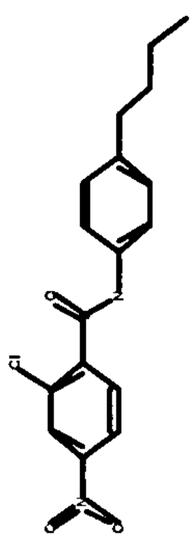
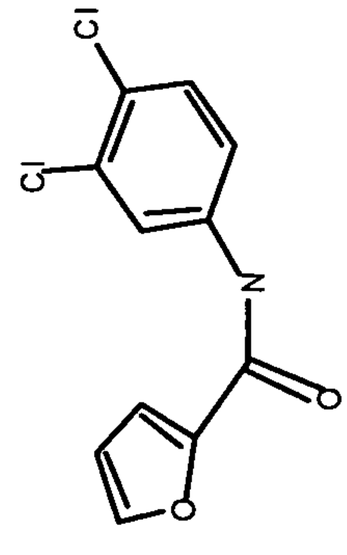
IPK 000 047 16		280.5	309.1	549.8	297.5	69.4	85.1	77.9	6.0	100.4	98.3	39.0	32.0
IPK 000 047 17		152.3	196.3	536.1	328.1	57.6	88.5	69.7	46.5	99.3	98.6	36.5	37.7
IPK 000 048 49		253.8	281.3	457.3	354.8	84.5	92.9	41.7	25.8	32.6	57.7	34.3	40.3

Table 1

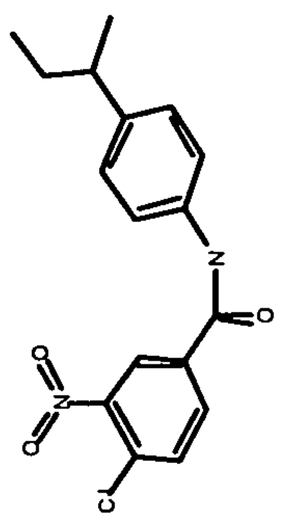
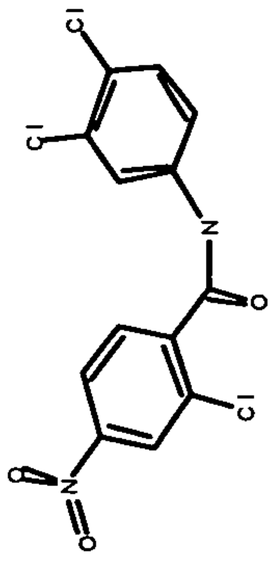
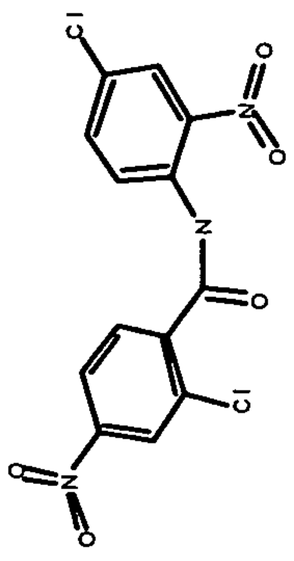
IPK 00048 71		56.5	98.1	331.5	255.1	62.1	70.0	22.6	4.5	85.2	72.7	44.3	39.8
IPK 00048 99		217.8	211.4	494.5	311.4	76.8	73.5	40.3	8.6	84.7	72.0	36.4	43.6
IPK 00049 00		108.0	212.4	503.6	349.5	32.1	74.2	72.2	24.4	86.2	73.9	38.7	48.1

Table 1

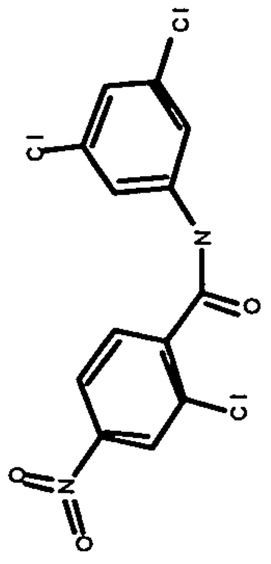
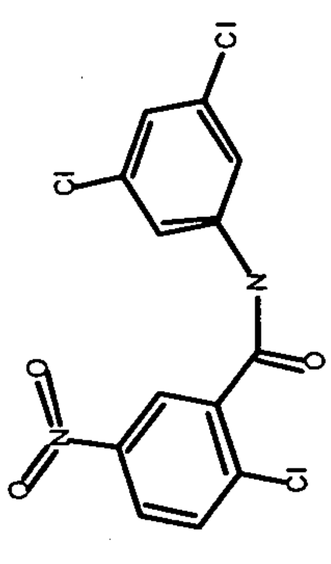
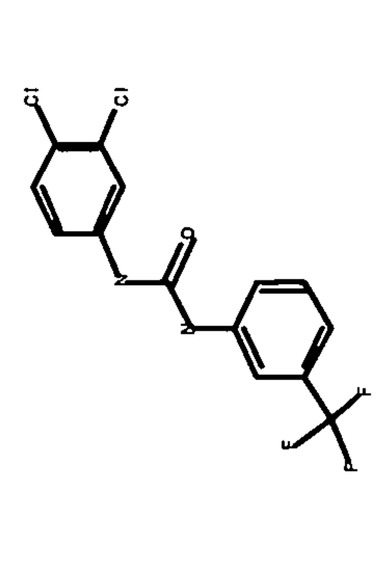
IPK 000 049 03		205.3	155.5
		481.3	157.0
		525.4	405.8
		370.8	296.1
		62.6	71.5
		48.6	88.6
		56.0	54.8
		23.4	36.9
		94.5	28.3
		69.1	27.0
		43.7	31.5
		41.3	36.0
IPK 000 049 20		44.0	44.0
		117.6	117.6
		289.6	289.6
		339.1	339.1
		76.4	45.0
		10.8	10.8
		33.8	33.8
		101.0	101.0
		70.4	70.4
		28.8	28.8
		30.9	30.9
IPK 000 052 50		339.1	339.1

Table 1

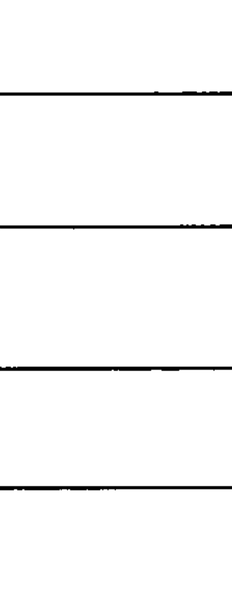
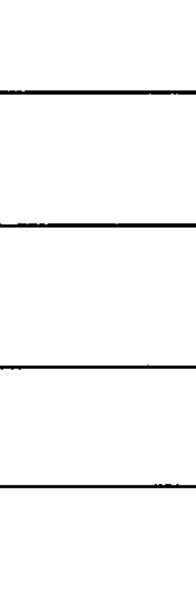
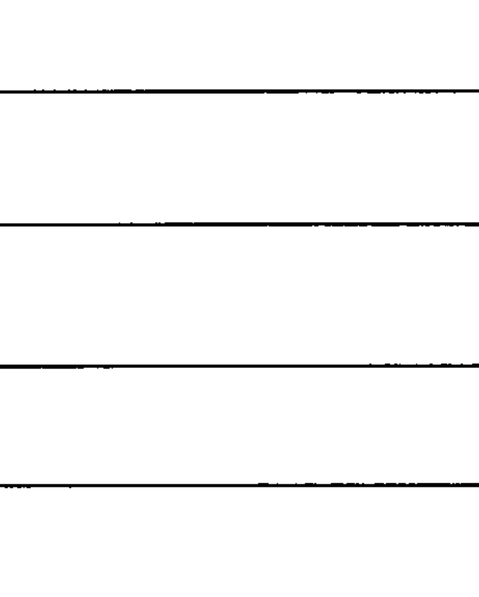
IPK 000 052 75		48.8	290.3	265.9	256.8	-77.4	10.1	13.3	14.7	85.6	66.9	43.8	43.3
IPK 000 057 78		177.0	292.4	203.8	252.1	-10.9	35.5	12.6	4.5	93.9	86.2	39.0	43.7
IPK 000 057 92		165.3	197.4	225.8	237.3	-30.7	27.2	23.0	26.2	89.8	81.6	29.1	33.5

Table 1

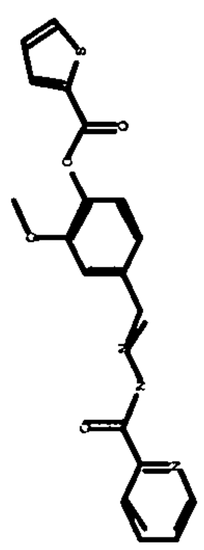
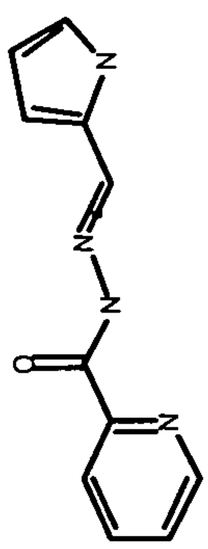
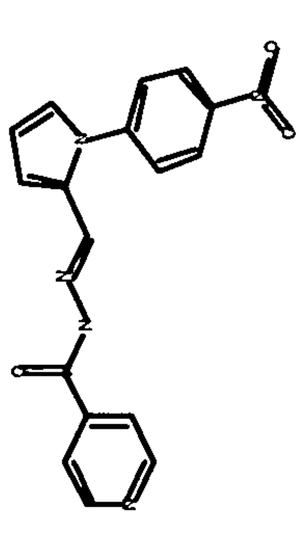
IPK 000 058 20		344.8	278.0	458.8	295.1	98.1	44.3	66.6	13.3	55.8	68.0	57.9	42.5
IPK 000 058 21		452.5	453.1	525.3	341.6	90.9	56.0	75.7	24.8	52.3	41.2	54.6	47.6
IPK 000 058 29		75.5	224.8	432.0	499.8	63.0	31.5	103.7	77.6	99.2	47.5	93.4	52.2

Table 1

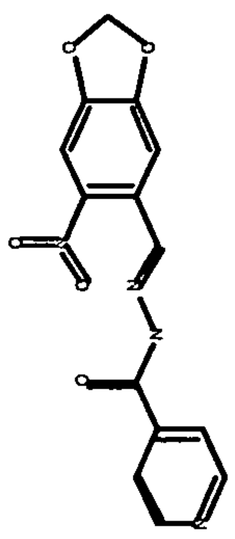
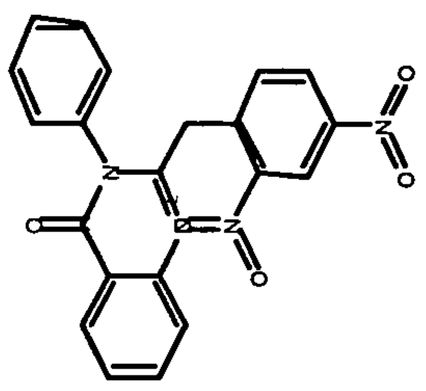
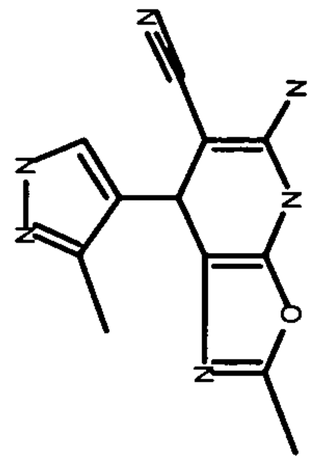
IPK 000 058 30	315.8 188.3 435.3 183.6 483.4 232.5 325.6 327.4 95.5 49.6 70.8 68.7 98.3 16.7 21.0 17.4 84.5 96.2 48.8 74.8 72.8 38.1 47.0 37.1		197.8 172.1 227.0 299.3 68.2 73.2 1.1 -16.3 14.7 35.2 38.1 29.1
IPK 000 063 24	188.3 183.6 232.5 327.4 49.6 68.7 16.7 96.2 17.4 74.8 38.1 37.1		
IPK 000 065 03			

Table 1

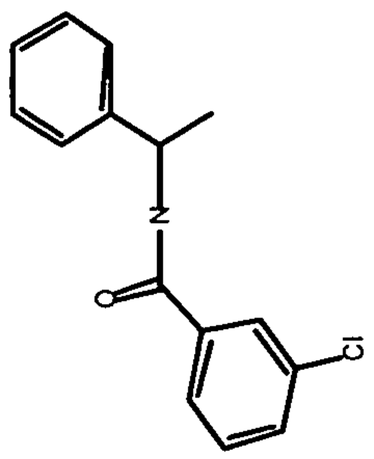
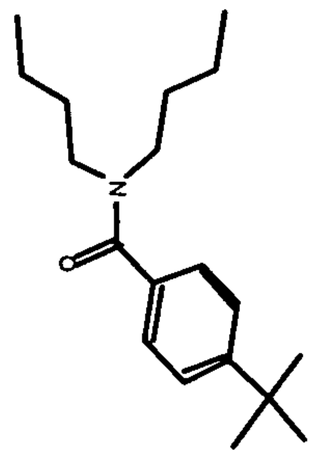
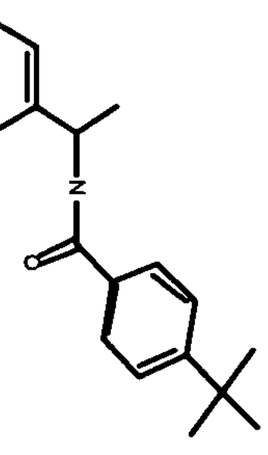
IPK 000067 51		142.3	164.5	301.5	367.1	75.2	73.2	17.9	11.0	37.4	59.5	39.9	39.0
IPK 000067 60		164.3	278.3	179.1	326.4	63.4	46.6	25.8	17.7	99.7	65.3	46.4	40.8
IPK 000067 61		182.0	478.4	340.5	305.0	84.3	68.5	29.2	14.3	99.3	70.6	44.5	39.6

Table 1

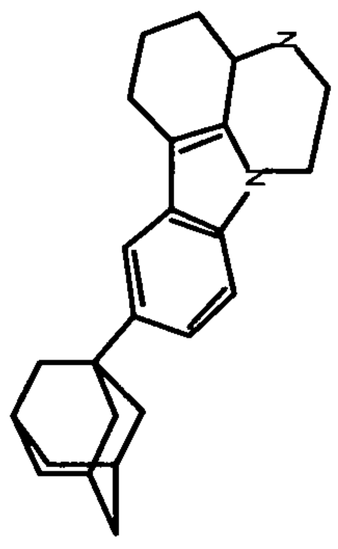
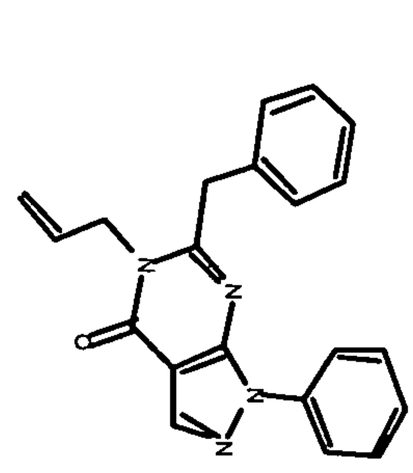
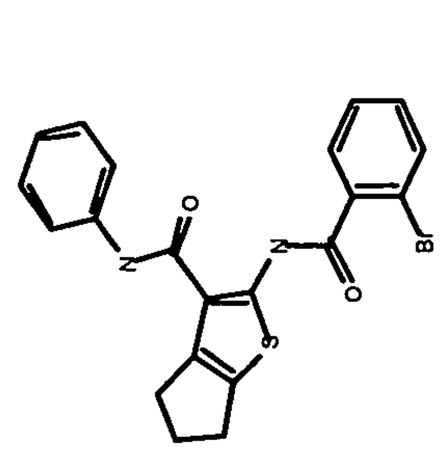
IPK 000 068 87		156.8	183.6	221.4	295.9	73.1	71.4	-12.0	-0.6	92.9	79.3	31.0	38.1	
IPK 000 073 11		147.8	224.4	168.9	256.1	49.1	56.1	-10.5	6.4	84.0	67.2	33.5	42.2	
IPK 000 073 29		230.5	193.1	551.9	315.5	78.6	86.6	55.1	1.3	94.1	74.9	73.9	50.0	

Table 1

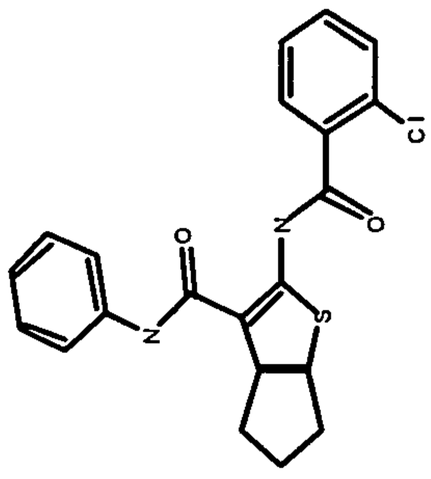
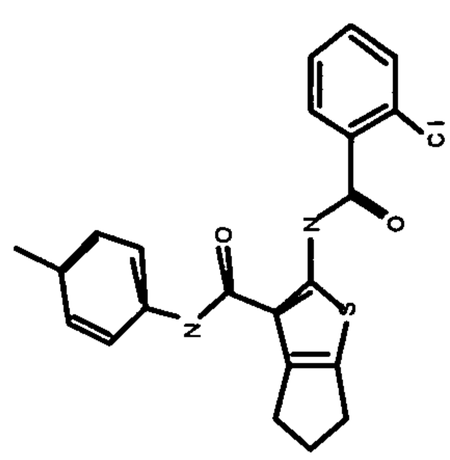
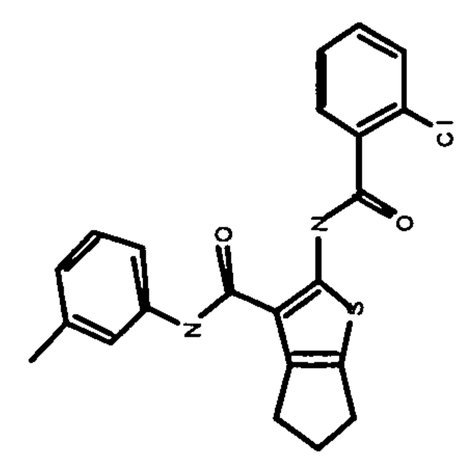
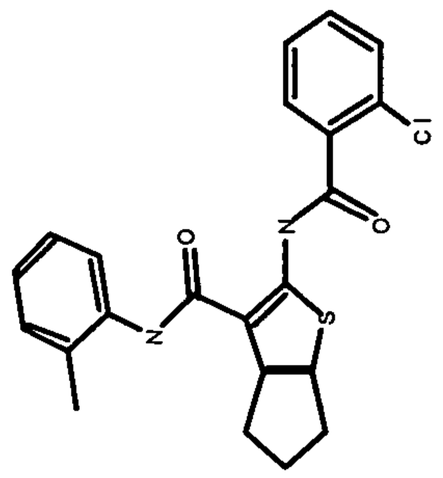
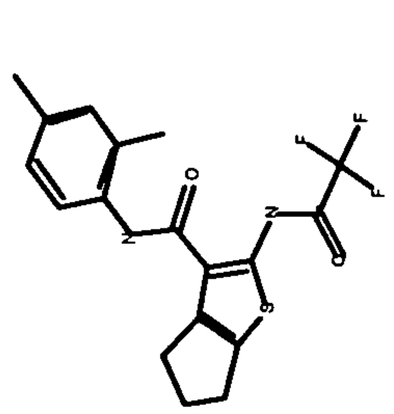
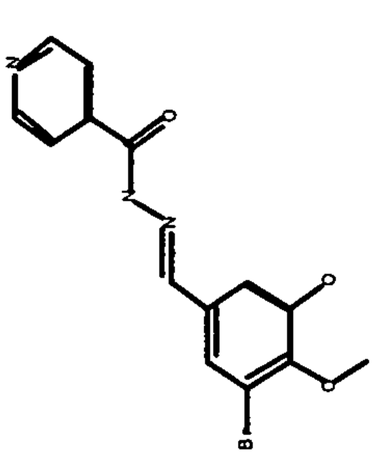
IPK 000 073 68		202.3	242.6	557.3	378.6	88.2	92.6	74.7	16.9	94.9	73.4	69.2	53.5
IPK 000 073 69		246.5	465.9	375.5	335.9	87.0	32.3	14.0	21.8	80.9	72.9	44.3	37.2
IPK 000 073 70		234.0	539.8	523.0	348.3	88.6	103.0	56.7	17.8	96.3	74.9	68.1	49.8

Table 1

IPK 000 073 71		218.3	534.3	467.8	244.5	87.5	65.2	29.8	-4.5	8.3	45.3	59.6	44.5
IPK 000 077 22		84.5	253.9	380.5	314.1	-33.0	46.6	33.3	35.6	95.4	68.6	45.7	45.0
IPK 000 078 30		388.3	540.5	495.6	250.6	108.5	105.0	97.0	8.2	96.6	69.7	62.0	39.2

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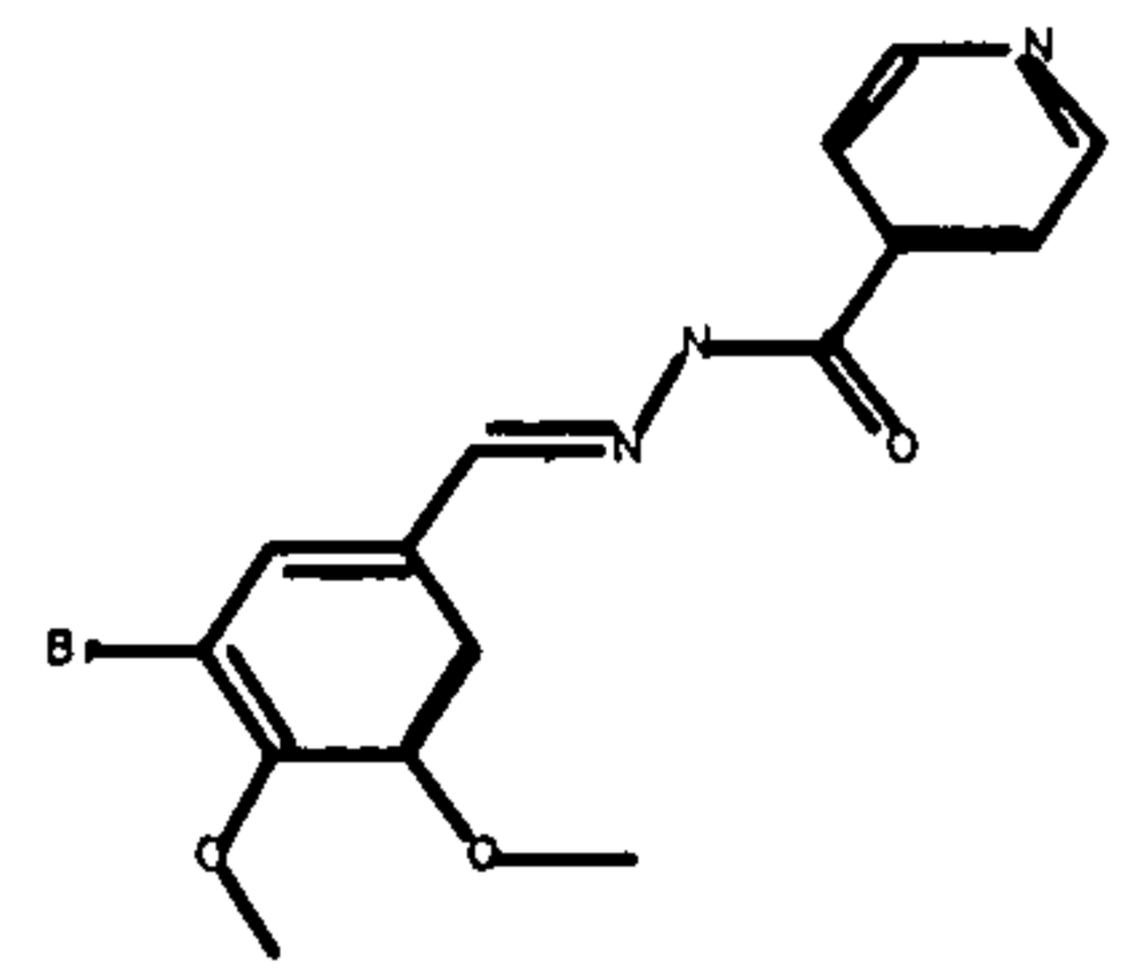
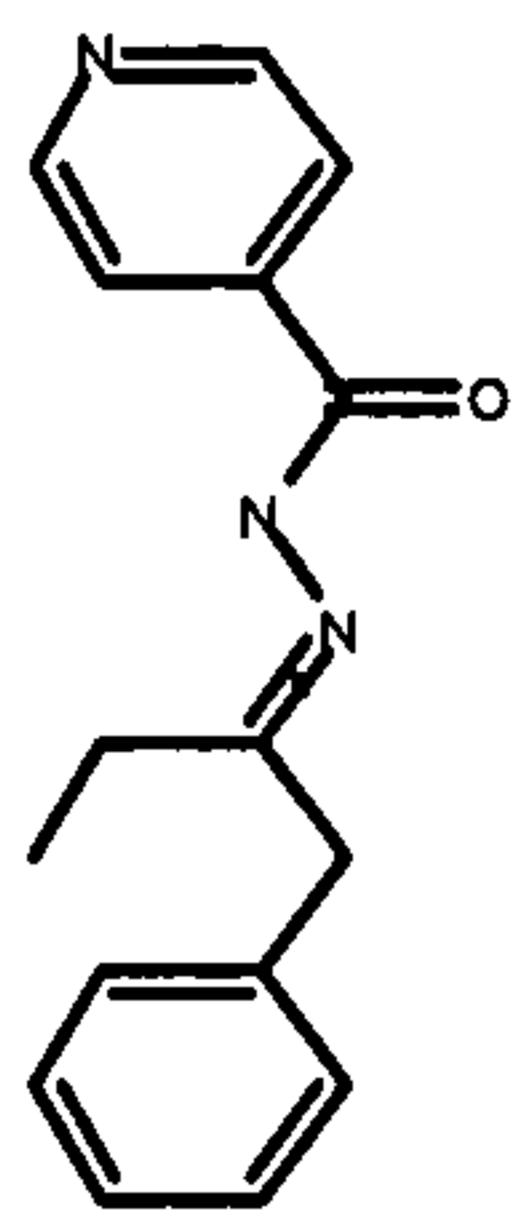
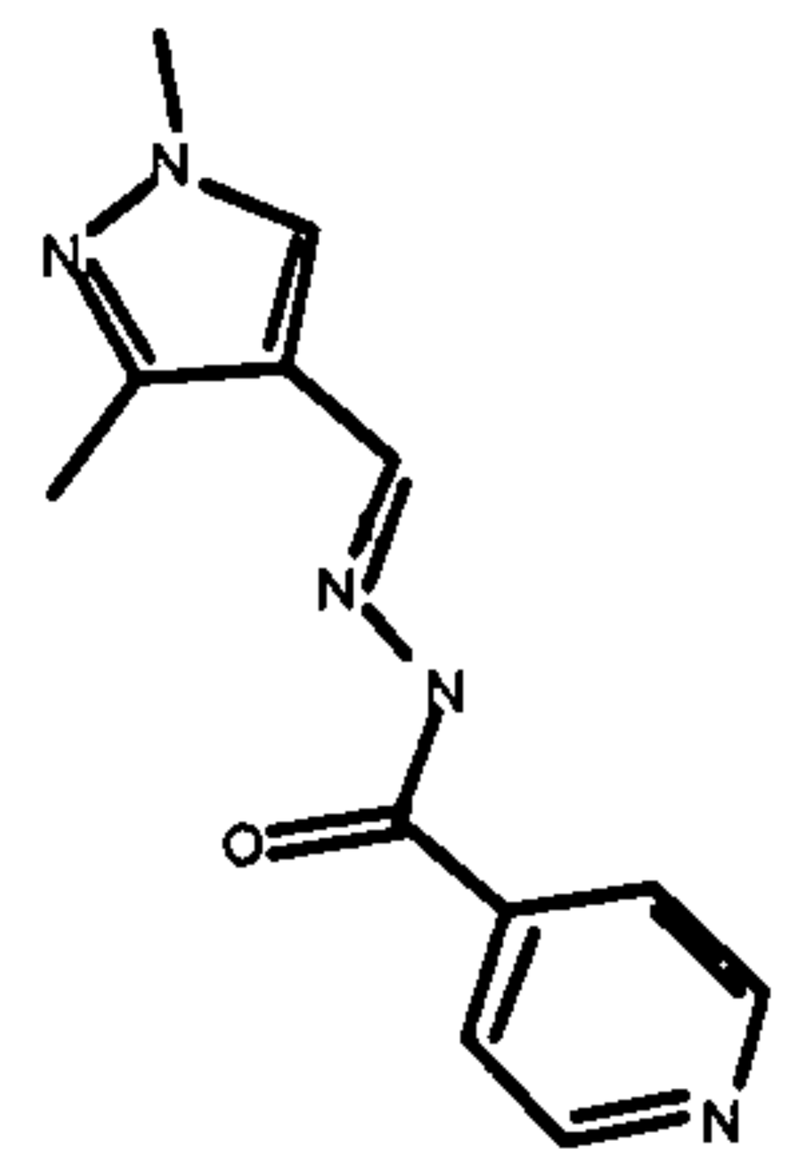
IPK 000 078 53		386.0	523.3	489.9	298.6	84.0	106.6	95.6	17.6	98.3	68.4	73.8	39.3
IPK 000 078 86		462.0	583.0	531.4	373.4	96.9	107.5	88.3	32.7	98.5	68.0	74.5	37.9
IPK 000 079 13		294.0	528.5	493.6	217.9	69.6	101.6	97.8	8.4	97.0	97.8	68.5	36.6

Table 1

Table 1

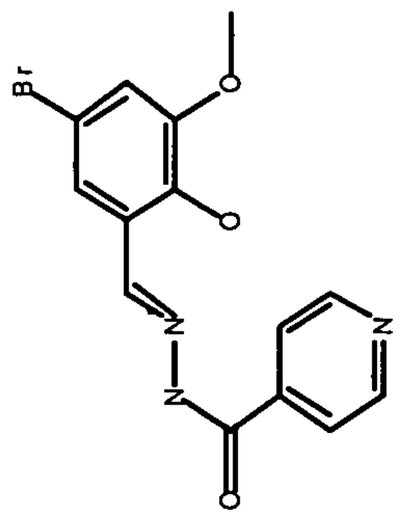
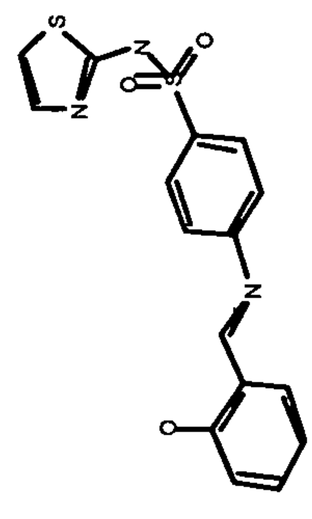
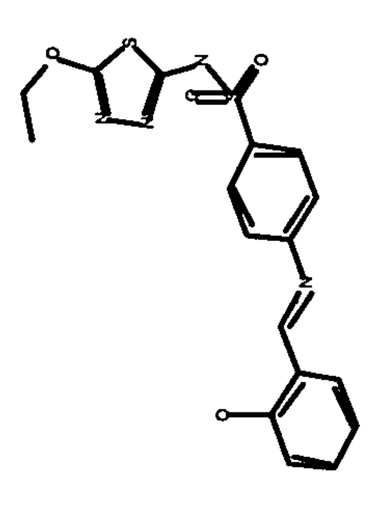
IPK 000 079 15		383.3 470.9 477.4 274.6 82.4 97.9 90.5 13.4 97.1 98.6 72.6 41.5	181.8 368.8 289.8 254.3 -69.2 28.1 7.5 0.9 92.2 88.3 34.9 39.3
IPK 000 079 88		409.8 469.1 387.1 360.5 14.3 56.6 27.4 20.1 96.1 70.4 47.4 39.5	
IPK 000 080 01			

Table 1

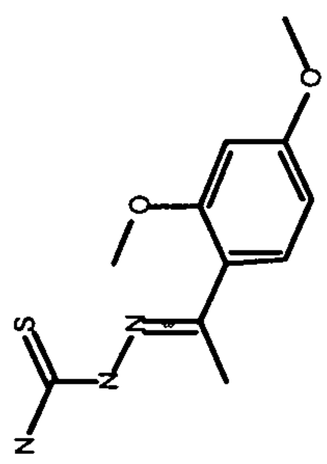
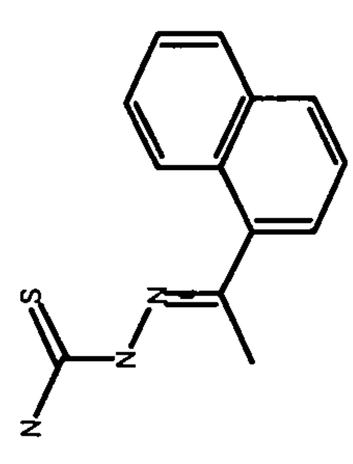
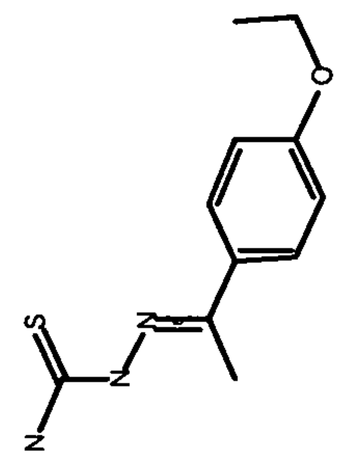
IPK 000 080 24		188.8	321.0	492.1	323.8	0.2	-91.1	23.0	67.6	13.0	99.5	73.0	62.4	45.3
IPK 000 080 36		79.8	251.4	559.6	514.4	-91.1	26.0	75.8	70.0	98.9	71.9	68.7	59.7
IPK 000 080 37		110.8	393.6	491.4	377.0	-44.0	36.0	62.1	55.4	99.6	78.6	80.6	63.1

Table 1

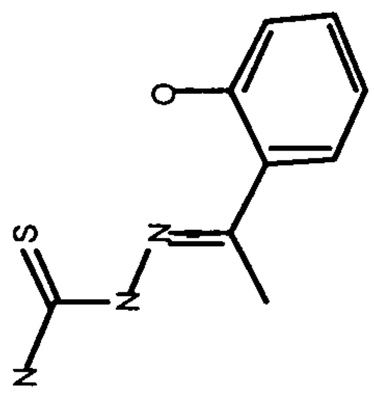
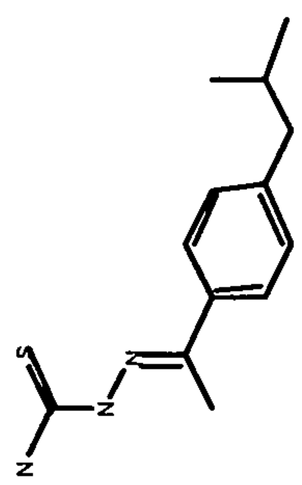
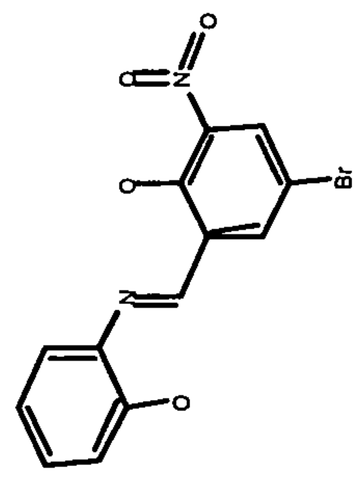
IPK 000 080 38		307.5	371.6	337.3	264.9	21.0	3.4	32.1	13.3	98.2	73.8	58.7	52.3
IPK 000 080 39		99.0	377.1	591.3	418.3	38.3	9.2	73.0	51.8	96.3	68.2	66.6	54.7
IPK 000 080 69		123.5	328.0	358.9	289.4	-42.5	16.5	37.6	27.5	99.1	71.8	47.6	46.9

Table 1

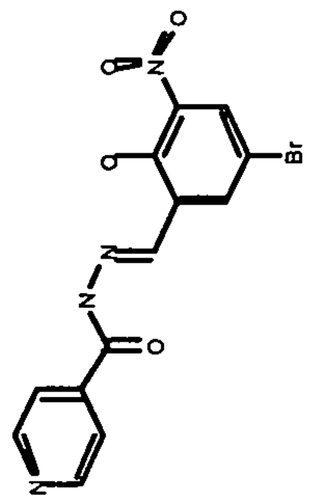
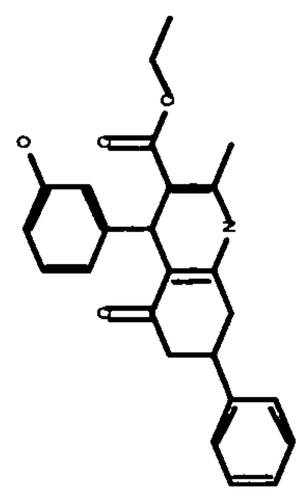
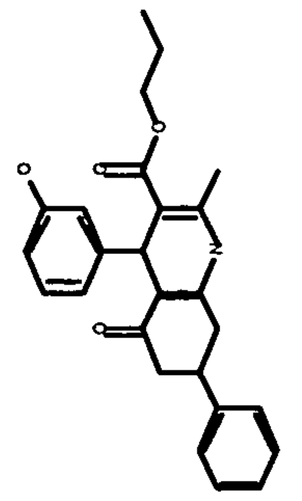
IPK 000 080 81		363.3	482.0	559.0	405.8	94.7	99.5	104.9	66.3	55.0	69.5	48.4	50.8
IPK 000 083 89		38.8	268.3	255.5	217.4	73.5	59.1	19.5	2.6	98.7	72.9	48.1	46.3
IPK 000 085 99		67.3	261.3	322.0	267.5	37.1	49.5	4.5	2.2	84.3	72.0	47.7	49.5

Table 1

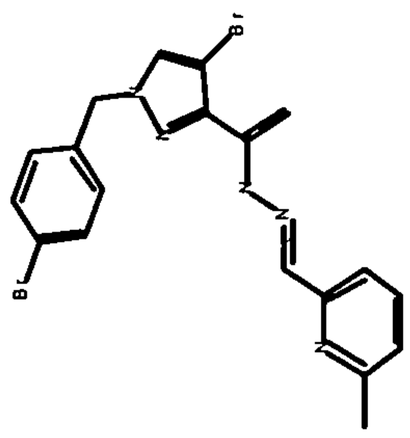
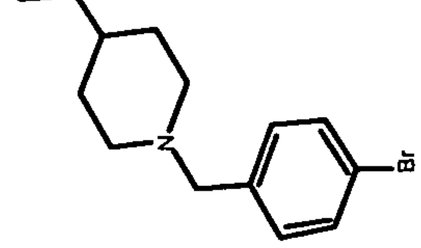
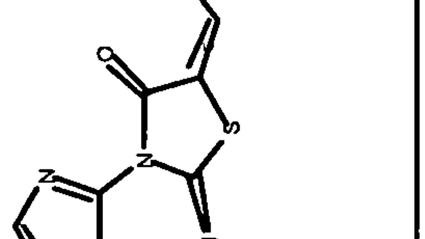
IPK 000 091 17		191.5	42.6
		110.5	37.3
		126.3	41.3
IPK 000 091 49		276.8	38.8
		228.6	33.4
		367.8	54.6
IPK 000 094 38		254.8	22.4
		265.4	65.5
		286.9	68.6
		319.9	-11.9
		388.5	94.1
		271.6	97.2
		75.6	50.6
		90.5	30.6
		46.0	-9.3
		75.7	35.4
		95.1	9.3
		6.0	13.6

Table 1

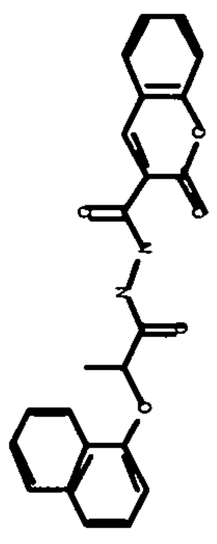
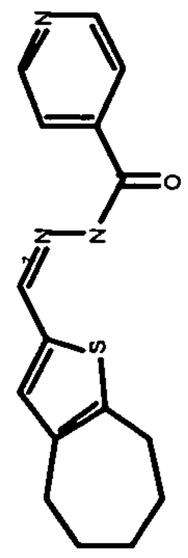
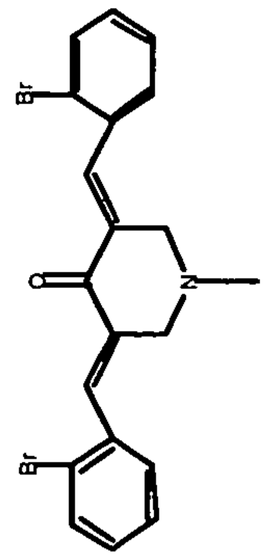
IPK 000 095 07		388.3	551.8	348.6	377.4	70.9	75.8	8.0	19.2	-3.7	39.6	46.1	45.0
IPK 000 102 07		116.0	124.8	516.3	476.1	94.7	84.7	99.5	79.6	99.2	100.1	84.3	77.4
IPK 000 102 36		193.3	194.8	224.0	291.8	65.6	66.5	4.4	13.2	-1.6	16.8	32.7	36.3

Table 1

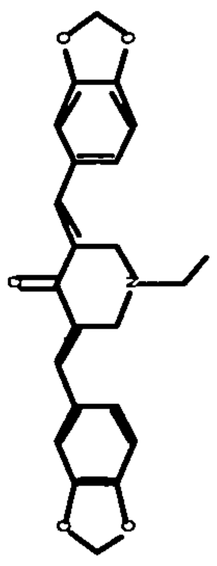
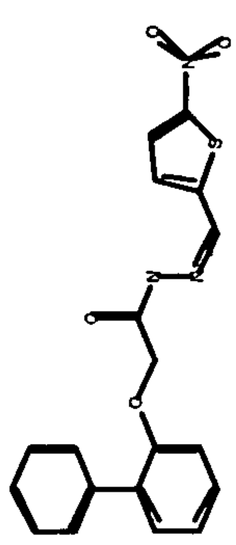
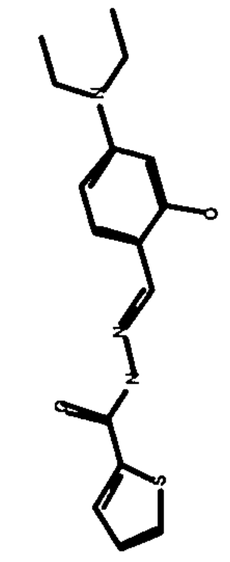
IPK 000 102 52		179.8 175.9 145.9 219.5 70.4 68.1 -7.0 12.6 18.3 19.1 40.8 36.7	134.3 164.6 419.5 263.8 71.1 71.3 49.5 11.6 5.0 48.2 47.6 39.8
IPK 000 103 28		134.3 164.6 419.5 263.8 71.1 71.3 49.5 11.6 5.0 48.2 47.6 39.8	47.8 122.4 109.5 143.1 85.7 74.5 -1.7 -13.2 45.6 50.2 50.0 39.0
IPK 000 103 76		47.8 122.4 109.5 143.1 85.7 74.5 -1.7 -13.2 45.6 50.2 50.0 39.0	

Table 1

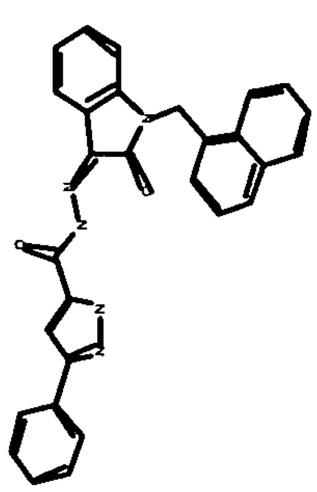
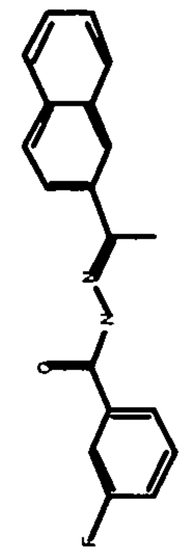
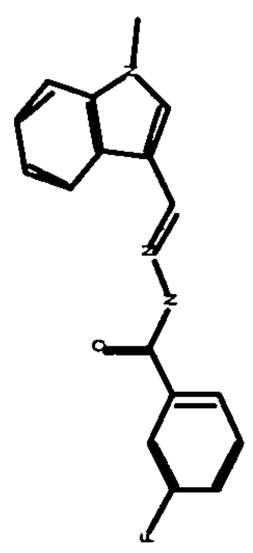
IPK 000 103 78		263.5	409.1	244.8	236.5	94.6	98.0	3.5	-3.9	90.8	94.0	34.8	31.6			
IPK 000 104 07		243.5	222.5	575.9	330.4	82.3	80.2	65.1	37.5	35.1	58.0	45.1	42.5			
IPK 000 104 11		203.8	370.3	278.4	240.4	89.7	83.3	19.9	10.2	36.1	52.8	42.4	26.9			

Table 1

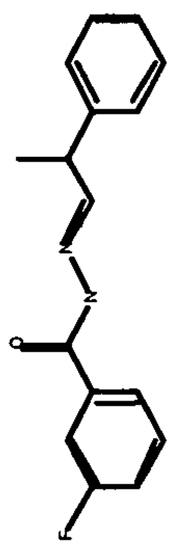
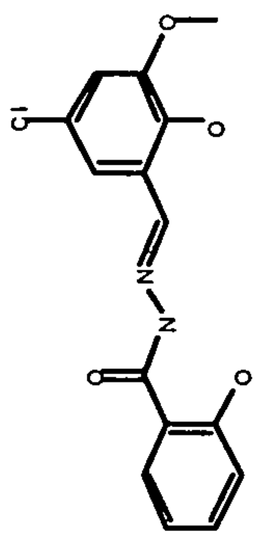
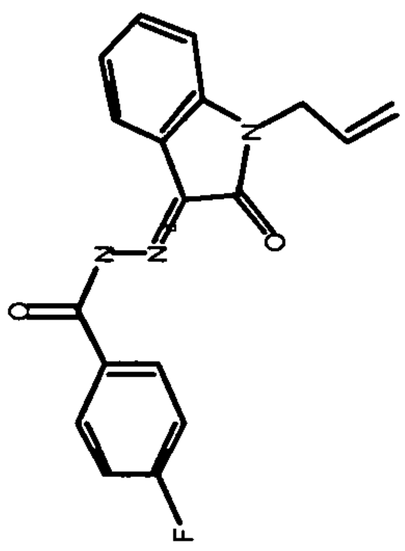
IPK 000 104 13		115.8	446.5	313.3	260.6	68.8	89.6	25.2	10.1	3.7	37.9	29.0	44.7
IPK 000 104 20		29.5	81.9	153.1	230.0	65.2	34.6	-2.9	5.3	67.1	90.6	56.2	40.4
IPK 000 104 67		183.0	211.4	332.9	283.1	33.8	73.7	16.1	11.9	67.7	86.2	34.2	36.4

Table 1

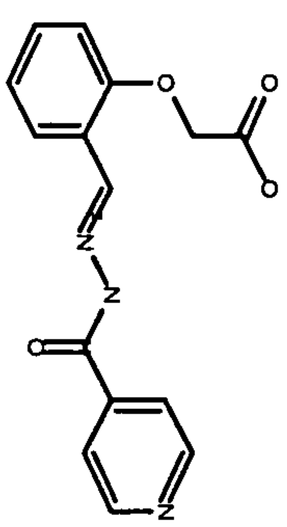
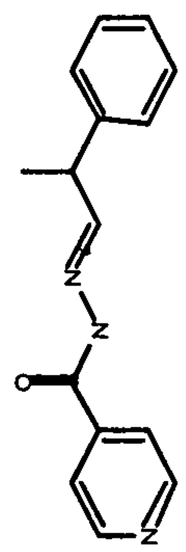
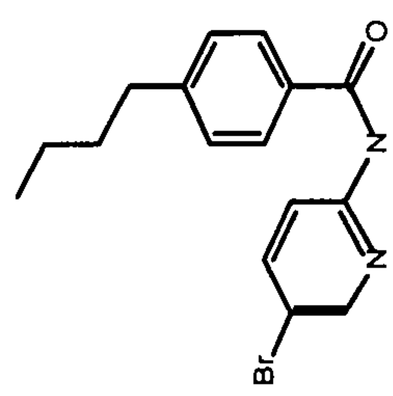
IPK 000 105 19		374.3 425.6 566.4 521.1 98.7 105.8 104.1 88.5 98.7 66.4 93.6 70.3	316.5 397.9 547.6 482.3 97.1 96.4 103.8 83.8 99.6 66.0 96.0 66.6
IPK 000 105 20		125.8 172.4 468.0 270.4 60.1 50.7 68.1 13.0 75.5 95.5 54.7 47.3	
IPK 000 105 47			

Table 1

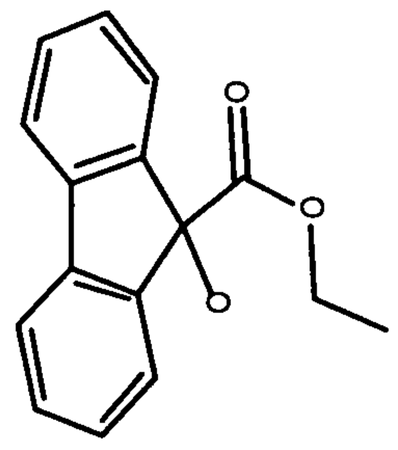
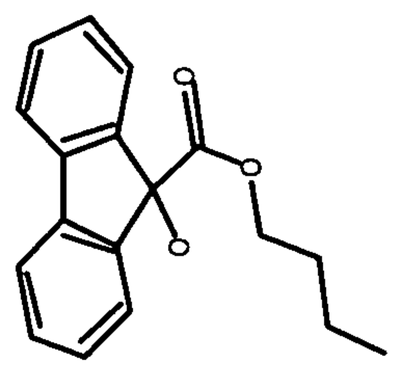
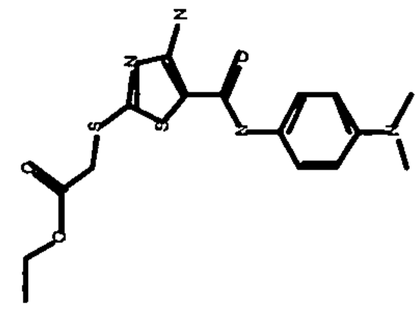
18.8	36.4	41.8
35.1	39.5	53.8
62.2	68.0	80.7
29.2	36.1	70.7
13.0	28.0	16.8
55.5	79.9	4.7
91.5	86.9	84.6
89.3	65.7	61.2
272.8	371.5	237.8
402.4	442.0	226.4
538.8	169.6	573.6
181.3	225.0	315.5
		
IPK 000 105 55	IPK 000 105 56	IPK 000 105 70

Table 1

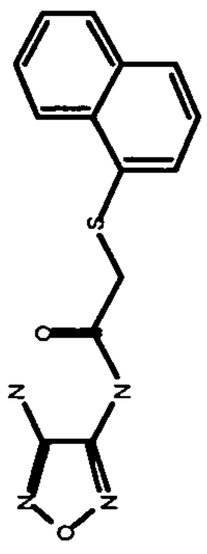
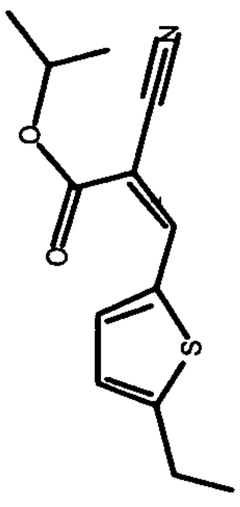
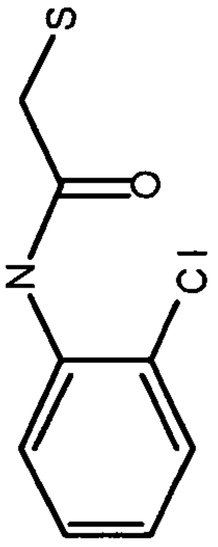
IPK 000 106 30		146.5 171.3 409.1 233.5 65.7 67.2 28.5 9.3 19.1 61.2 36.4 17.1	
IPK 000 107 90		494.3 577.5 498.0 339.3 79.3 89.6 52.8 23.7 7.0 42.6 39.6 37.4	
IPK 000 108 27		171.0 369.9 332.0 321.6 54.7 44.1 19.0 14.1 95.0 71.0 47.6 49.0	

Table 1

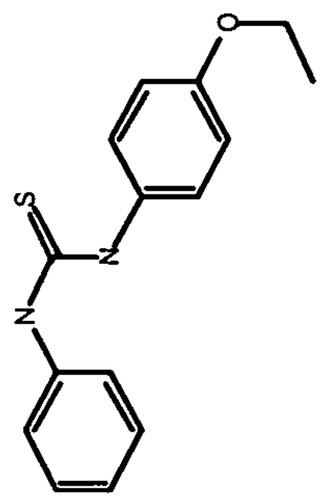
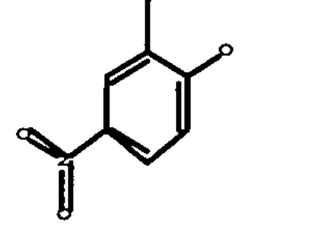
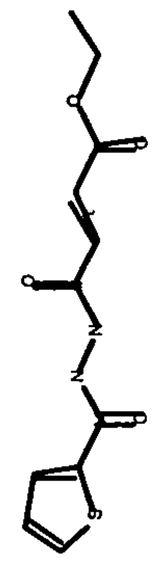
IPK 000 10878		200.3 287.6 400.0 339.8 78.8 68.8 38.3 28.2 20.9 43.1 39.2 38.4	87.8 171.8 348.5 312.4 -7.8 8.2 23.8 15.6 98.4 72.2 55.2 47.6
IPK 000 10900		183.0 170.0 174.3 332.1 8.5 28.1 20.3 22.6 101.3 99.5 90.5 29.6	
IPK 000 10999			

Table 1

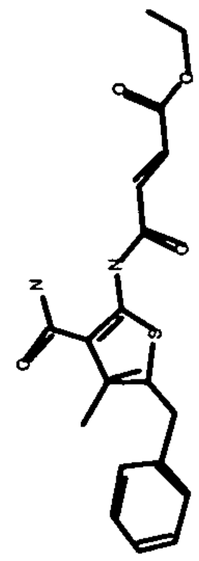
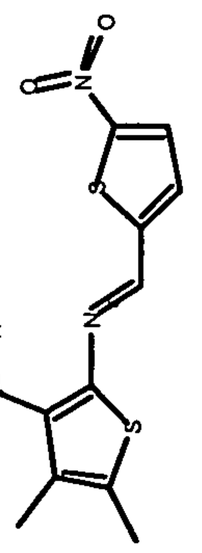
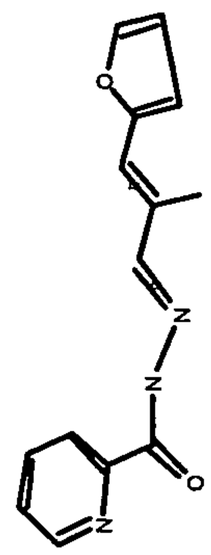
IPK 000 110 16		179.5 204.9 224.6 288.0 59.0 72.9 179.5 204.9 224.6 288.0 59.0 55.6 155.5 173.4 221.5 292.3 55.6 47.2 155.5 173.4 221.5 292.3 55.6 47.2 97.3 92.6 23.9 12.9 71.6 97.3 92.6 23.9 12.9 71.6 83.4 49.5 19.7 44.6 22.8 83.4 49.5 19.7 44.6 22.8 34.0 32.4 260.1 337.0 72.9 32.4 260.1 337.0 462.3 459.5 462.3 459.5 179.5 204.9 224.6 288.0 59.0 36.7 96.3 99.4 8.1 6.0 86.0 44.2	
IPK 000 110 17			
IPK 000 110 79			

Table 1

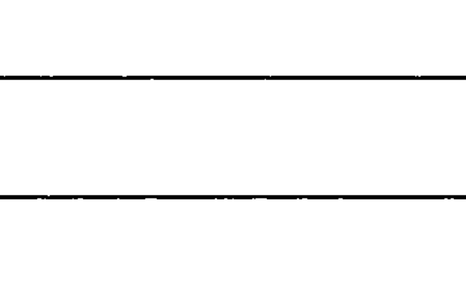
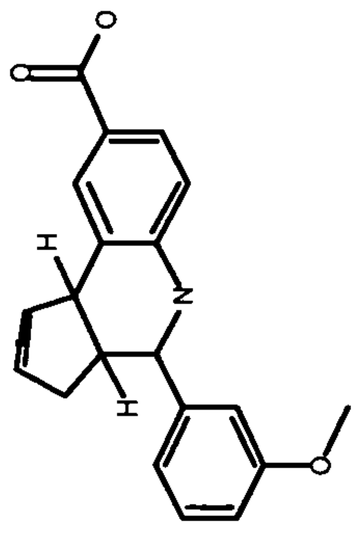
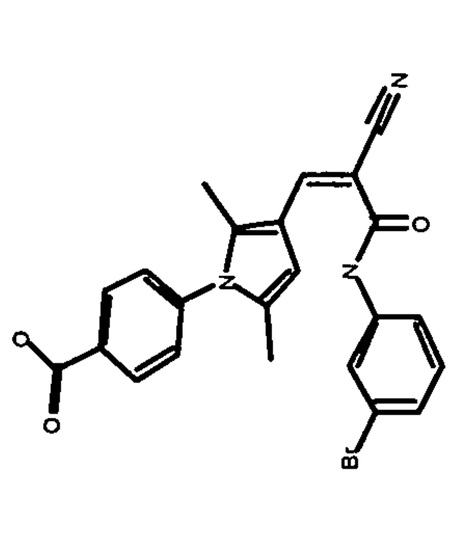
IPK 000 112 67		250.8	383.9	356.4	188.8	-36.5	32.6	27.6	8.2	91.3	68.9	59.6	43.8
IPK 000 112 80		152.0	183.0	189.0	279.9	41.1	55.6	10.7	7.7	98.3	72.1	32.5	35.9
IPK 000 113 05		162.0	107.9	309.0	264.5	55.4	56.4	31.6	10.0	74.9	67.9	45.3	41.6

Table 1

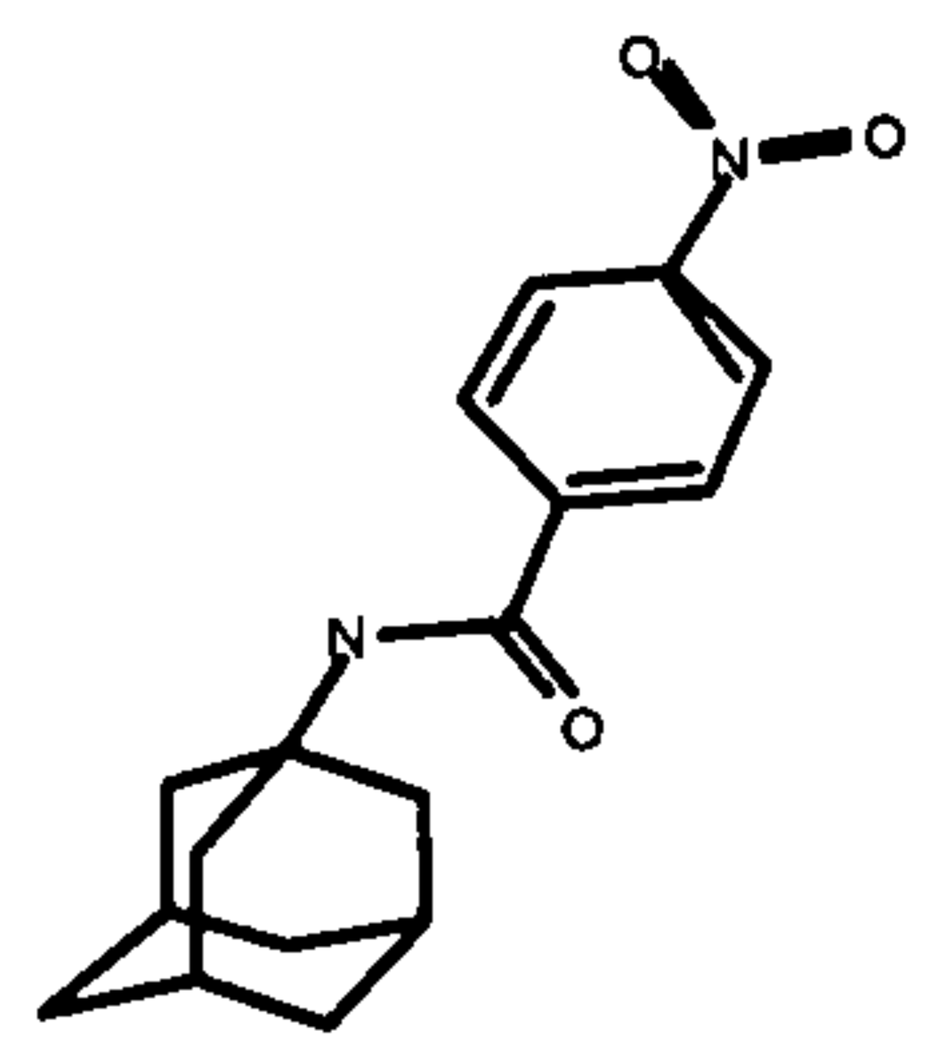
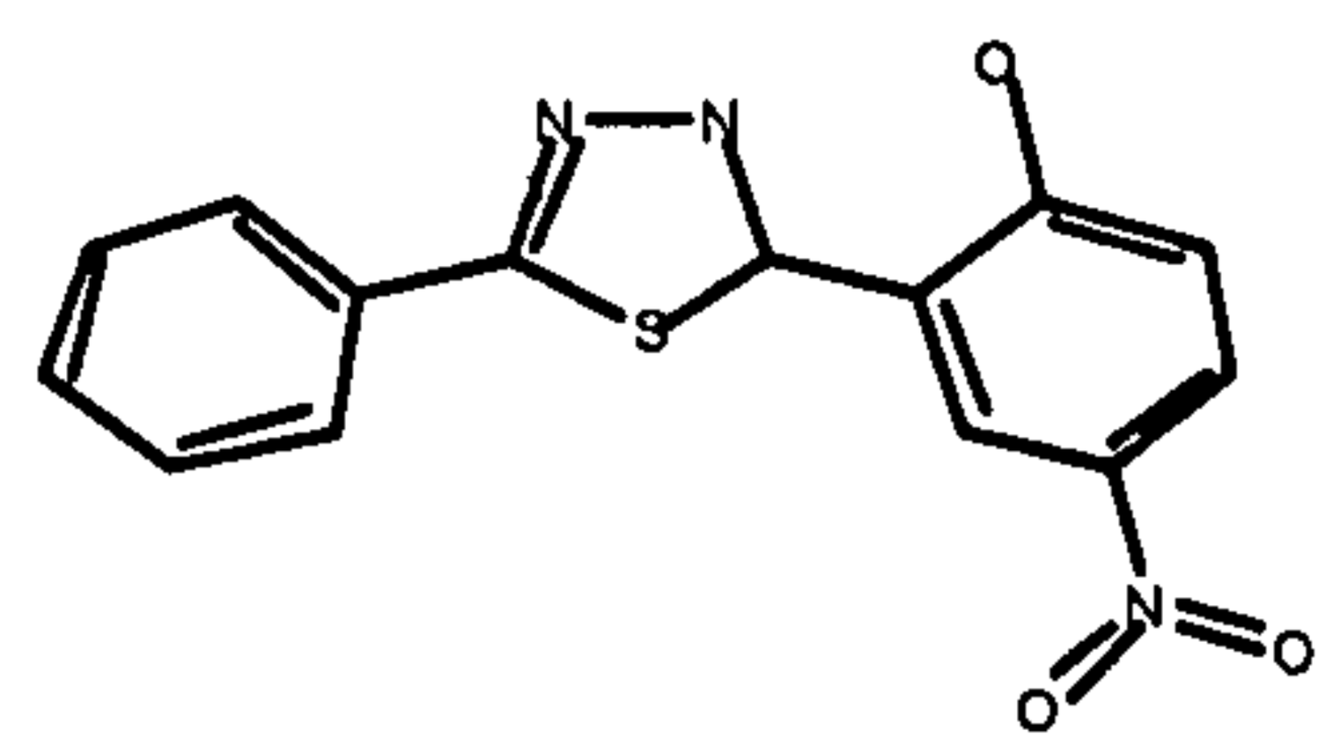
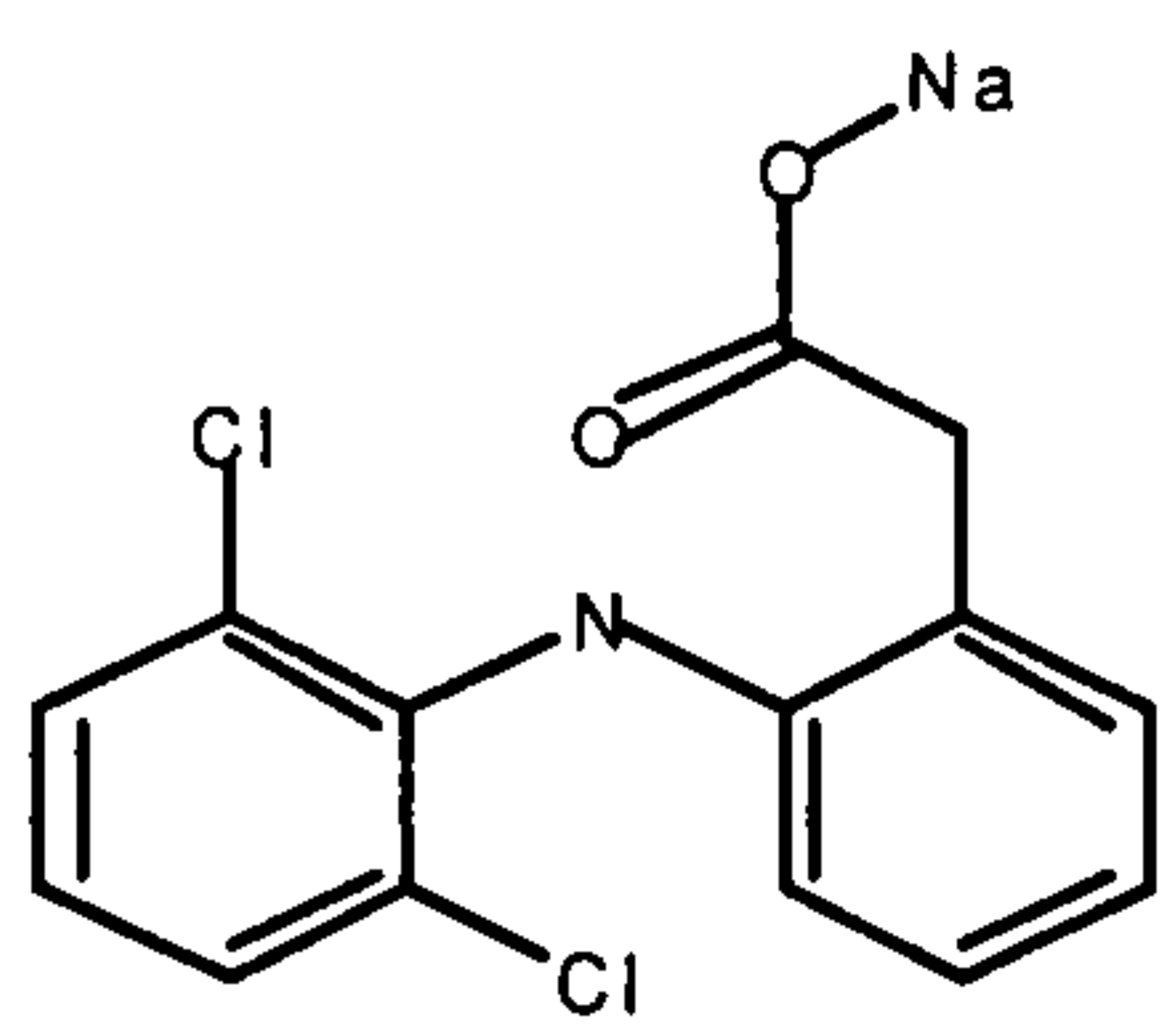
IPK 000 113 77		319.8	523.4	437.4	319.8	60.9	74.3	48.4	26.6	66.7	82.9	59.5	45.0
IPK 000 114 01		163.5	236.9	198.4	233.3	3.2	-1.5	7.3	18.3	73.9	71.1	51.4	53.6
IPK 000 117 05		656.5	578.0	540.9	503.5	79.8	80.5	45.4	49.9	12.9	46.3	44.5	39.6

Table 1

IPK 000117 14	546.0	548.6	349.0	329.5	69.1	66.5	15.6	11.2	100.8	98.6	82.8	33.9
	131.5	92.6	328.4	254.9	62.8	65.1	26.2	13.0	90.7	67.9	37.7	26.4
	411.8	347.8	427.0	270.5	76.8	78.2	45.3	24.6	23.9	32.0	38.2	42.1
IPK 000122 62												
IPK 000123 02												

Table 1

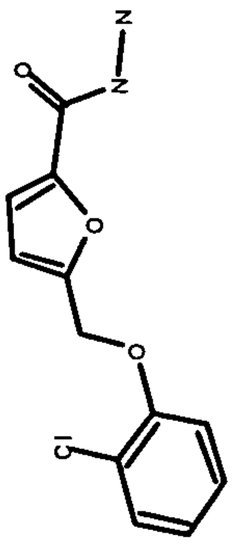
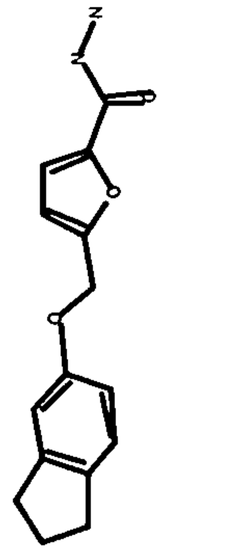
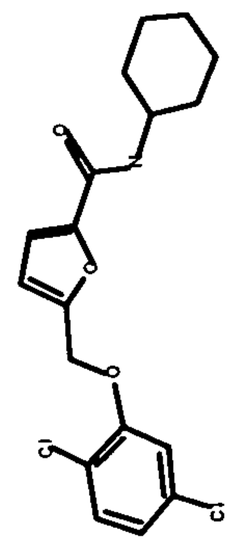
IPK 000 123 03		540.8	36.5
IPK 000 123 30		296.8	38.0
IPK 000 123 90		205.0	40.5
		467.6	35.8
		390.9	36.6
		226.5	43.2
		319.6	34.7
		495.6	37.0
		374.3	49.4
		234.1	21.2
		263.1	20.9
		230.8	35.1
		78.3	-8.6
		77.2	22.6
		73.1	-6.0
		72.1	12.0
		64.1	72.5
		87.7	26.7

Table 1

IPK 000 123 92	31.5 46.7 77.0 96.4 12.0 32.3 15.4 45.3 277.3 409.8 127.8 135.8	44.0 42.2 66.0 96.6 15.5 26.2 64.7 35.6 279.0 305.5 110.6 168.3
IPK 000 124 43	34.8 37.0 38.9 22.7 21.3 16.4 75.0 75.5 320.1 268.3 591.0 503.3	
IPK 000 124 54		

Table 1

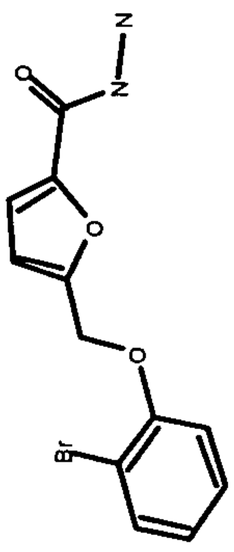
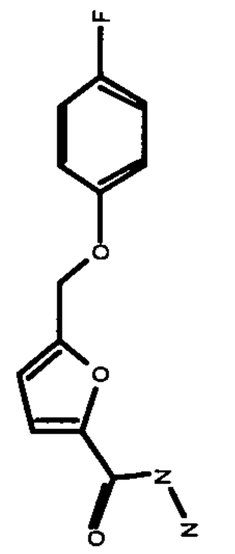
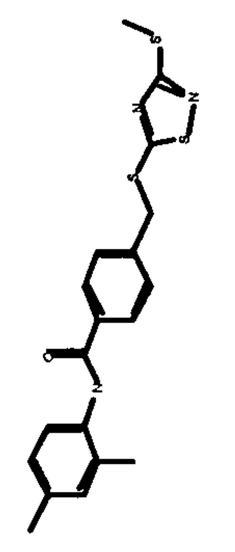
IPK 000 124 64		501.5 421.5 343.1 256.5 79.9 78.2 54.7 22.6 -0.6 25.1 31.1 33.9	490.8 577.0 416.9 267.0 77.1 94.3 45.0 7.2 5.2 38.6 38.6 36.9
IPK 000 124 65		490.8 577.0 416.9 267.0 77.1 94.3 45.0 7.2 5.2 38.6 38.6 36.9	261.5 254.5 476.3 388.9 72.8 65.1 46.9 28.2 74.4 65.1 32.8 36.9
IPK 000 125 08		261.5 254.5 476.3 388.9 72.8 65.1 46.9 28.2 74.4 65.1 32.8 36.9	

Table 1

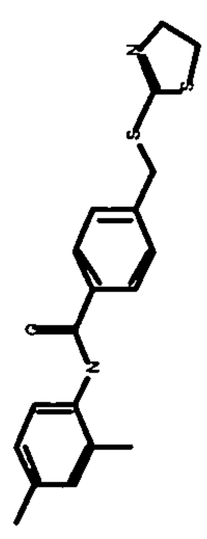
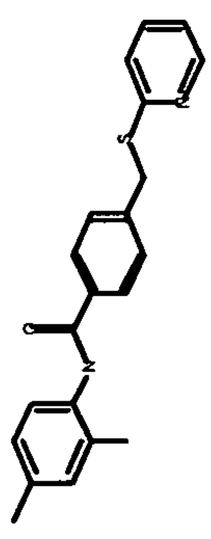
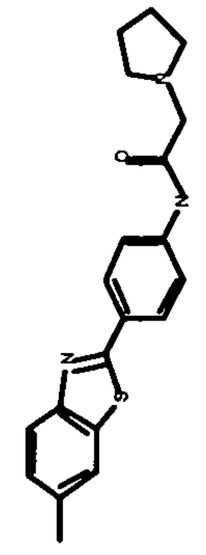
IPK 000 125 15			
233.8	265.5	55.5	
153.1	254.5	112.0	
186.4	194.1	293.5	
237.5	274.0	280.8	
87.1	75.4	44.1	
88.7	82.0	85.8	
4.0	-11.9	15.7	
4.4	3.1	2.8	
95.2	81.0	89.0	
55.2	73.2	25.3	
40.3	40.0	26.3	
40.9	38.5	31.2	
IPK 000 125 22	IPK 000 125 61		

Table 1

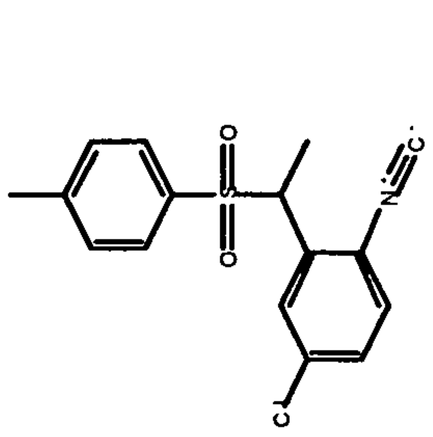
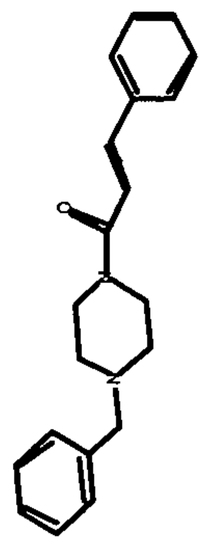
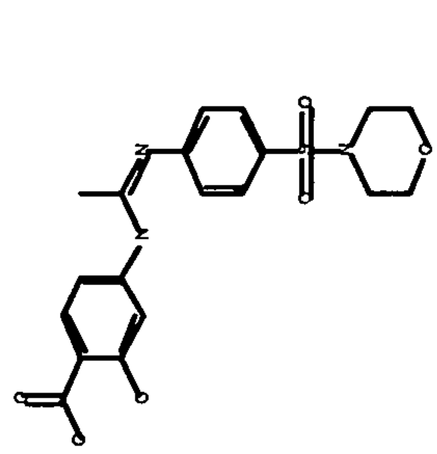
IPK 000 126 33		123.3	524.3
		86.8	271.6
		126.8	149.0
		270.9	225.6
		80.7	85.7
		73.4	60.8
		12.5	5.7
		24.9	3.3
		98.7	78.1
		99.6	76.0
		66.8	55.4
		41.4	35.5
IPK 000 126 73		519.8	402.6
		274.4	312.4
		43.8	26.5
		8.4	6.5
		90.9	71.8
		95.9	90.6
IPK 000 128 37			

Table 1

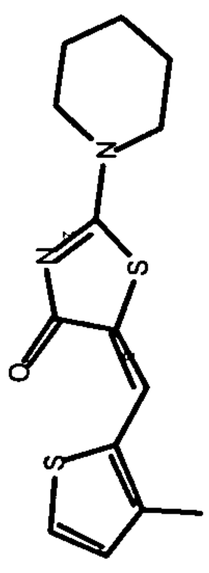
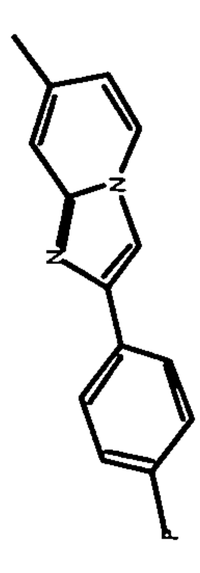
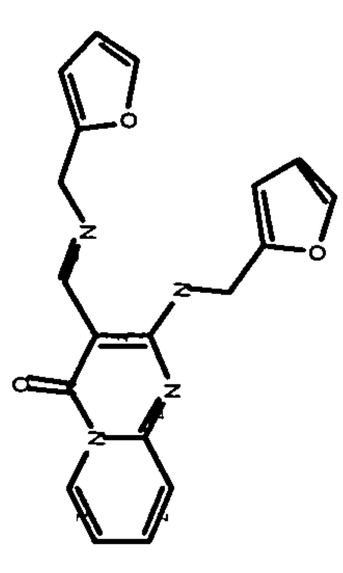
IPK 000 129 72		479.5	540.9	272.3	289.8	61.8	75.4	16.8	14.1	98.1	69.4	44.9	46.3
IPK 000 129 91		436.3	477.9	426.5	372.0	-36.0	38.2	25.4	25.1	69.3	74.8	51.4	52.1
IPK 000 130 26		476.8	385.9	270.4	340.1	66.3	29.9	14.5	17.8	100.0	70.1	79.7	53.0

Table 1

IPK 000 130 54	539.5	507.4	515.3	384.9	66.7	65.8	45.8	25.3	34.8	45.1	56.7	46.5
IPK 000 133 02	194.8	255.4	472.4	360.5	-44.8	37.2	27.6	18.1	90.2	68.1	49.0	35.7
IPK 000 133 46	473.3	534.5	397.5	243.9	69.6	94.4	45.3	22.5	40.1	57.0	38.3	40.2

Table 1

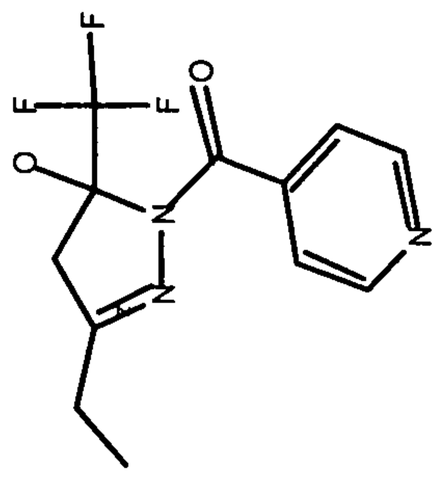
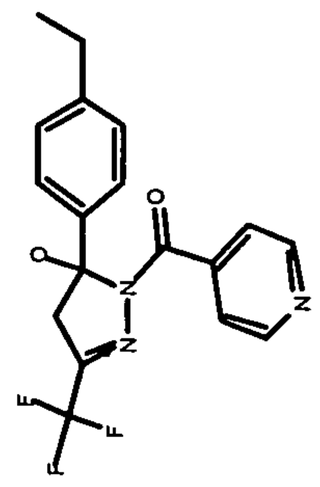
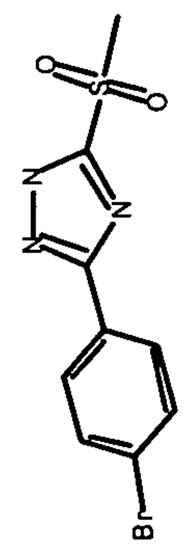
IPK 000 134 50		560.0	526.9	490.5	255.9	99.7	100.7	70.4	0.1	95.1	64.5	71.7	34.1
IPK 000 134 51		415.5	559.0	545.1	423.9	90.2	101.5	66.7	34.9	84.3	48.7	56.3	51.4
IPK 000 134 62		207.8	406.9	296.6	308.6	-56.1	25.5	11.5	13.0	89.8	68.6	44.8	46.8

Table 1

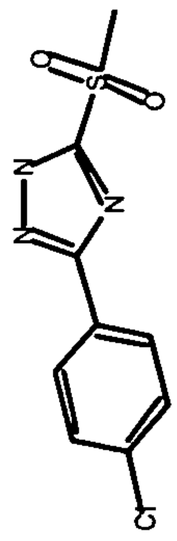
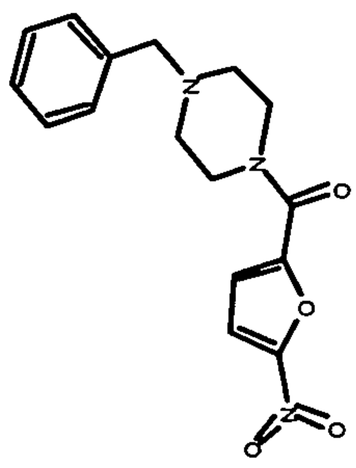
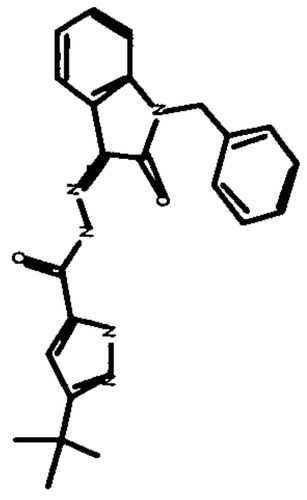
IPK 000134 63			
275.3	337.5	480.8	
294.1	255.6	501.4	
416.9	223.4	420.9	
316.6	219.5	343.8	
-18.3	53.3	96.8	
12.6	26.6	104.7	
23.9	17.5	92.8	
33.9	2.9	16.4	
94.1	99.0	98.8	
67.3	73.2	67.5	
54.0	56.9	90.8	
49.9	46.4	37.7	
IPK 000135 28	IPK 000138 12		

Table 1

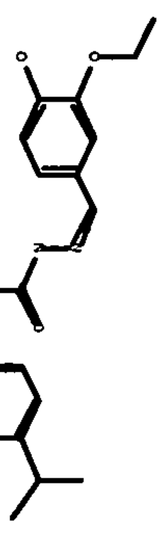
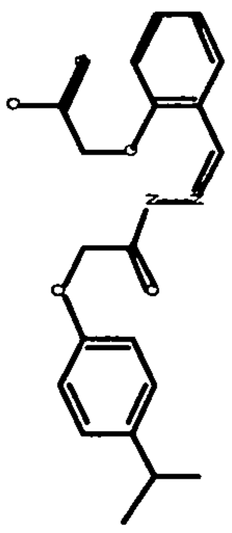
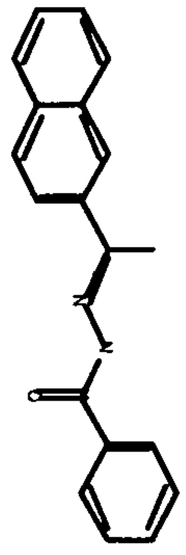
IPK 000138 40		569.8	44.6
IPK 000138 43		514.0	48.3
IPK 000139 17		199.8	52.3
		575.8	47.7
		521.3	22.9
		195.3	54.4
		285.0	52.4
		361.5	47.9
		414.0	70.4
		230.3	-1.0
		276.6	16.0
		411.5	33.8
		75.0	2.8
		76.3	6.6
		72.9	40.8
		86.4	35.4
		78.9	25.3
		65.8	38.1

Table 1

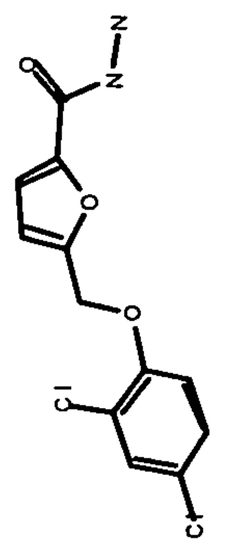
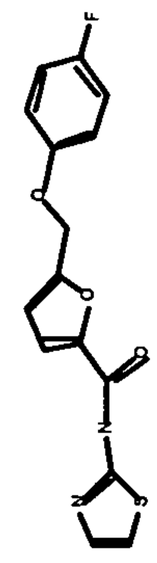
IPK 000140 81		208.5	384.4	460.8	276.8	94.6	91.8	51.8	11.0	12.0	37.9	35.4	35.4
IPK 000140 87		269.5	421.0	551.0	245.0	92.2	91.1	71.8	5.9	17.0	41.7	41.3	30.4
IPK 000141 08		529.8	625.5	270.9	200.4	67.5	84.0	11.7	4.6	13.8	34.5	47.8	41.3

Table 1

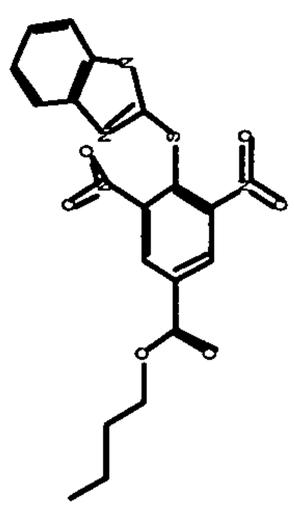
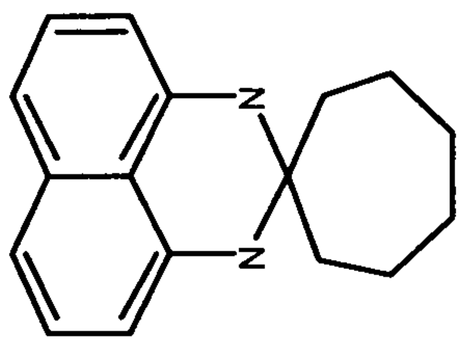
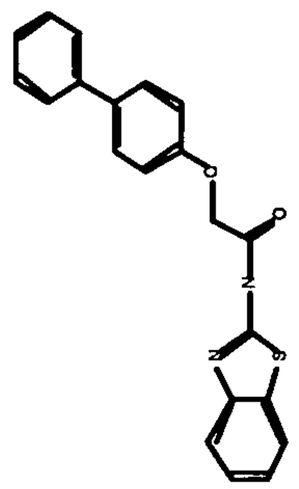
IPK 000141 58		42.9 45.3 95.9 92.7 -14.1 -2.2 59.2 58.2 184.0 239.1 167.0 191.5
IPK 000141 61		45.5 46.7 83.4 87.4 10.3 23.0 50.8 4.2 225.5 378.3 337.6 308.3
IPK 000142 17		42.9 35.1 54.3 14.6 28.7 18.5 82.3 84.3 340.4 361.8 581.1 635.8

Table 1

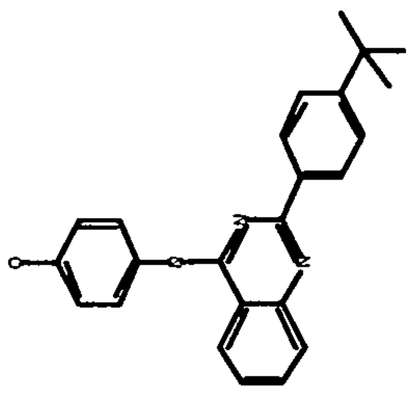
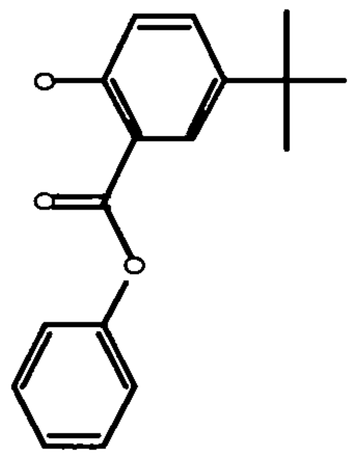
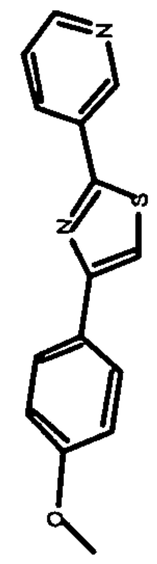
IPK 000 142 18		167.8	132.6	372.4	234.3	73.2	79.4	20.6	0.5	-1.3	38.3	39.8	39.4
IPK 000 143 45		581.8	625.4	467.4	282.6	81.2	83.5	60.3	14.4	11.5	72.5	50.0	38.3
IPK 000 144 22		262.5	377.4	452.9	318.4	-9.8	30.0	38.5	16.6	72.1	82.4	38.7	35.9

Table 1

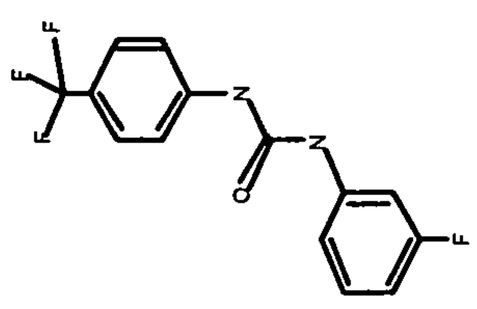
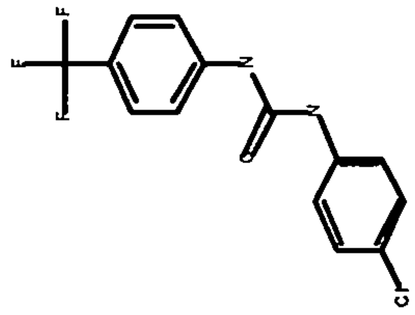
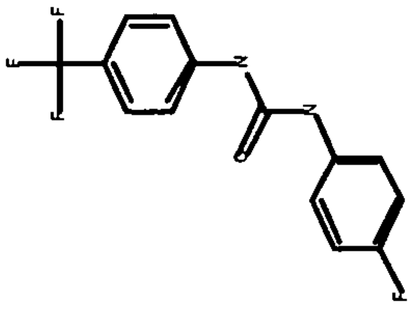
IPK 000146 91		40.7	37.6	45.1
IPK 000146 98		40.9	35.1	40.4
IPK 000147 17		100.7	70.3	74.3
		99.5	100.3	98.7
		4.4	-2.8	22.9
		57.2	26.2	47.2
		70.0	26.5	40.8
		57.0	55.8	11.9
		229.5	264.0	330.8
		201.1	283.5	186.5
		132.9	167.8	177.6
		167.5	101.3	81.0

Table 1

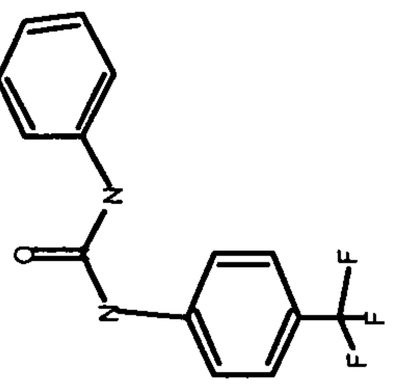
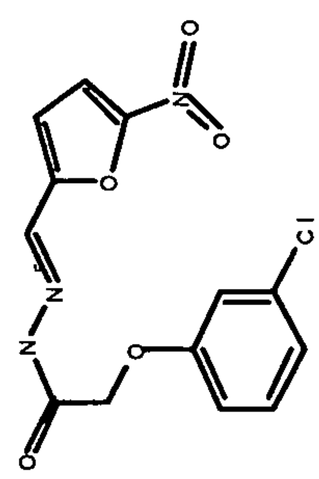
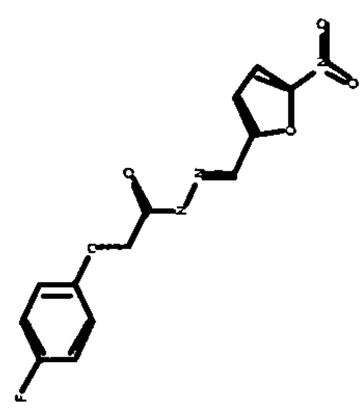
IPK 000 147 54		45.3 388.4 316.0 364.1 -10.9 6.3 21.8 59.3 26.8 40.7 17.7 15.4 98.0 99.3 67.6 98.1 46.8 37.2 48.9	67.5 82.4 375.9 250.9 6.3 72.3 40.7 13.0 26.0 92.6 66.8 48.9 42.9
IPK 000 147 98		48.0 63.8 313.4 317.6 21.8 59.3 26.8 40.7 17.7 15.4 98.0 99.3 67.6 98.1 46.8 37.2 48.9	67.5 82.4 375.9 250.9 6.3 72.3 40.7 13.0 26.0 92.6 66.8 48.9 42.9
IPK 000 148 04		48.0 63.8 313.4 317.6 21.8 59.3 26.8 40.7 17.7 15.4 98.0 99.3 67.6 98.1 46.8 37.2 48.9	67.5 82.4 375.9 250.9 6.3 72.3 40.7 13.0 26.0 92.6 66.8 48.9 42.9

Table 1

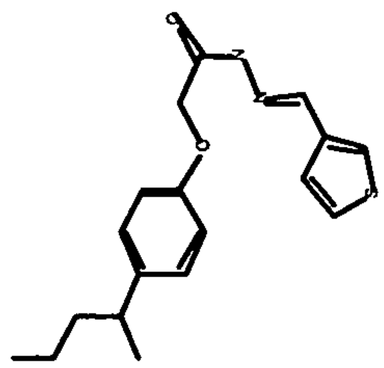
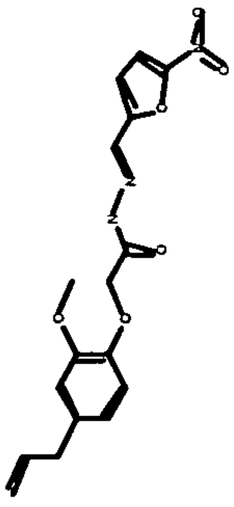
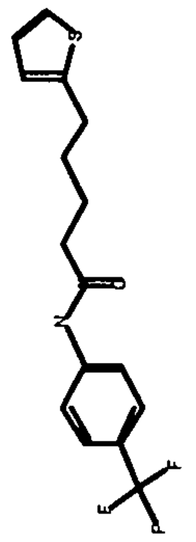
IPK 000 148 11		333.0	379.3
		577.0	516.6
		347.5	427.3
		341.8	261.9
		65.4	72.5
		86.6	71.4
		25.3	40.3
		31.9	3.7
		14.0	29.2
		36.6	42.0
		43.7	35.3
		49.4	46.5
IPK 000 148 44		58.8	58.8
		364.3	364.3
		268.6	268.6
		264.9	264.9
		-15.1	-15.1
		98.4	98.4
		11.0	11.0
		14.9	14.9
		8.2	8.2
		67.1	67.1
		43.4	43.4
		41.7	41.7
IPK 000 148 64		379.3	379.3
		516.6	516.6
		427.3	427.3
		261.9	261.9
		72.5	72.5
		71.4	71.4
		40.3	40.3
		3.7	3.7
		29.2	29.2
		42.0	42.0
		35.3	35.3
		46.5	46.5

Table 1

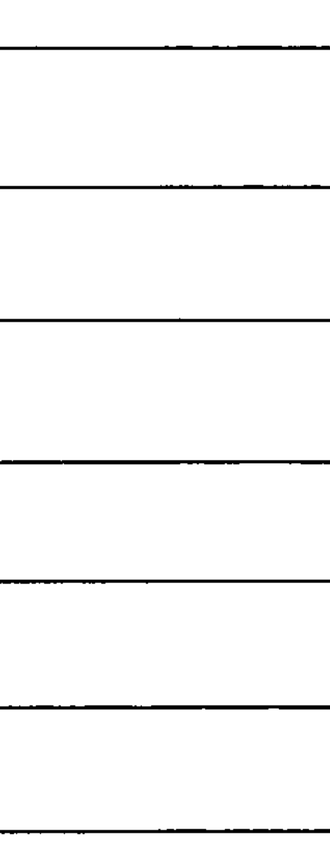
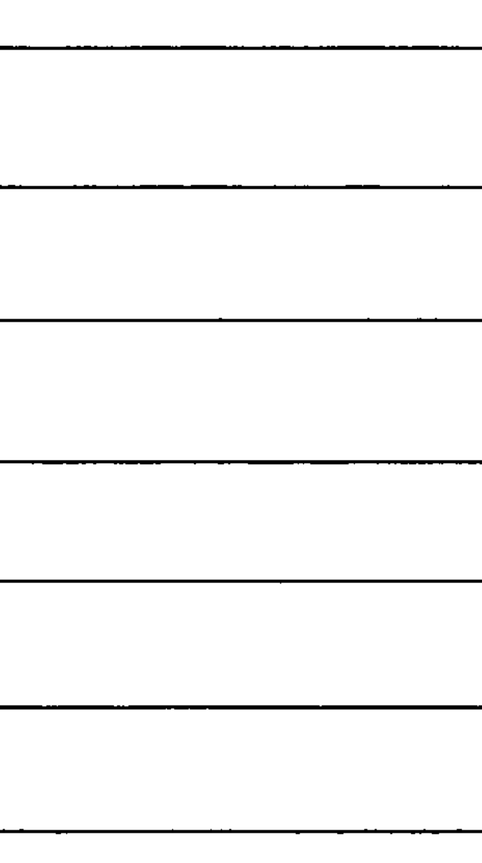
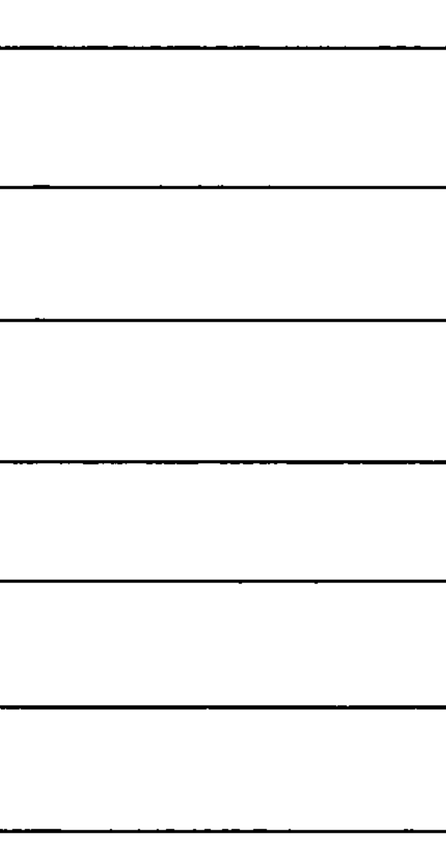
IPK 000 148 65		47.8	73.8	338.0	247.8	41.2	29.1	17.1	6.4	99.1	65.1	43.4	43.1
IPK 000 149 02		97.0	406.3	130.0	250.3	23.5	19.1	49.7	13.5	99.1	66.8	69.8	45.8
IPK 000 149 44		259.5	465.9	298.1	200.1	57.6	47.1	21.2	-10.4	100.0	70.4	43.0	27.1

Table 1

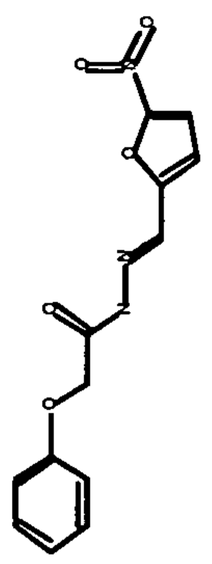
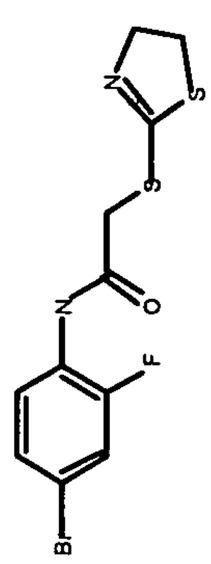
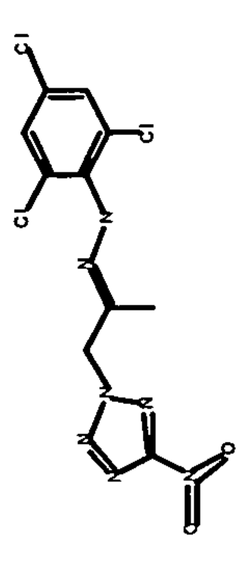
IPK 000 149 78		36.0	373.8
		328.1	539.6
		331.9	366.1
		289.0	285.3
		15.2	78.3
		3.6	83.3
		12.3	24.2
		9.6	5.2
		99.6	31.8
		74.4	44.6
		56.2	46.3
		49.9	35.9
IPK 000 150 41		61.0	333.6
		209.5	349.3
		-96.3	-2.3
		16.1	26.4
		95.6	69.8
		52.3	46.4
IPK 000 150 48			

Table 1

IPK 000 150 85	416.3 183.8 321.3	397.3 182.1 227.9	299.6 201.4 377.4
IPK 000 155 36	183.8	182.1	201.4
IPK 000 157 51	321.3	227.9	377.4

IPK 000 150 85	230.3	72.3	72.2
IPK 000 155 36	232.0	67.8	74.0
IPK 000 157 51	393.8	96.0	46.2

IPK 000 150 85	33.8	23.0	93.2
IPK 000 155 36	43.7	88.3	68.3
IPK 000 157 51	100.9	99.3	47.4

IPK 000 150 85	5.1	58.8	40.4
IPK 000 155 36	-8.6	29.0	33.2
IPK 000 157 51	39.7	82.2	47.2

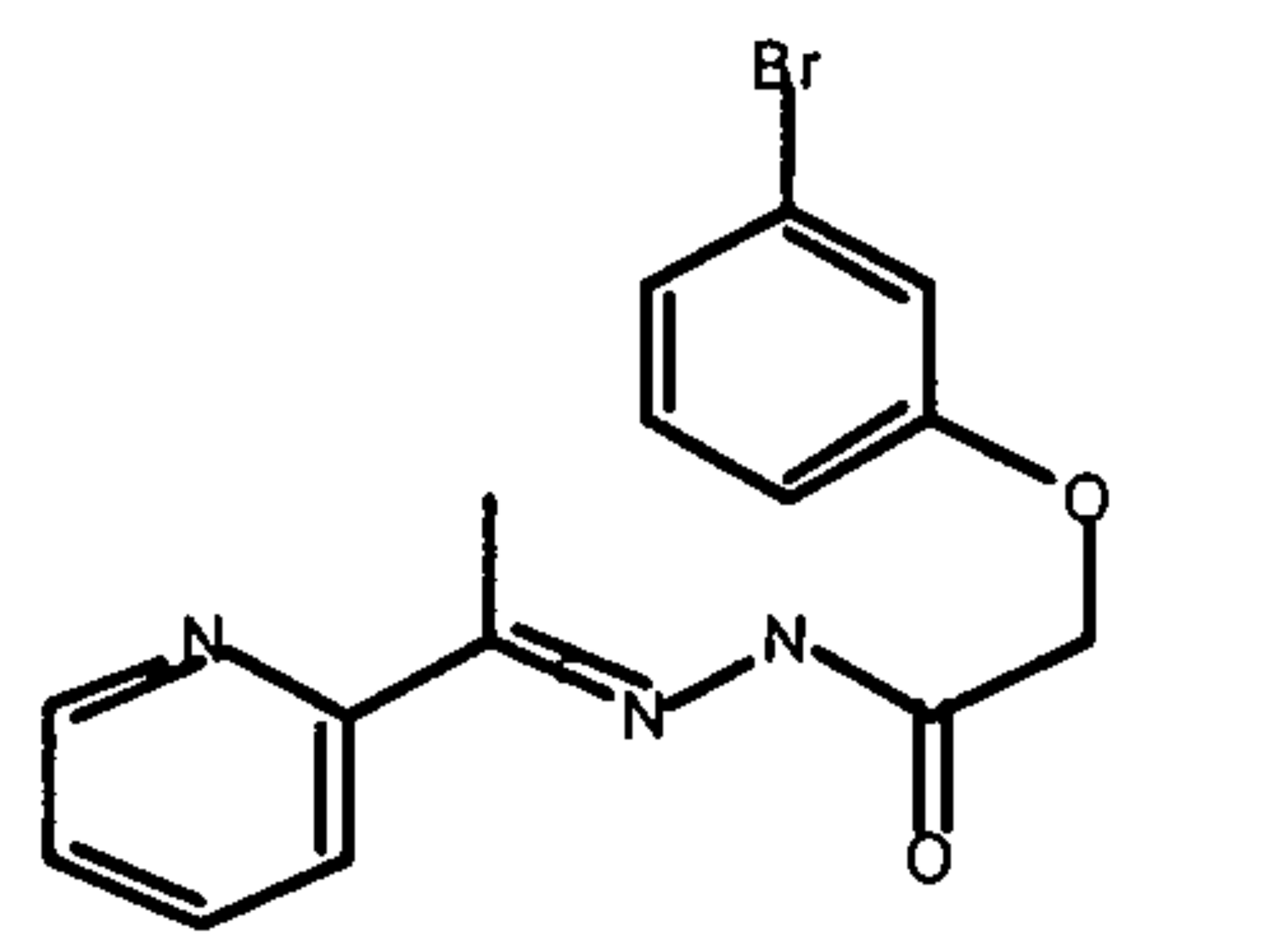
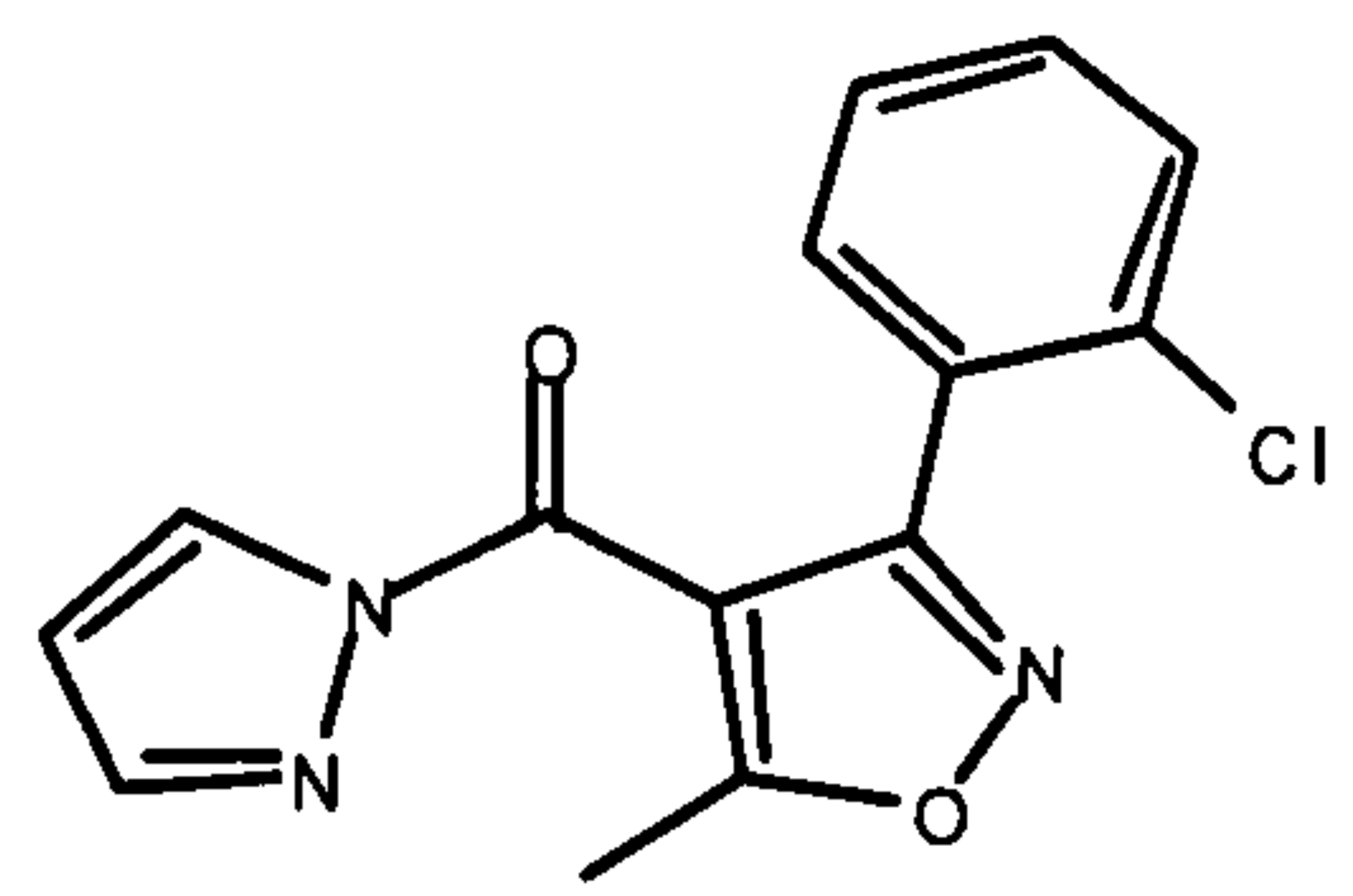
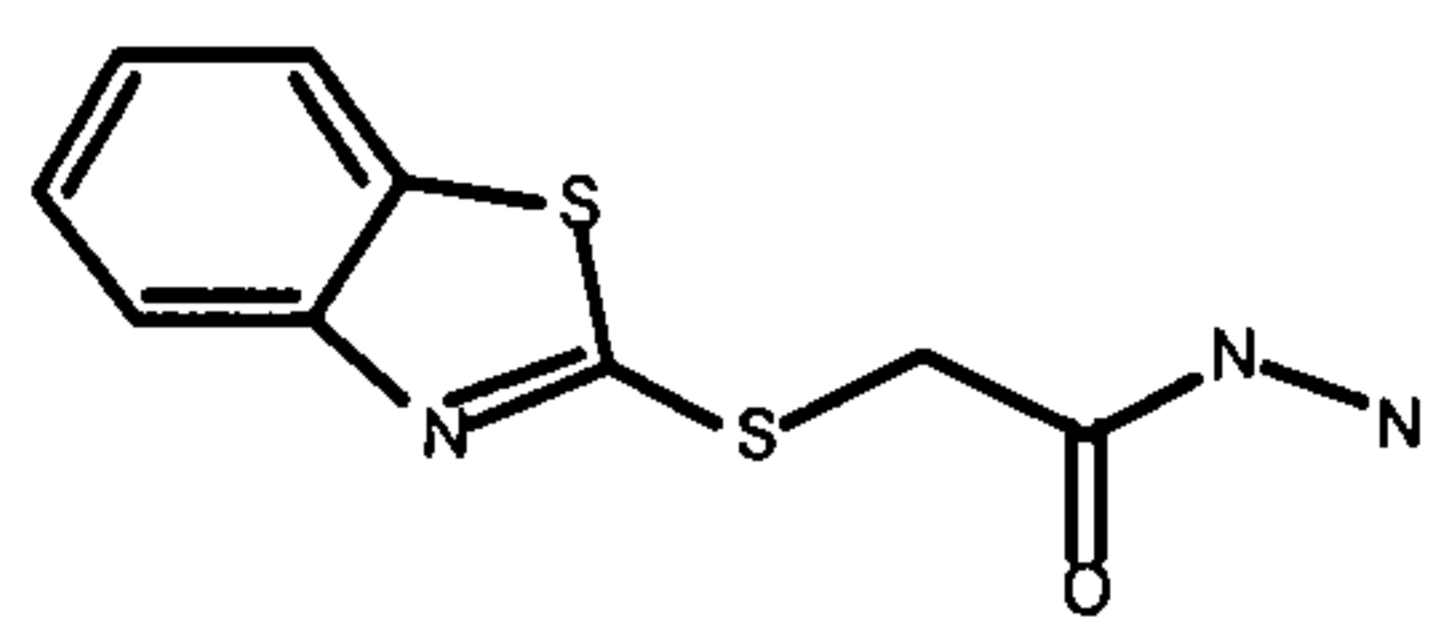
IPK 000 157 55		108.3	277.0	278.0	396.4	22.0	19.9	77.5	39.0	97.6	77.0	55.2	45.1
IPK 000 158 49		96.0	266.6	168.3	248.6	-3.0	4.8	-0.8	8.4	66.6	69.1	44.3	43.2
IPK 000 160 45		436.3	371.4	285.5	296.5	66.0	70.7	43.2	31.7	17.6	25.1	30.9	45.0

Table 1

Table 1

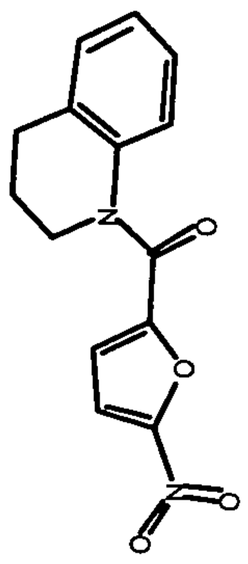
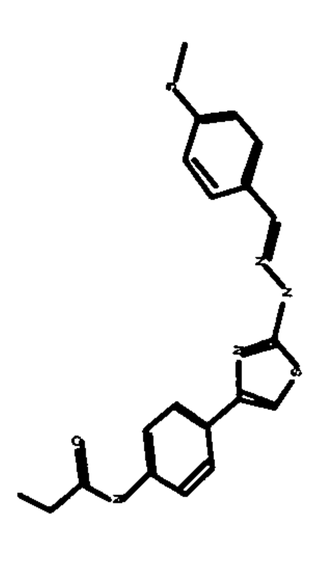
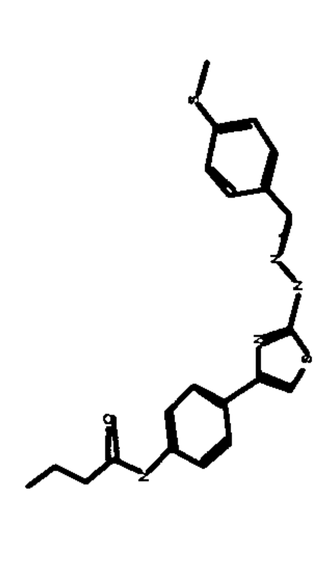
IPK 000 161 32		100.3	534.3
		65.1	263.4
		194.0	474.9
		245.4	393.4
		-37.9	69.5
		17.8	17.9
		25.1	71.8
		9.4	33.5
		99.1	86.4
		63.7	45.6
		95.8	55.6
		52.1	47.7
IPK 000 163 27		447.5	52.8
		377.5	57.2
		491.4	68.6
		335.8	92.9
		89.4	40.8
		82.7	50.0
IPK 000 163 51			

Table 1

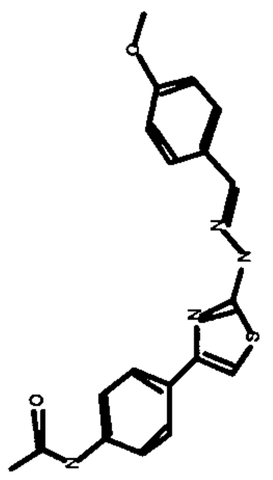
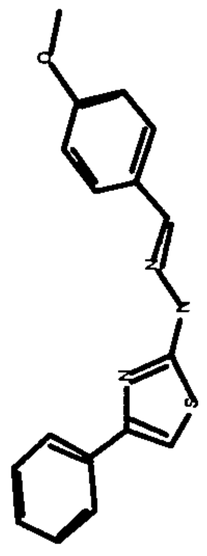
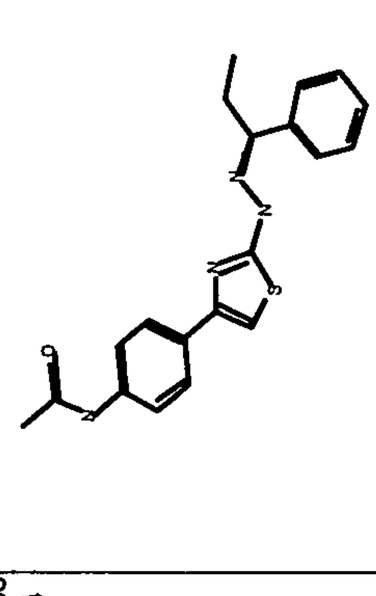
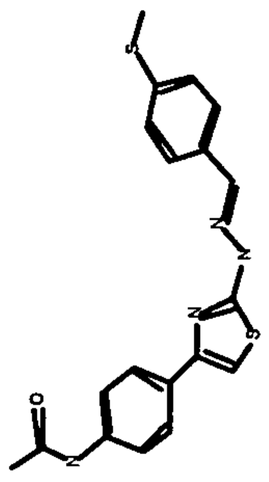
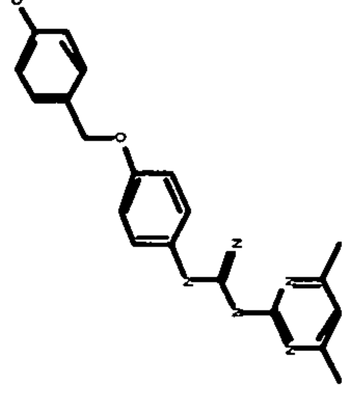
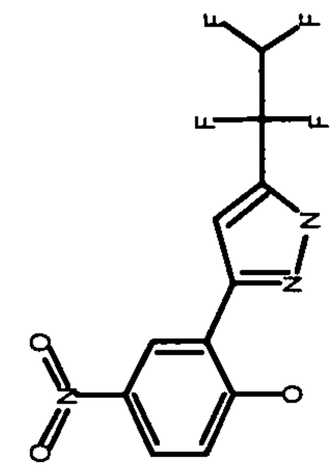
IPK 000 163 52		446.5	473.3	447.6	428.4	86.2	70.7	61.4	43.7	83.0	64.6	46.4	44.3
IPK 000 163 62		246.5	599.8	485.8	451.9	81.3	107.2	59.4	49.8	95.9	68.1	54.8	47.8
IPK 000 163 64		486.3	576.1	394.9	302.8	74.9	74.2	26.7	14.7	20.8	63.3	47.4	49.9

Table 1

IPK 000 163 67		47.9	31.3
IPK 000 163 93		44.7	34.2
IPK 000 164 52		65.3	50.1
		76.2	30.4
		27.4	45.5
		70.7	36.3
		99.5	89.9
		68.1	66.7
		340.0	339.0
		520.1	249.4
		481.6	123.6
		517.5	74.0

38.7	
36.5	
86.8	
91.8	
0.1	
-2.2	
80.0	
64.7	
286.8	
204.8	
171.8	
202.3	

Table 1

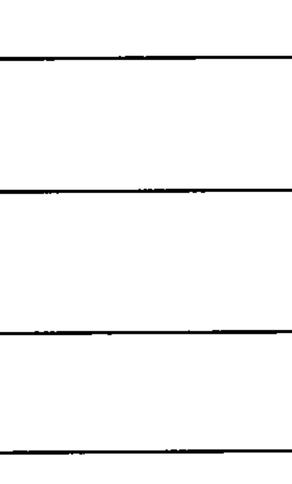
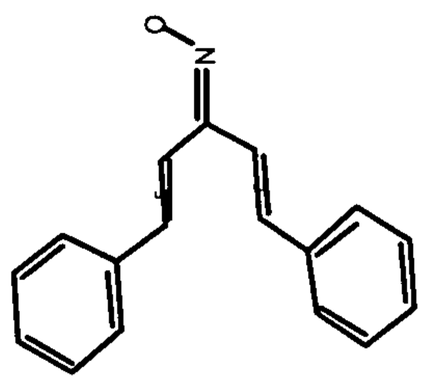
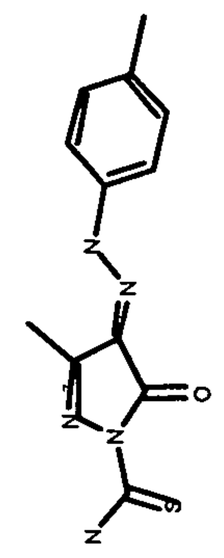
IPK 000 167 54		33.3 34.3 98.3 74.3 23.2 27.7 16.5 19.5 248.0 184.8 117.3 60.3	43.5 38.1 54.4 100.2 8.2 17.9 65.6 38.0 294.4 137.1 185.3 106.5
IPK 000 168 10		32.3 38.8 29.3 15.9 15.7 20.6 78.1 83.9 256.1 159.9 254.9 178.8	
IPK 000 168 31			

Table 1

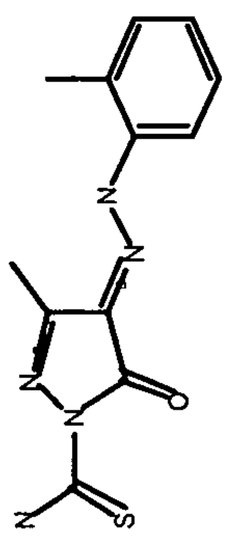
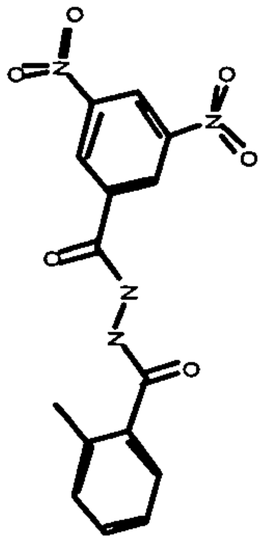
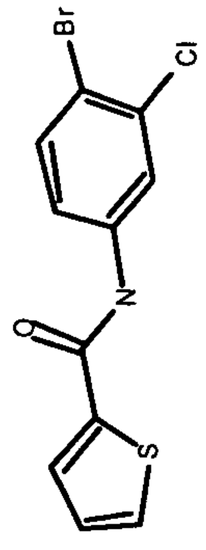
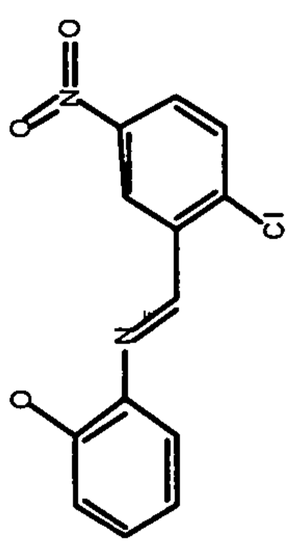
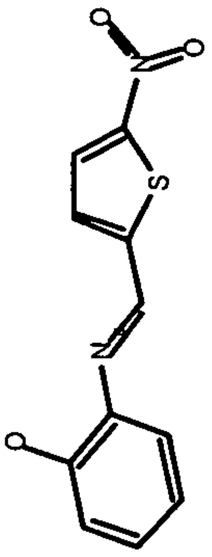
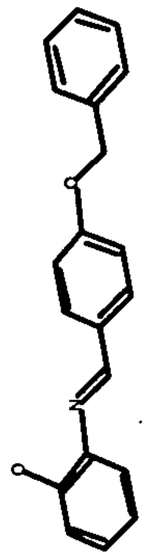
IPK 000 168 32		167.5	254.9	352.9	312.6	75.7	81.9	37.2	18.3	50.5	29.5	31.5	35.6
IPK 000 169 30		220.0	291.1	313.0	269.3	56.2	73.7	12.4	8.8	99.3	99.4	62.8	42.8
IPK 000 169 42		106.0	218.3	410.4	361.9	-64.0	76.9	32.9	8.5	95.2	45.0	44.0	44.8

Table 1

IPK 000 169 68			
	97.8	62.0	119.3
	114.9	137.6	174.4
	183.8	188.0	191.5
	289.1	301.6	359.9
	-5.0	-71.9	0.5
	-5.3	16.7	13.0
	-5.9	2.1	14.4
	16.8	16.4	31.1
	99.1	99.6	100.9
	65.3	100.3	100.3
	43.9	31.4	43.7
	45.5	30.7	34.8

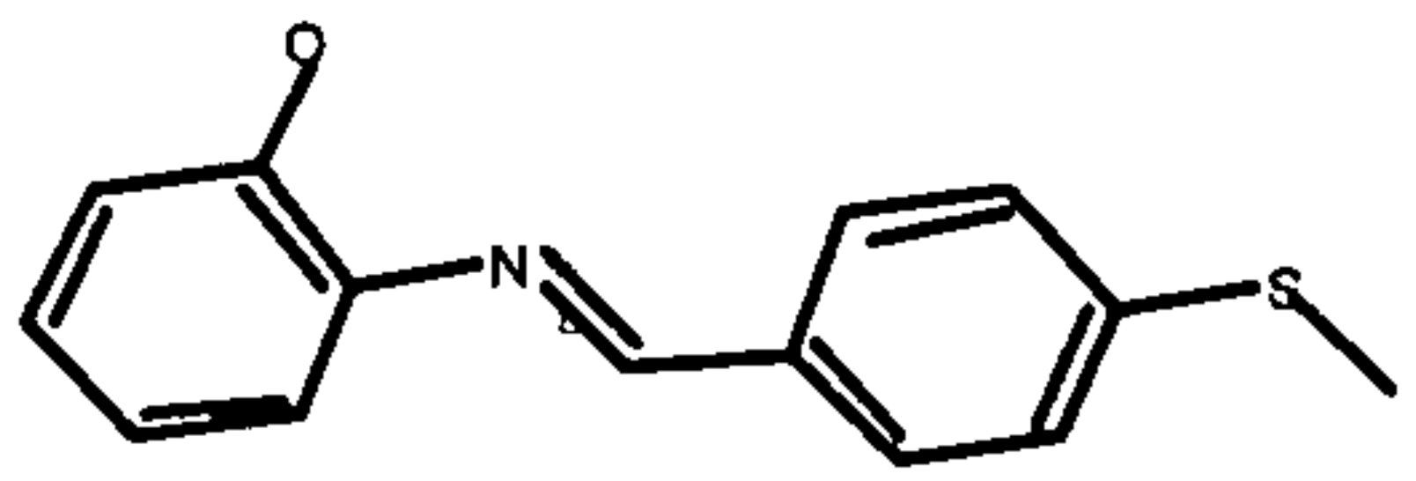
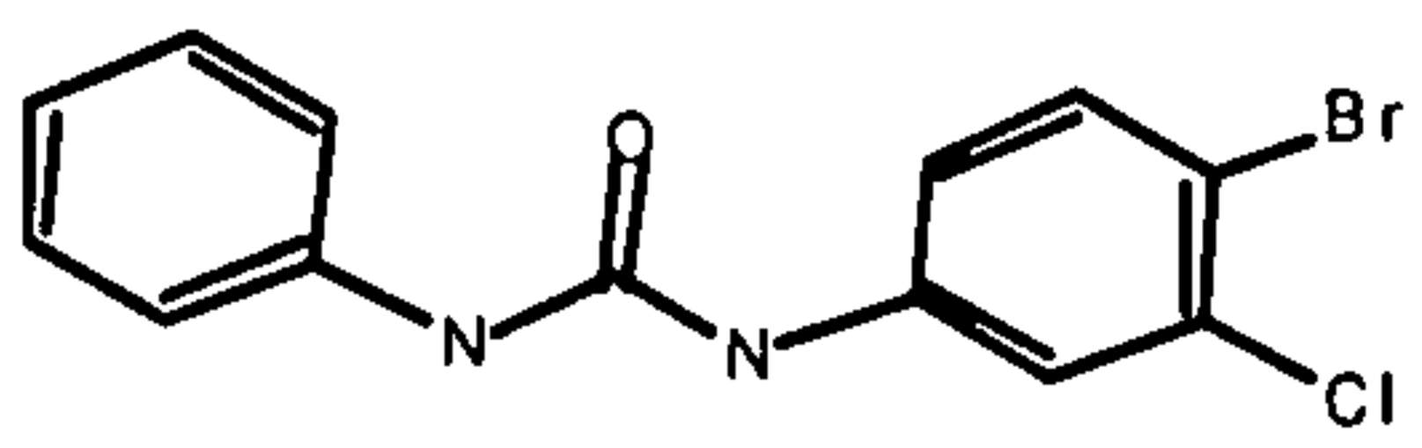
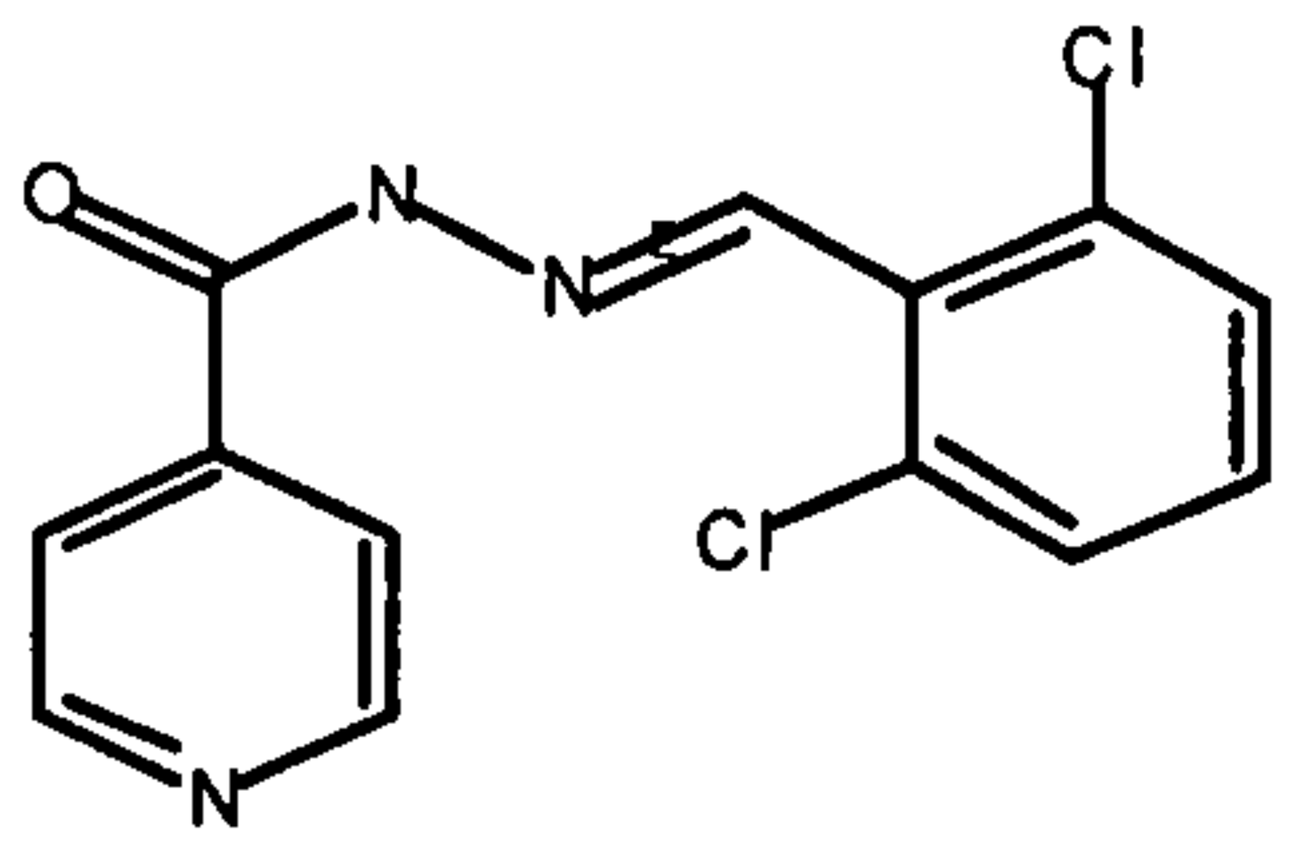
IPK 000 169 96		64.8	140.4	134.5	193.4	-50.2	14.7	27.5	17.0	103.8	100.6	68.8	34.4
IPK 000 170 27		59.0	118.9	319.5	499.9	-72.4	18.9	8.1	46.9	80.7	76.3	34.0	33.5
IPK 000 170 33		143.0	353.9	525.0	501.1	49.5	96.3	102.5	92.7	103.5	100.2	97.4	75.7

Table 1

Table 1

IPK 000 170 72	67.0	283.3	55.0
	72.1	176.9	108.5
	321.0	201.4	82.0
	275.8	276.8	236.5
	-29.5	95.2	24.3
	-21.3	95.2	72.1
	27.0	-1.5	0.9
	17.4	9.0	11.0
	76.0	99.2	100.0
	82.7	82.5	59.5
	30.3	59.9	73.5
	5.9	40.9	39.6

Table 1

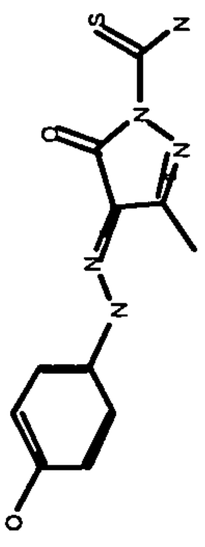
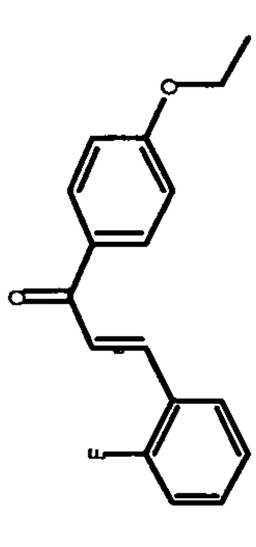
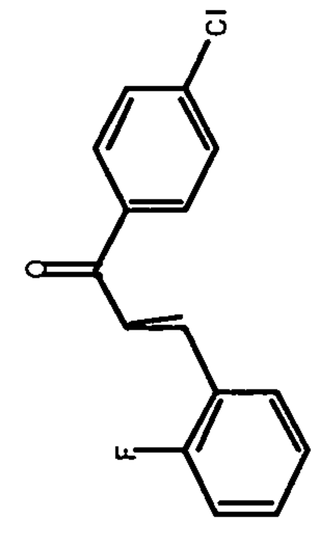
IPK 000171 84		31.0	46.1	98.8	99.0	16.9	29.4	13.3	-79.6	298.4	129.5	93.9	30.0
IPK 000172 34		37.8	45.1	69.9	98.2	7.7	18.2	12.5	-82.4	298.4	158.4	69.5	40.8
IPK 000172 35		41.1	53.6	81.2	101.4	28.5	55.2	59.9	0.9	345.6	470.5	185.4	104.3

Table 1

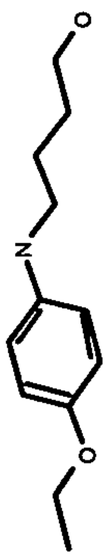
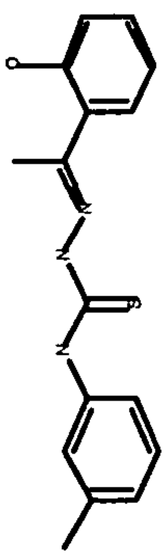
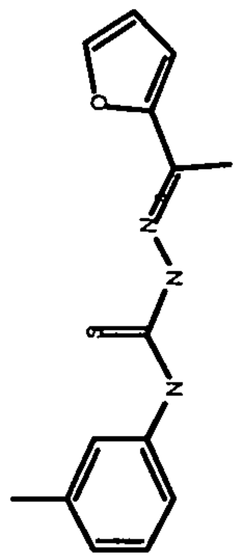
IPK 000 172 54		92.0	221.3	190.0	226.6	4.1	40.4	14.5	11.4	82.1	92.0	40.3	36.2
IPK 000 173 06		54.3	314.4	224.9	308.4	44.0	15.8	9.2	26.9	74.8	73.6	58.5	44.2
IPK 000 173 45		113.0	304.0	305.5	292.8	14.9	19.2	25.3	24.8	103.7	71.5	72.0	46.7

Table 1

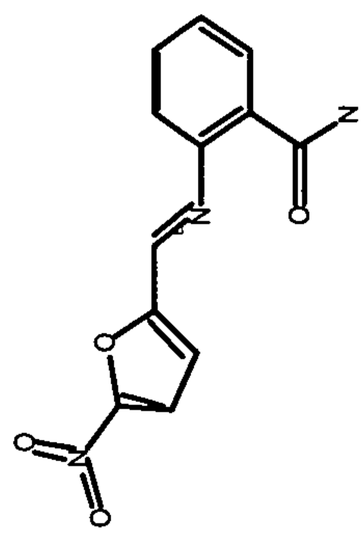
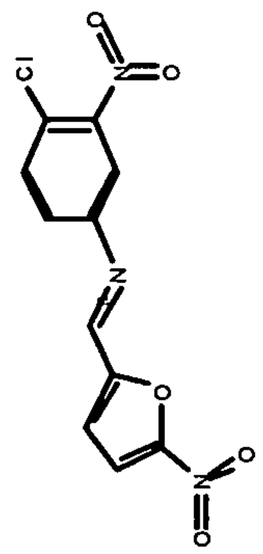
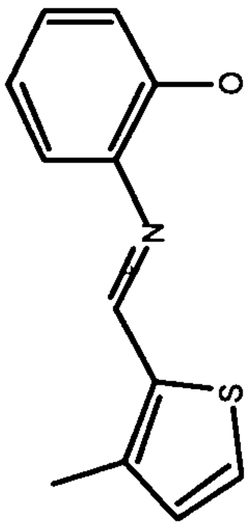
IPK 000 175 27		39.9 46.5 70.7 95.8 25.1 -3.3 25.8 -69.7 302.8 131.8 177.0 44.3
IPK 000 178 24		34.0 37.9 97.0 74.0 1.3 6.9 -4.1 -26.4 199.5 158.6 140.4 111.8
IPK 000 179 05		24.9 81.6 100.6 101.0 27.5 30.0 16.6 -31.7 352.4 194.5 190.0 124.3

Table 1

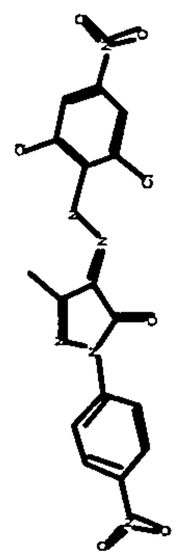
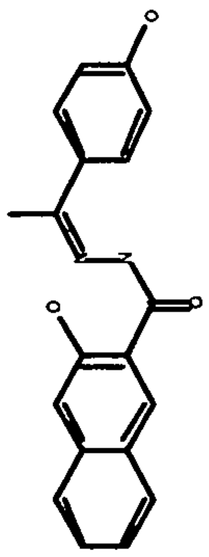
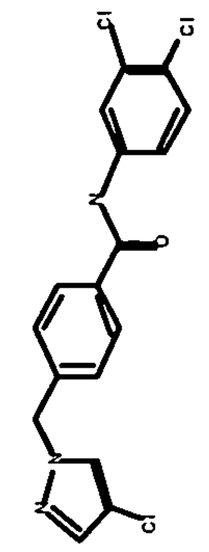
IPK 000 179 49		187.0	257.9	163.8	300.4	65.7	44.4	54.5	16.2	17.6	-8.9	78.5	48.0
IPK 000 180 11		447.8	451.5	309.9	309.1	68.2	40.6	28.9	14.8	9.7	26.0	69.7	44.8
IPK 000 180 16		217.0	234.4	323.8	332.3	46.1	41.3	68.9	93.8	13.5	12.6	69.8	43.5

Table 1

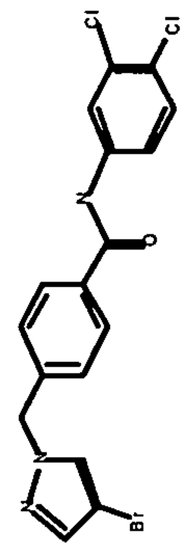
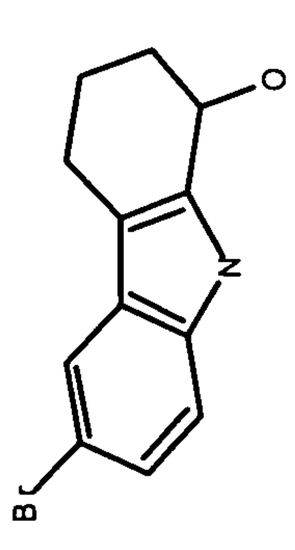
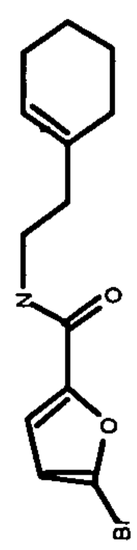
IPK 000 180 17		199.3 229.5 265.0 377.5 63.2 20.2 34.5 8.6 93.8 72.0 29.6 50.6	467.8 565.3 347.6 289.9 70.6 37.5 92.1 43.8 90.0 63.8 40.8 32.9
IPK 000 180 76		256.3 215.1 342.5 391.5 85.1 45.0 72.7 34.4 63.3 67.3 46.9 46.5
IPK 000 184 56			

Table 1

IPK 000192 45	219.8	386.4	376.8	334.0	88.8	65.4	22.0	16.2	-9.0	42.7	43.3	42.7
IPK 000192 59	470.5	551.3	344.4	246.3	65.3	81.1	30.1	10.8	-8.7	41.9	31.7	42.7
IPK 000193 76	355.5	420.4	497.1	520.4	81.4	97.4	100.4	91.9	98.6	68.4	85.6	63.9

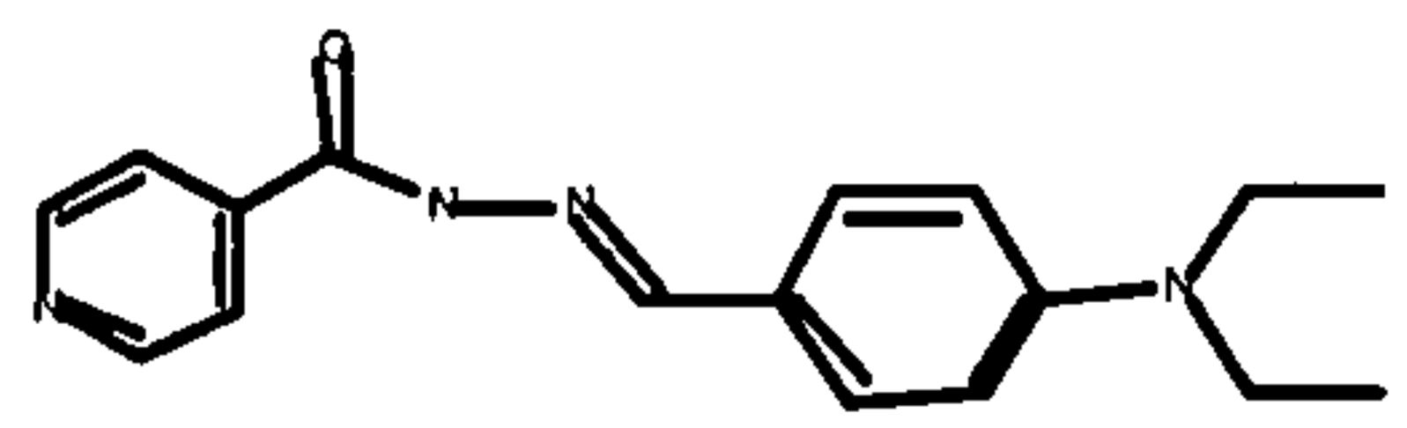
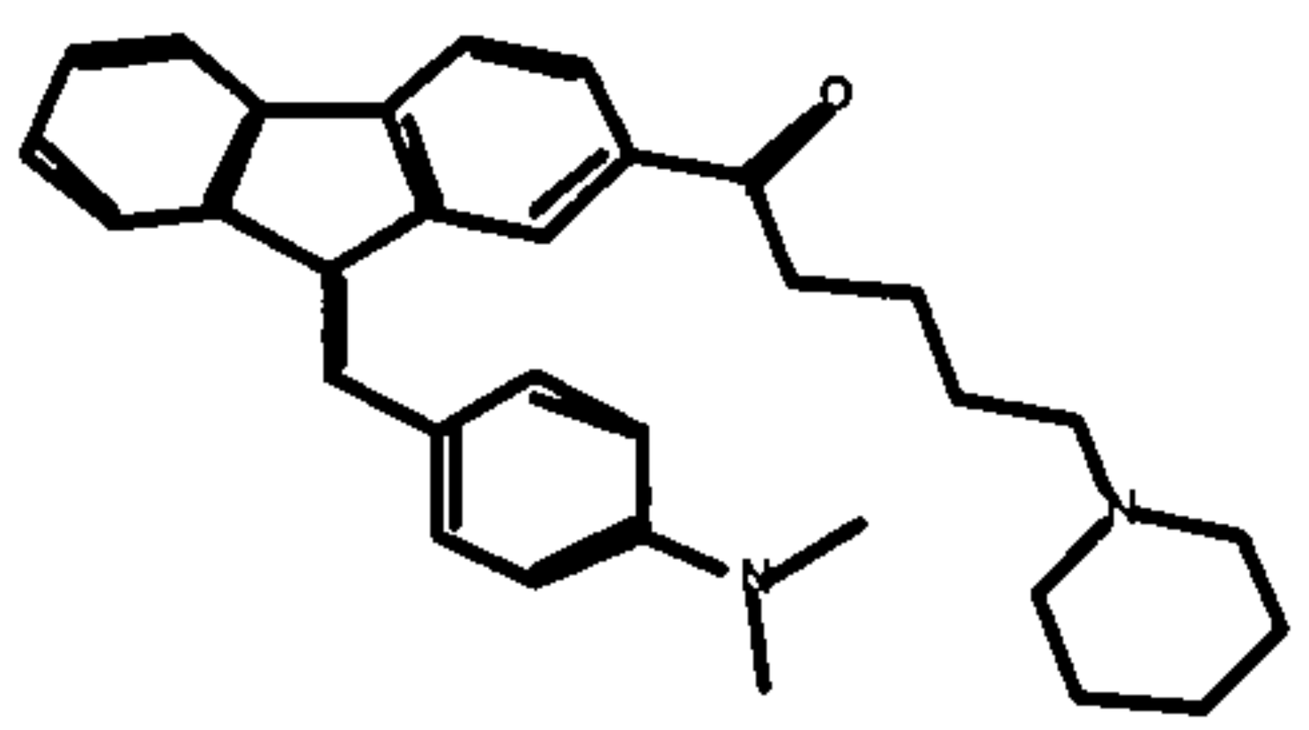
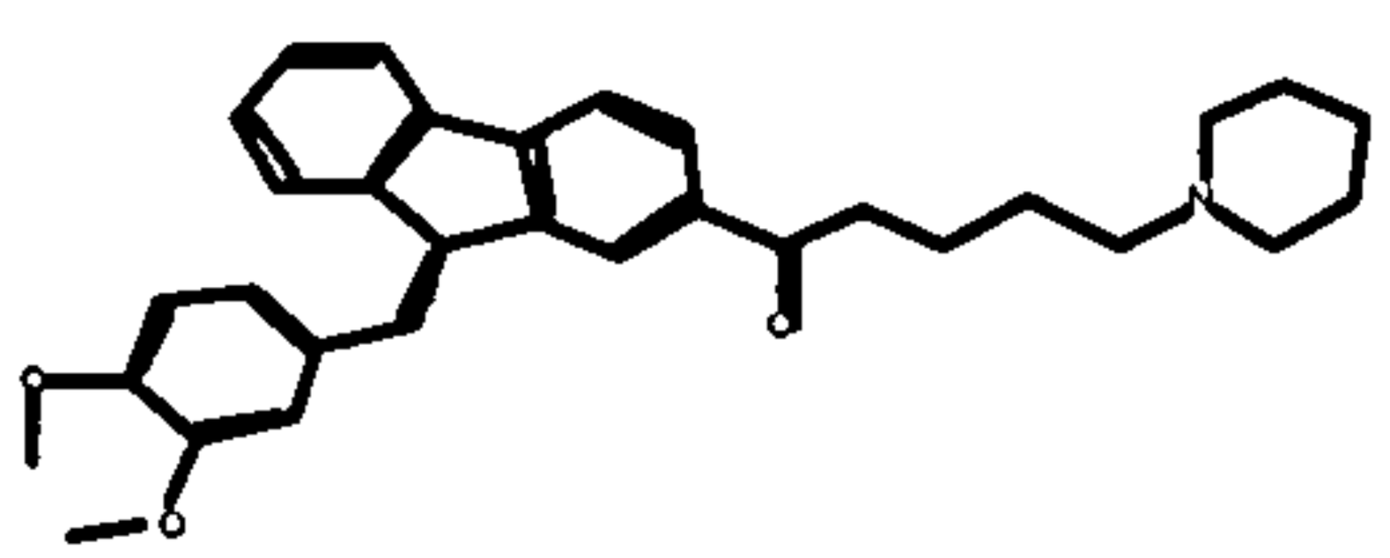
IPK 000 195 99		305.0	488.8	537.1	477.8	79.1	69.5	105.7	96.1	98.8	74.2	97.1	70.8
IPK 000 198 53		129.5	217.9	192.3	210.1	58.5	41.9	10.2	1.8	97.3	66.2	29.5	34.9
IPK 000 198 54		153.0	189.3	394.4	309.5	38.6	77.9	27.3	20.3	88.8	55.5	44.6	31.6

Table 1

Table 1

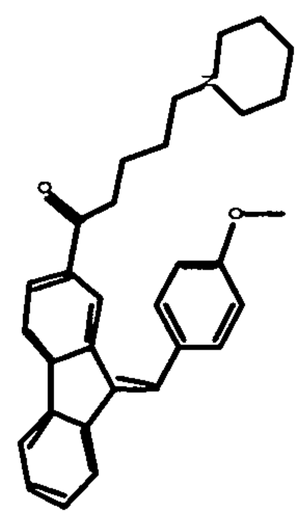
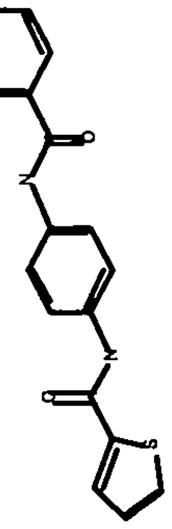
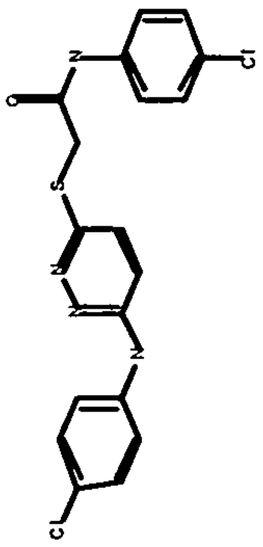
IPK 000198 56		131.5	278.1	272.5	276.0	15.1	64.7	28.7	21.2	95.9	68.3	39.0	34.3
IPK 000199 70		227.5	256.5	301.6	345.8	70.5	69.6	11.2	23.0	-8.2	39.8	43.5	49.3
IPK 000200 16		265.0	430.5	238.4	235.3	73.3	76.8	2.7	1.6	-16.9	50.3	42.3	40.2

Table 1

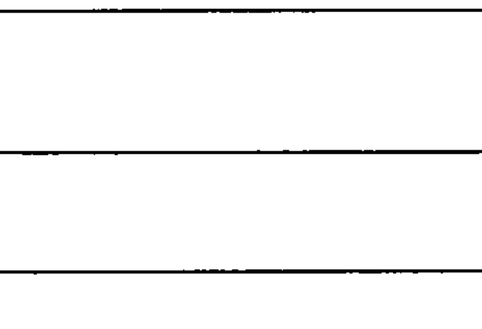
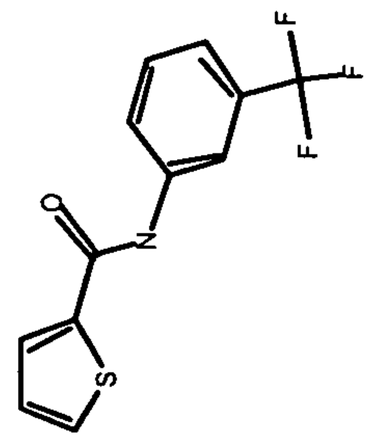
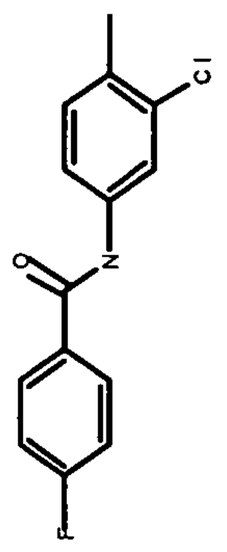
IPK 000 200 47		146.0 309.6 294.9 370.5 6.3 85.1 19.1 69.7 3.5 33.2 15.8 6.2 90.3 38.2 95.0 47.4 58.9 50.0 37.1 45.5	283.0 279.9 354.1 396.1 65.7 67.4 18.1 23.7 4.6 28.7 38.5 39.4
IPK 000 202 08			280.0 319.8 414.0 313.6 85.1 69.7 33.2 6.2 38.2 47.4 50.0 45.5
IPK 000 205 22			

Table 1

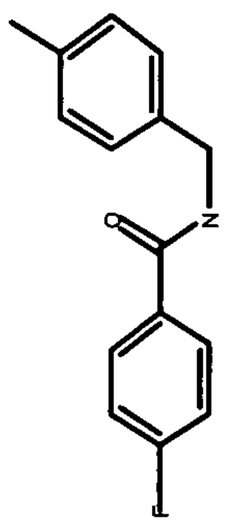
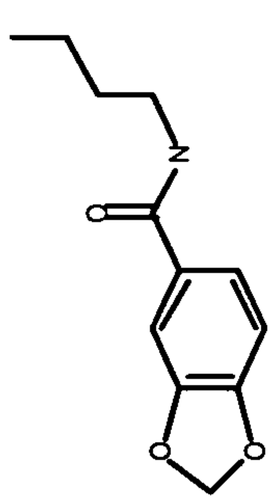
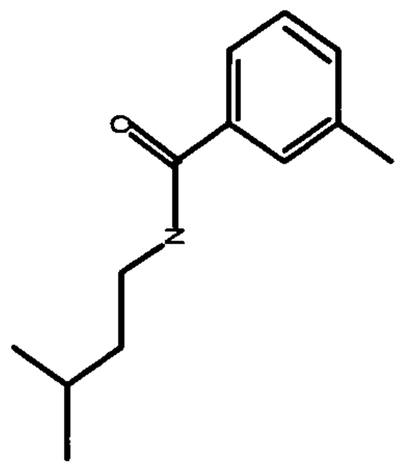
IPK 000 205 42		205.3	51.8
		314.8	65.1
		430.8	47.0
		330.6	44.7
		71.5	19.9
		69.3	58.7
		58.7	69.3
		19.9	71.5
		44.7	330.6
		47.0	430.8
		65.1	314.8
		51.8	205.3
IPK 000 208 53		166.8	44.0
		232.5	57.3
		319.1	56.4
		275.9	46.0
		71.6	-4.0
		23.3	65.1
		20.2	23.3
		76.4	71.6
		344.3	275.9
		425.6	319.1
		304.9	232.5
		191.8	166.8
IPK 000 210 74		191.8	50.0
		304.9	66.8
		425.6	43.9
		344.3	49.9
		76.4	32.6
		88.9	88.9
		20.2	20.2
		71.6	76.4
		275.9	344.3
		319.1	425.6
		232.5	304.9
		166.8	191.8

Table 1

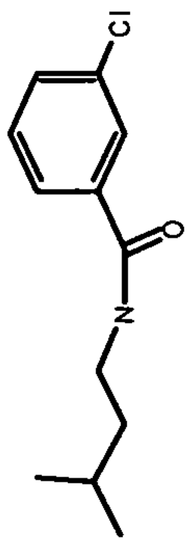
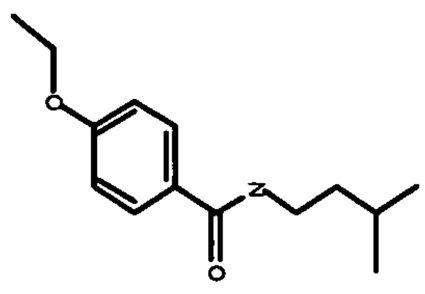
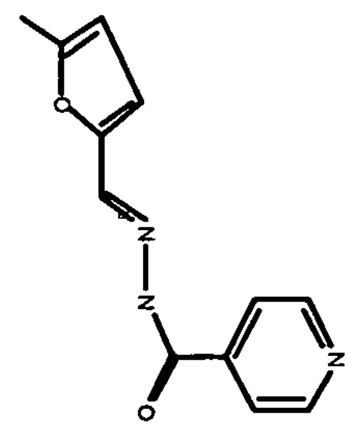
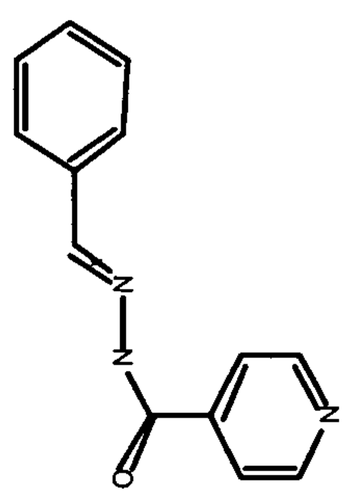
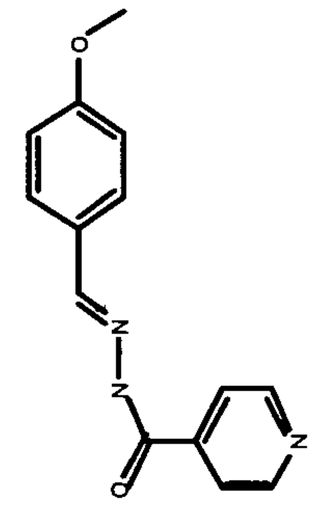
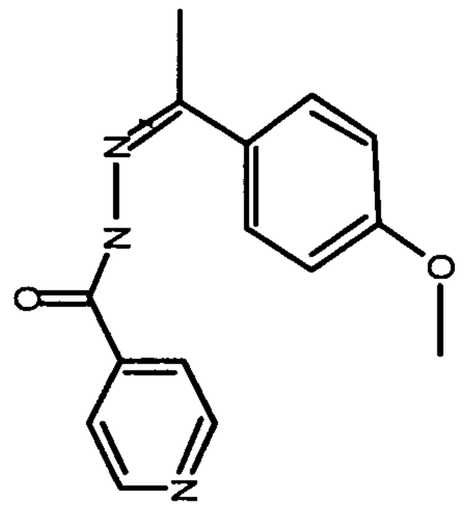
IPK 000 210 79		273.8 200.8	548.0
		255.0 190.5	218.4
		350.9 320.4	476.1
		353.6 213.9	435.6
		273.8 200.8	548.0
IPK 000 210 83		255.0 190.5	218.4
		350.9 320.4	476.1
		353.6 213.9	435.6
		273.8 200.8	548.0
IPK 000 219 26		255.0 190.5	218.4
		350.9 320.4	476.1
		353.6 213.9	435.6
		273.8 200.8	548.0
		71.5 71.4	98.6
		28.7 48.2	51.4
		77.2 70.4	91.1
		43.5 49.8	98.6
		25.1 -5.0	63.8
		48.6 52.8	46.0
		58.2 62.8	84.5
		49.2 50.6	45.3

Table 1

IPK 000 219 27		586.3 623.3	47.6
		384.6 240.8	85.0
		513.9 480.5	70.3
		443.3 433.8	98.7
		96.9 106.5	78.1
		57.4 46.2	104.8
		104.8 98.1	57.4
		78.1 57.6	96.9
		98.7 98.0	443.3
		70.3 40.9	513.9
		85.0 83.1	384.6
		47.6 42.4	586.3
IPK 000 219 28			
IPK 000 219 29			
		623.5	46.5
		423.8	84.5
		502.5	32.9
		221.3	99.3
		107.4	18.5
		67.6	95.1
		95.1	67.6
		18.5	107.4
		99.3	221.3
		32.9	502.5
		84.5	423.8
		46.5	623.5

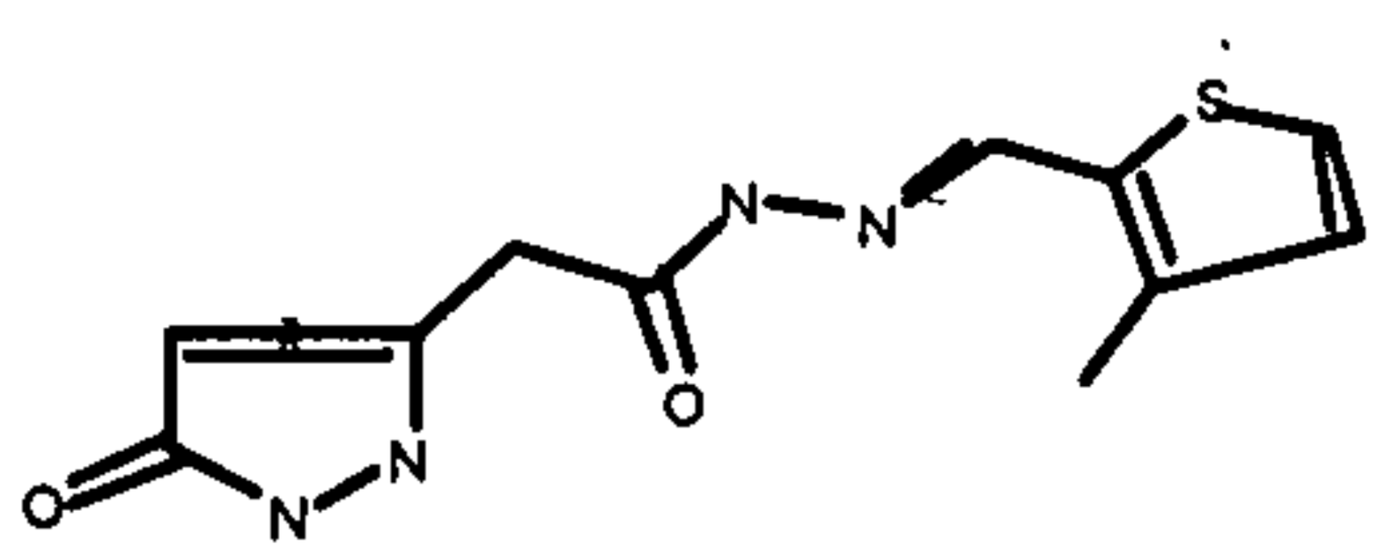
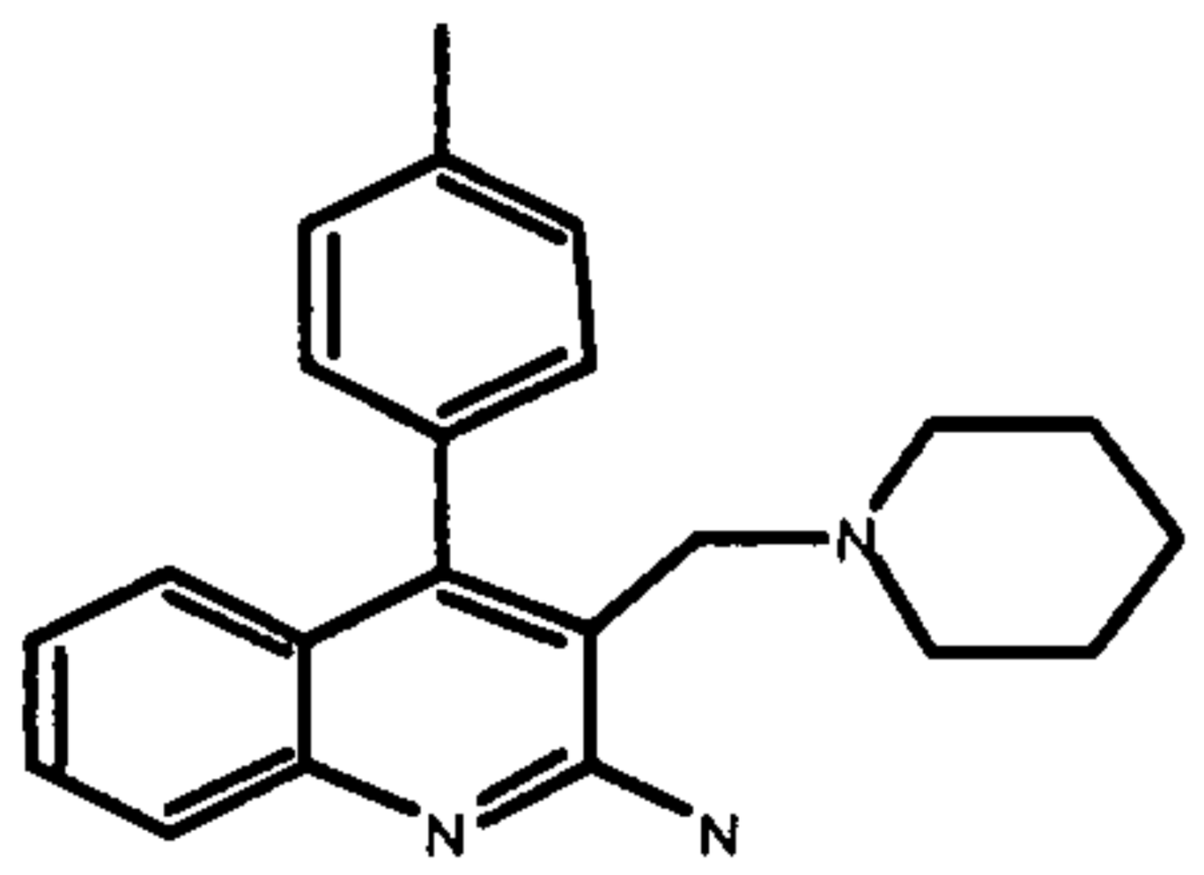
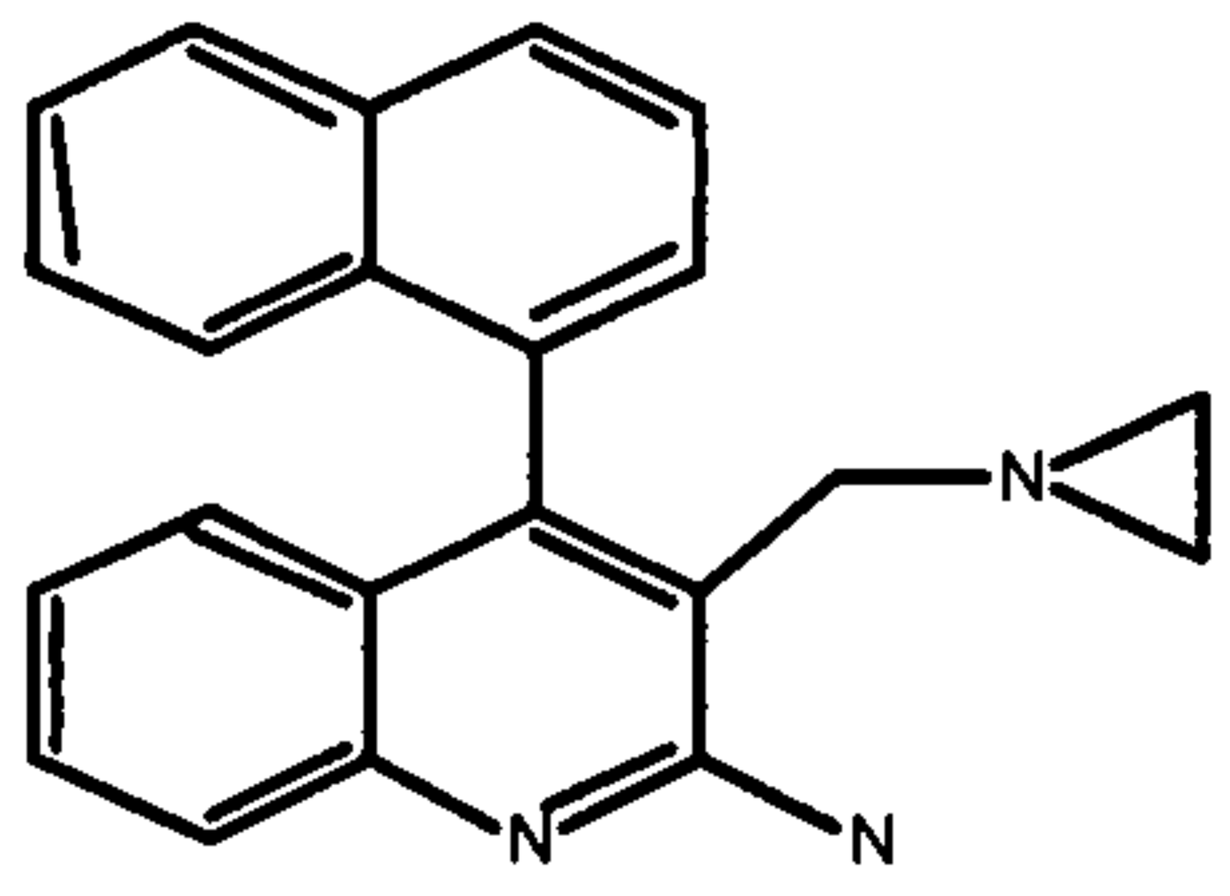
IPK 000 219 30		700.8	300.0	564.3	356.9	103.5	93.0	101.1	13.0	99.5	99.7	82.9	44.9
IPK 000 222 00		148.3	279.5	218.5	282.6	74.4	86.2	29.9	3.6	82.3	19.7	26.8	30.8
IPK 000 222 04		181.3	124.9	210.8	261.9	65.4	85.8	69.0	40.1	54.9	78.1	21.1	30.0

Table 1

Table 1

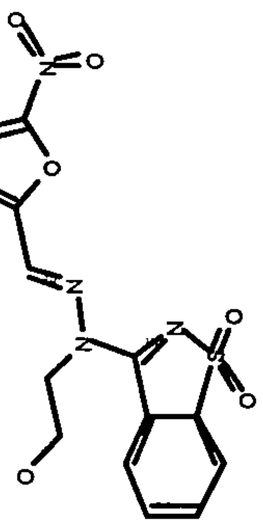
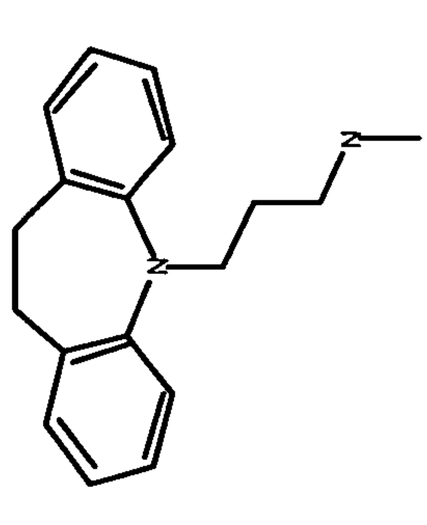
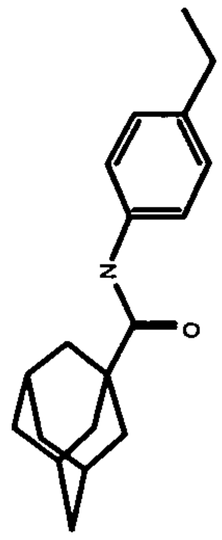
IPK 000 222 32		124.8 114.3 227.8 294.8 16.2 72.9 63.2 86.2 53.1 41.8 14.4 14.9 102.4 30.0 96.7 31.5 47.9 37.7 40.7 45.7	492.8 261.5 431.4 376.6 22.5 47.1 37.8 67.1 38.9 97.5 41.0 25.0
IPK 000 224 59		60.3 95.1 248.2 333.3	
IPK 000 228 46			

Table 1

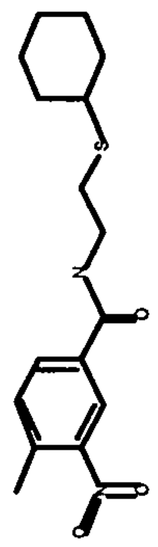
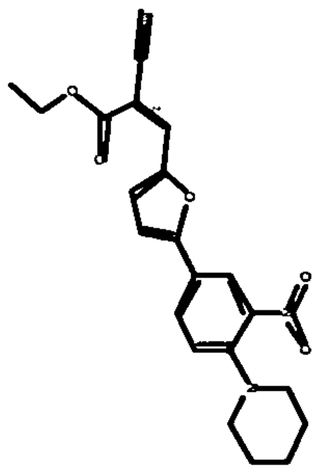
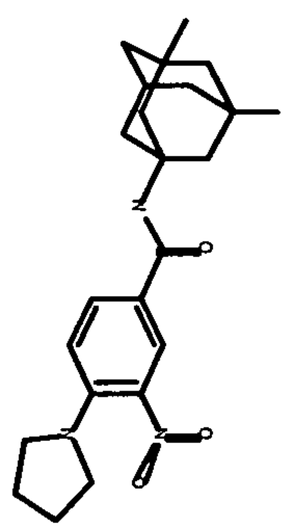
IPK 000 229 50		488.0	36.6
		300.3	27.1
IPK 000 229 72		223.5	40.5
IPK 000 230 02		277.6	44.6
		322.5	41.9
		386.8	95.7
		352.8	19.0
		483.6	78.8
		412.9	40.5
		322.8	-7.7
		453.1	
		388.4	
		277.6	
		223.5	

Table 1

IPK 000 234 61	156.8	207.9	209.1	292.6	-61.0	-1.6	-5.8	14.6	67.9	76.3	42.7	37.4		
IPK 000 235 09	91.8	251.8	346.0	276.6	46.2	36.4	32.6	3.9	98.8	45.4	70.1	22.4		
IPK 000 235 12	39.8	254.0	325.5	333.5	-67.9	26.9	31.9	34.9	99.4	71.1	48.7	40.9		

Table 1

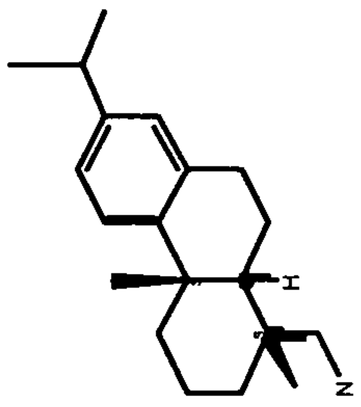
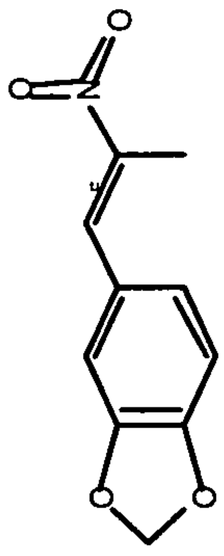
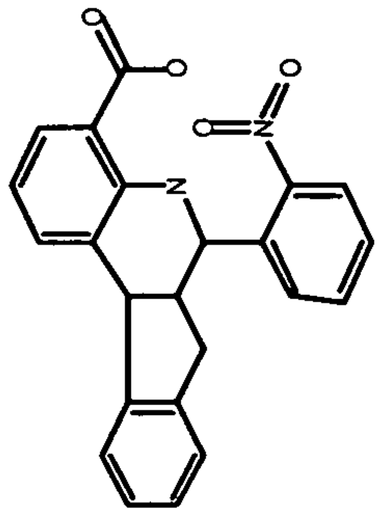
IPK 000 238 91		379.0	132.8
		382.4	156.5
		145.6	116.5
		202.6	213.9
		99.3	17.0
		27.8	67.5
		78.4	11.6
		-6.0	-10.9
		96.7	104.1
		70.1	76.2
		43.2	37.9
		28.6	30.6
IPK 000 240 37		132.8	175.3
		156.5	102.4
		116.5	487.5
		213.9	421.1
		17.0	-43.3
		67.5	28.0
		11.6	67.1
		-10.9	53.6
		104.1	100.4
		76.2	97.2
		37.9	92.6
		30.6	68.9
IPK 000 241 72		175.3	175.3
		102.4	102.4
		487.5	487.5
		421.1	421.1
		-43.3	-43.3
		28.0	28.0
		67.1	67.1
		53.6	53.6
		100.4	100.4
		97.2	97.2
		92.6	92.6
		68.9	68.9

Table 1

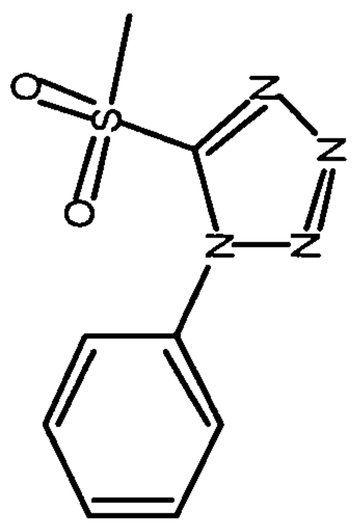
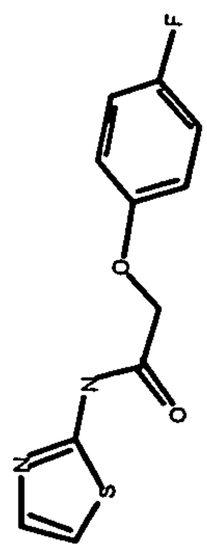
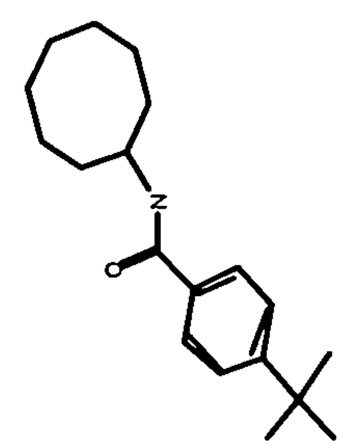
IPK 000 244 12		136.0 257.8 249.4 270.0 29.9 89.1 11.8 60.1 0.5 12.3 98.4 -12.2 75.7 23.8 66.8 30.8 42.3 34.7	584.0 332.8 158.6 174.5 89.1 97.1 17.5 12.3 -12.2 23.8 30.8 34.7
IPK 000 247 44		150.8 256.0 486.9 334.6 79.5 79.0 73.4 21.5 99.2 41.6 70.4 40.5	
IPK 000 248 71			

Table 1

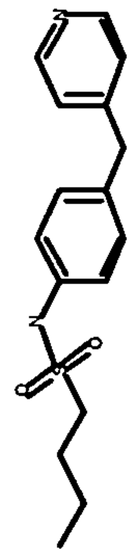
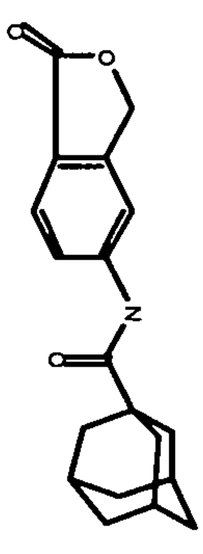
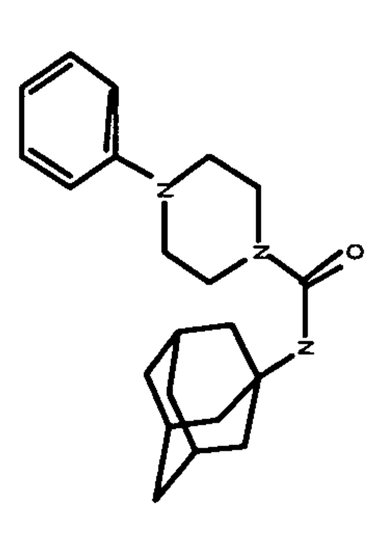
IPK 000 249 12		130.8 124.3 138.9 172.9 23.6 78.2 53.2 41.8 7.8 94.3 86.2 56.8 41.7	404.3 211.3 407.6 332.9 45.0 68.6 41.9 21.0 96.3 37.4 40.3 43.3
IPK 000 249 14		163.3 173.8 295.4 325.3 78.2 80.9 -4.9 21.4 86.0 64.6 46.3 45.8	
IPK 000 249 84			

Table 1

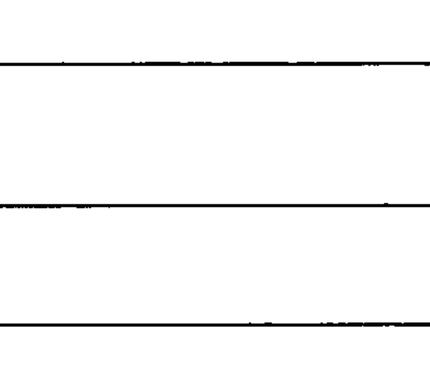
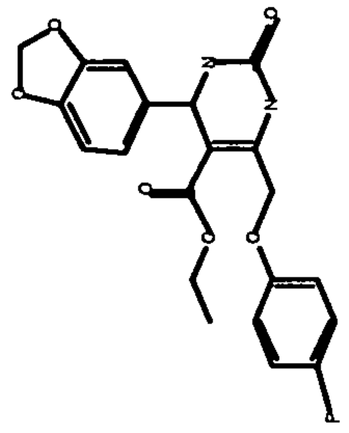
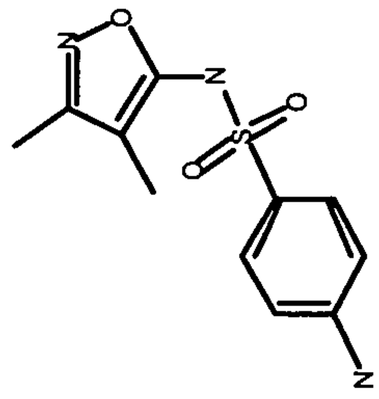
IPK 000 251 49		162.5	282.1	327.6	285.9	27.0	15.0	18.0	0.8	92.6	65.1	55.1	44.5
IPK 000 251 80		321.0	322.1	217.8	294.5	25.5	38.2	9.2	3.9	77.6	69.2	64.3	48.0
IPK 000 254 12		179.8	351.4	306.5	225.3	30.3	13.2	13.1	-11.6	90.9	66.4	43.8	43.0

Table 1

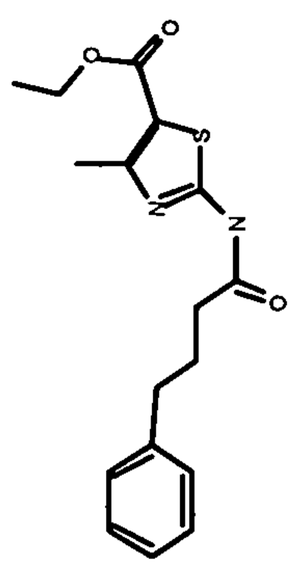
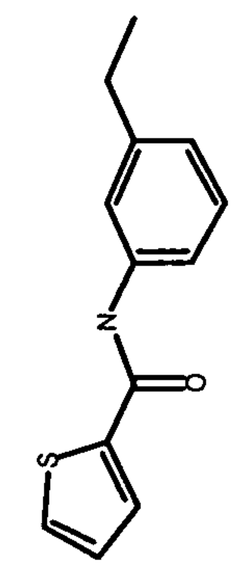
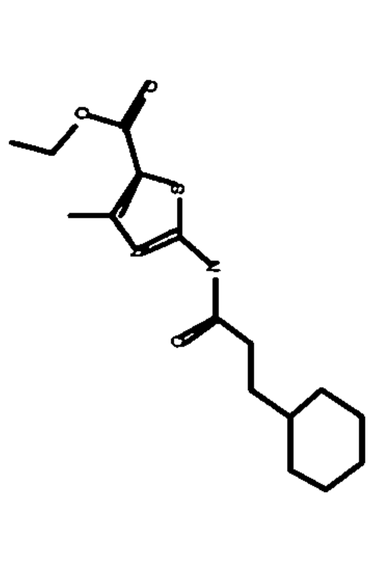
IPK 000254 25		
49.1	31.8	33.2
53.2	64.7	28.1
75.6	68.7	60.5
100.5	96.3	31.8
20.5	32.6	15.8
20.0	54.7	42.1
18.5	53.1	79.9
32.5	25.0	71.5
356.9	408.3	314.1
356.3	499.5	381.8
367.0	279.6	226.9
243.3	192.3	285.0
IPK 000255 46		IPK 000257 61

Table 1

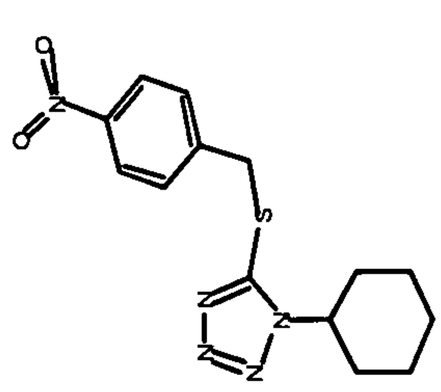
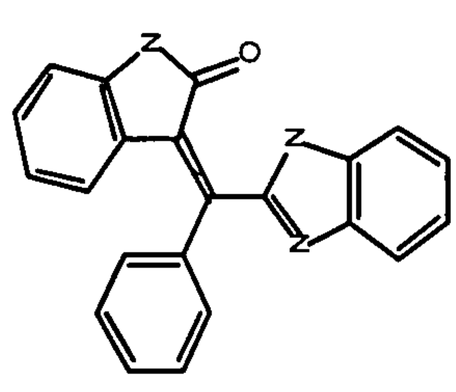
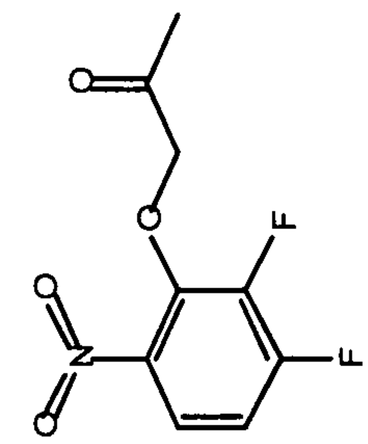
IPK 000 258 07		37.0	36.8	47.9	98.4	9.4	26.2	72.7	49.9	240.9	380.6	174.8	142.3
IPK 000 259 35		40.2	42.5	28.4	10.9	5.3	12.0	68.8	66.0	335.9	262.1	78.8	64.5
IPK 000 259 78		30.2	25.0	83.6	91.4	14.9	11.0	58.8	4.9	292.8	271.1	194.3	115.8

Table 1

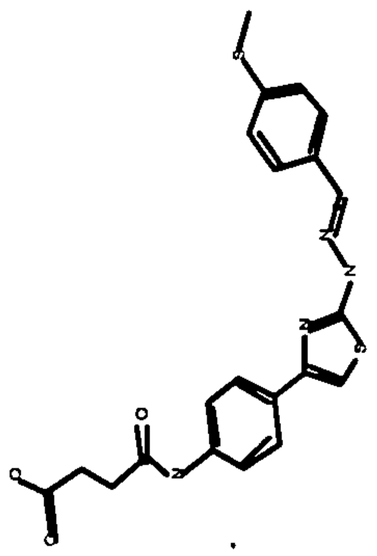
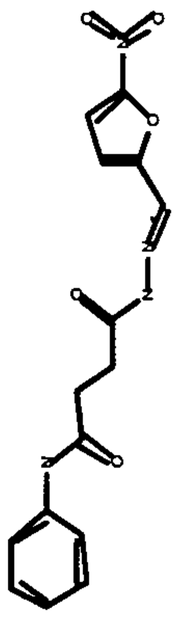
IPK 000 262 07	IPK 000 262 39
	
242.0	93.3
309.8	273.3
461.8	164.5
383.3	349.1
34.3	75.4
99.0	98.5
40.1	20.4
89.0	48.7
7.9	29.5
80.3	2.1
61.4	60.0
45.9	43.8

Table 2

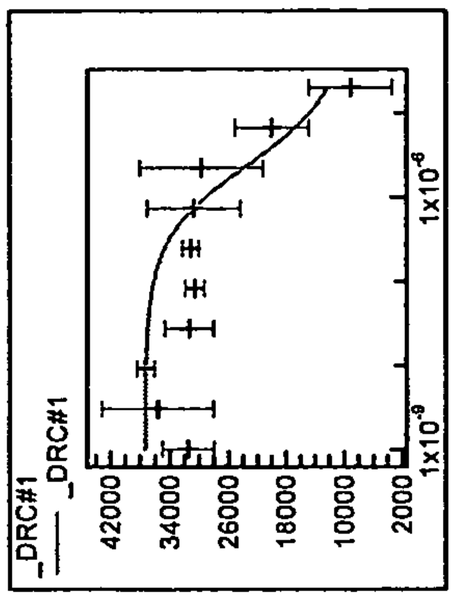
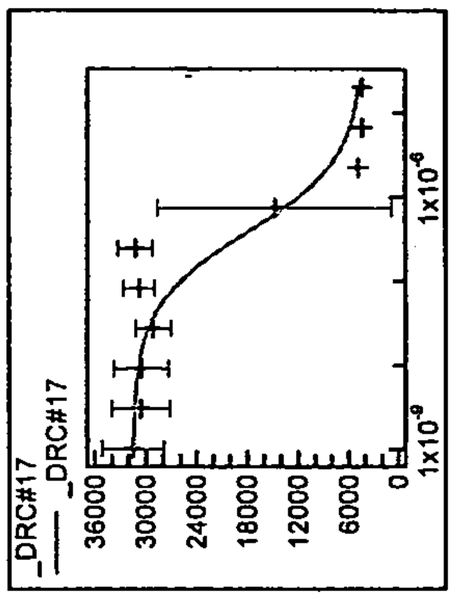
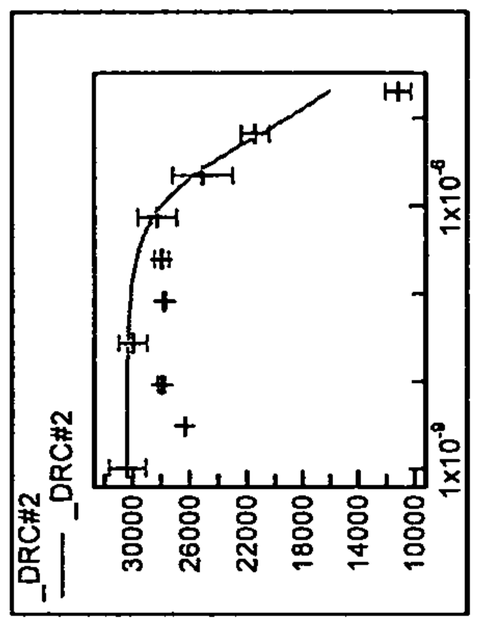
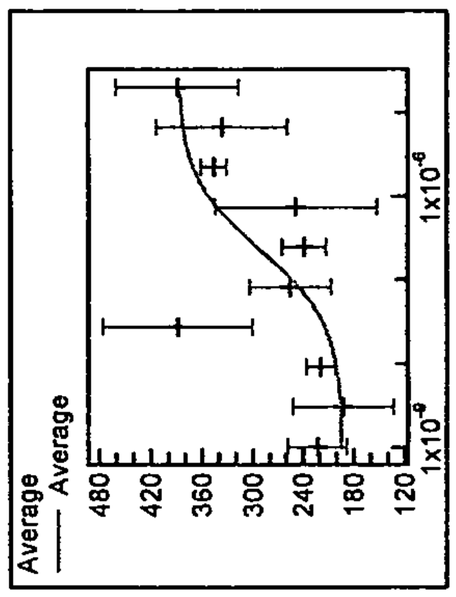
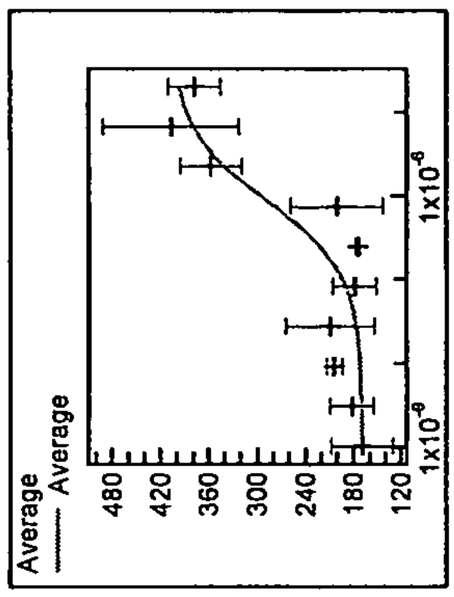
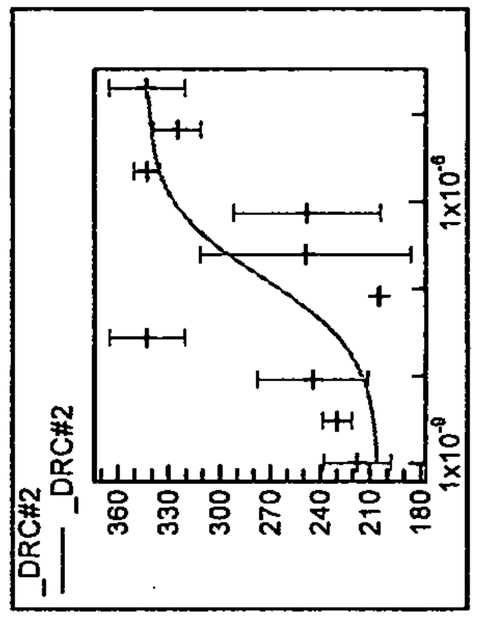
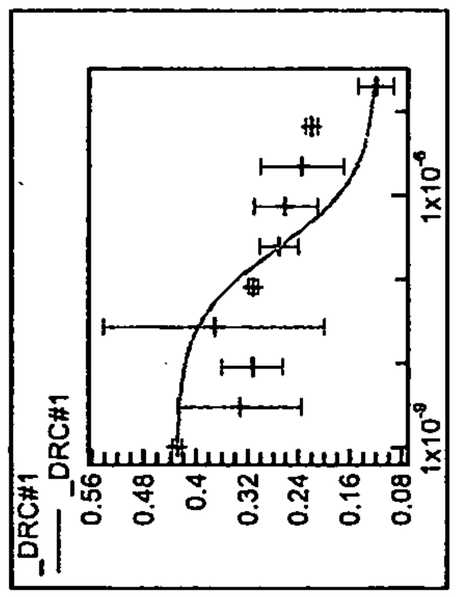
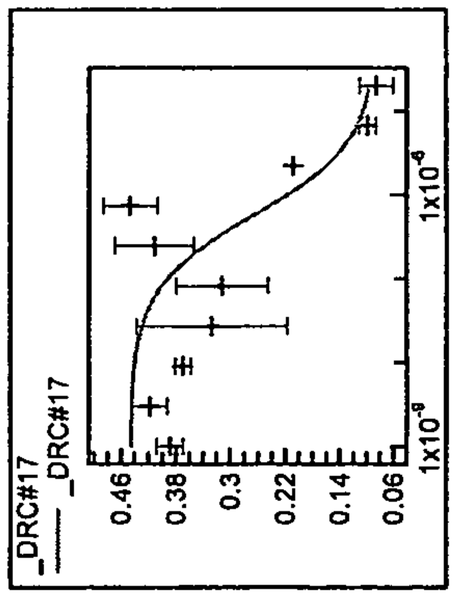
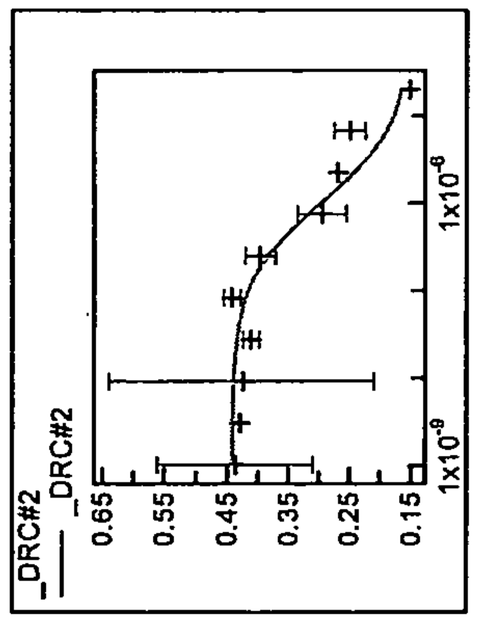
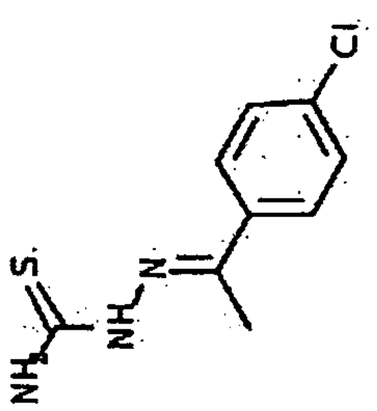
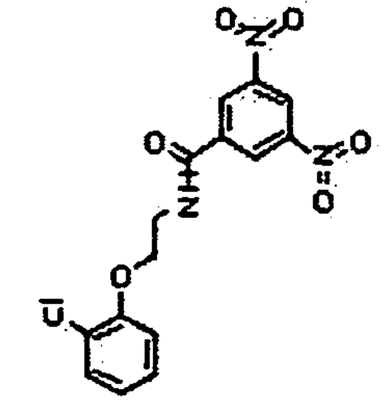
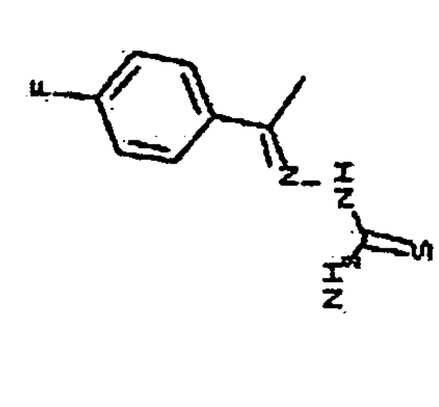
	QUM		
Cell number			
			
	QIM		
			
	IPK00000217	Scaffold IV	IPK00002772	Scaffold II	IPK00000219	Scaffold IV
						

Table 2

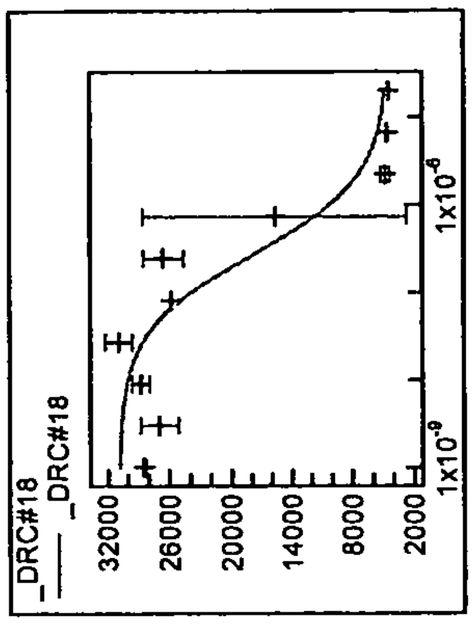
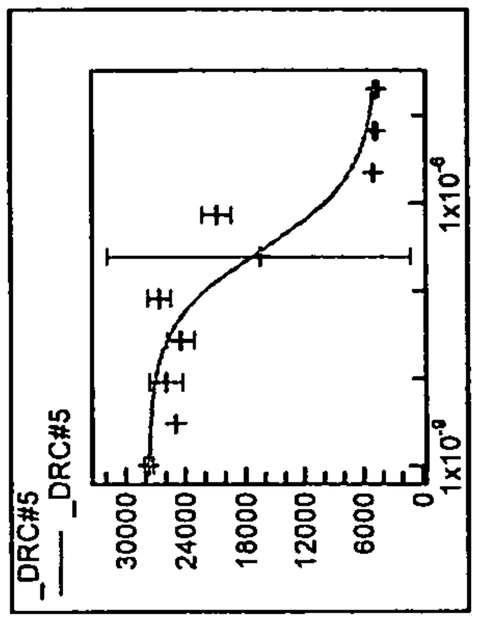
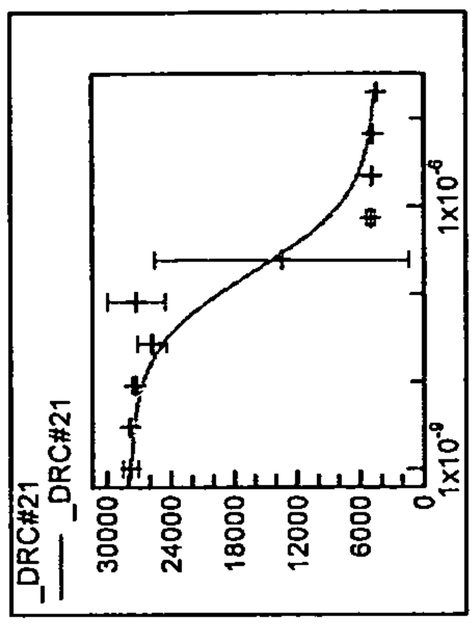
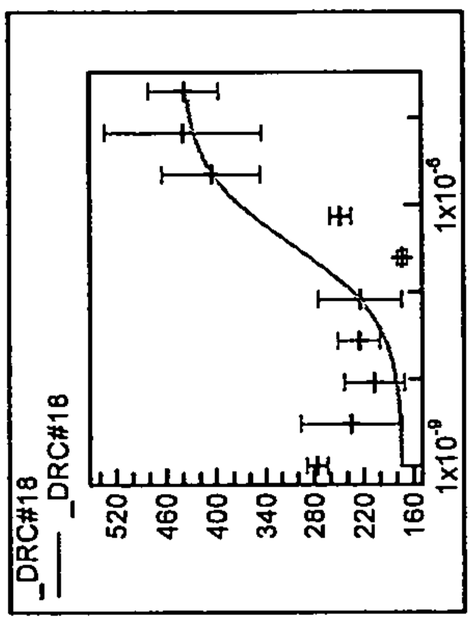
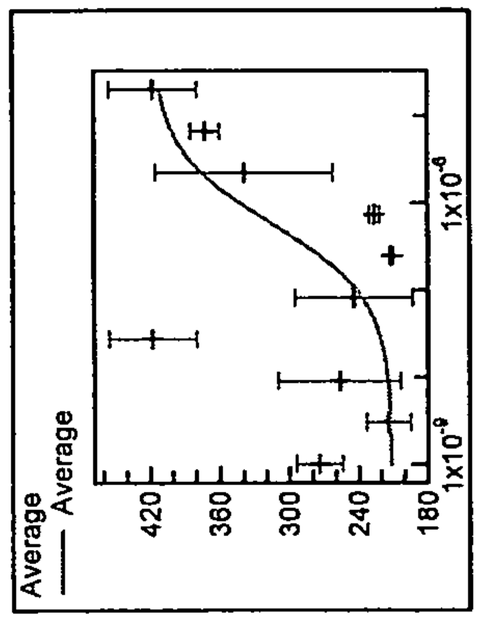
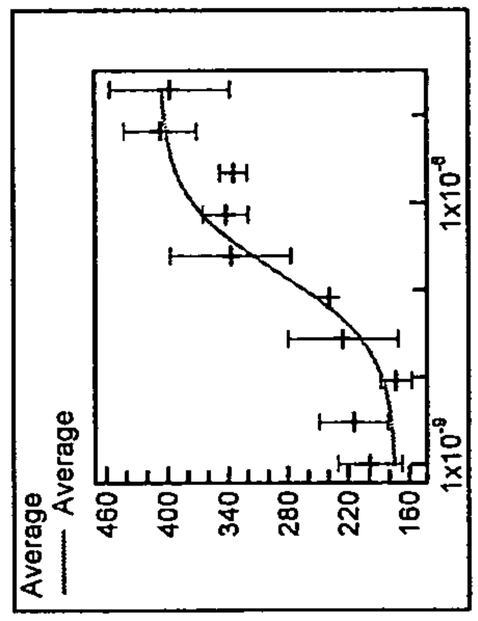
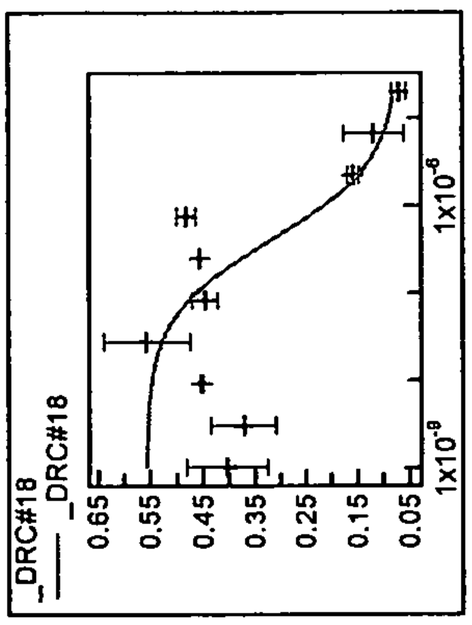
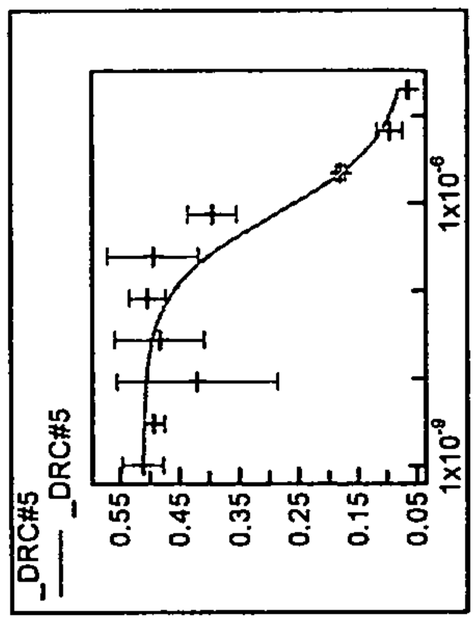
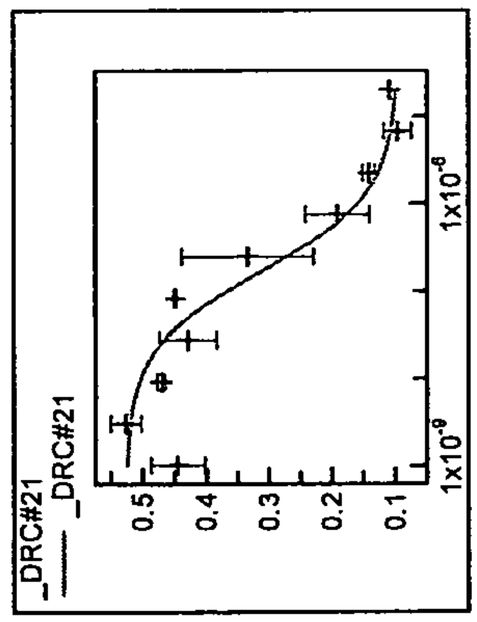
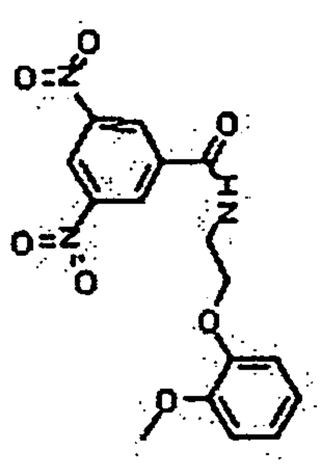
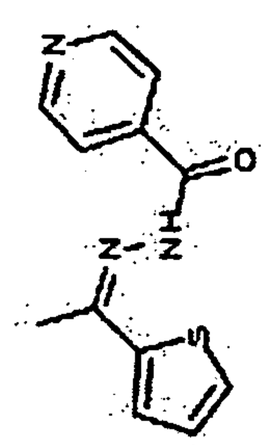
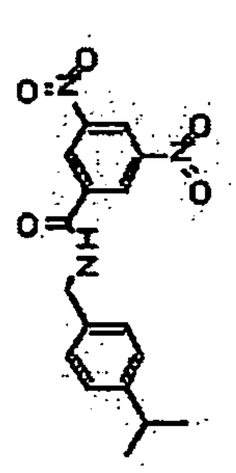
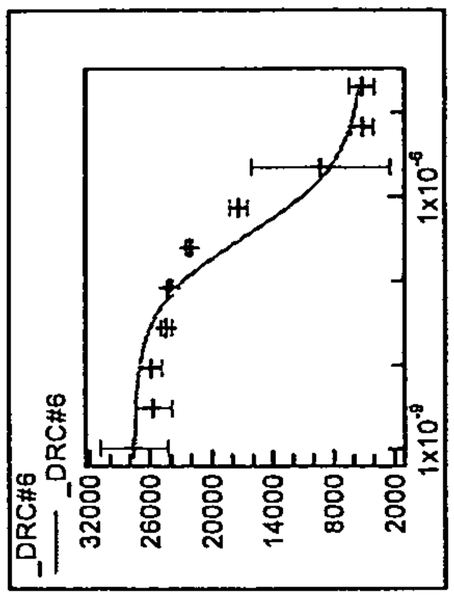
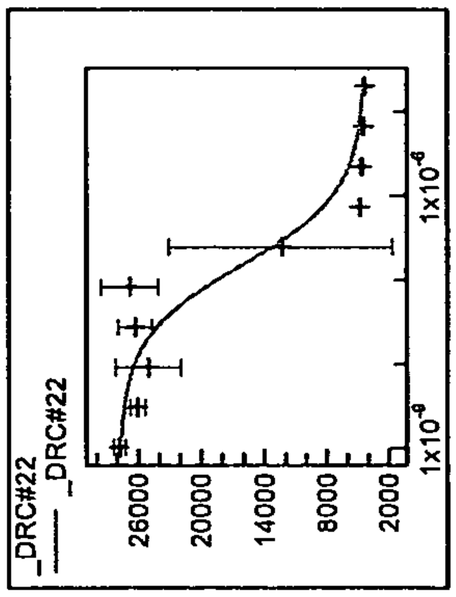
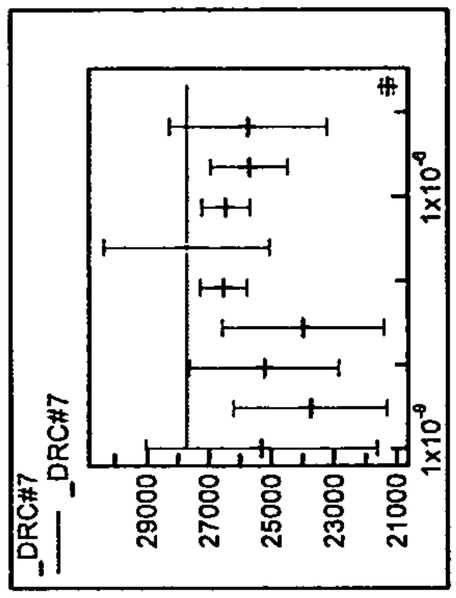
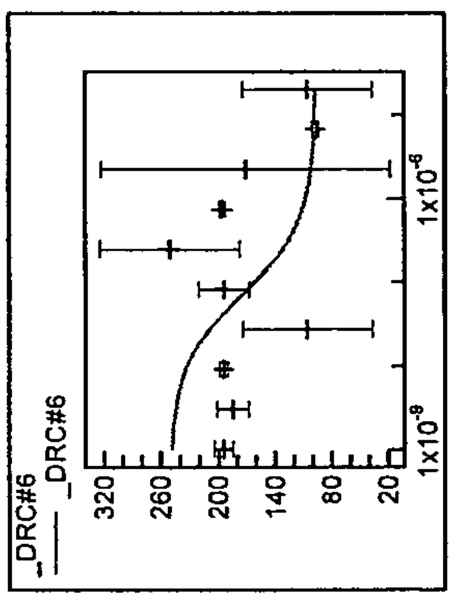
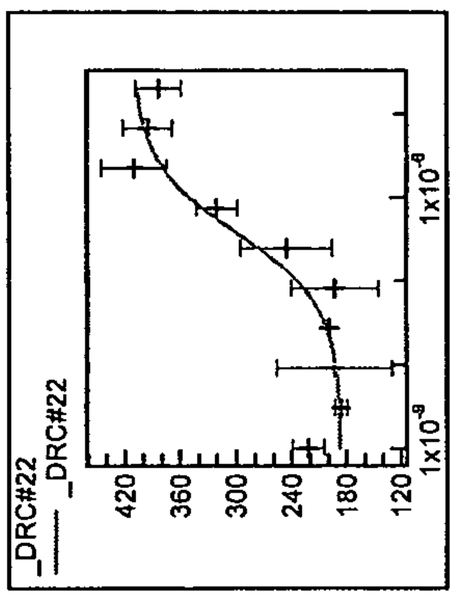
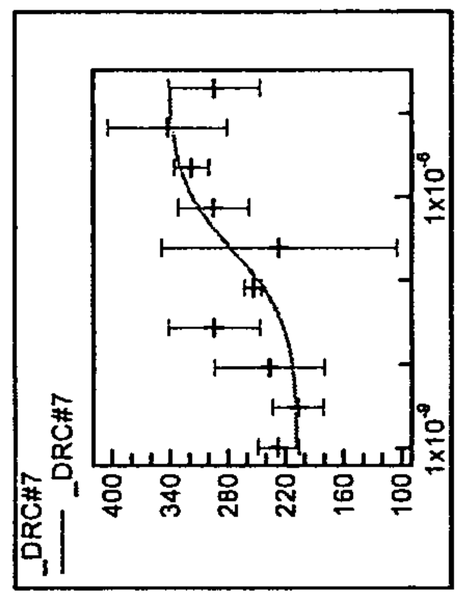
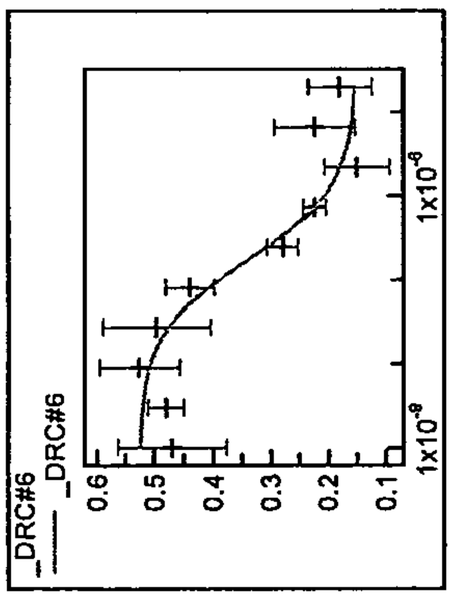
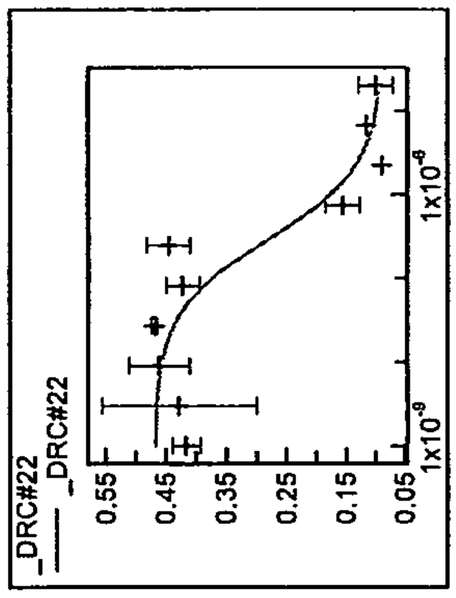
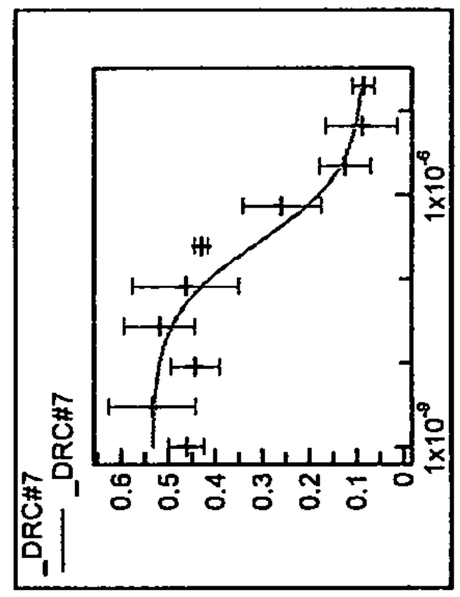
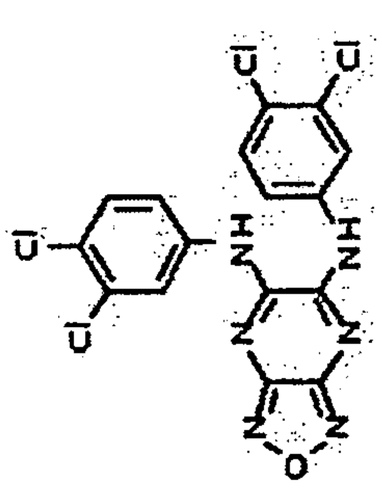
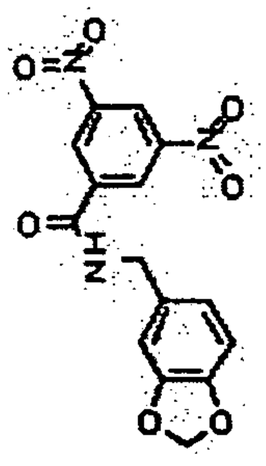
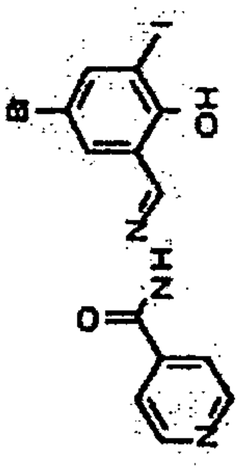
QUM			
Cell number			
QIM			
	IPK00002774	Scaffold II	IPK00000301	Scaffold I	IPK00002778	Scaffold II
						

Table 2

	IPK00000389	Scaffold XX	IPK00002785
QUM			
Cell number			
QIM			
		Scaffold II		Scaffold I	

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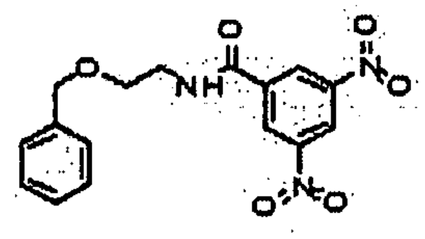
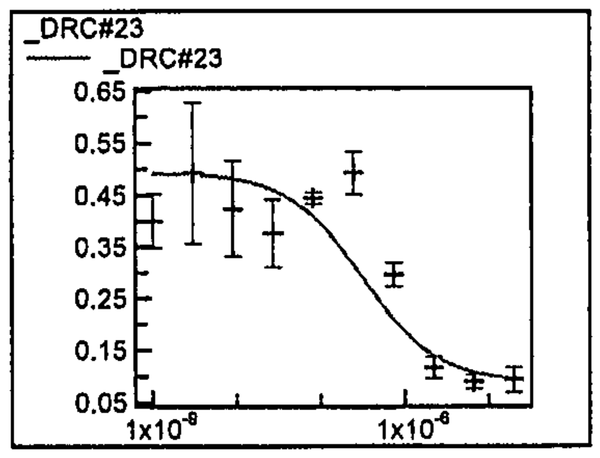
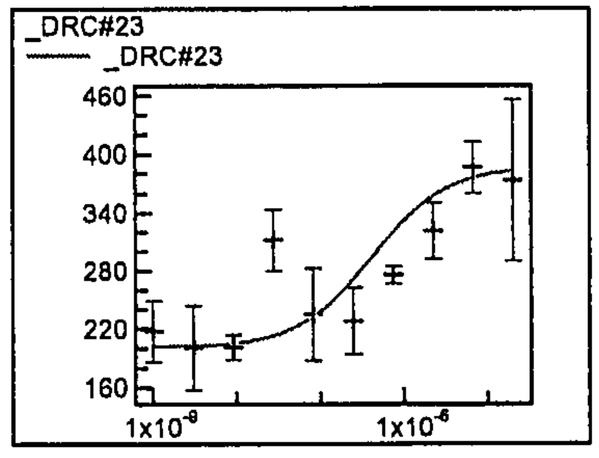
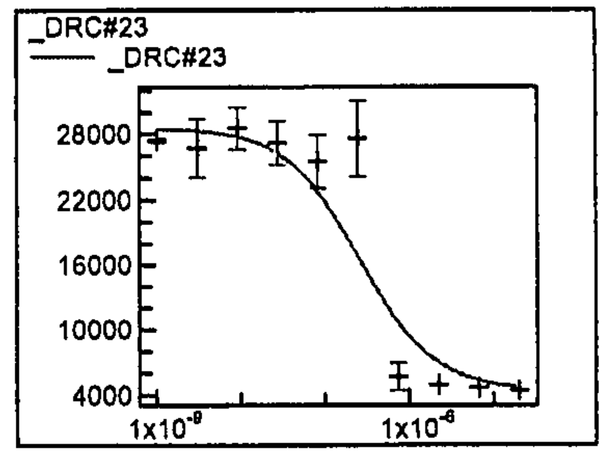
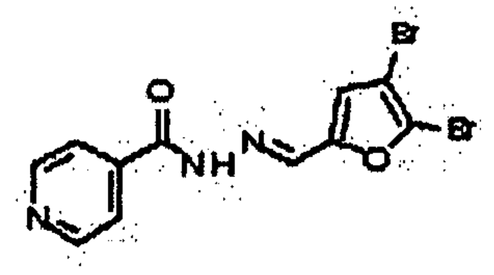
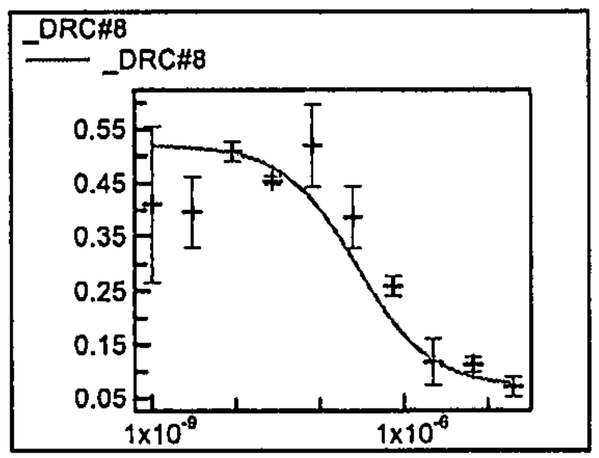
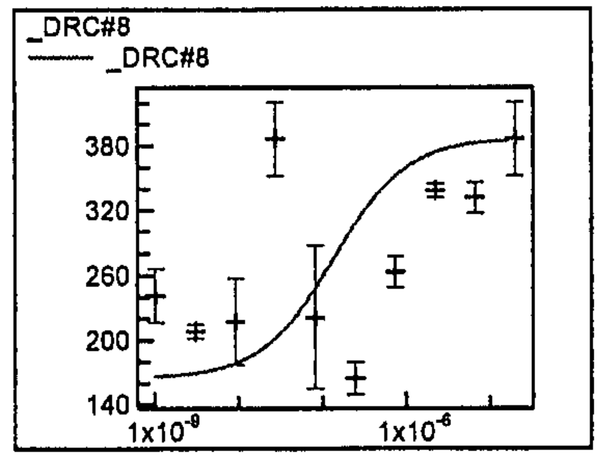
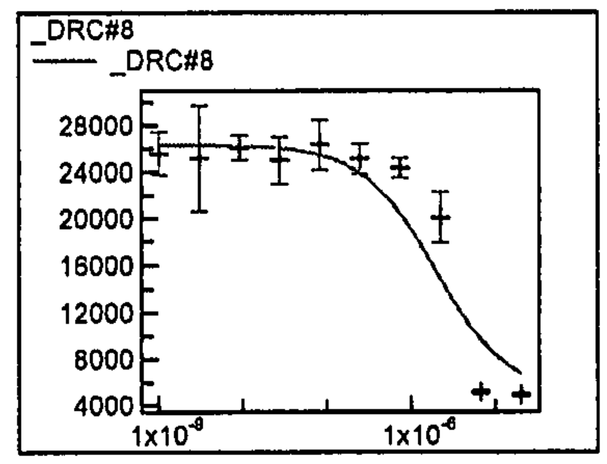
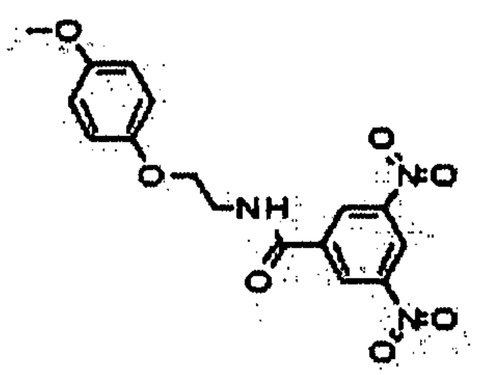
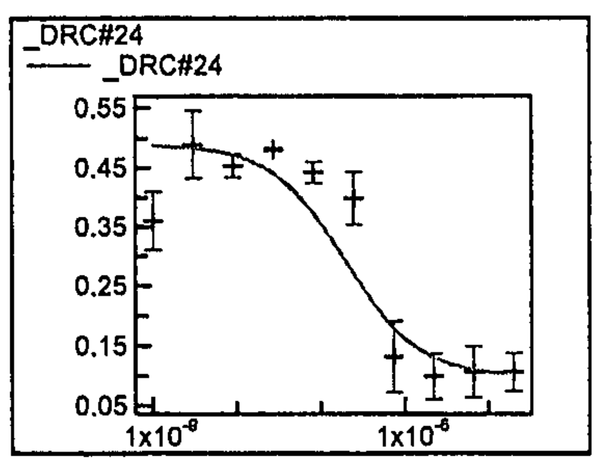
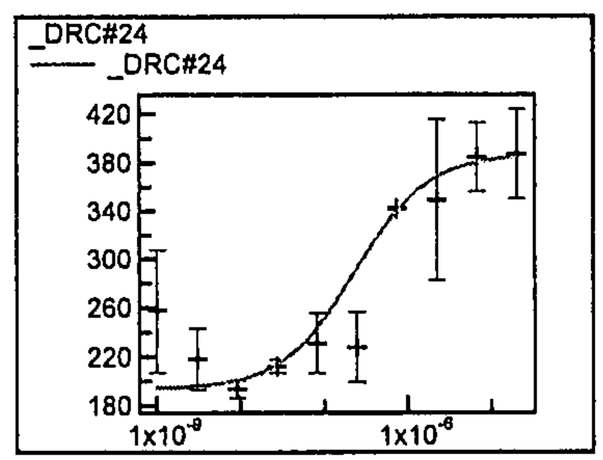
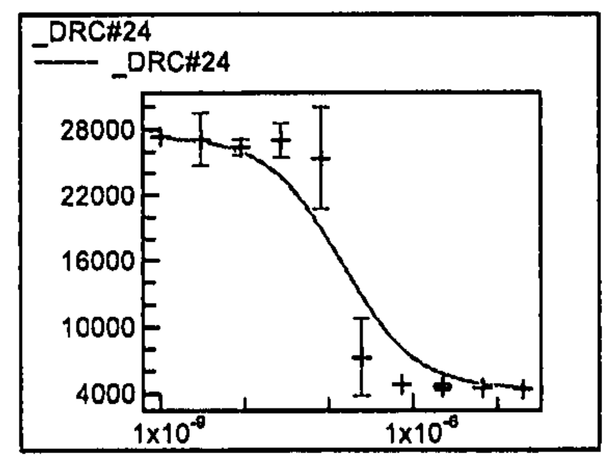
IPK00002791  Scaffold II			
IPK00000941  Scaffold I			
IPK00002835  Scaffold II			

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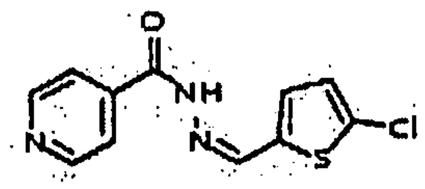
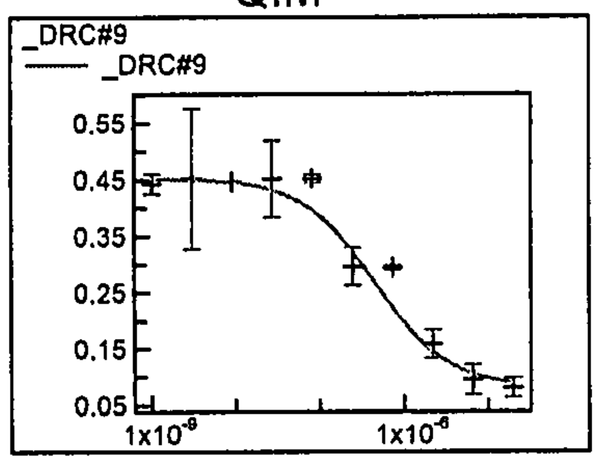
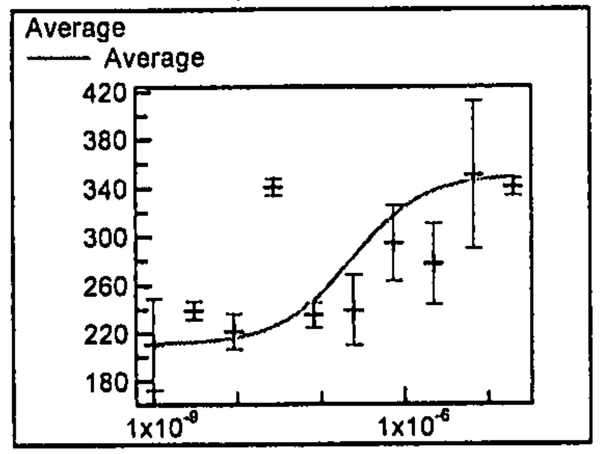
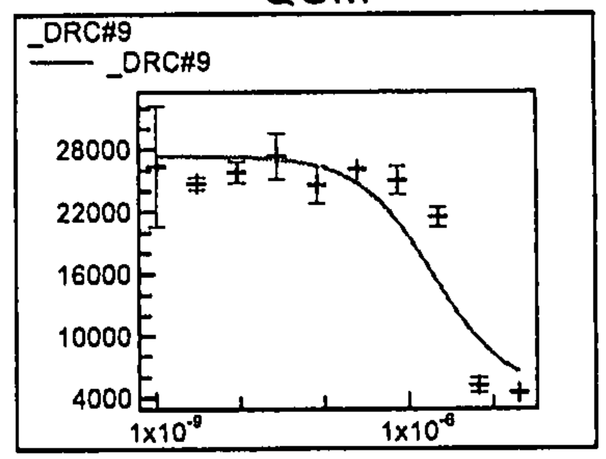
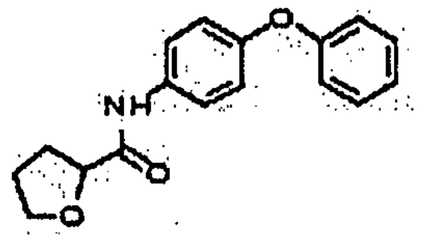
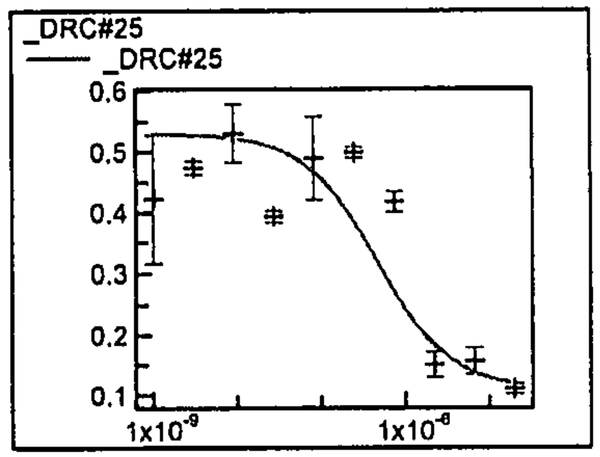
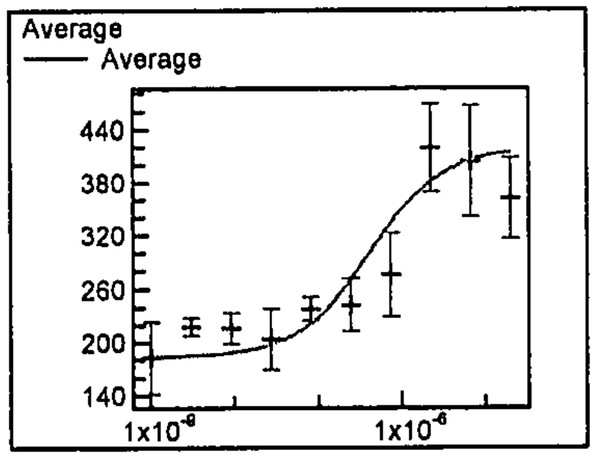
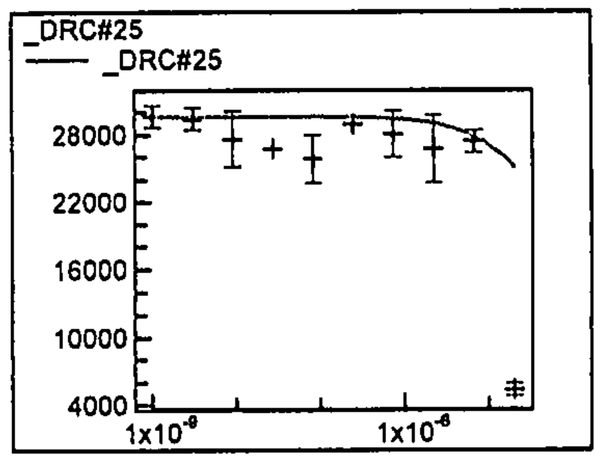
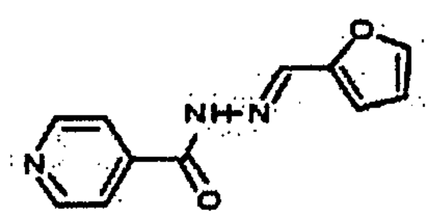
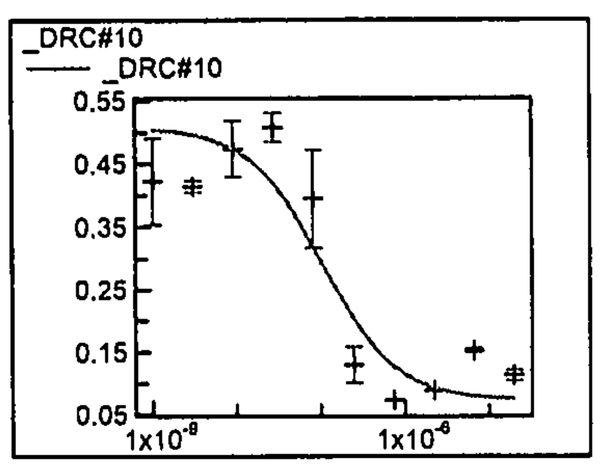
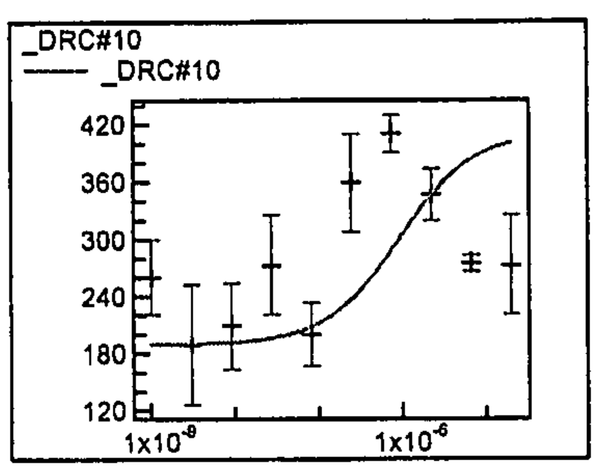
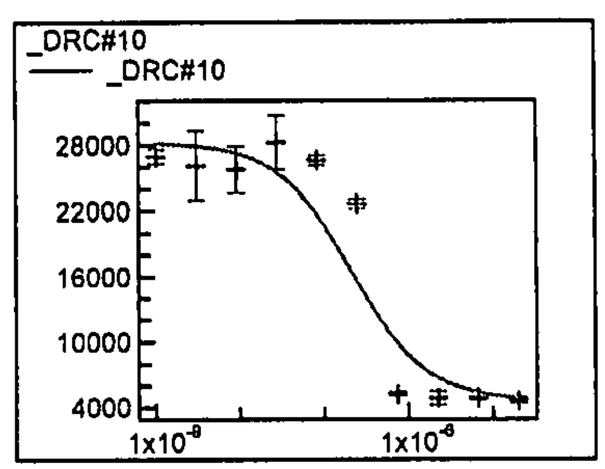
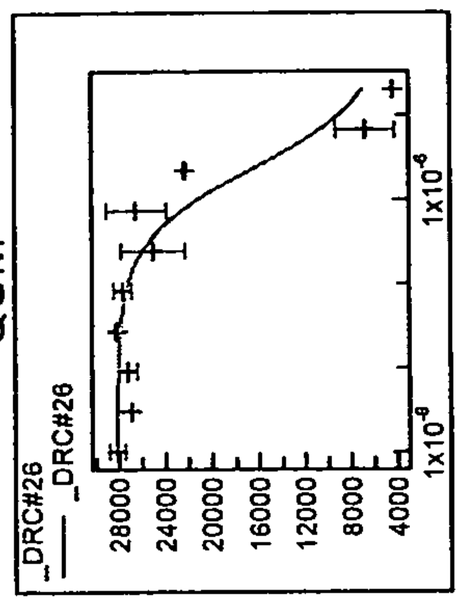
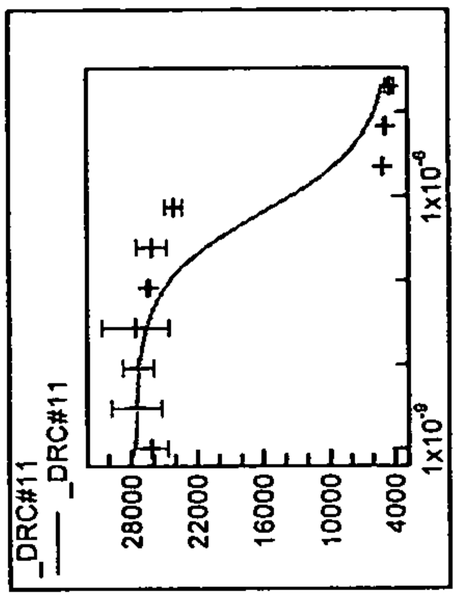
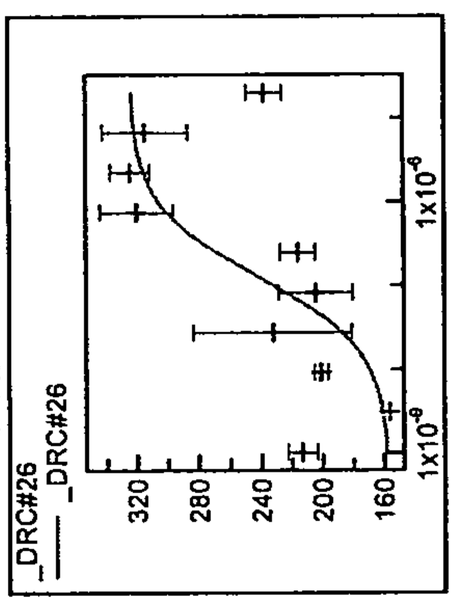
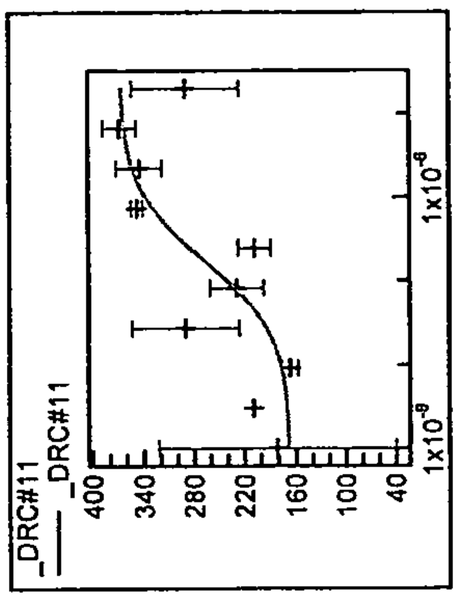
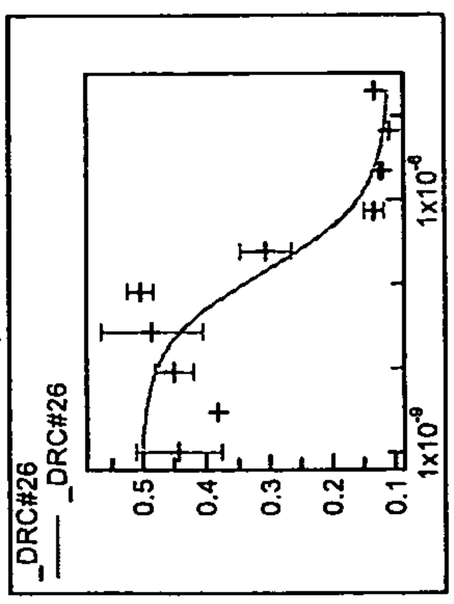
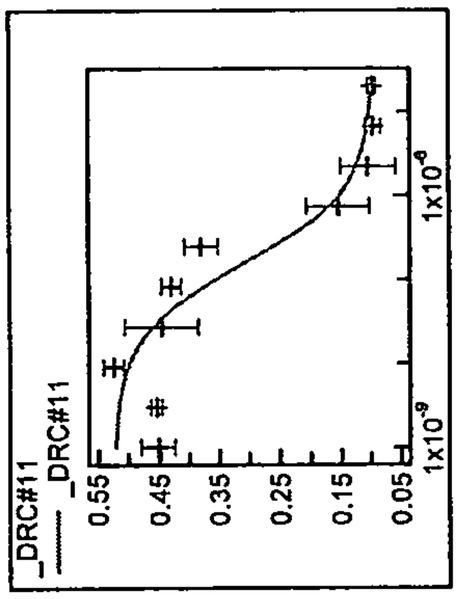
IPK00000942  Scaffold I			
IPK00003316  Scaffold XIX			
IPK00001367  Scaffold I			

Table 2

Table 2

QUM		
Cell number		
QIM		
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	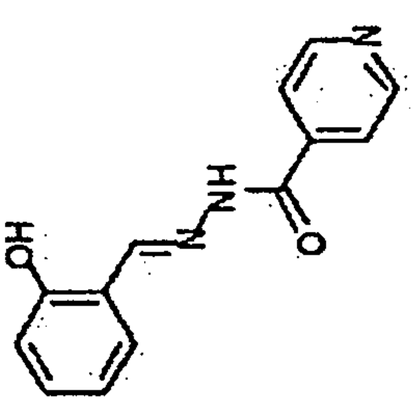	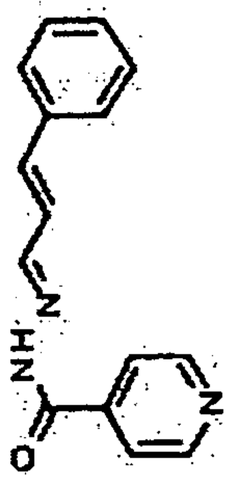
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	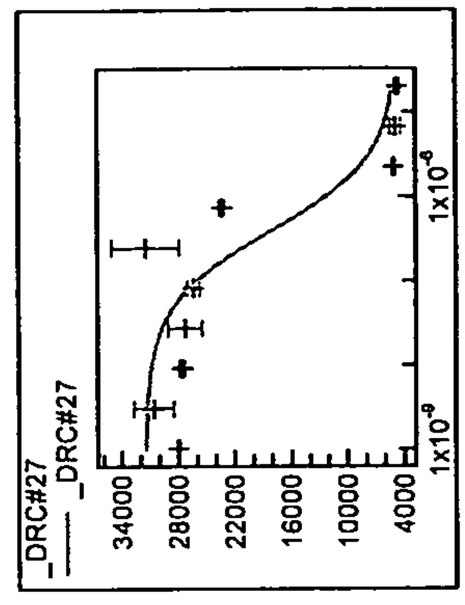
	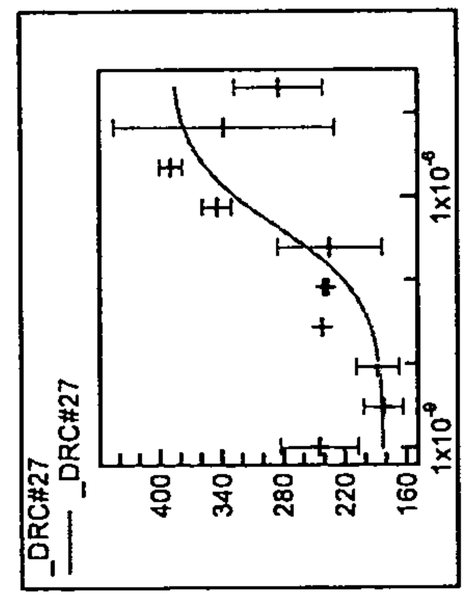
	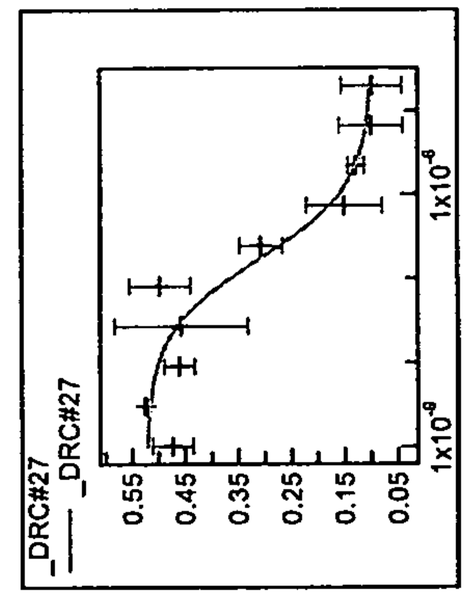
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	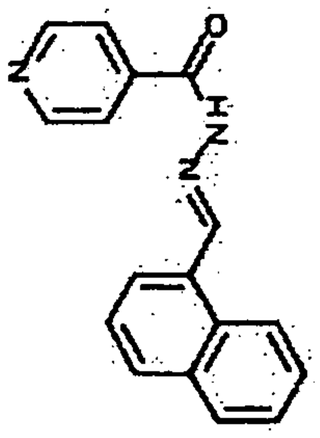
	Scaffold I

Table 2

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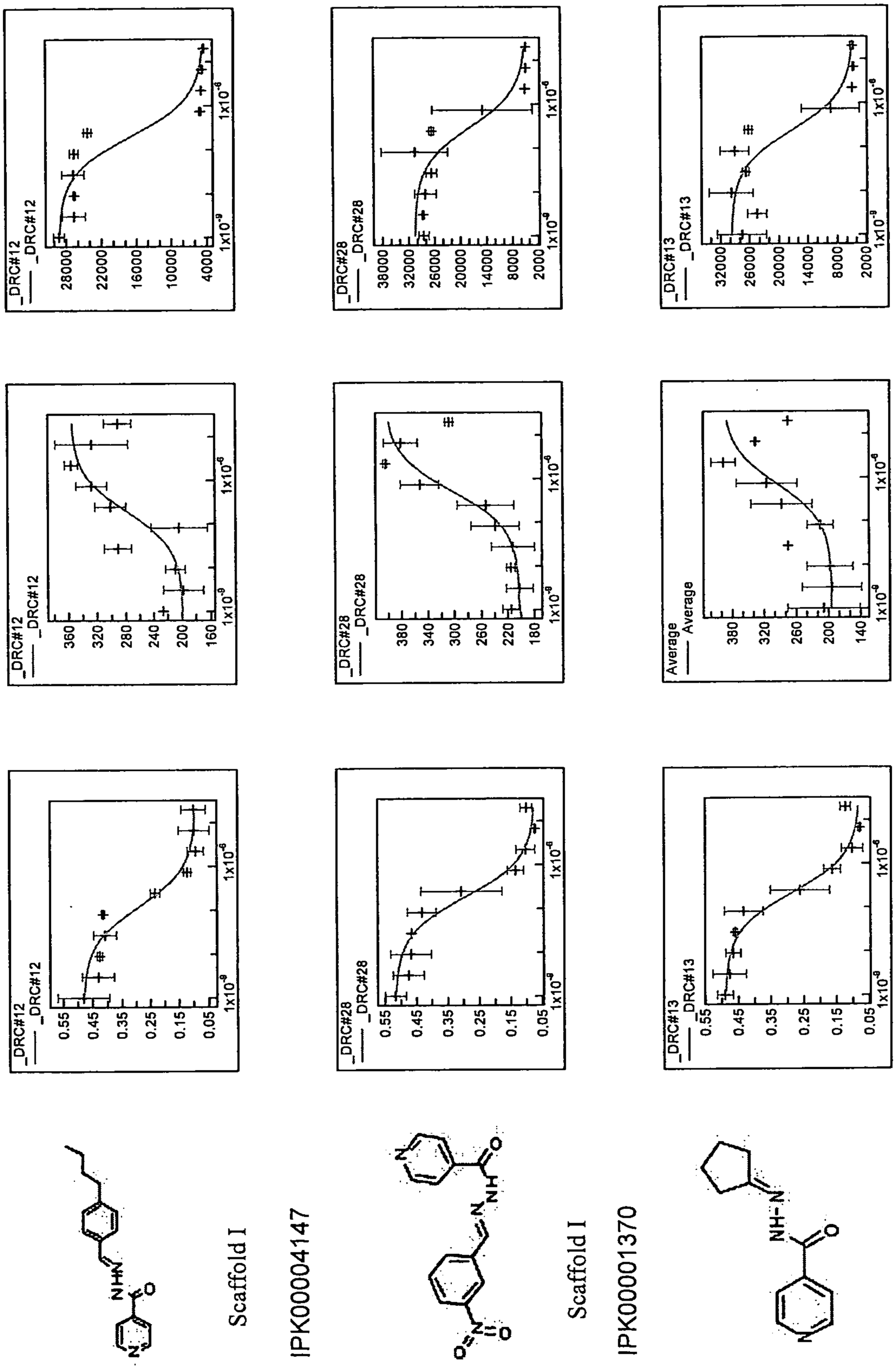
IPK00001370	Scaffold I		



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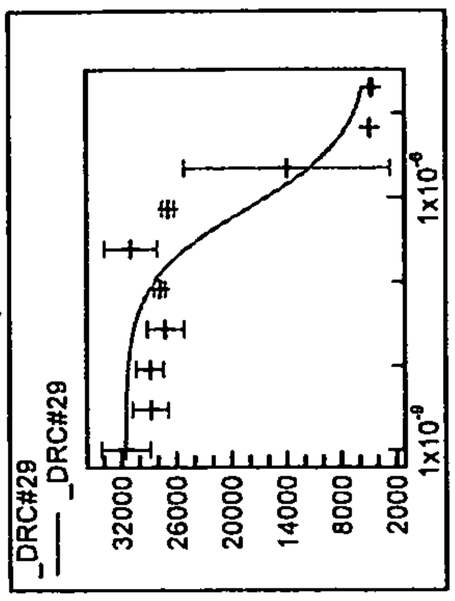
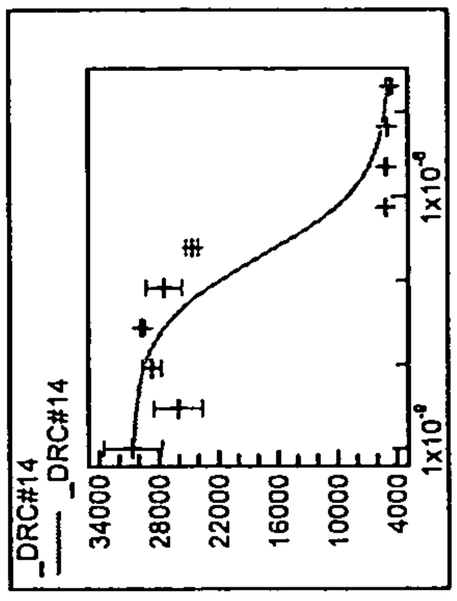
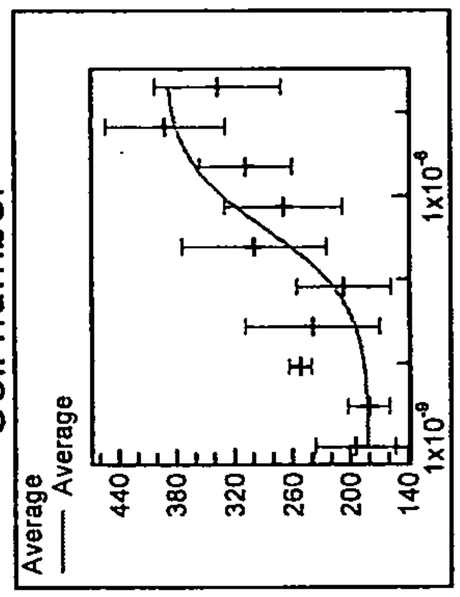
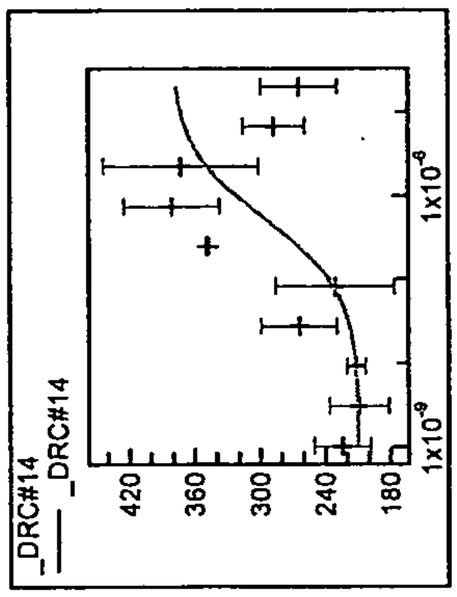
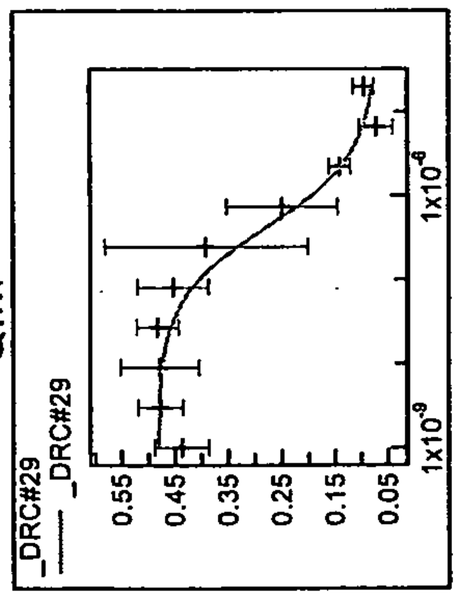
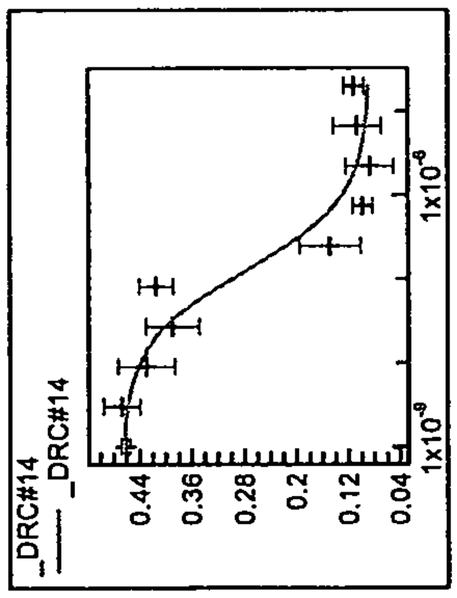
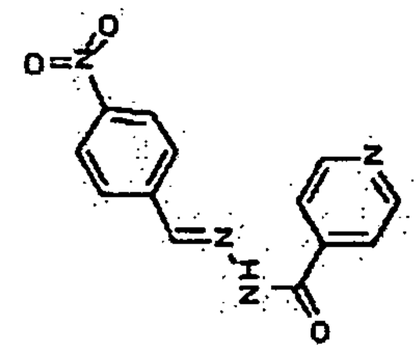
QUM		
Cell number		
QIM		
IPK00004148		
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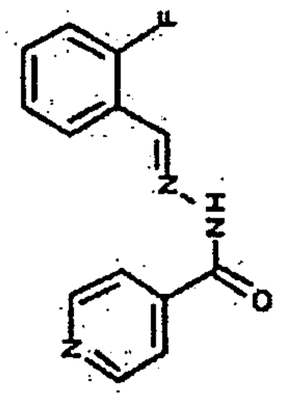
IPK00001371		
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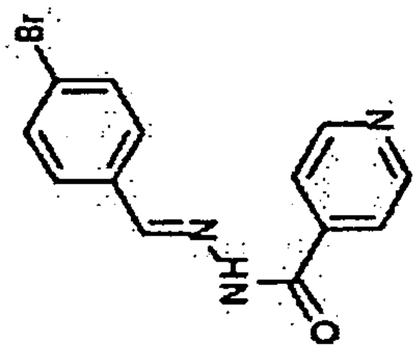
IPK00004151		
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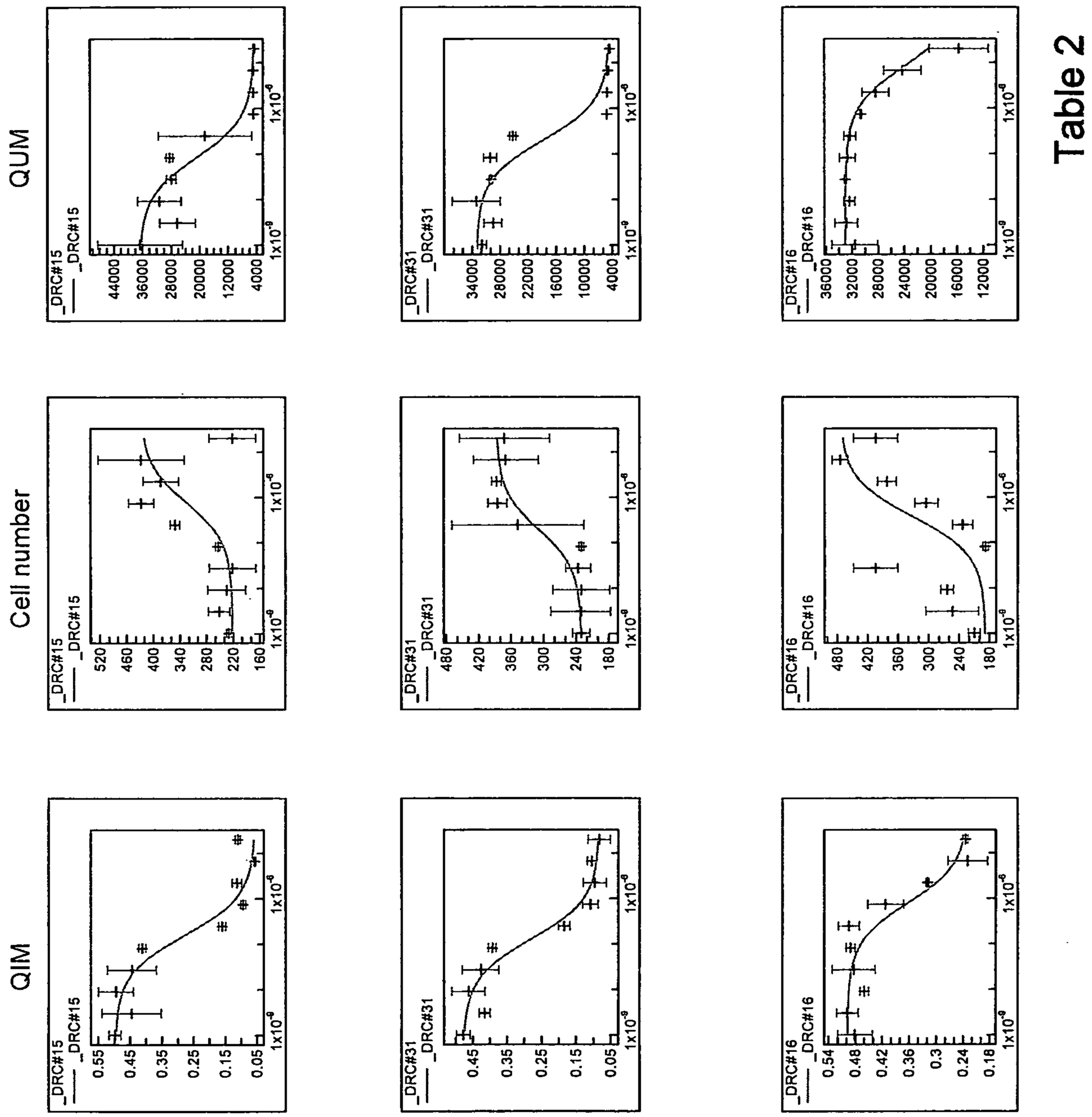
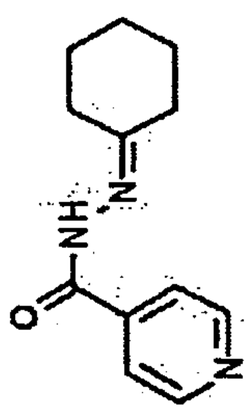
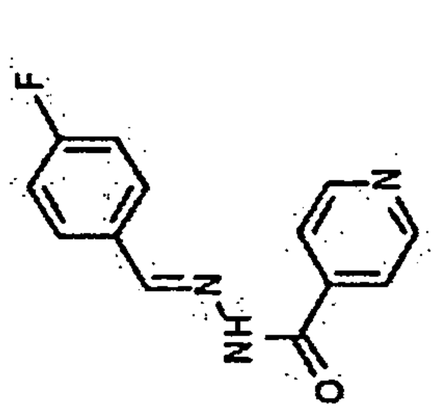




Scaffold I

IPK00004152



Scaffold I

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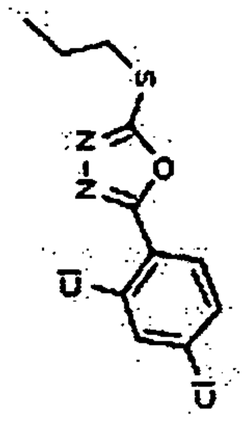
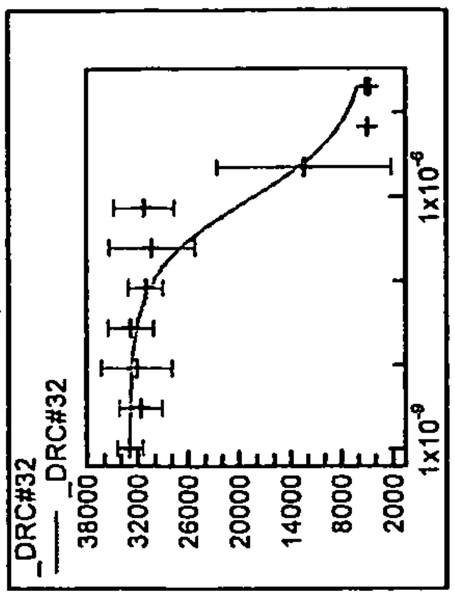
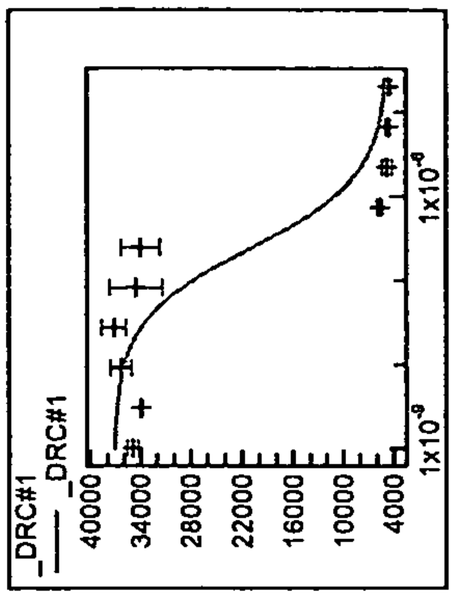
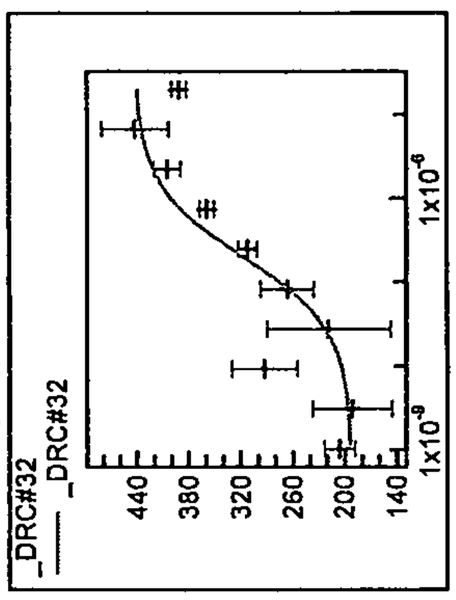
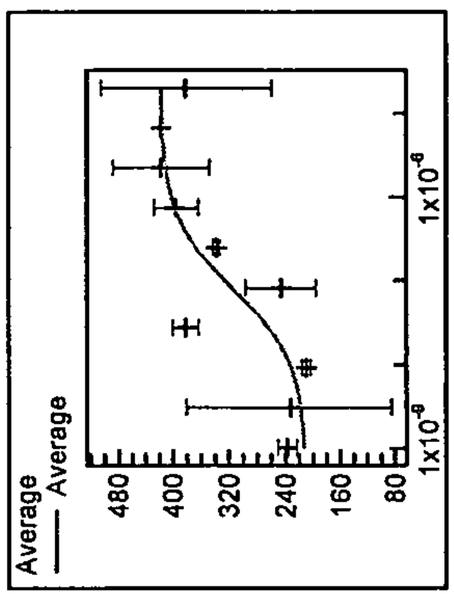
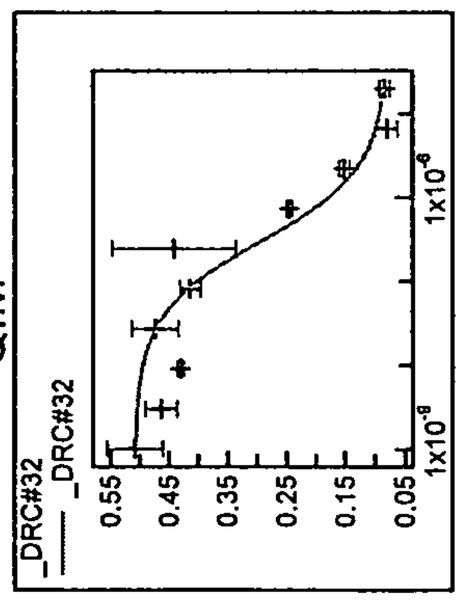
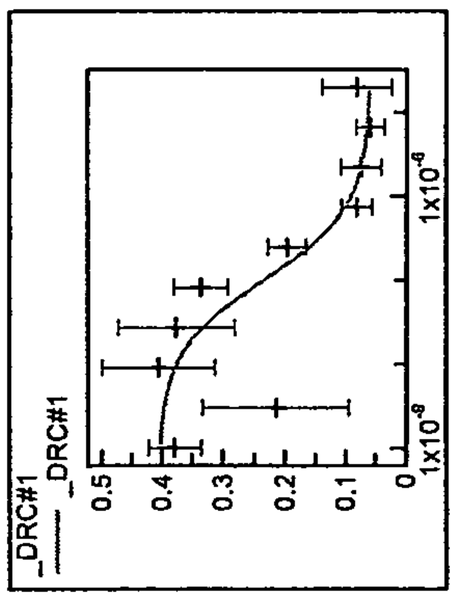


Scaffold XII

Table 2

QUM		
Cell number		
QIM		
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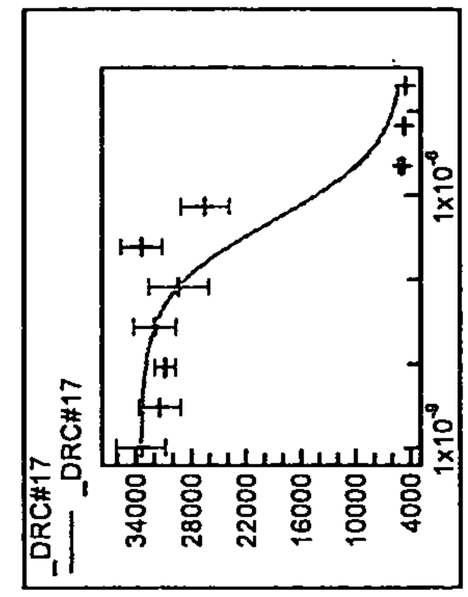
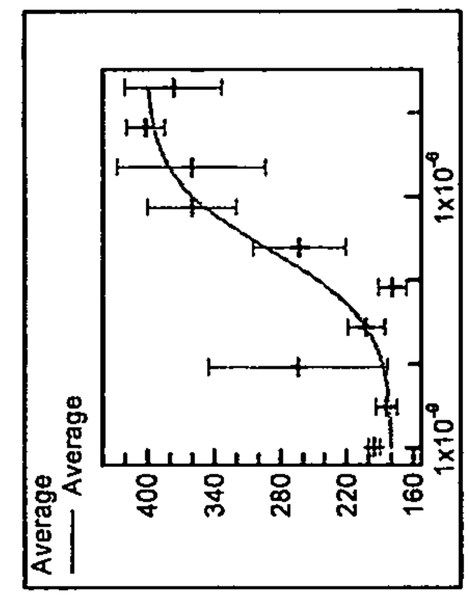
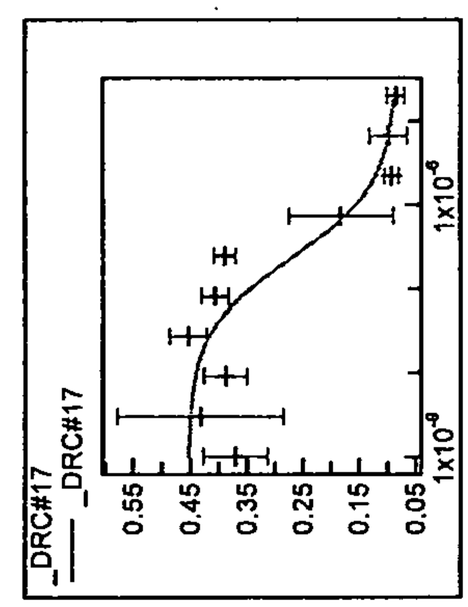
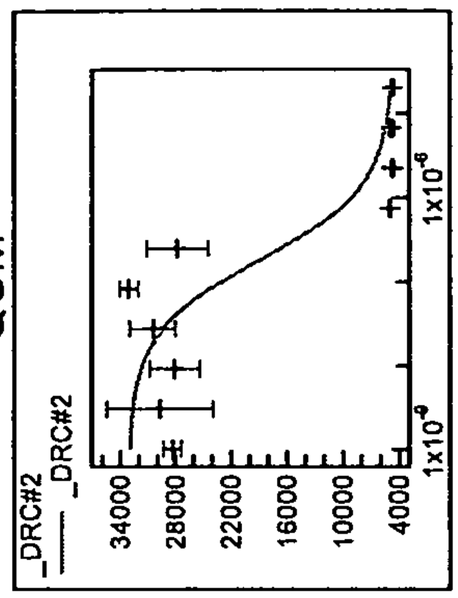
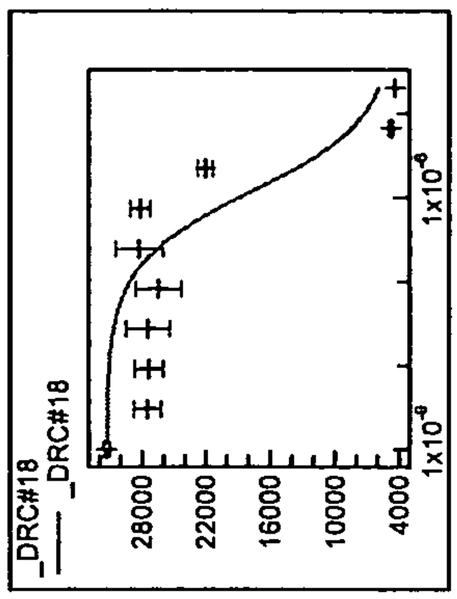
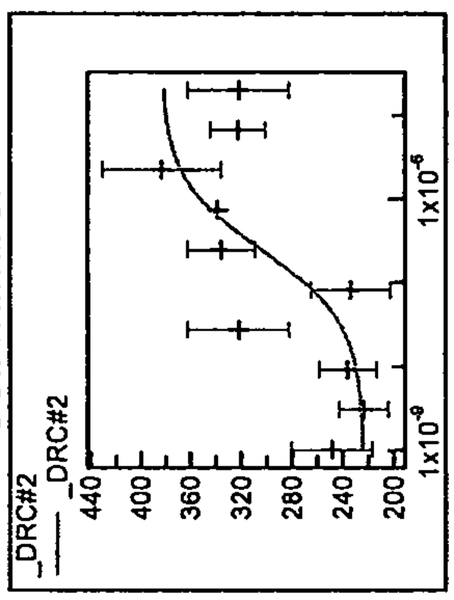
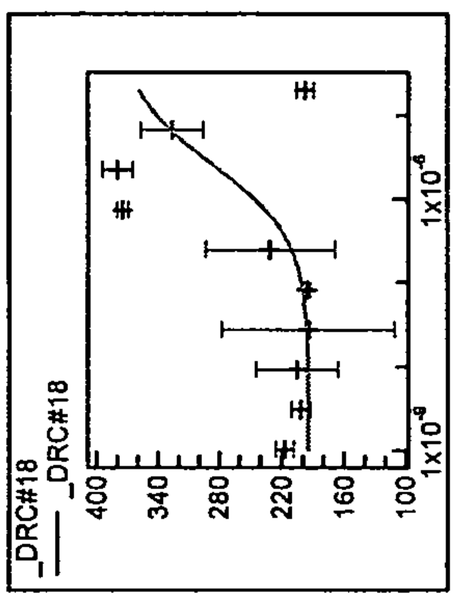
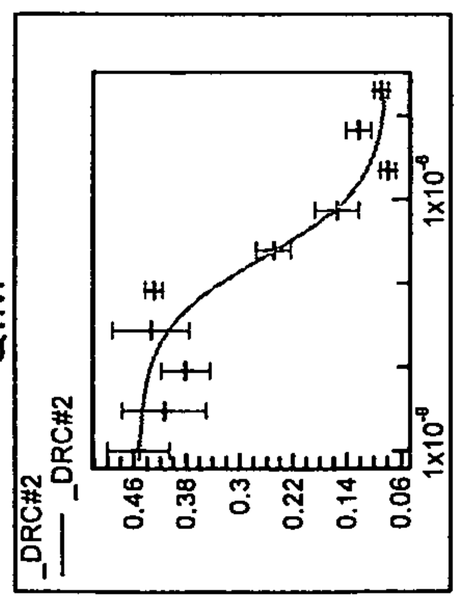
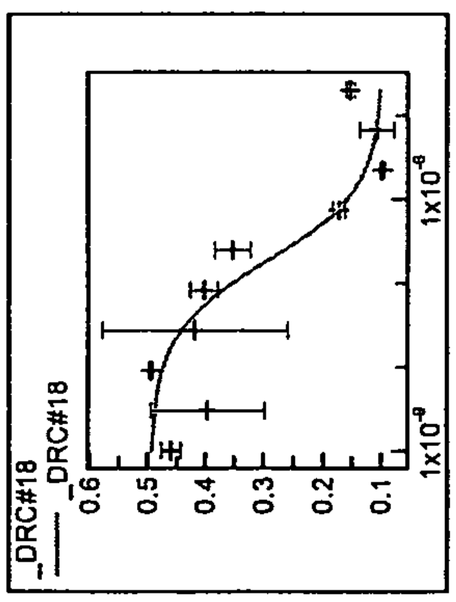
	
	Scaffold I IPK00004307
	Scaffold I

Table 2

QUM		
Cell number		
QIM		
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	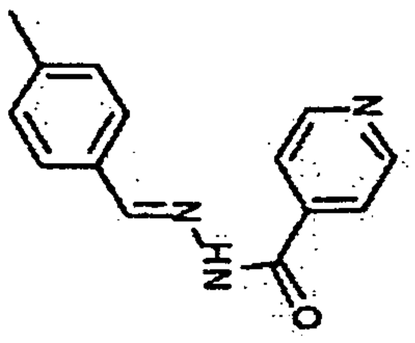	
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	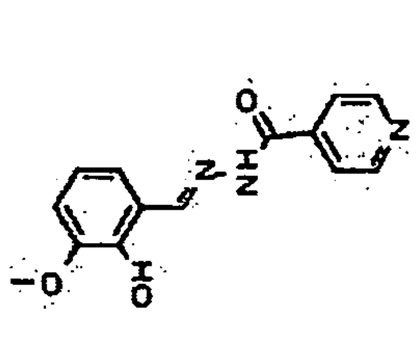	
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	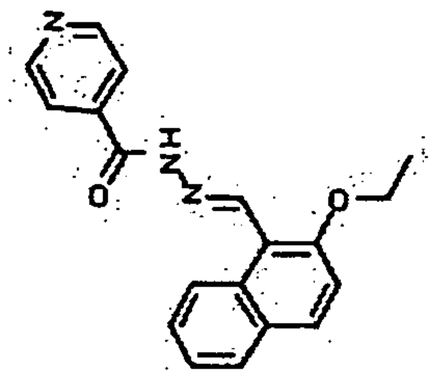	

	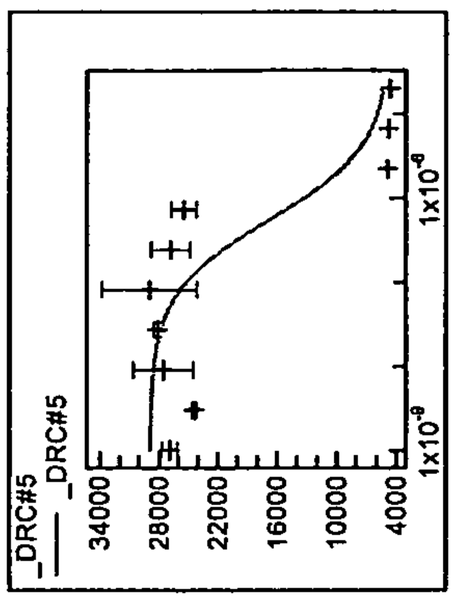	
	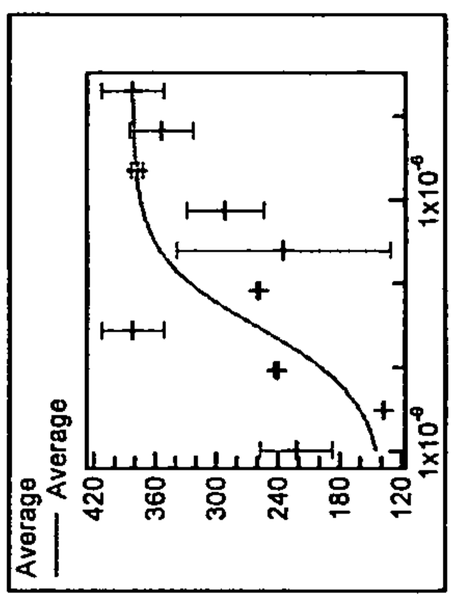	
	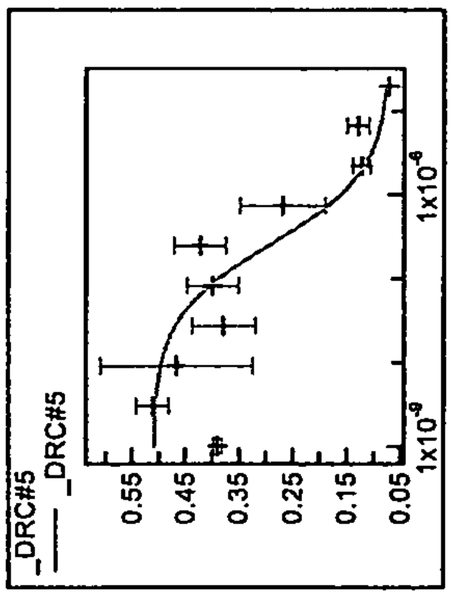	

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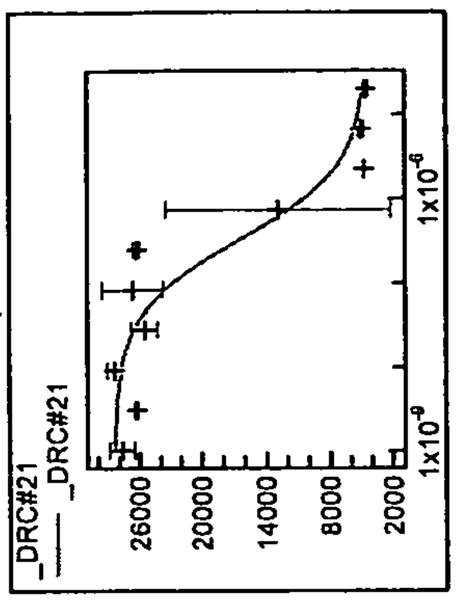
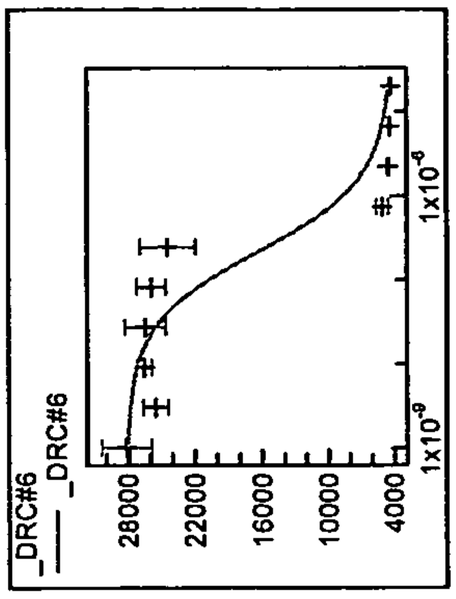
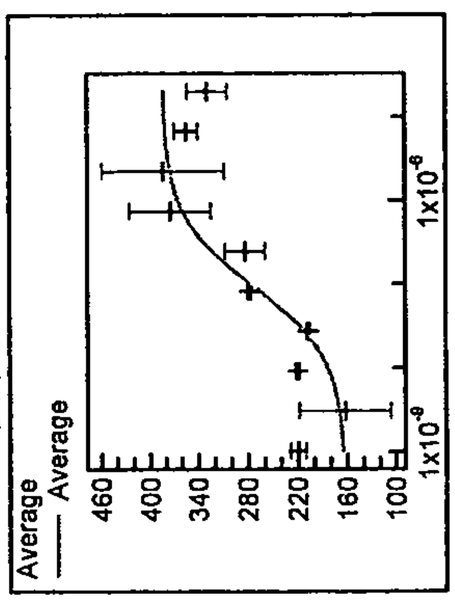
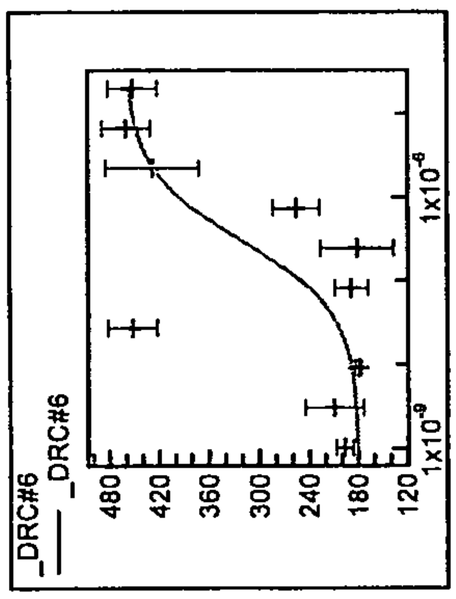
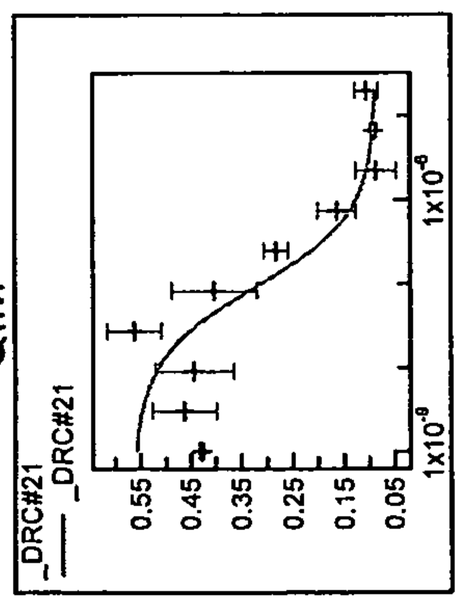
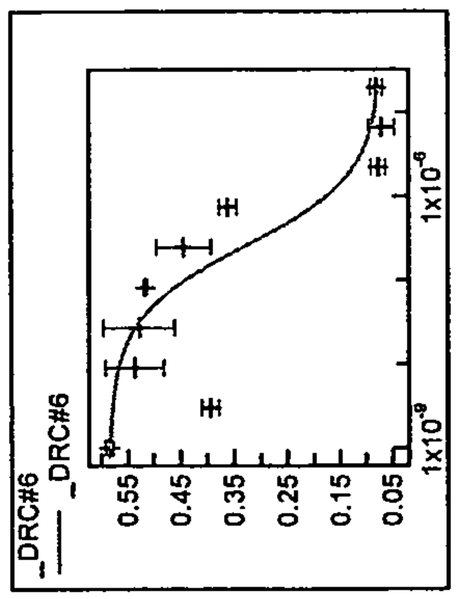
QUM		
Cell number		
QIM		
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	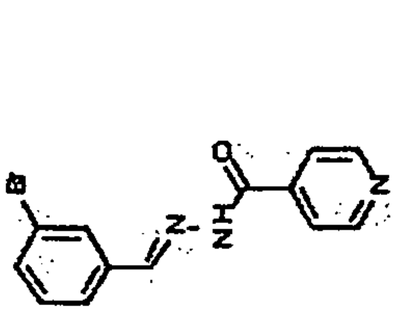	
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	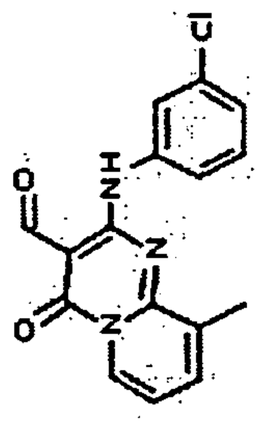	
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	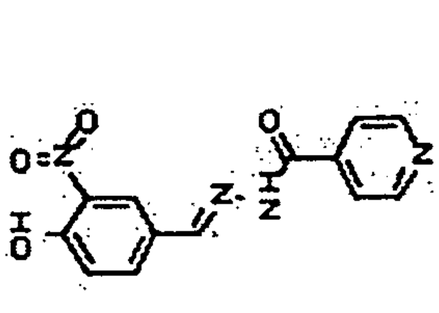	

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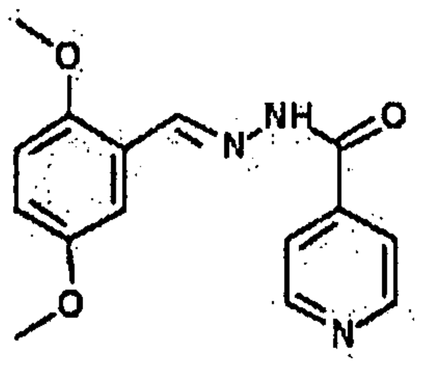
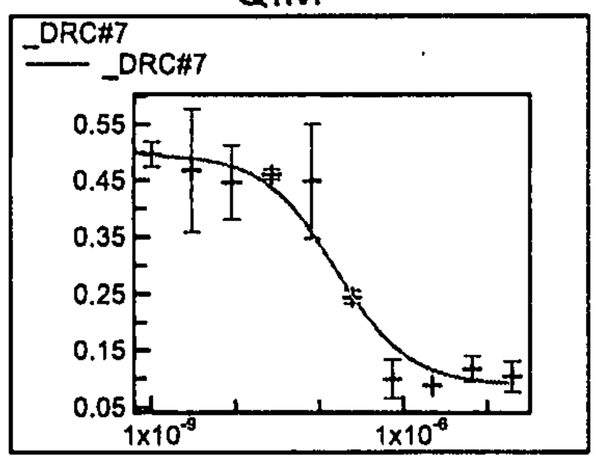
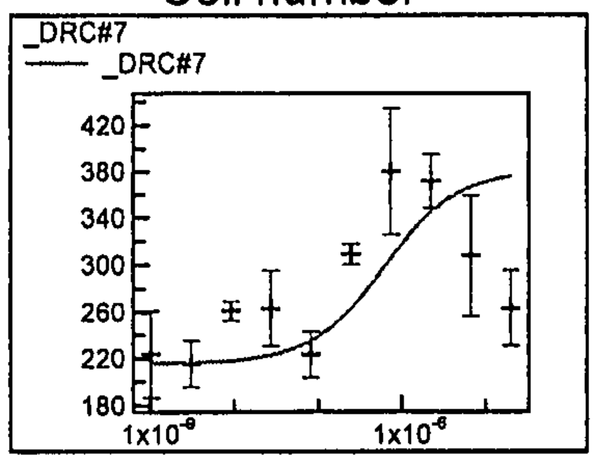
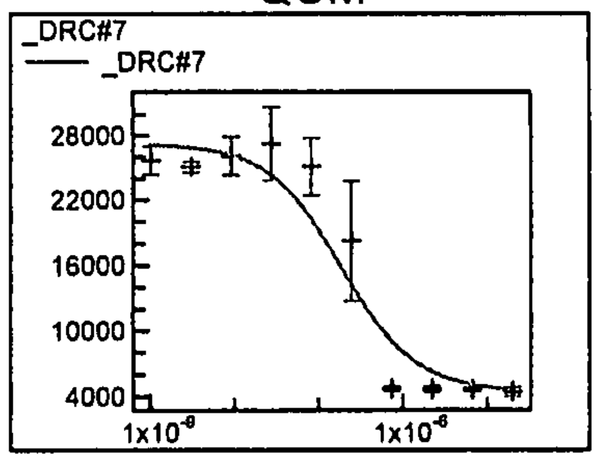
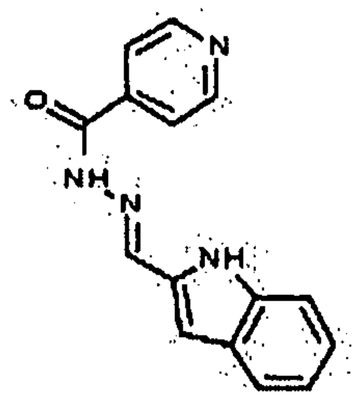
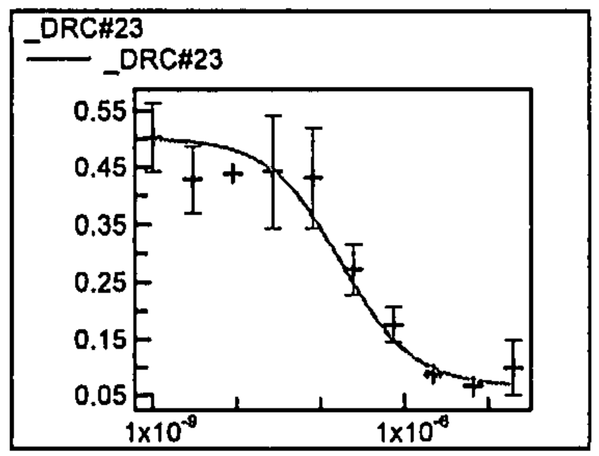
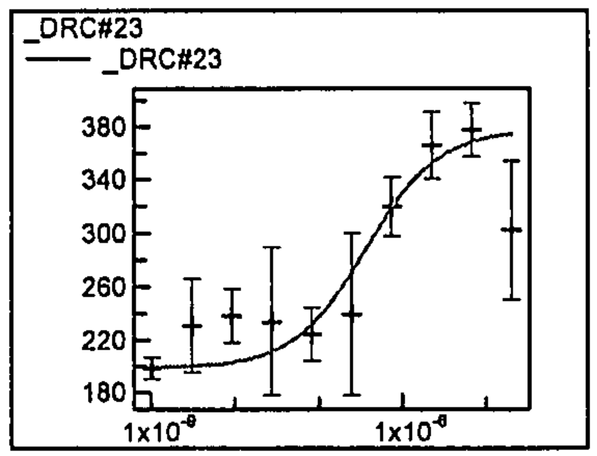
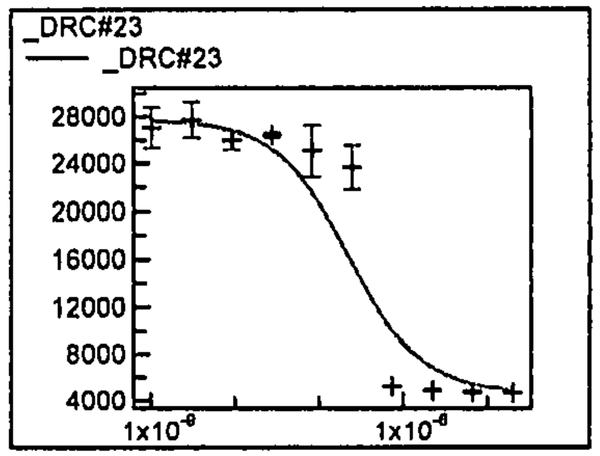
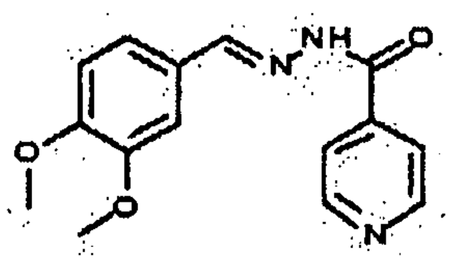
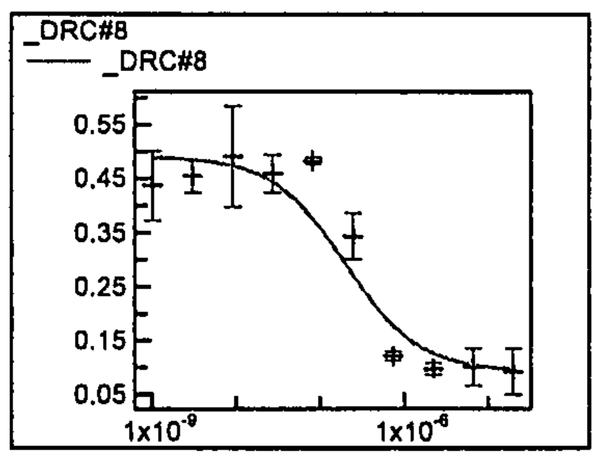
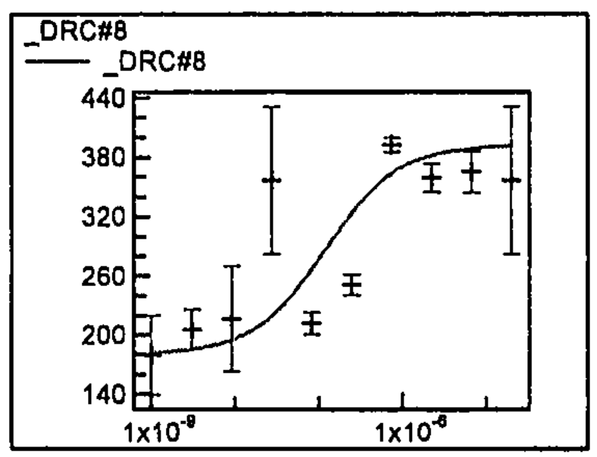
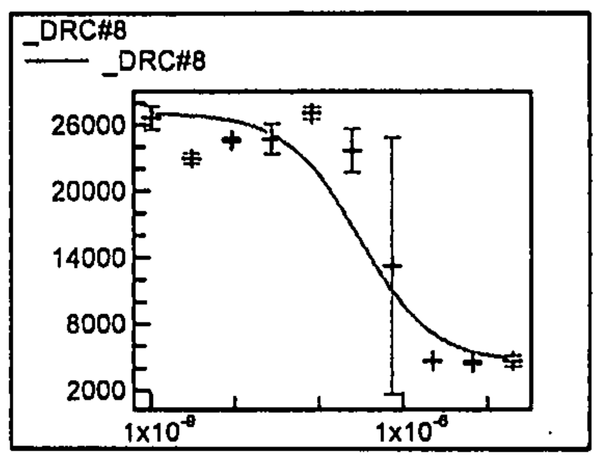
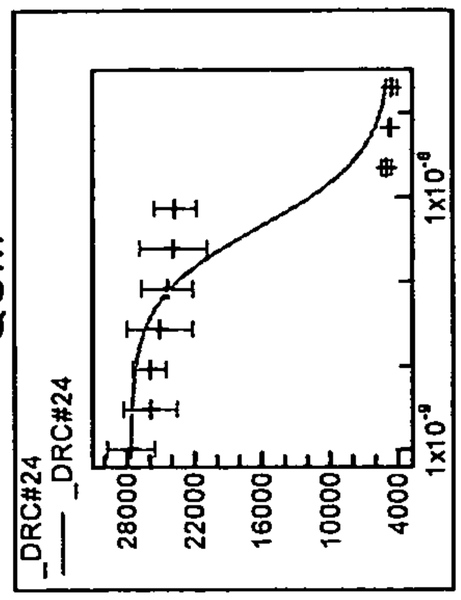
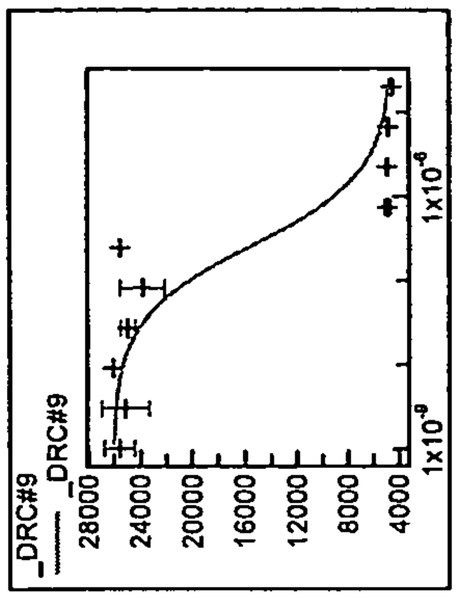
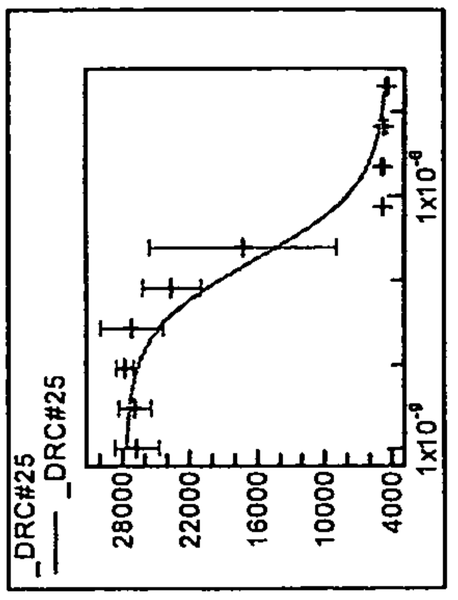
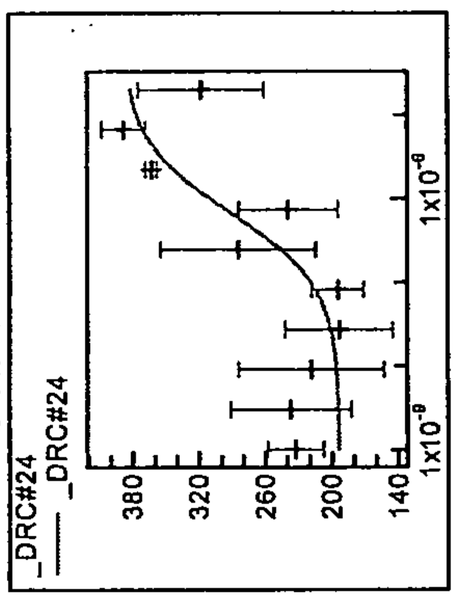
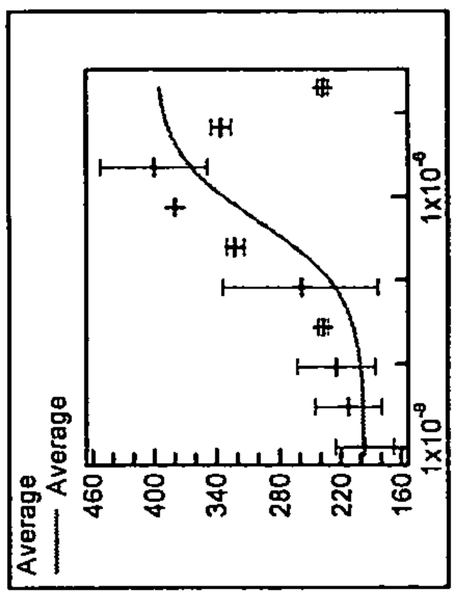
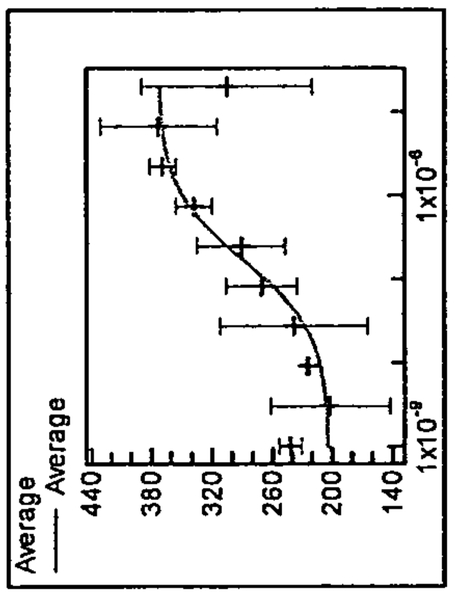
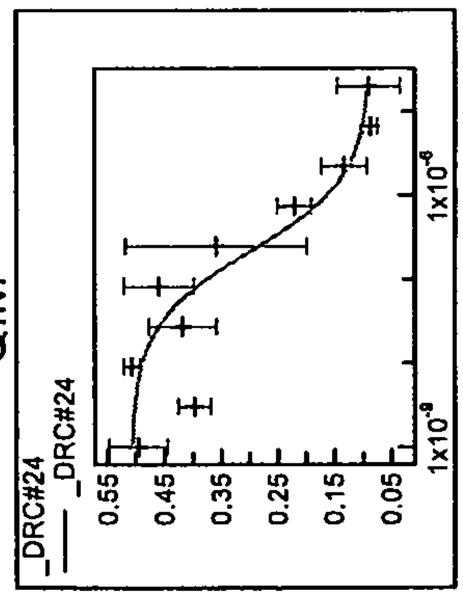
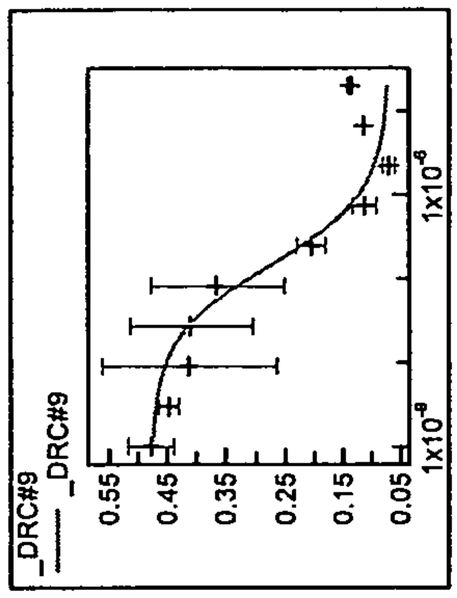
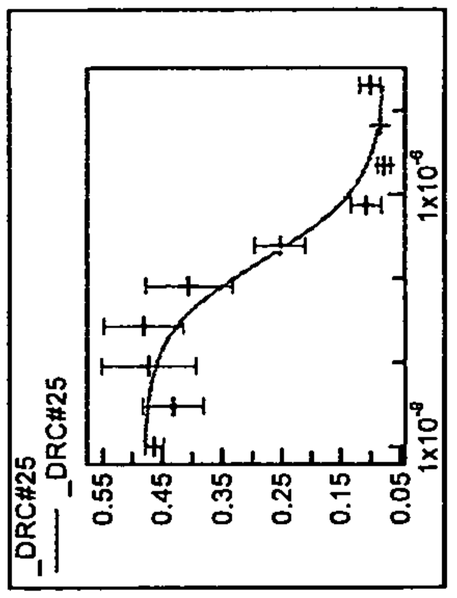
IPK00004293  Scaffold I			
IPK00004312  Scaffold I			
IPK00004295  Scaffold I			

Table 2

Table 2

QUM			
Cell number			
QIM			
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	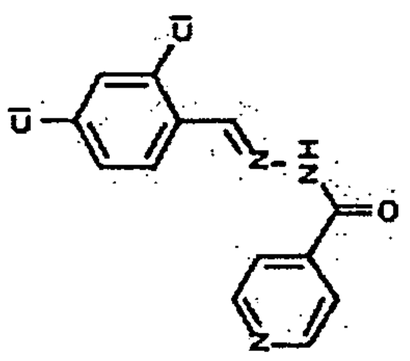		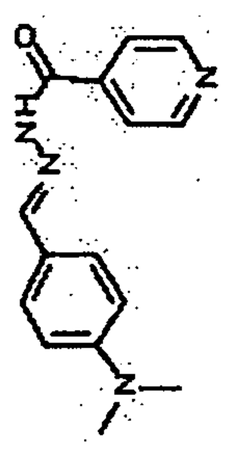		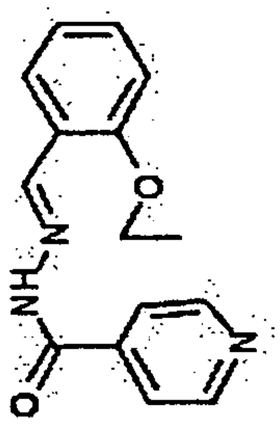	

Table 2

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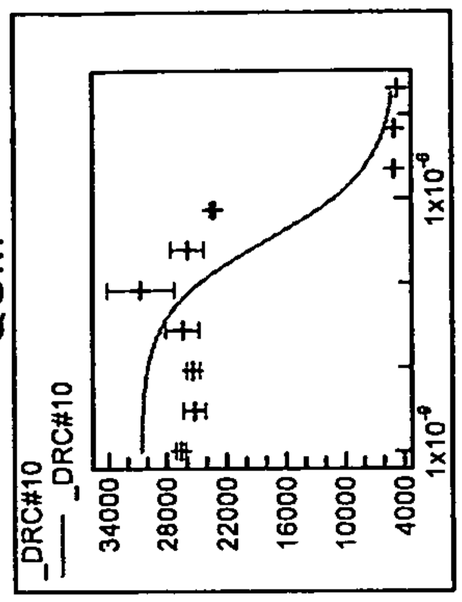
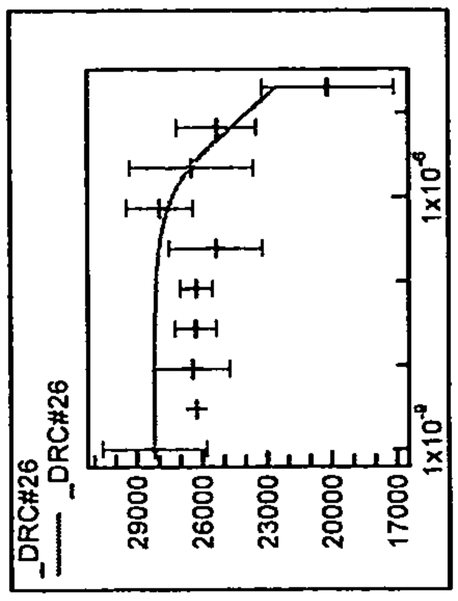
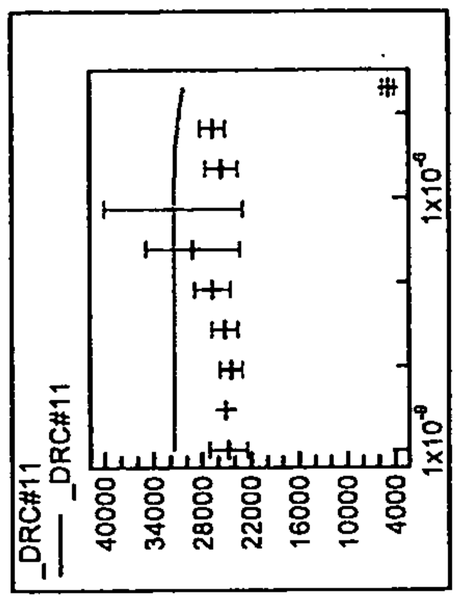
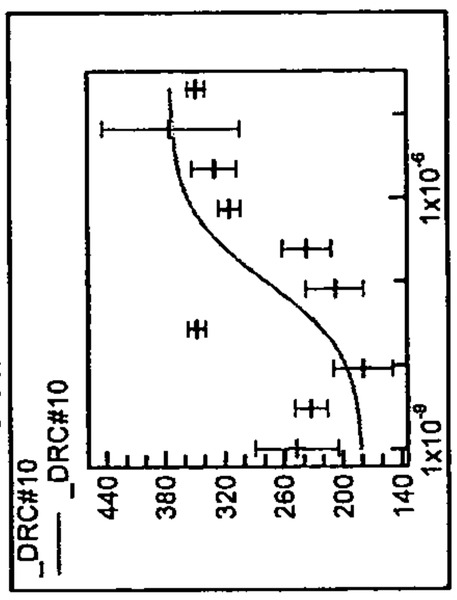
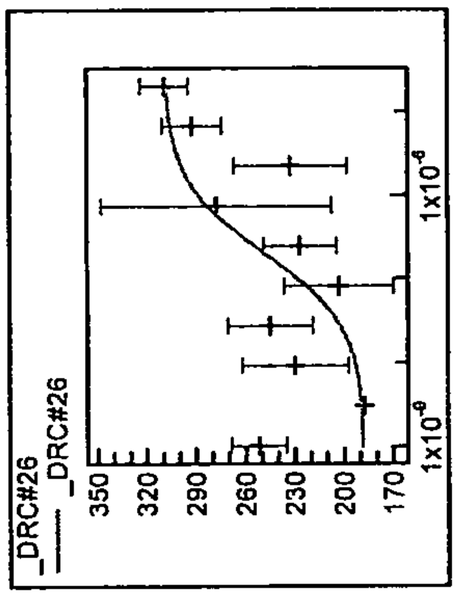
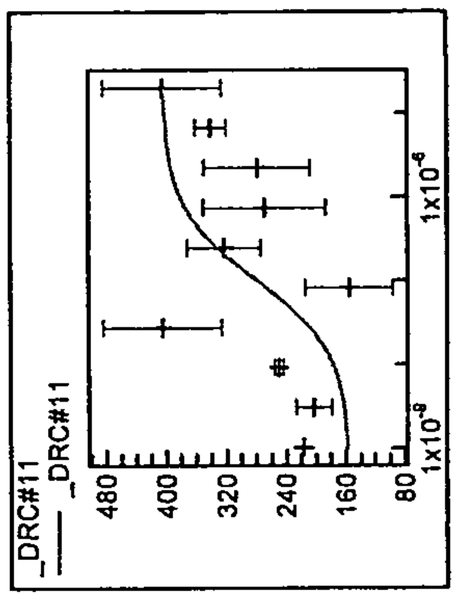
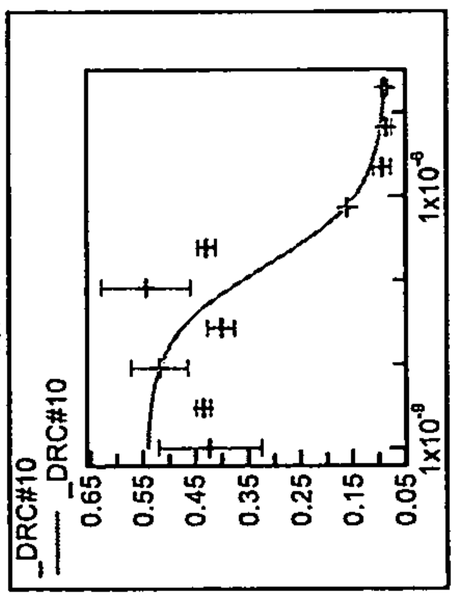
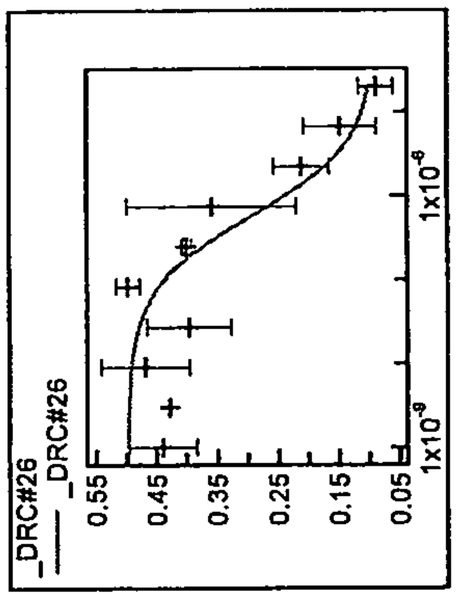
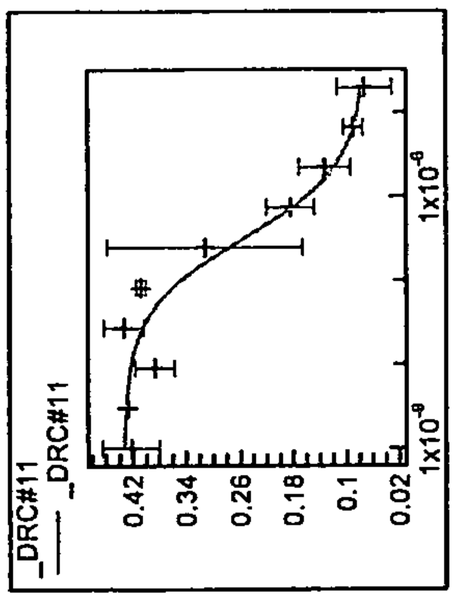
QUM			
Cell number			
QIM			

Table 2

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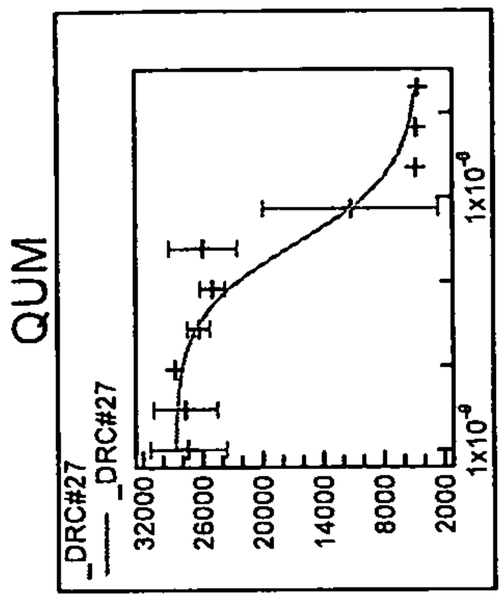
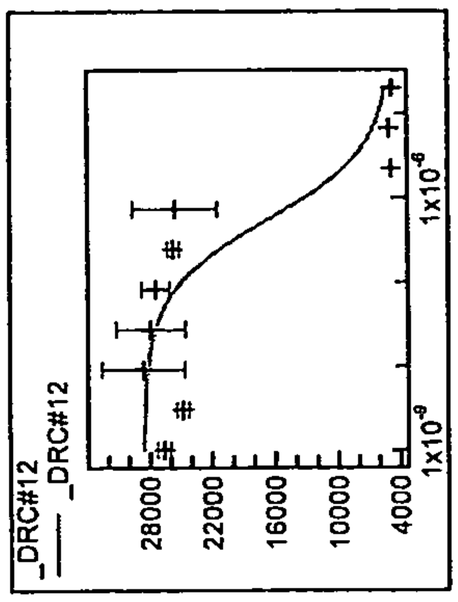
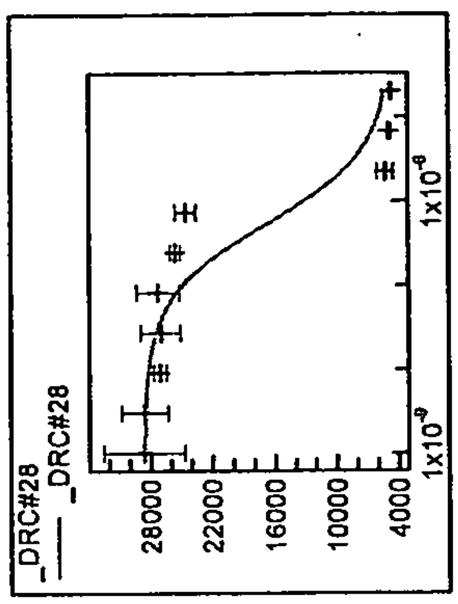
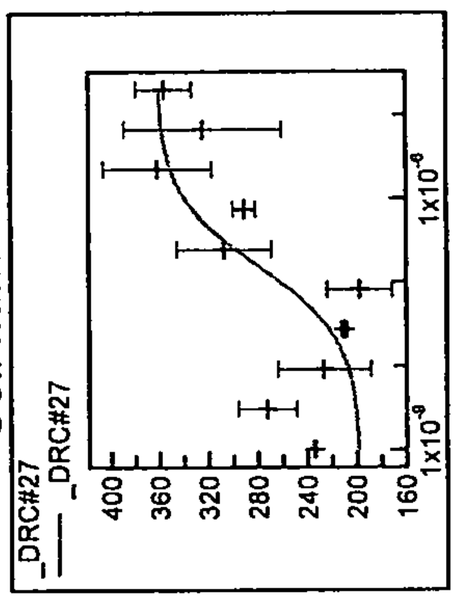
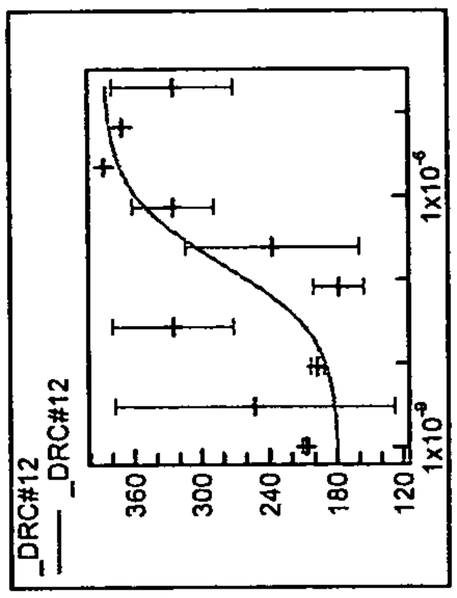
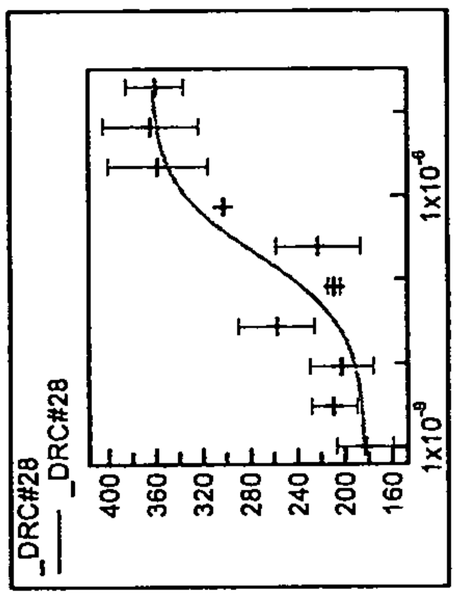
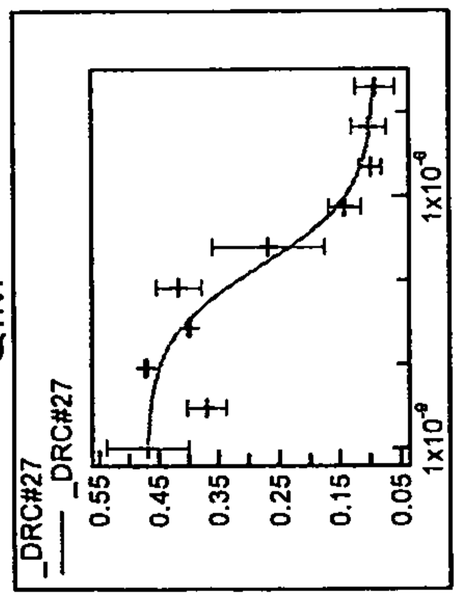
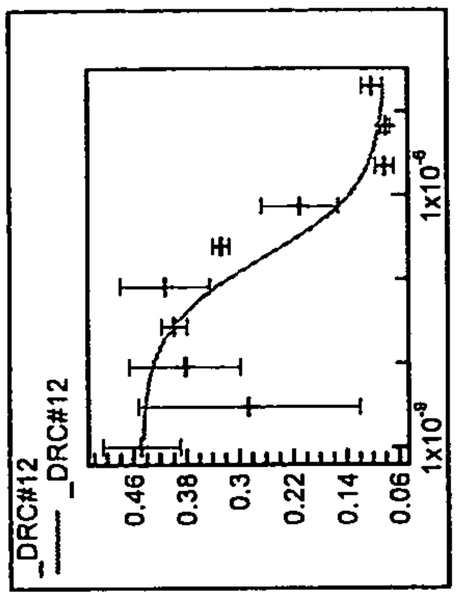
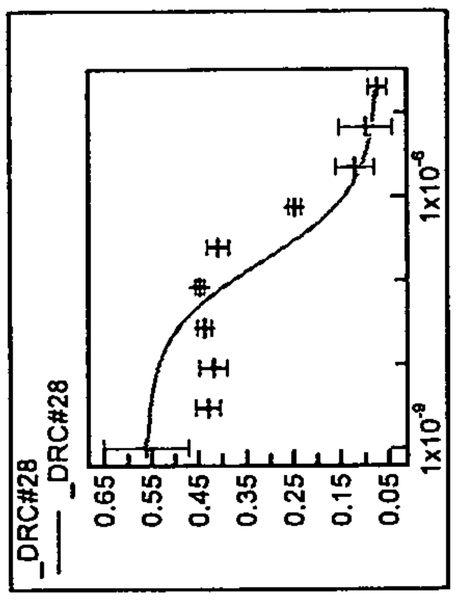
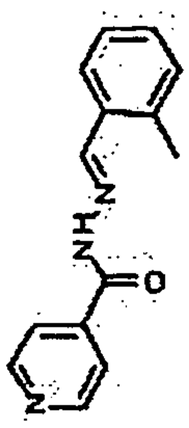
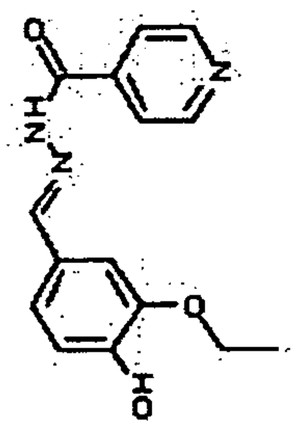
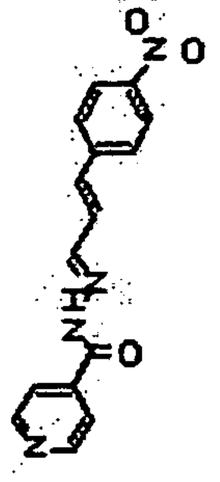
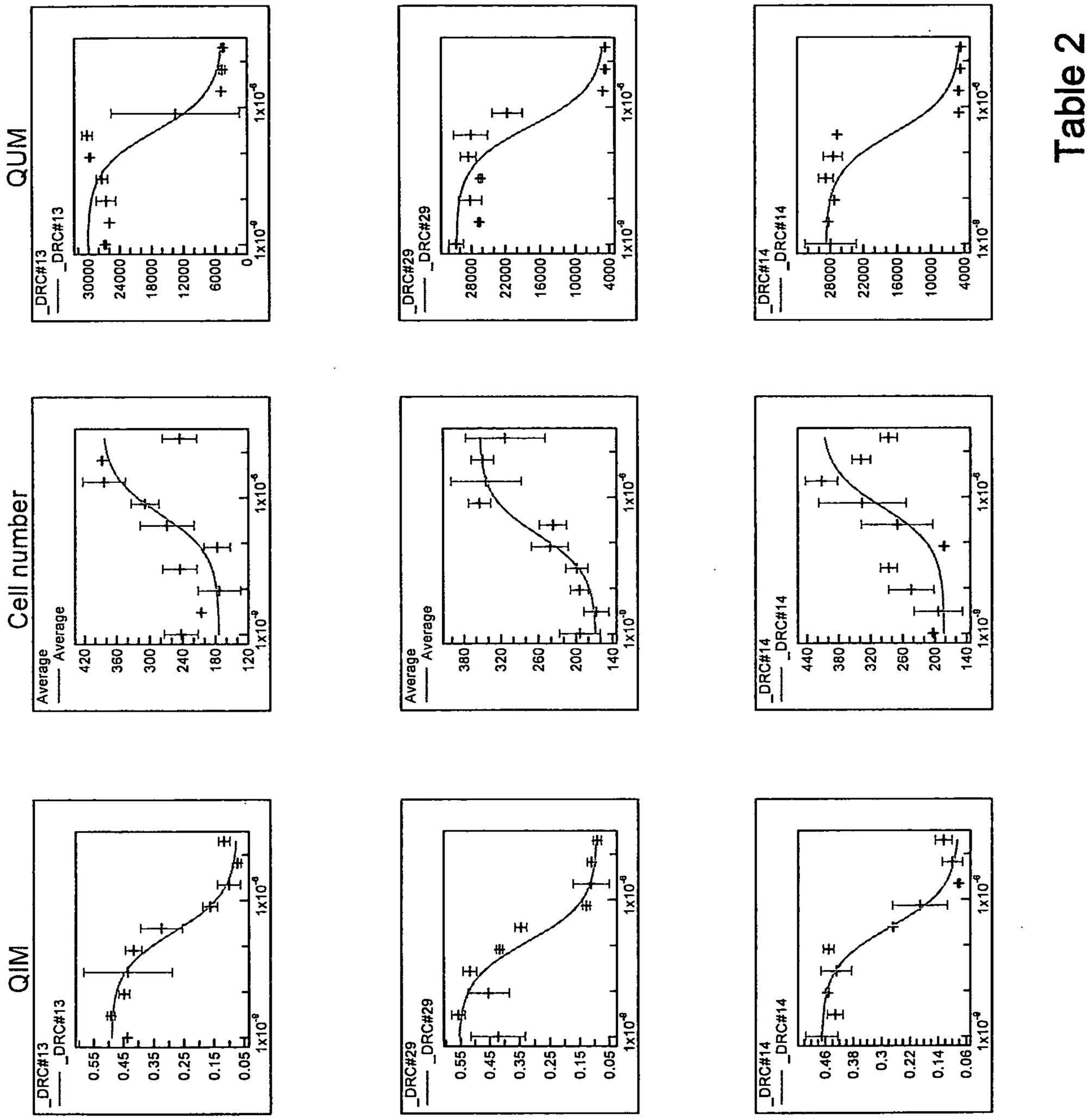
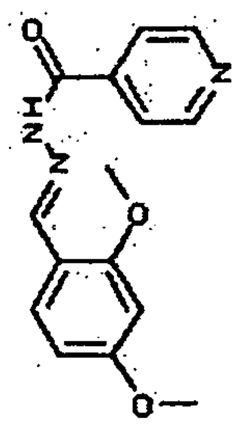
QUM			
Cell number			
QIM			
			

Table 2

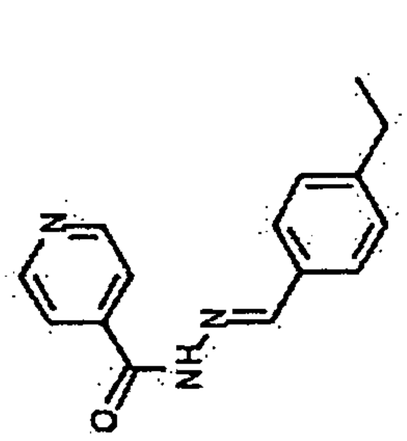


IPK0000430U



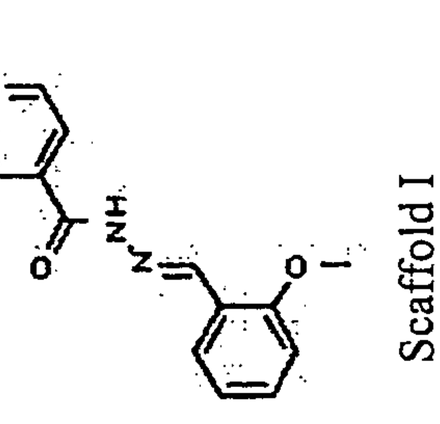
Scaffold I

IPK0000433 1



Scaffold I

IPK0000430 1



Scaffold I

Table 2

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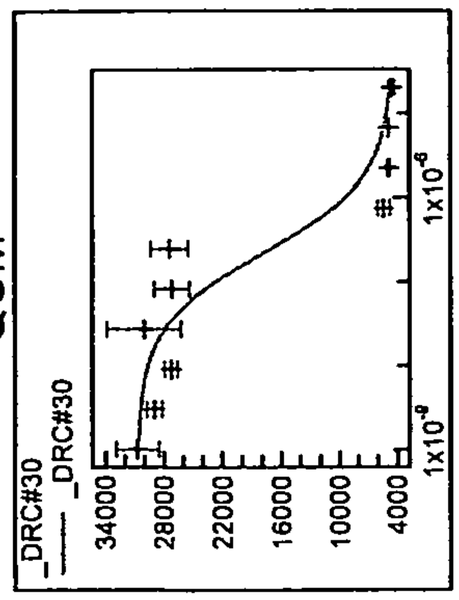
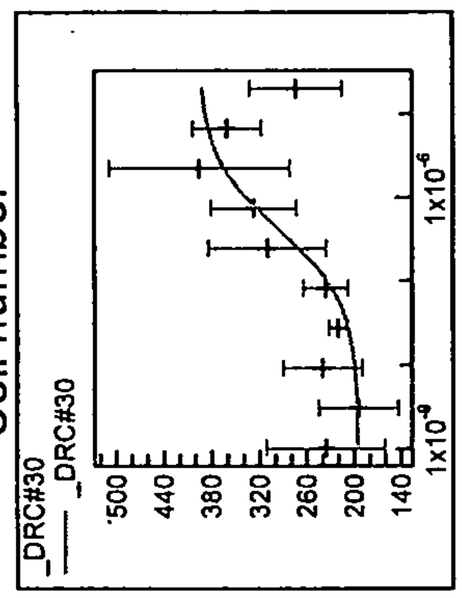
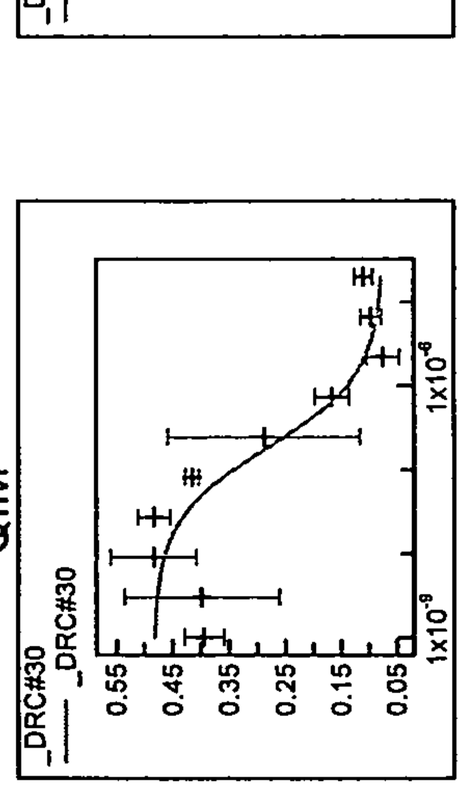
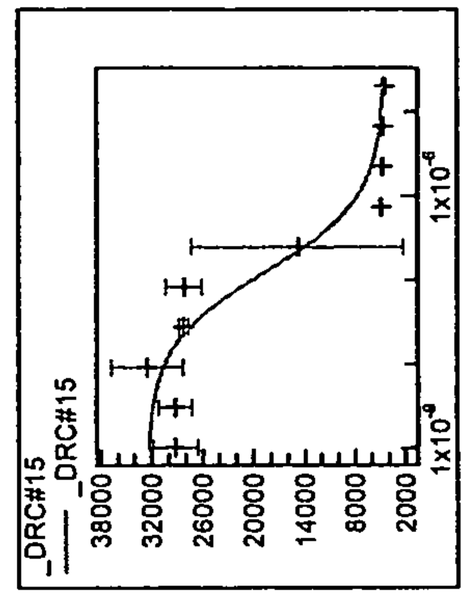
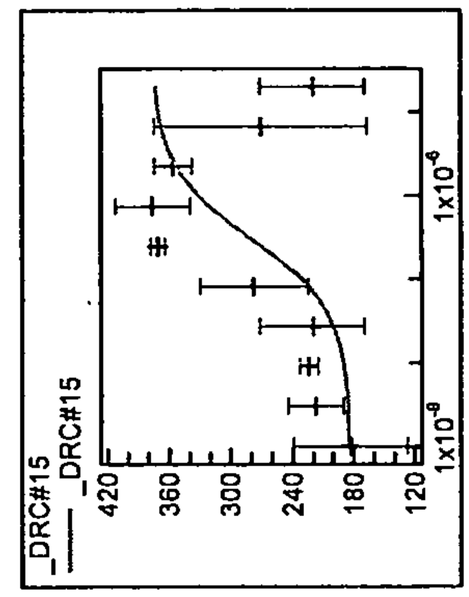
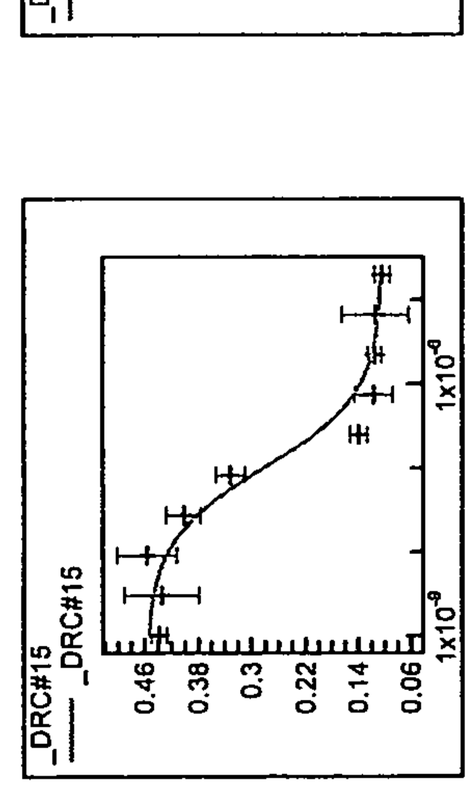
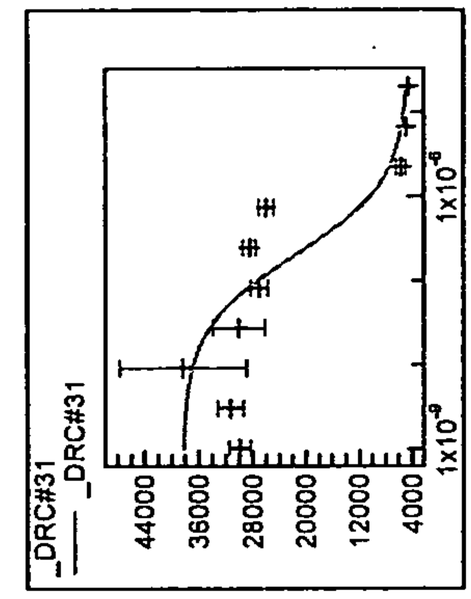
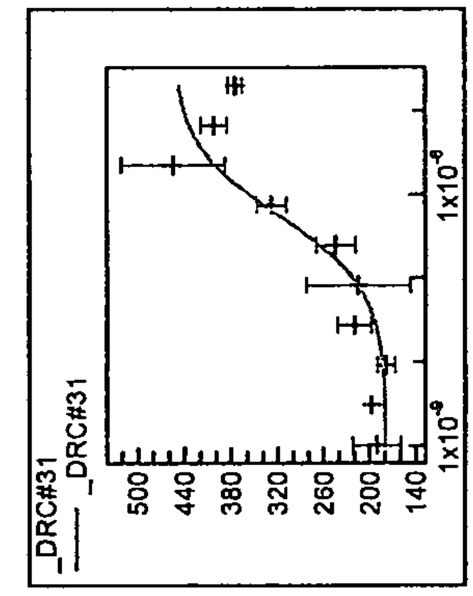
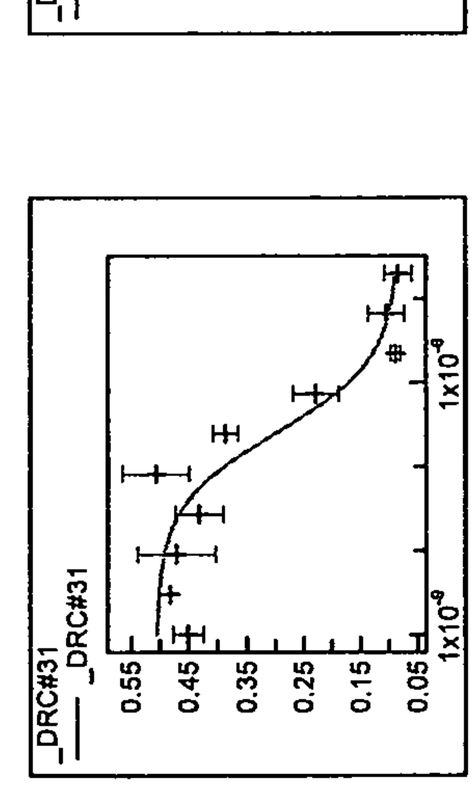
IPK00004332			
Scaffold I			
IPK00004305			
Scaffold I			
IPK00004333			
Scaffold I			

Table 2

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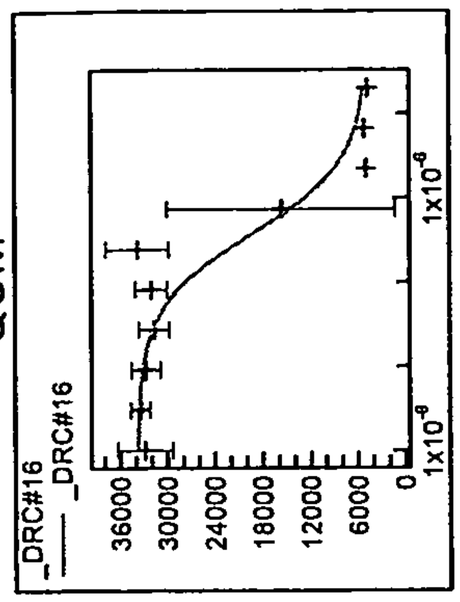
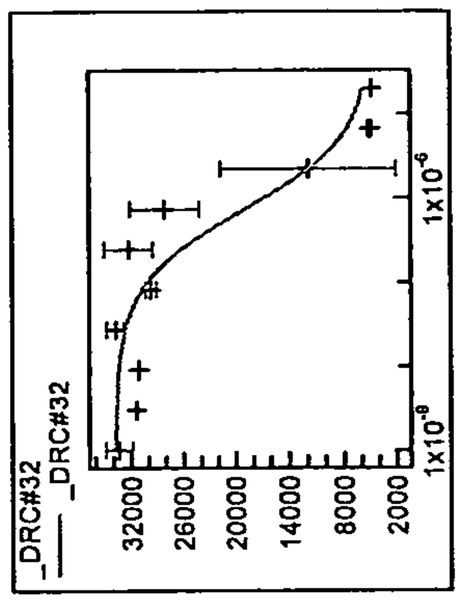
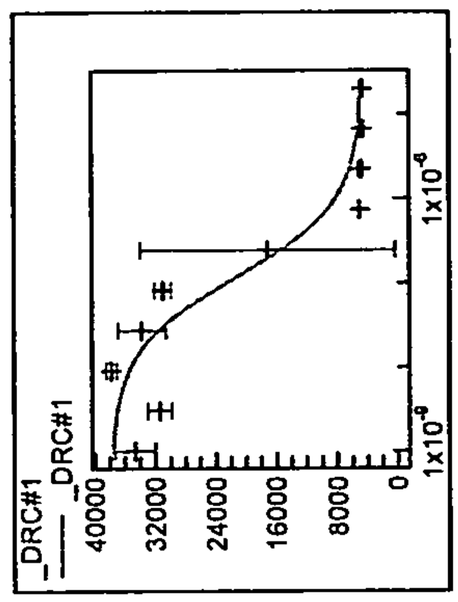
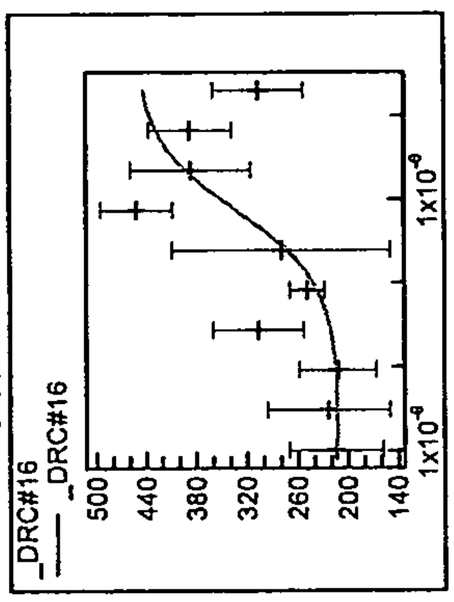
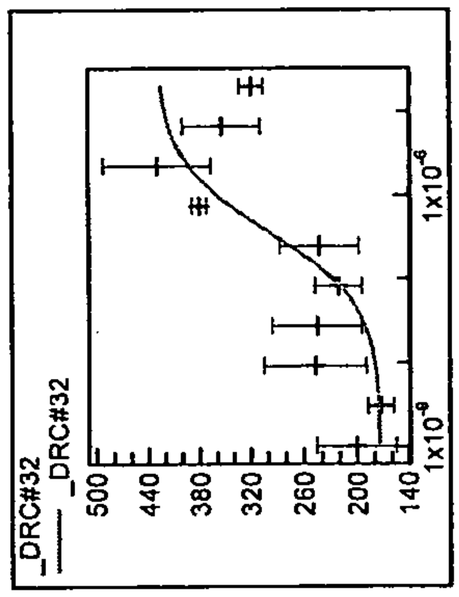
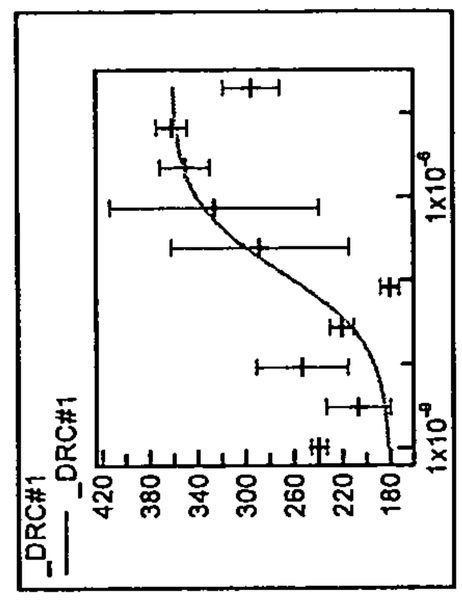
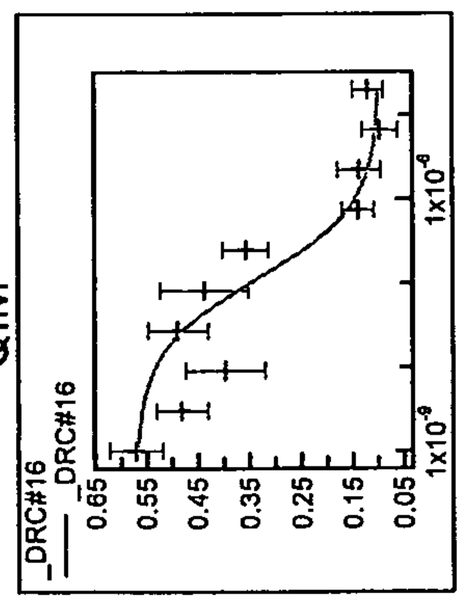
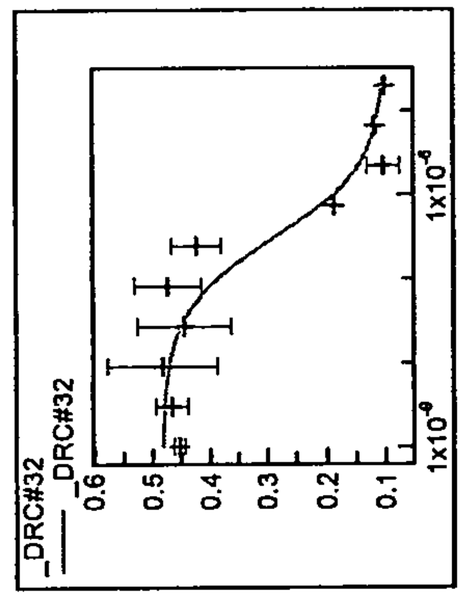
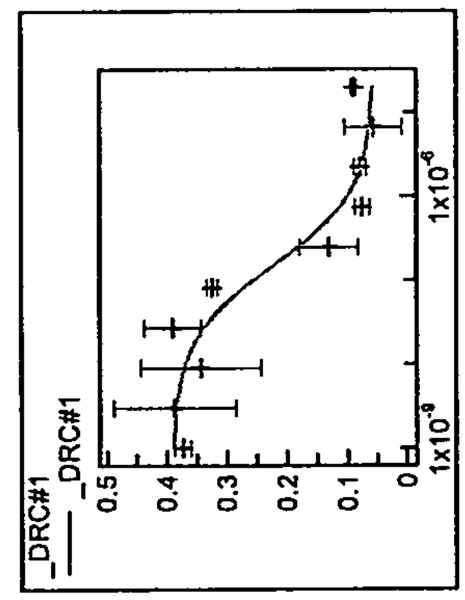
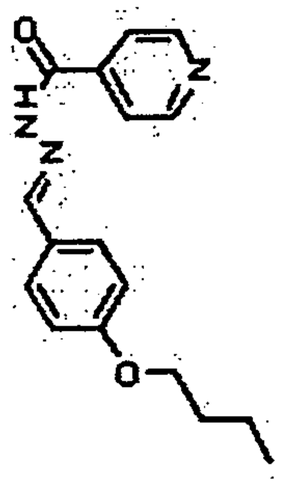
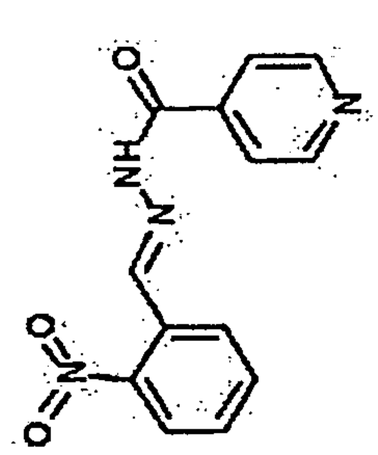
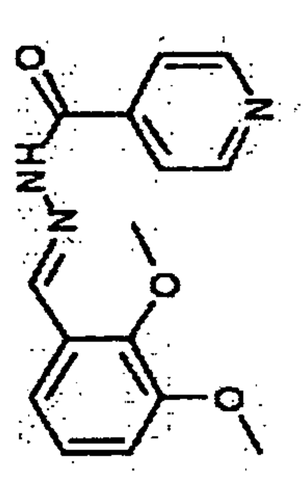
QUM			
Cell number			
QIM			
			

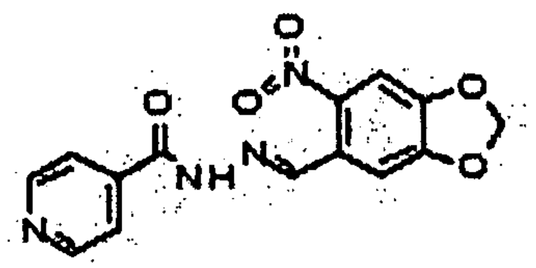
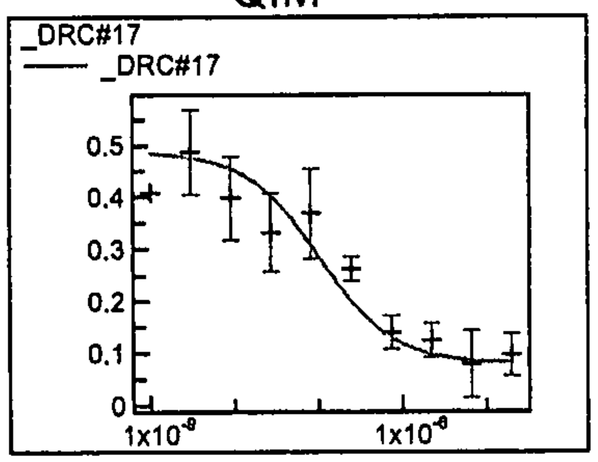
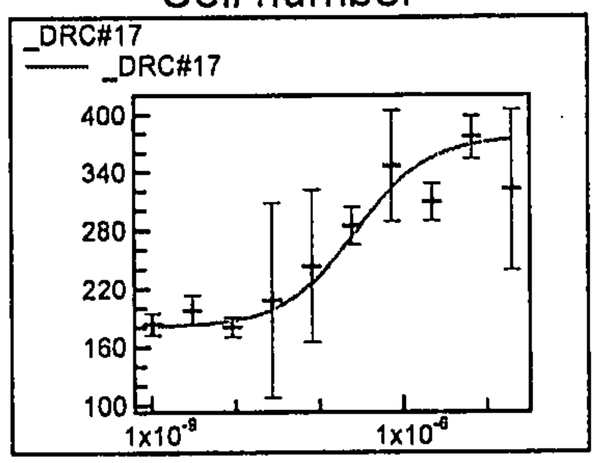
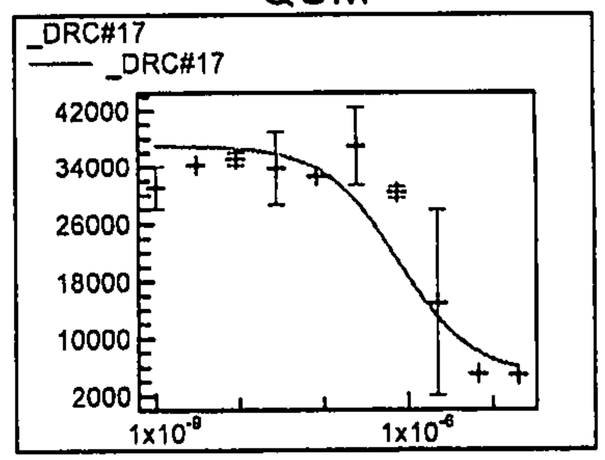
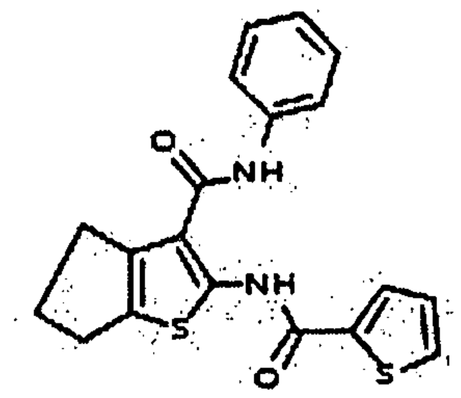
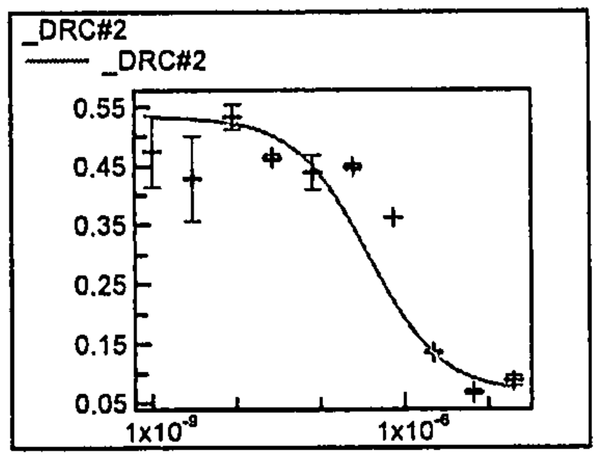
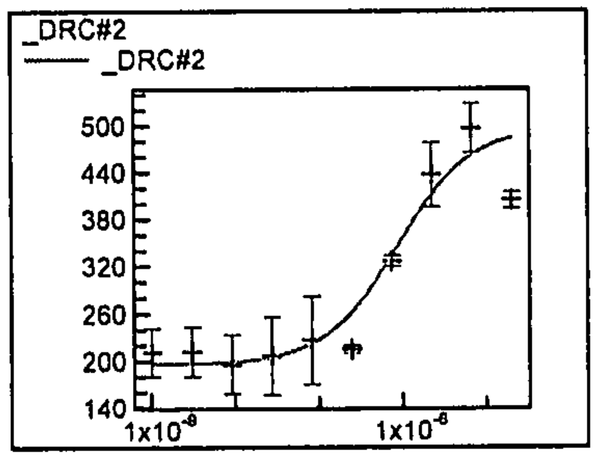
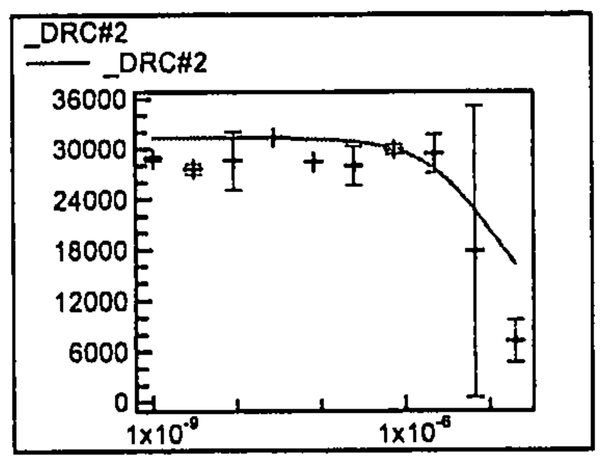
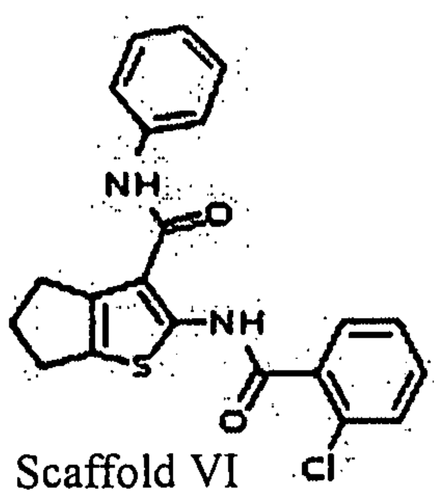
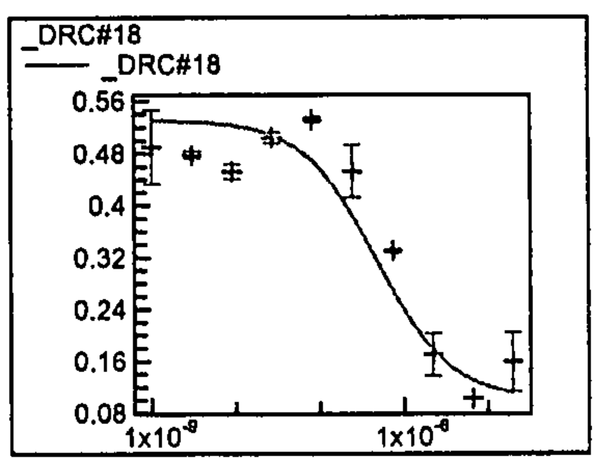
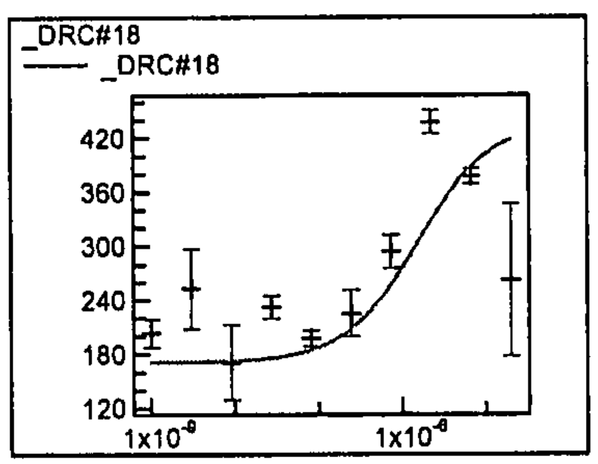
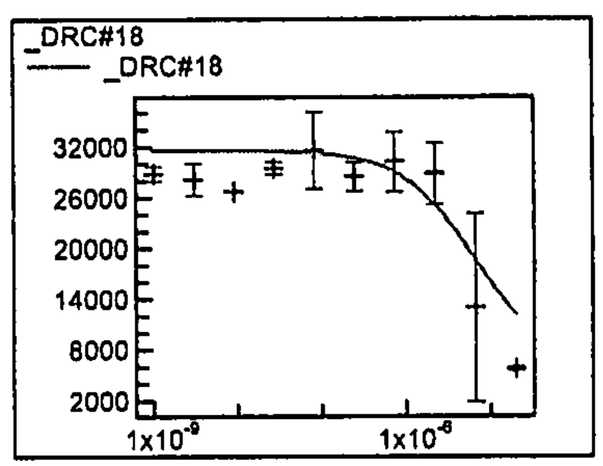
	QIM	Cell number	QUM
IPK00005830  Scaffold I			
IPK00004230  Scaffold VI			
IPK00007368  Scaffold VI			

Table 2

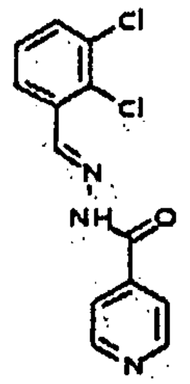
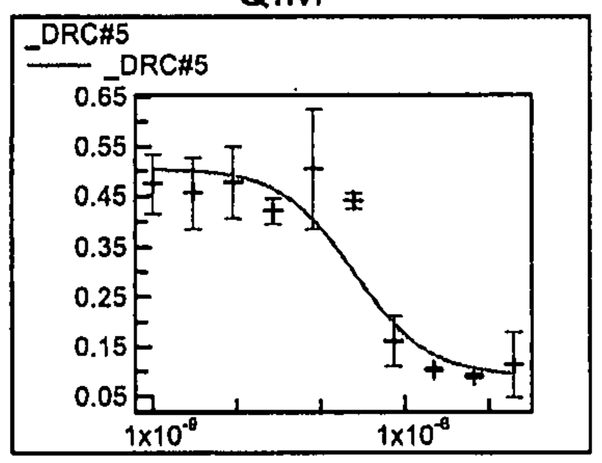
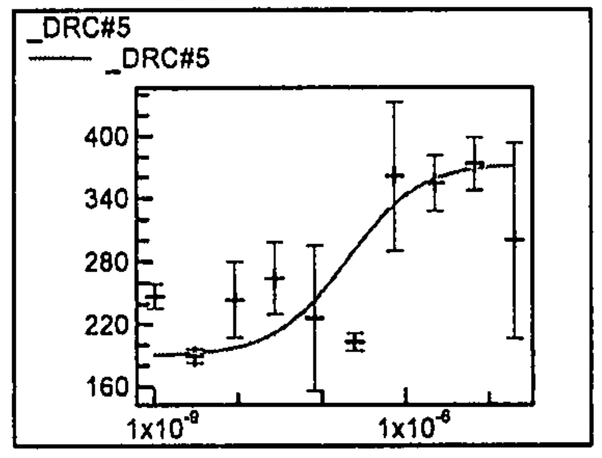
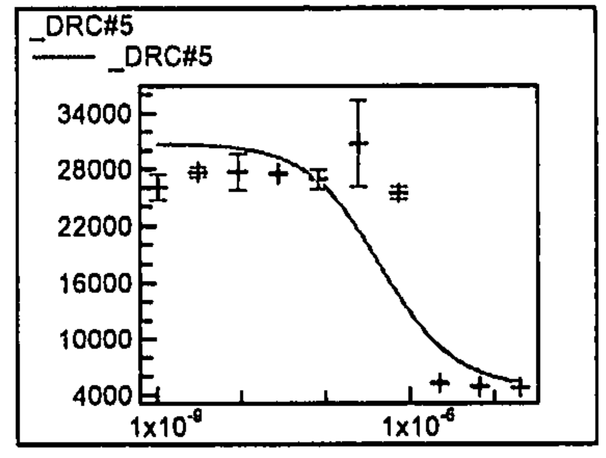
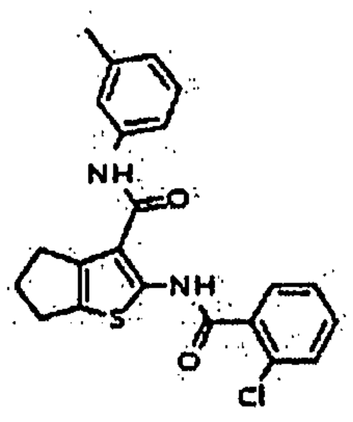
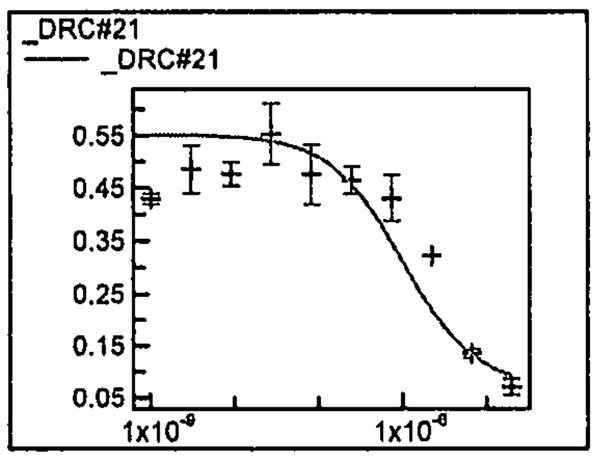
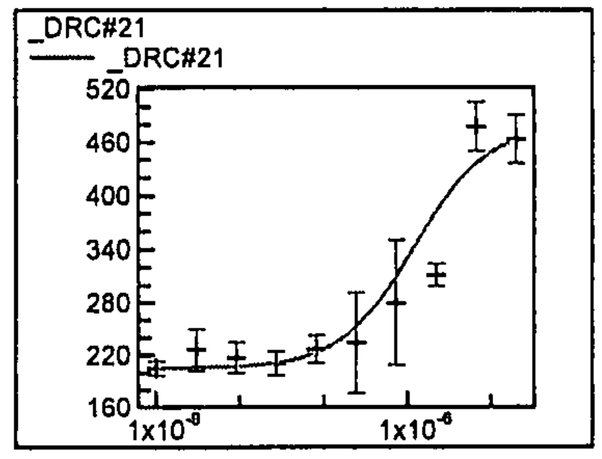
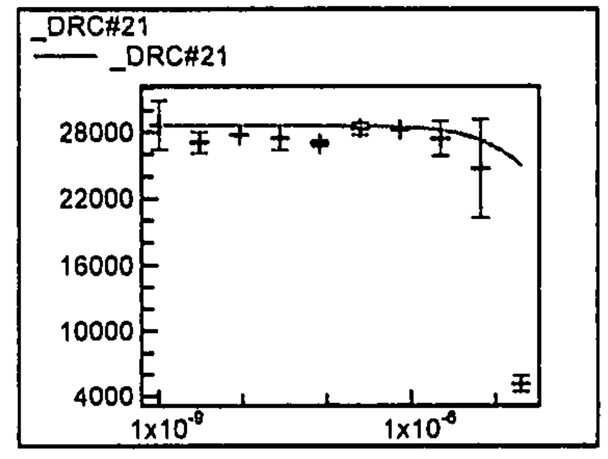
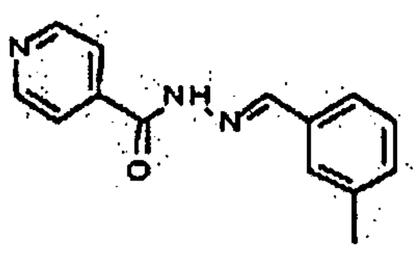
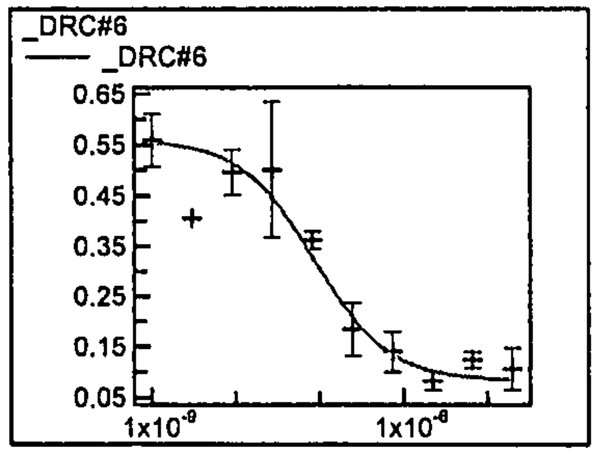
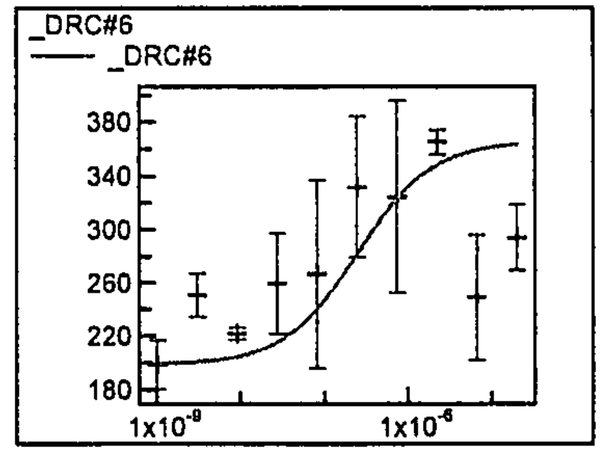
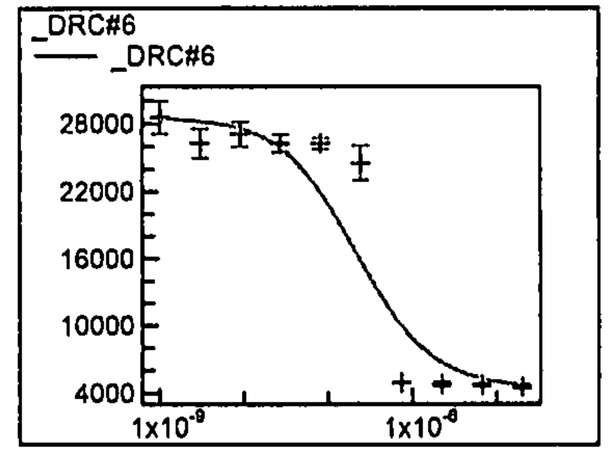
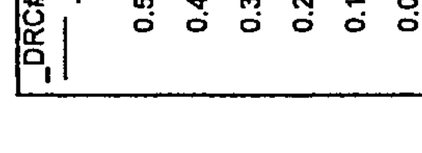
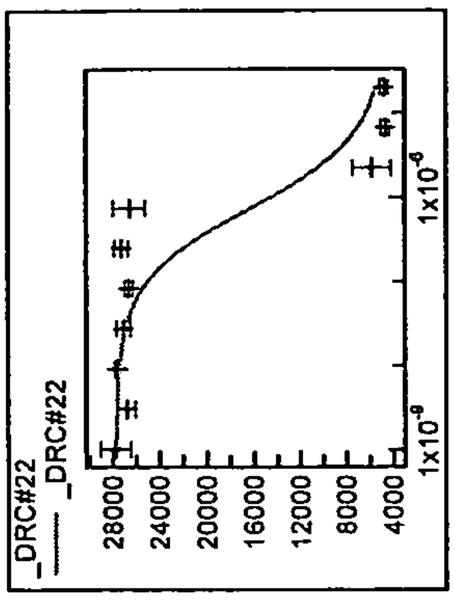
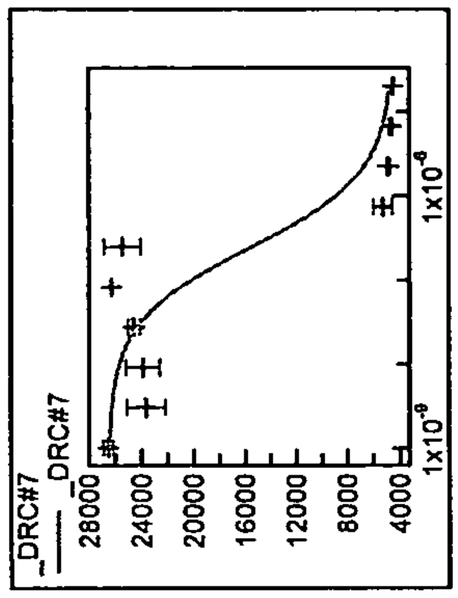
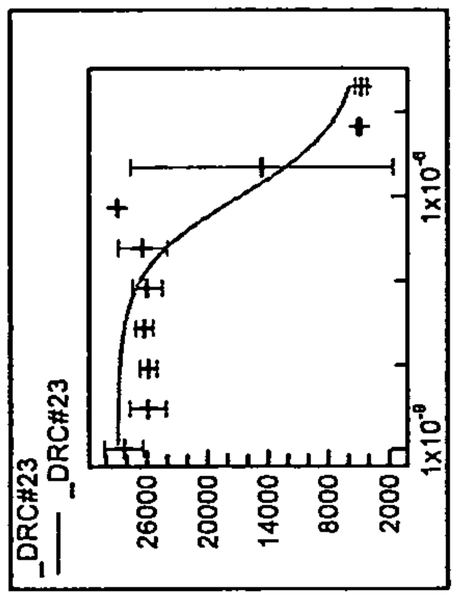
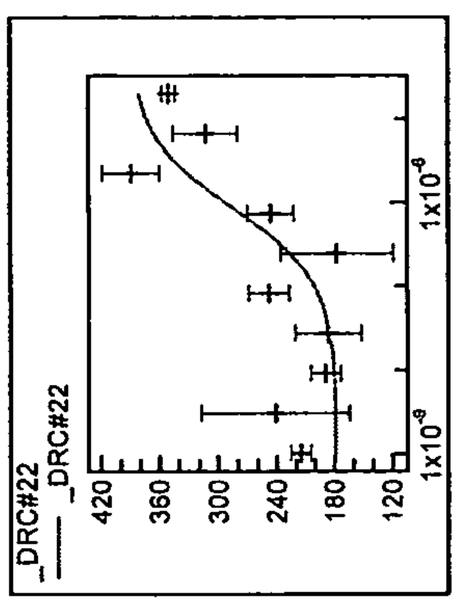
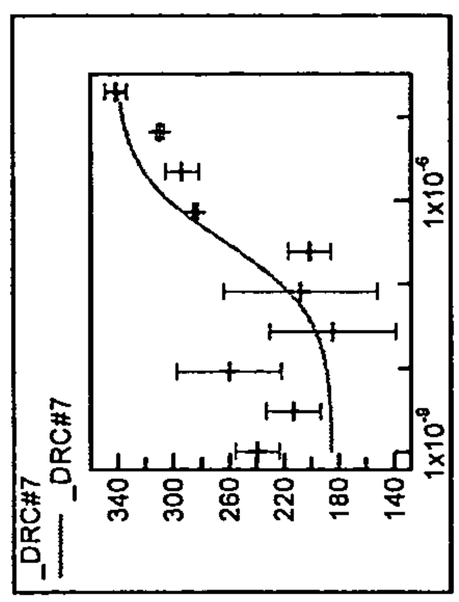
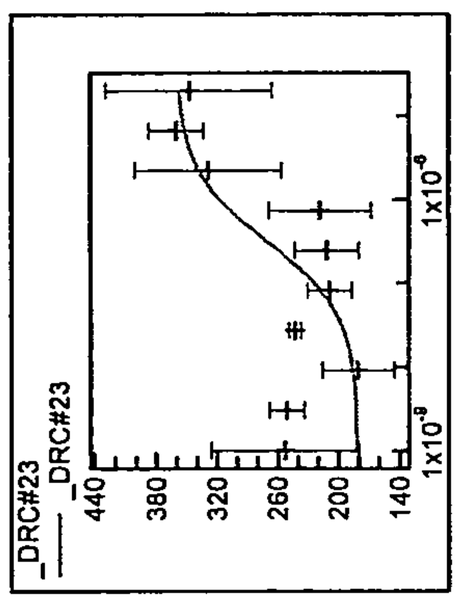
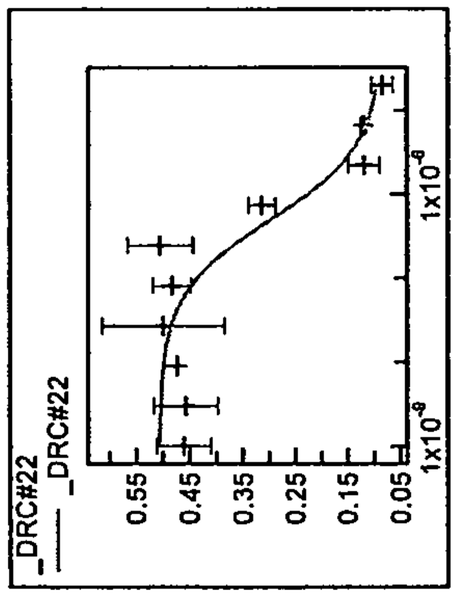
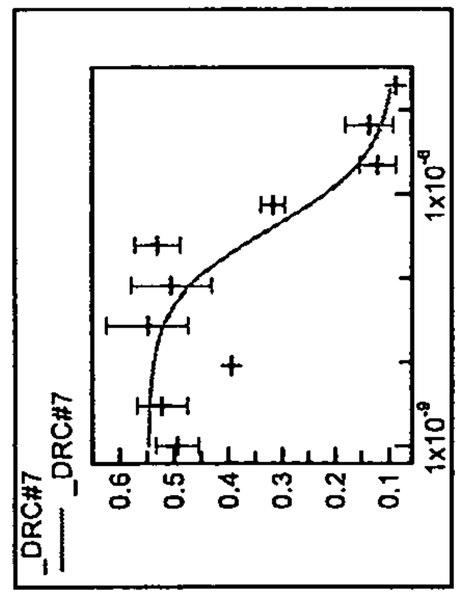
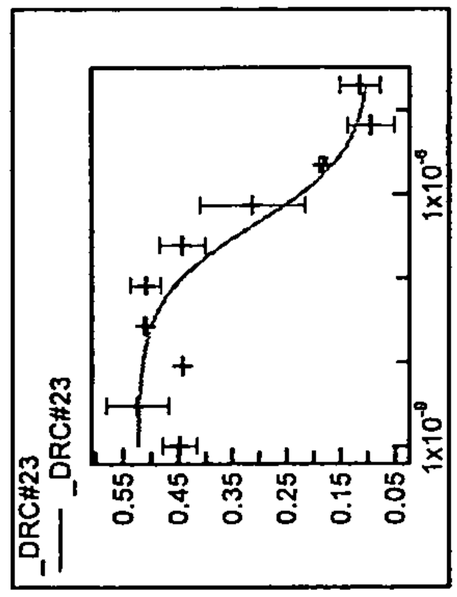
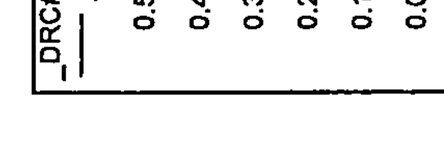
	QIM	Cell number	QUM
IPK00004310  Scaffold I			
IPK00007370  Scaffold VI			
IPK00004326  Scaffold I			

Table 2

Table 2

	IPKUUU7886	Scaffold I	
QUM			
Cell number			
QIM			
	IPK00004683	Scaffold II	
		IPK00007913	Scaffold I

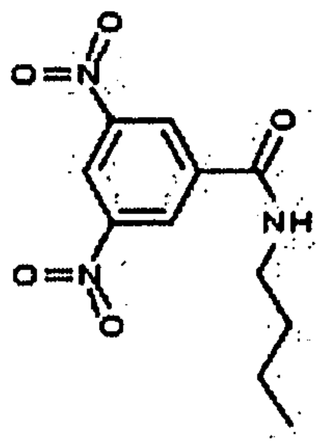
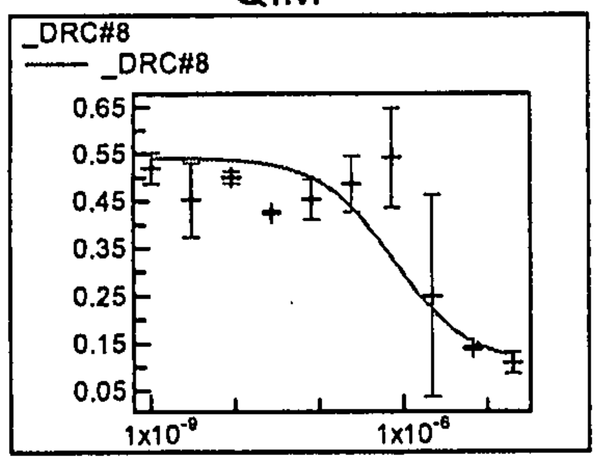
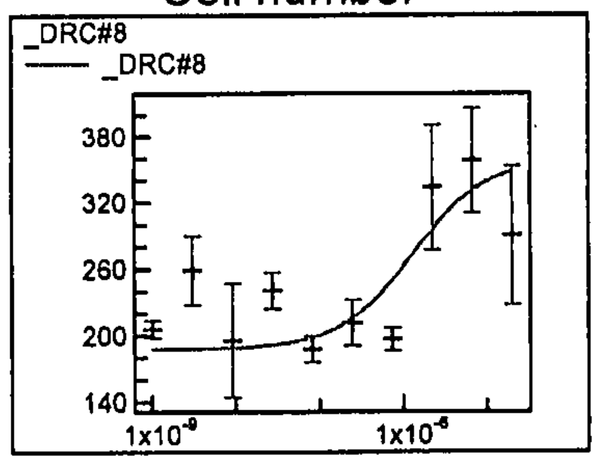
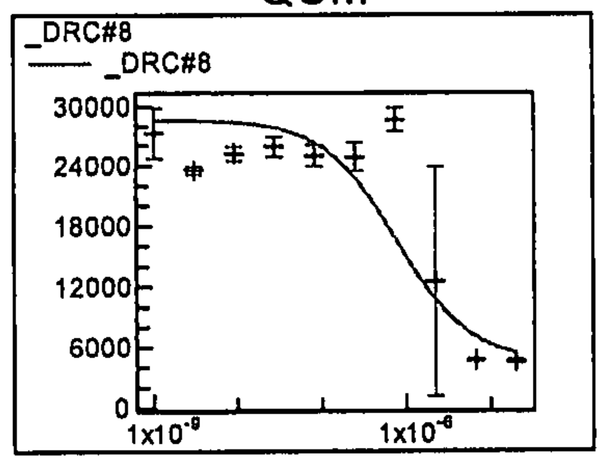
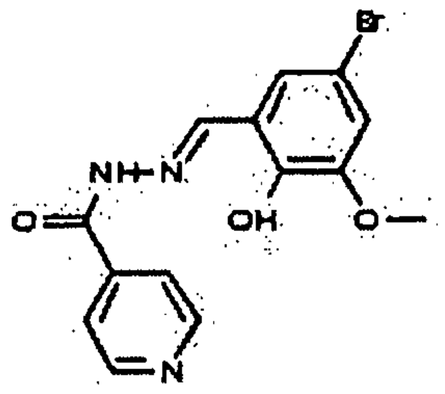
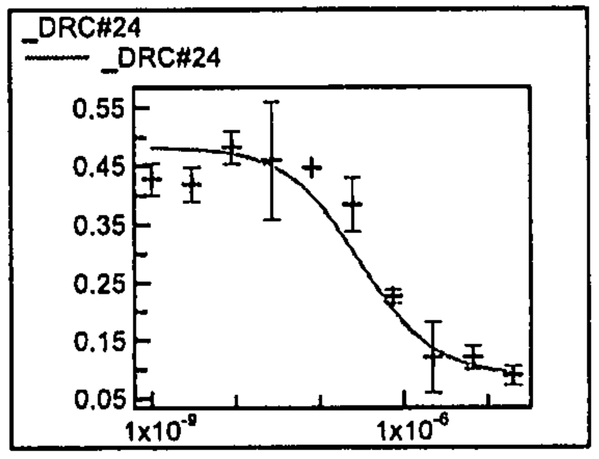
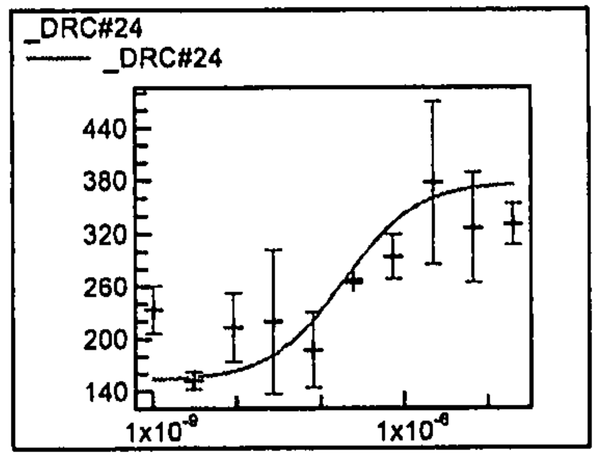
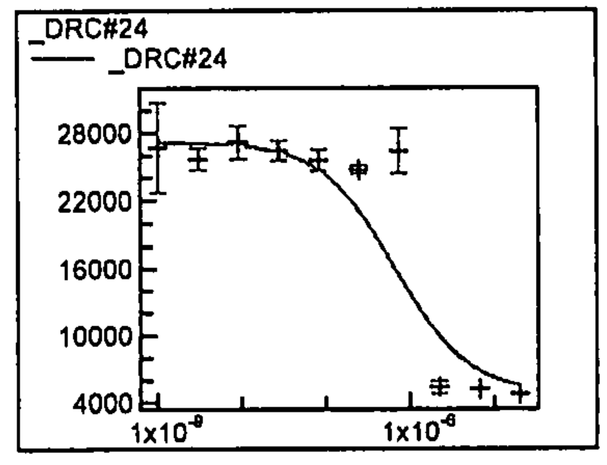
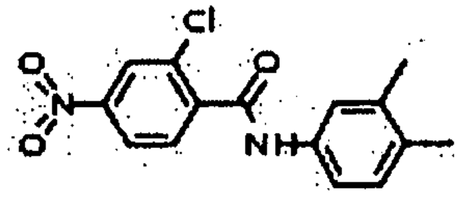
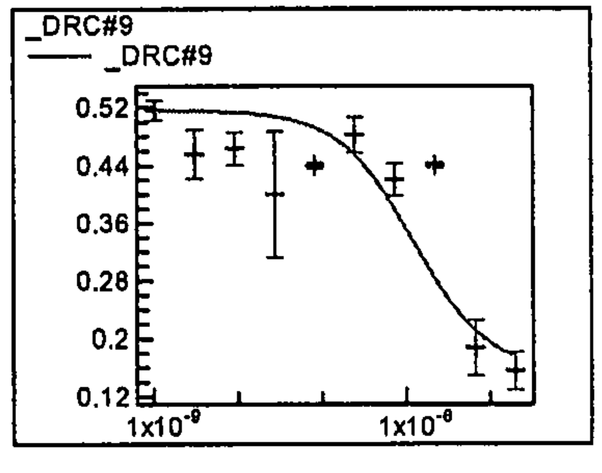
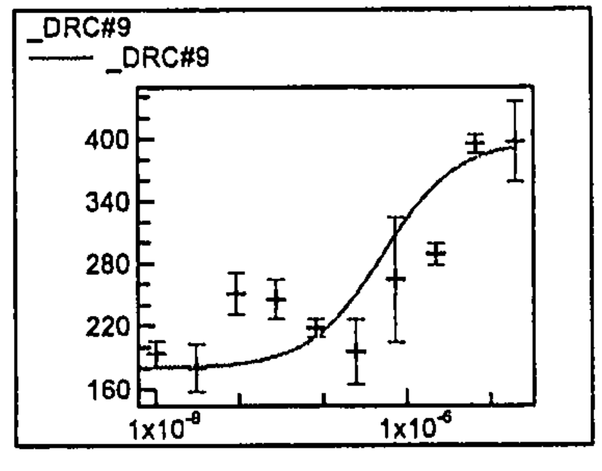
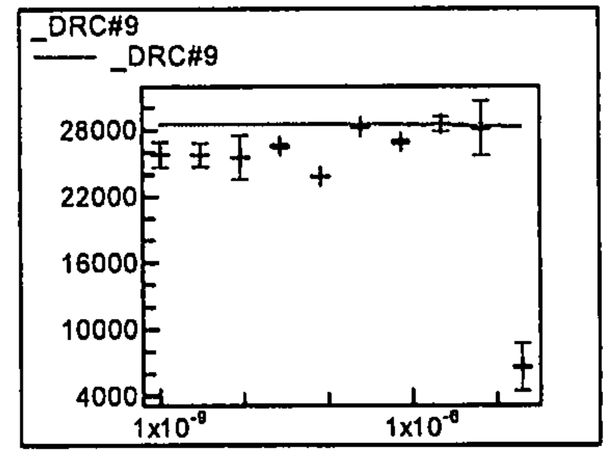
	QIM	Cell number	QUM
IPK00004687  Scaffold II			
IPK00007915  Scaffold I			
IPK00004715  Scaffold II			

Table 2

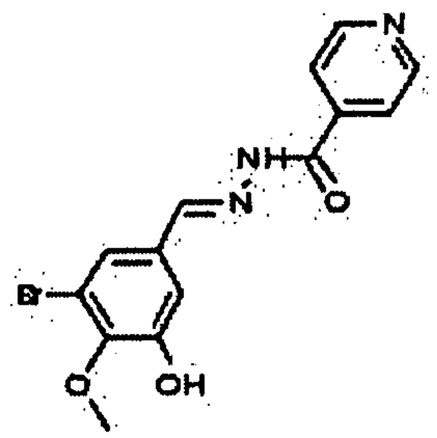
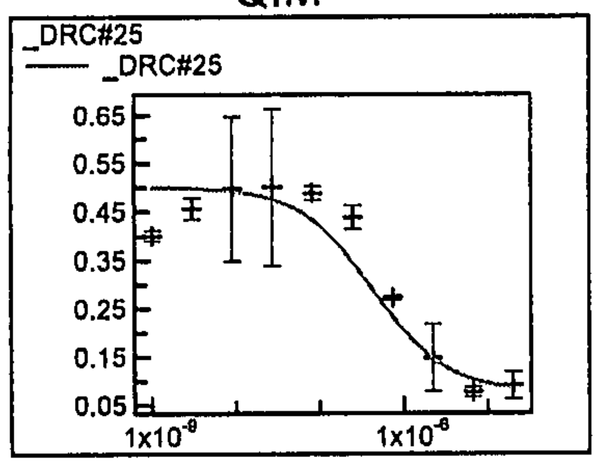
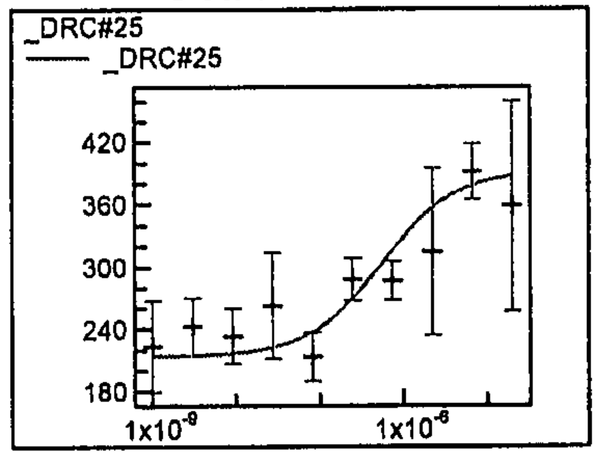
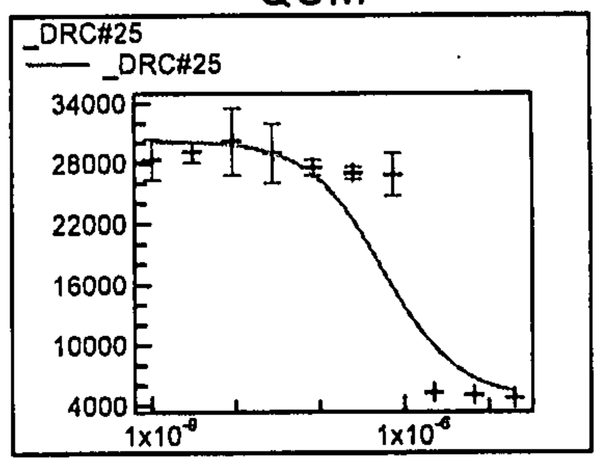
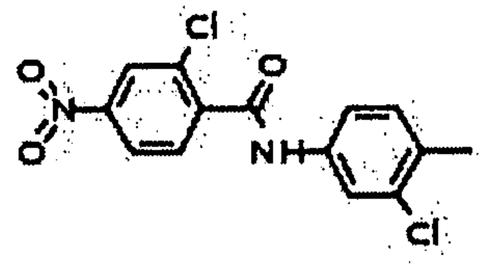
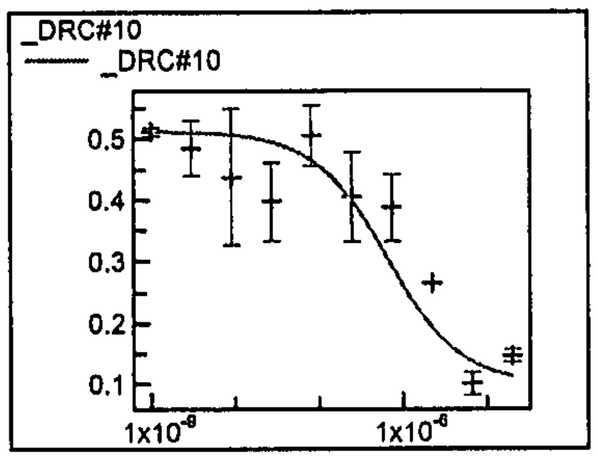
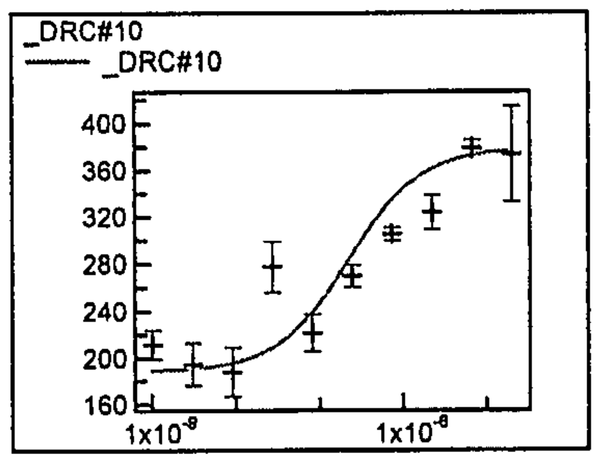
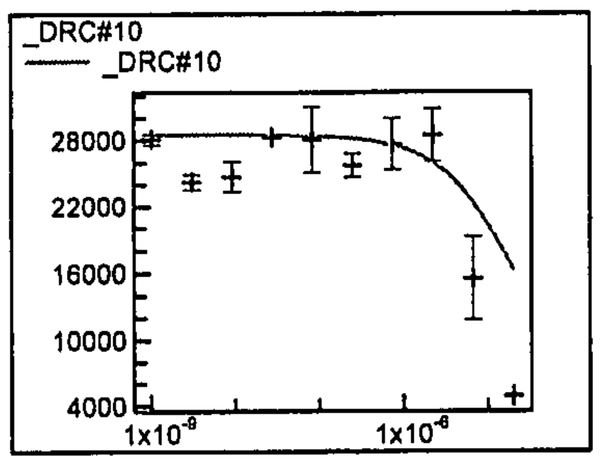
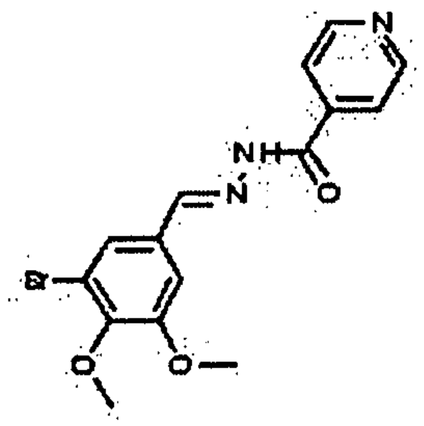
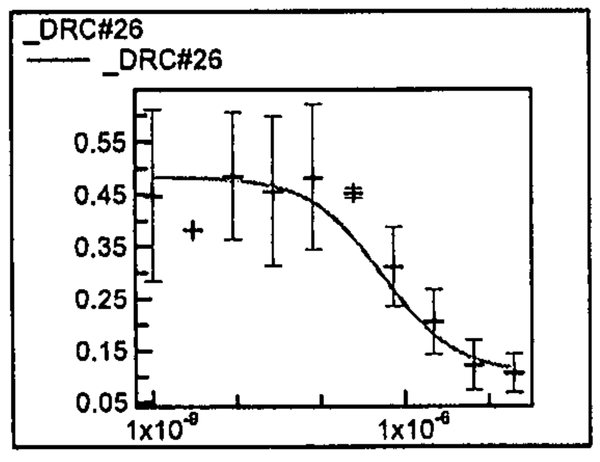
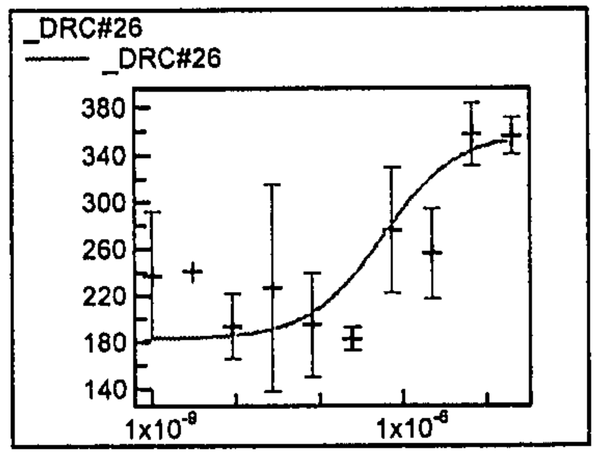
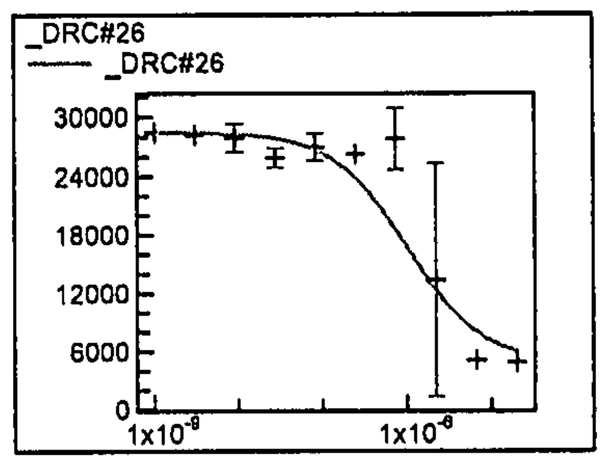
IPK00007830	QIM	Cell number	QUM
			
Scaffold I			
IPK00004716			
			
Scaffold II			
IPK00007853			
			
Scaffold I			

Table 2

Table 2

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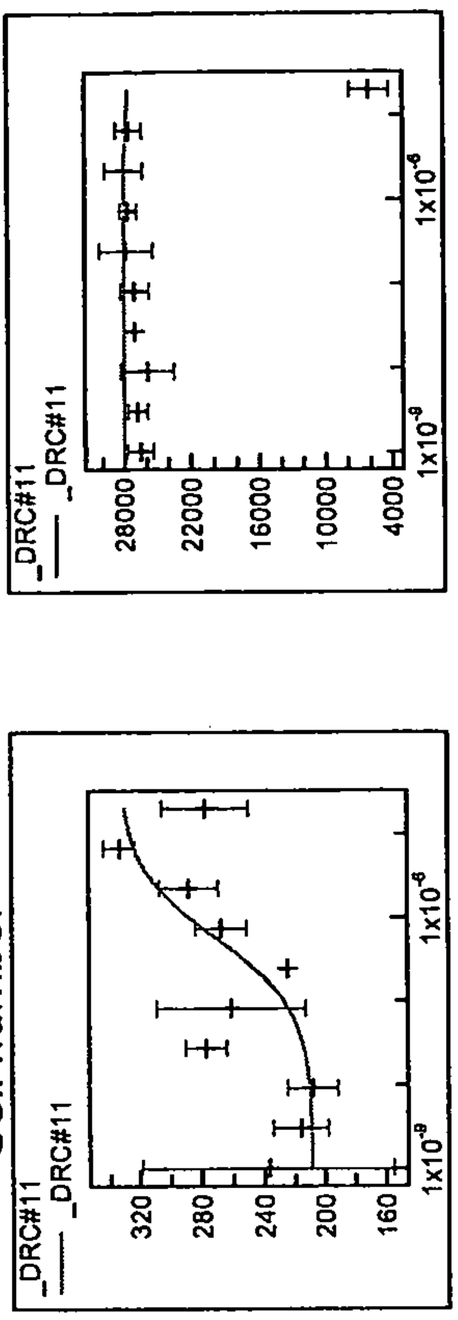
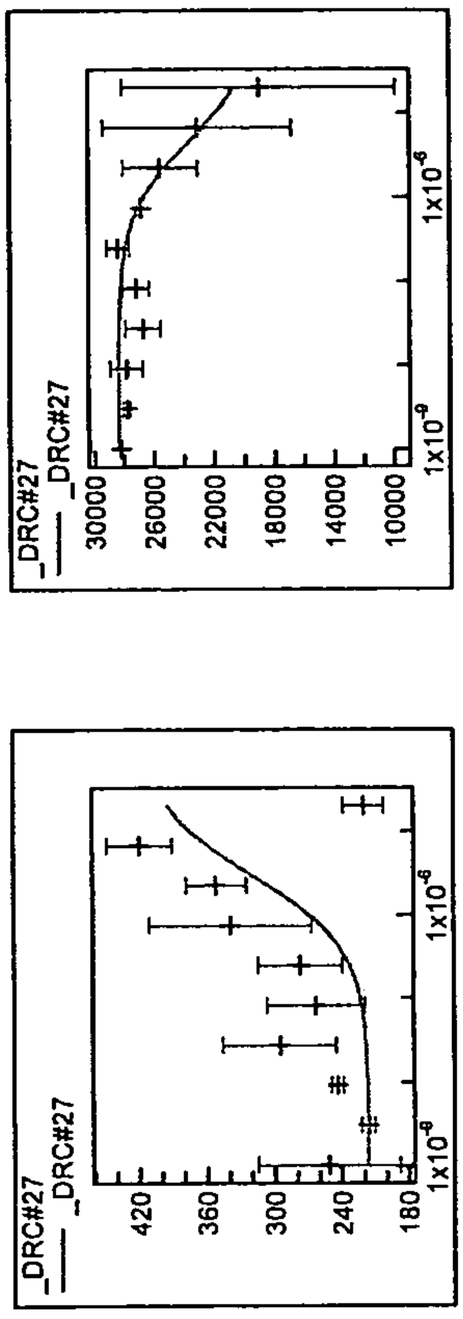
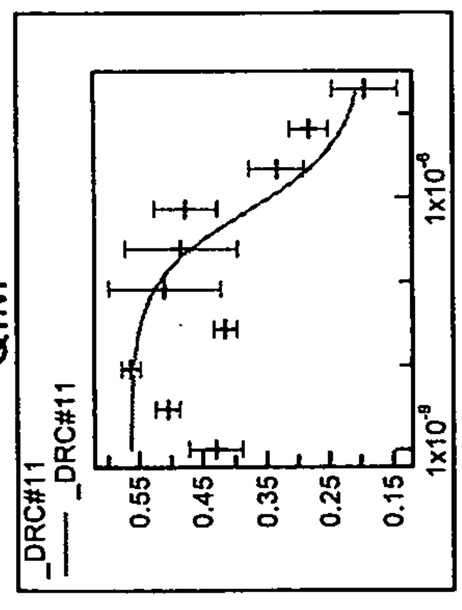
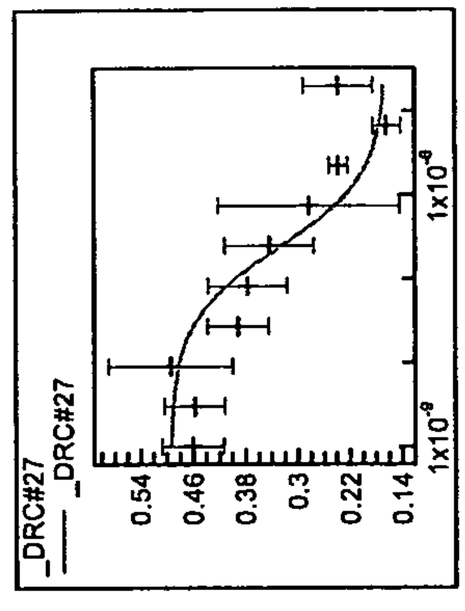
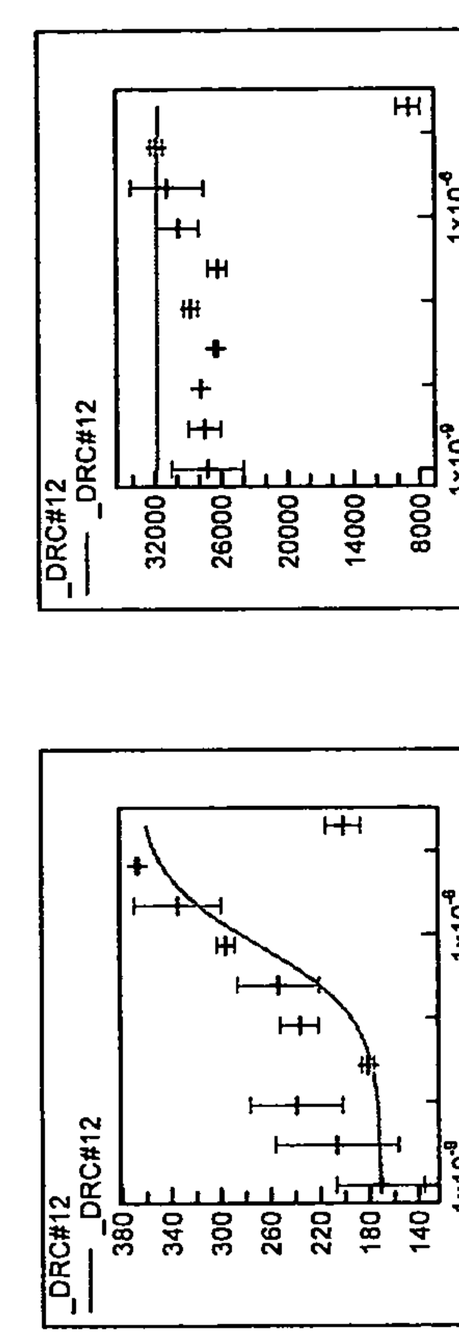
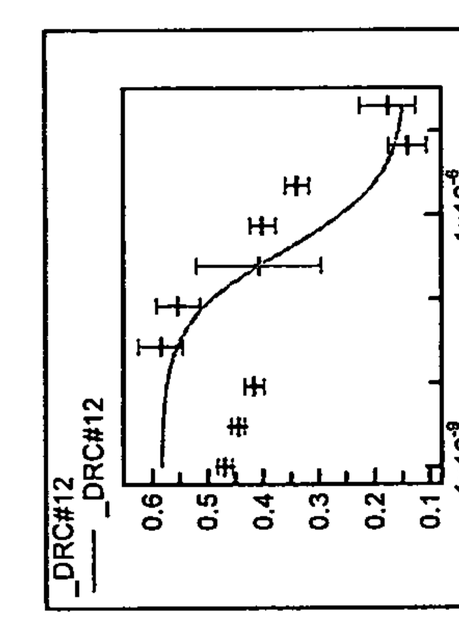
Cell number			
QIM			

	Scaffold IV	IPK00004900	Scaffold II
Cell number			
QIM			

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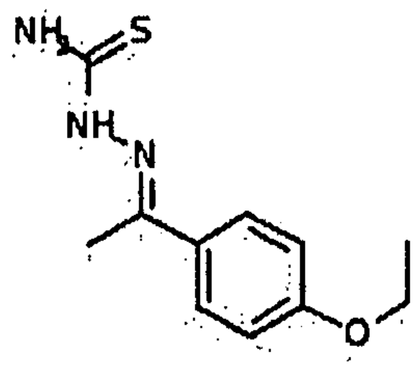
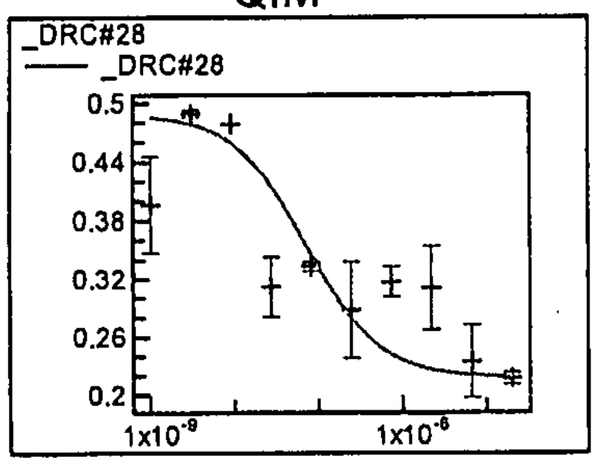
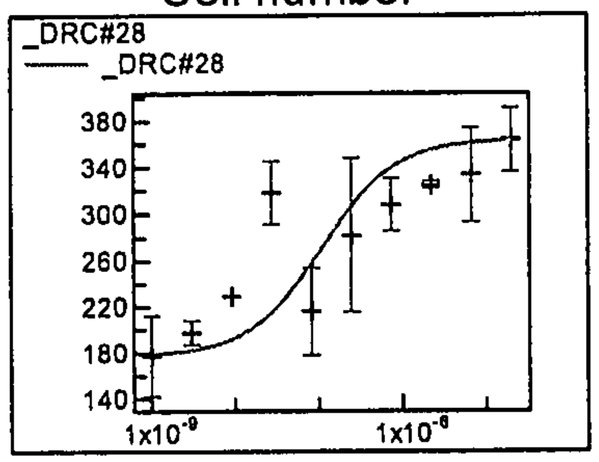
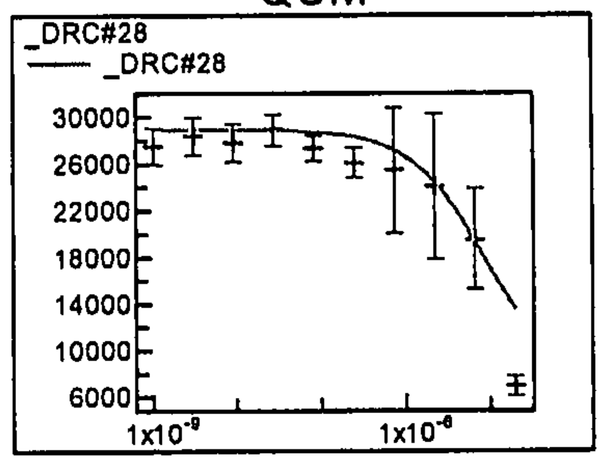
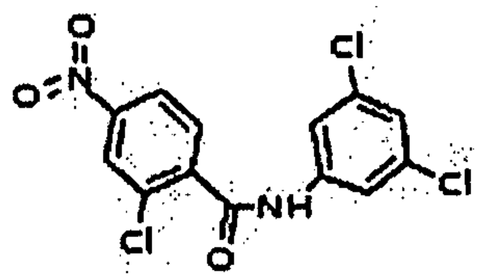
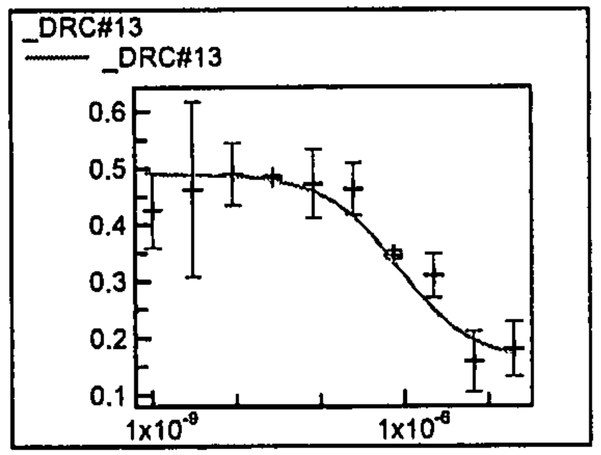
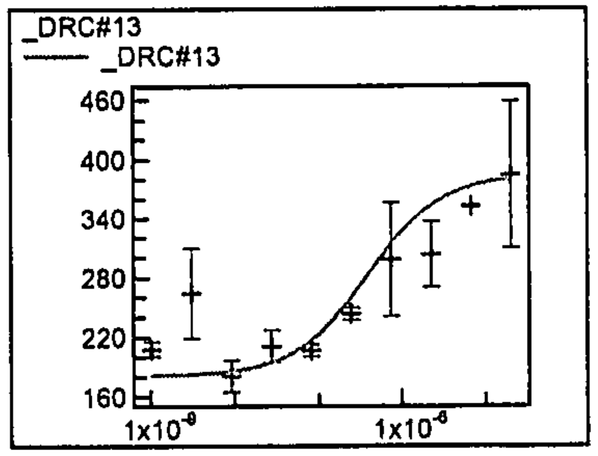
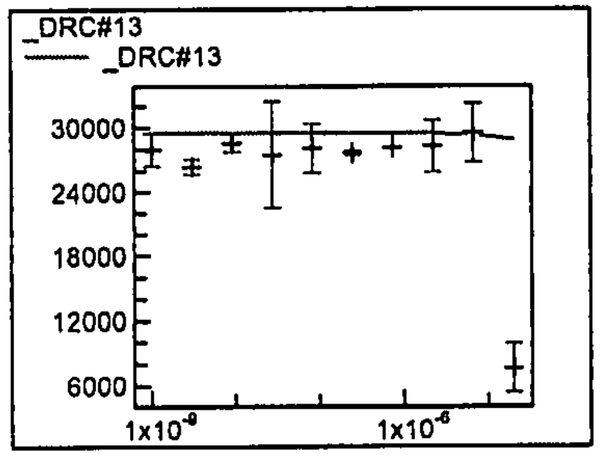
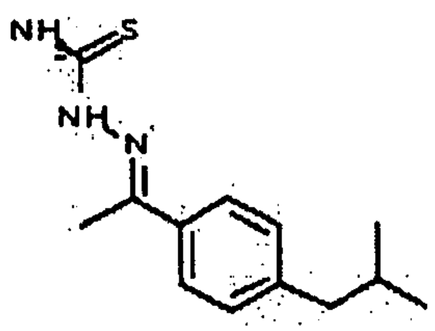
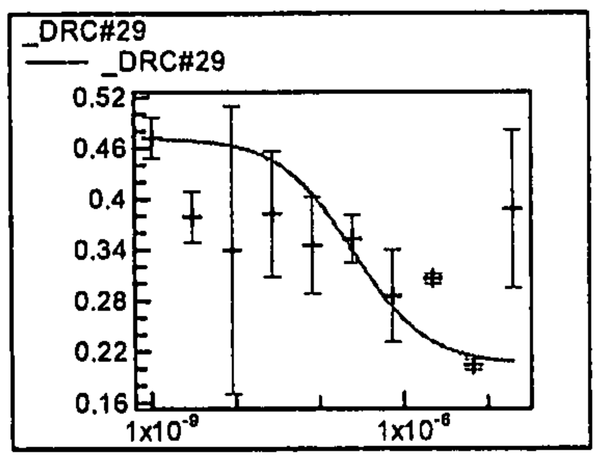
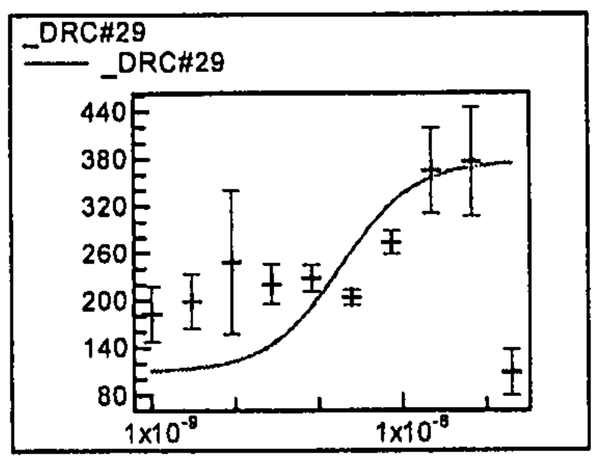
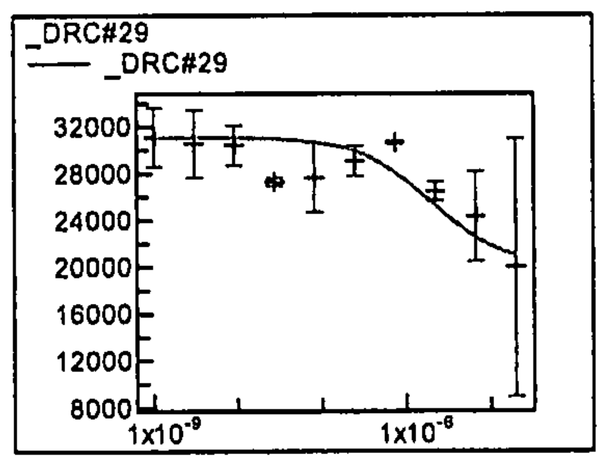
IPK00008037  Scaffold IV			
IPK00004903  Scaffold II			
IPK00008039  Scaffold IV			

Table 2

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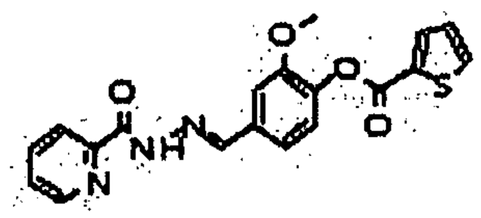
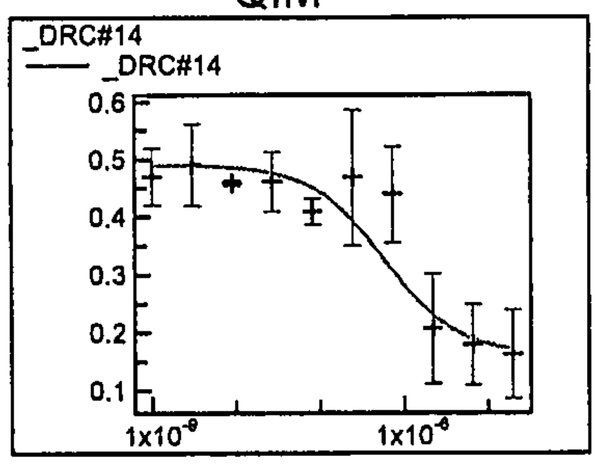
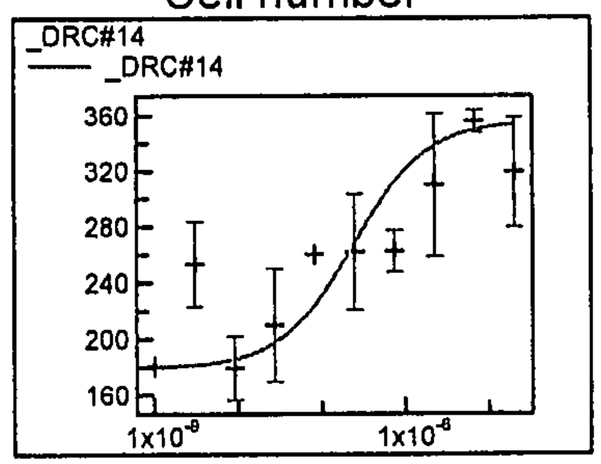
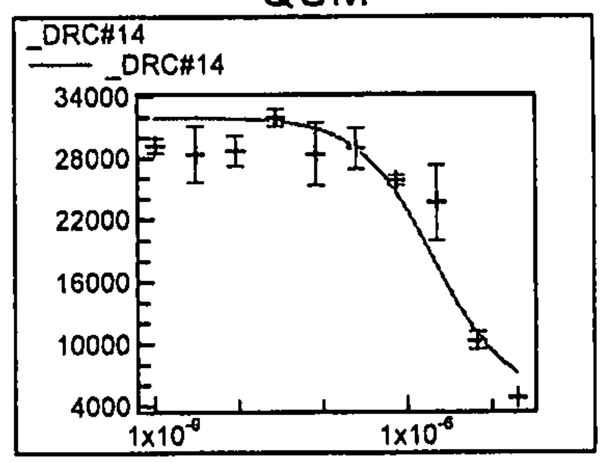
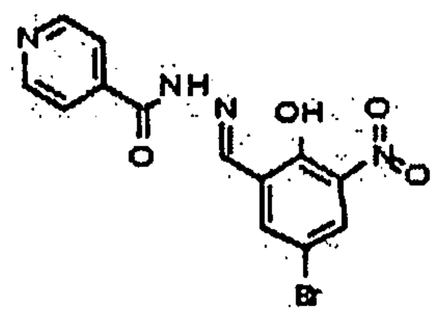
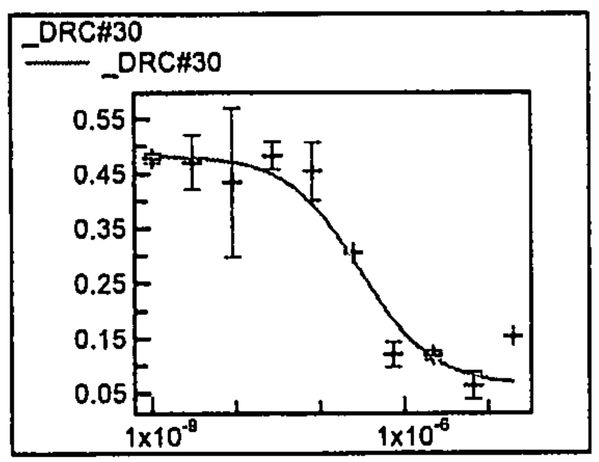
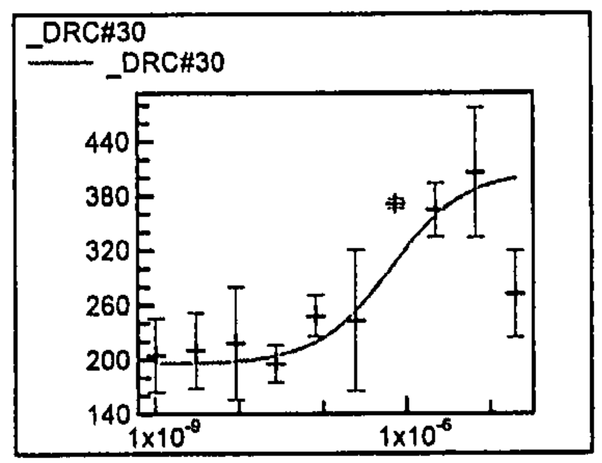
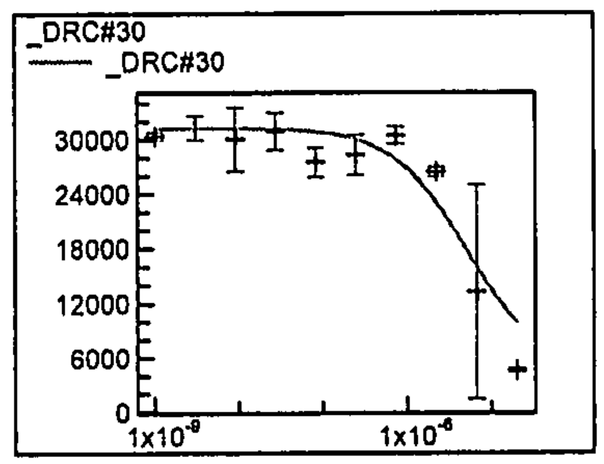
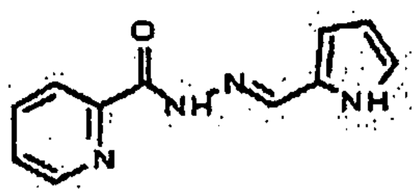
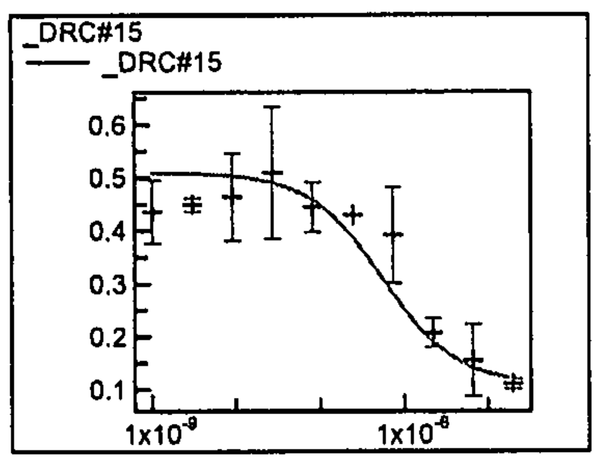
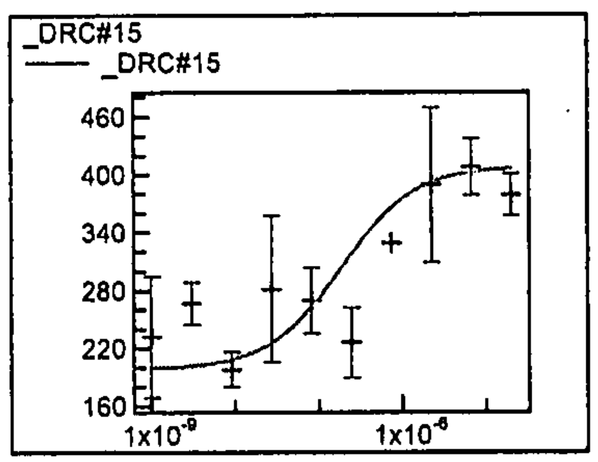
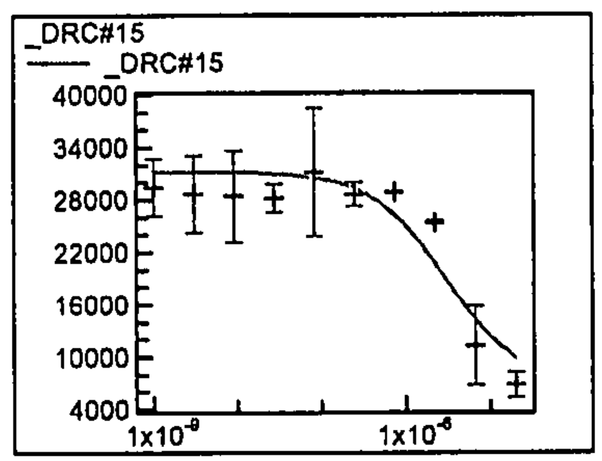
IPK00005820  Scaffold I			
IPK00008081  Scaffold I			
IPK00005821  Scaffold I			

Table 2

Table 2

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Cell number			
QIM			
	IPK00010207	Scaffold I	IPK00005829
	Scaffold I	IPK00010407	Scaffold I

Table 2

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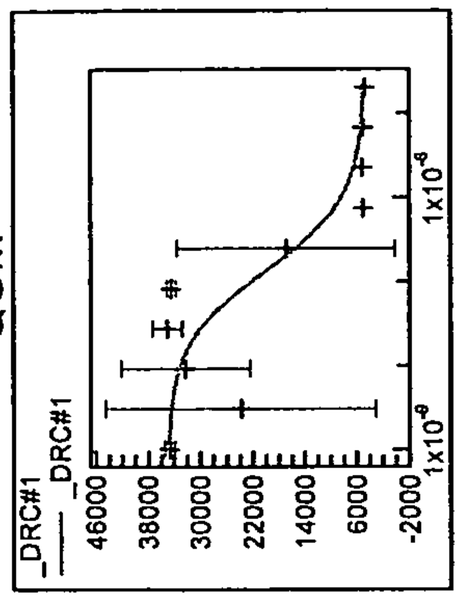
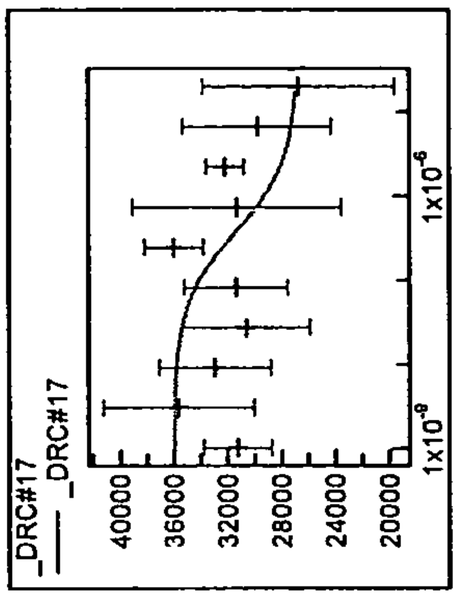
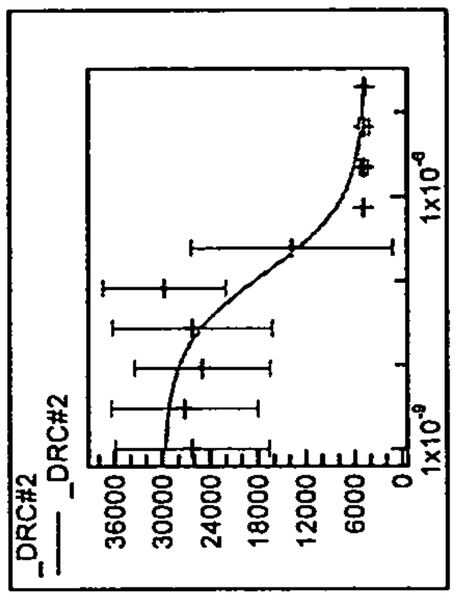
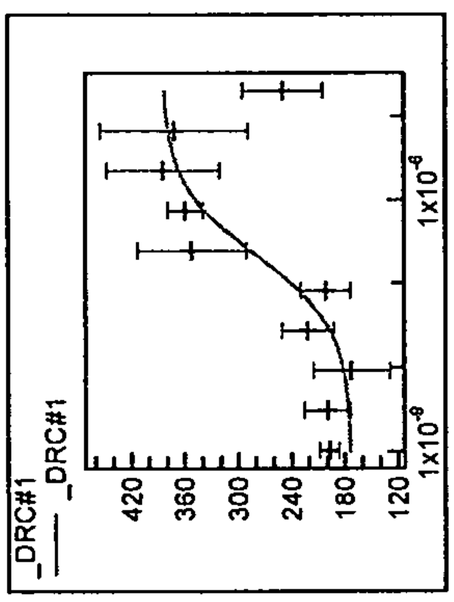
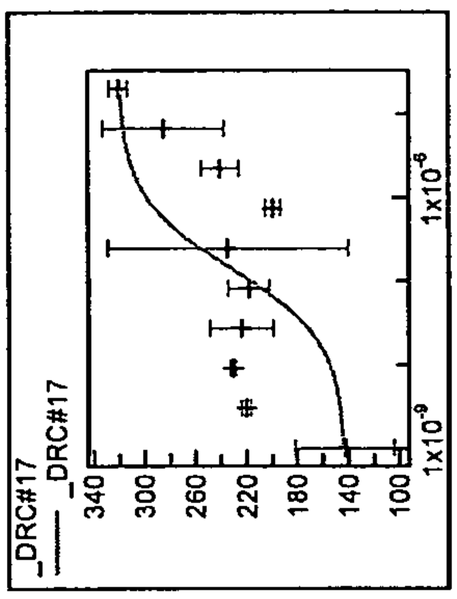
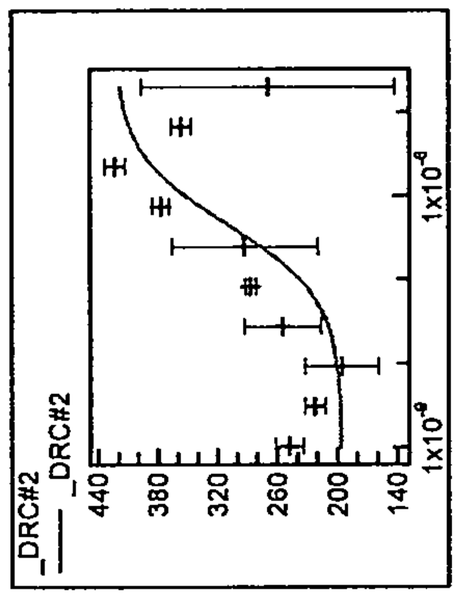
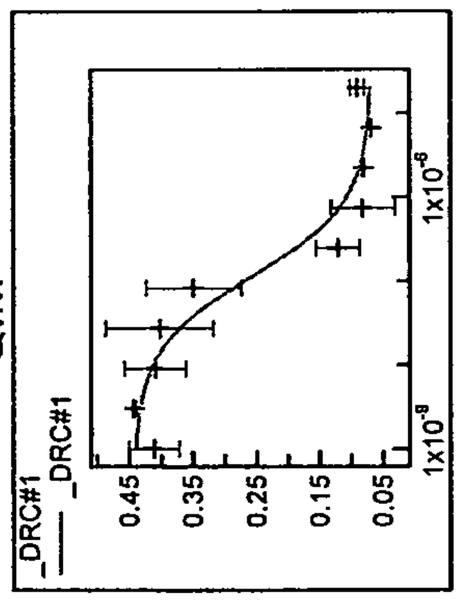
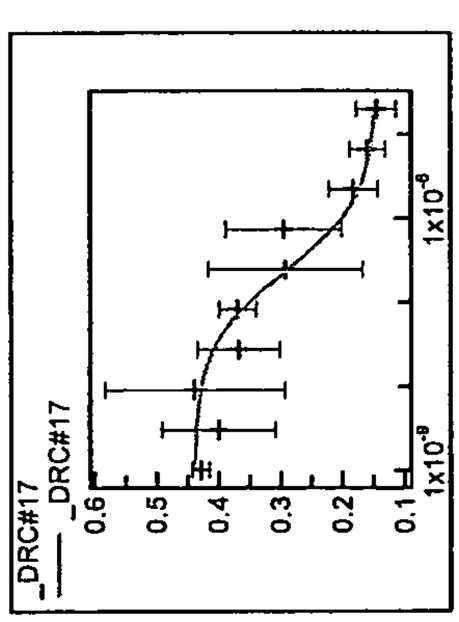
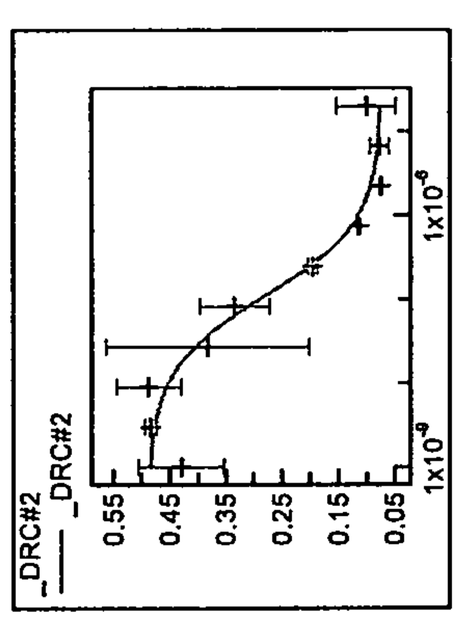
QUM			
Cell number			
QIM			

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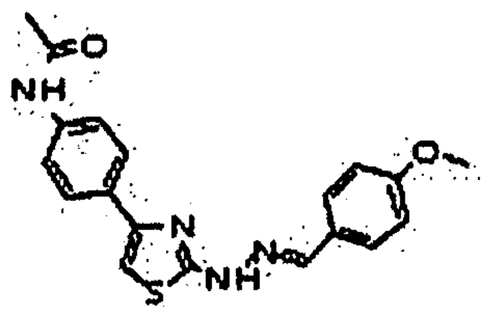	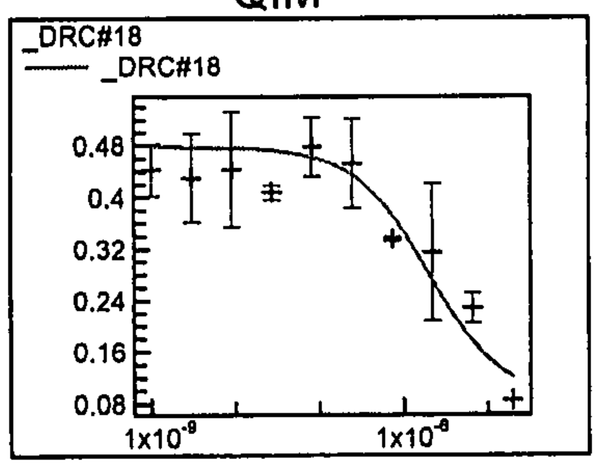	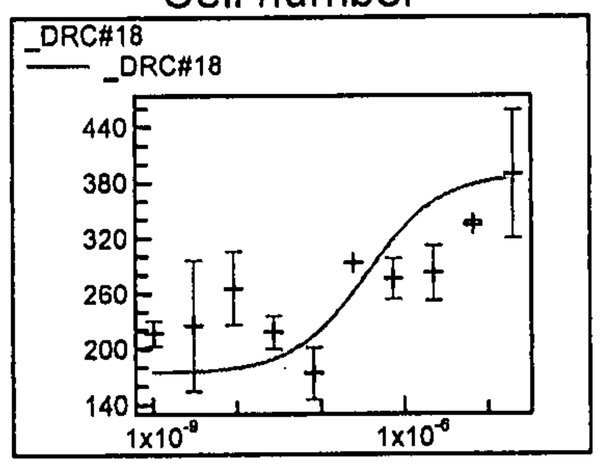	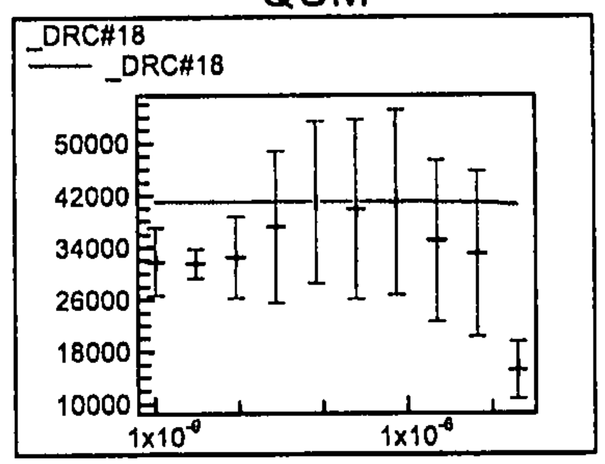
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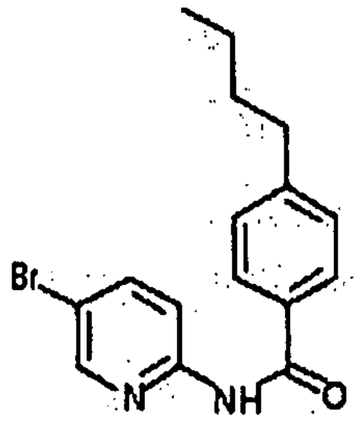	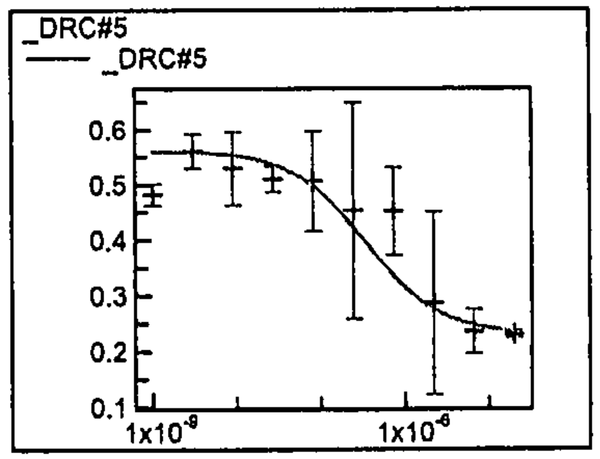	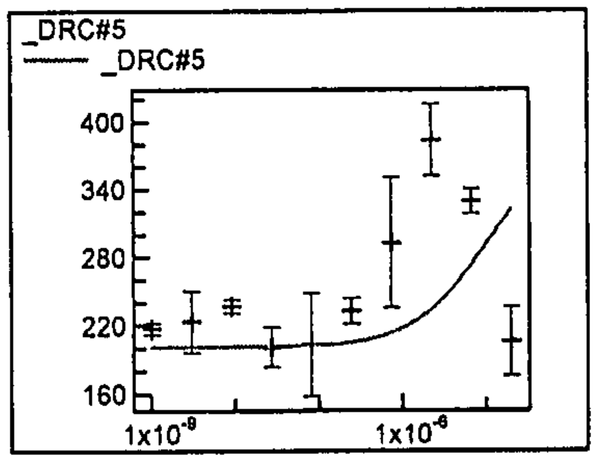	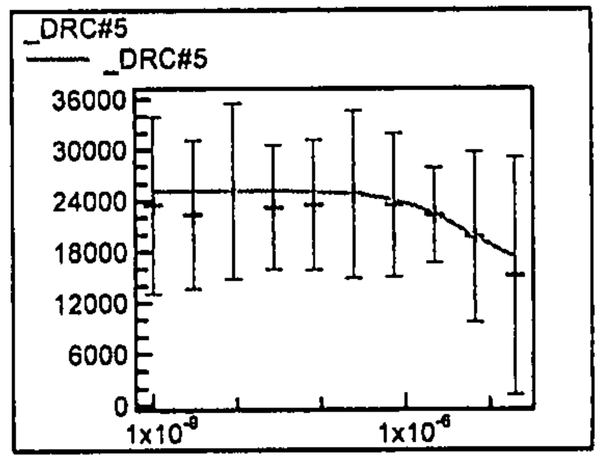
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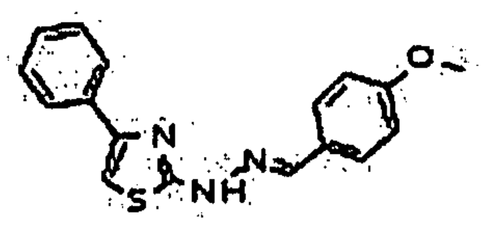
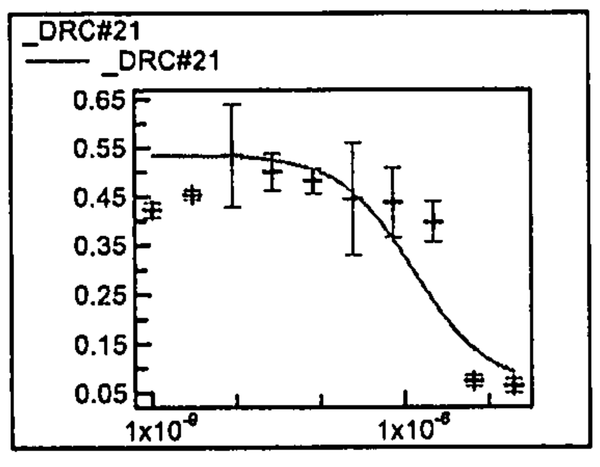
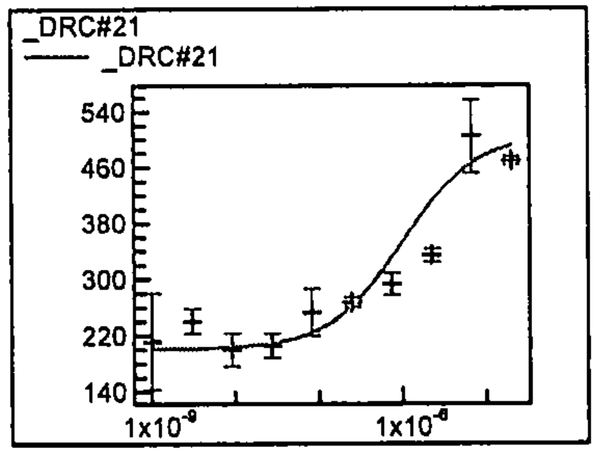
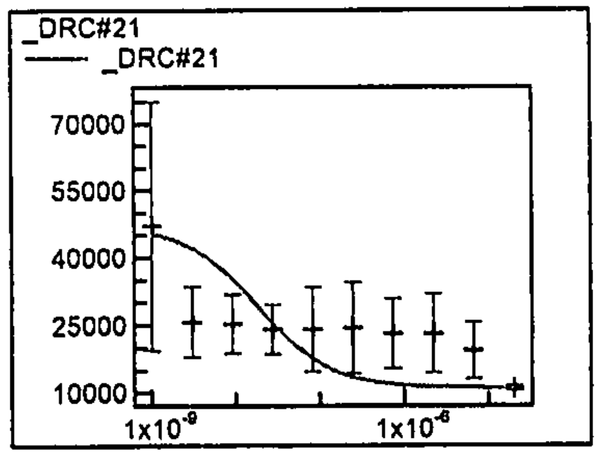
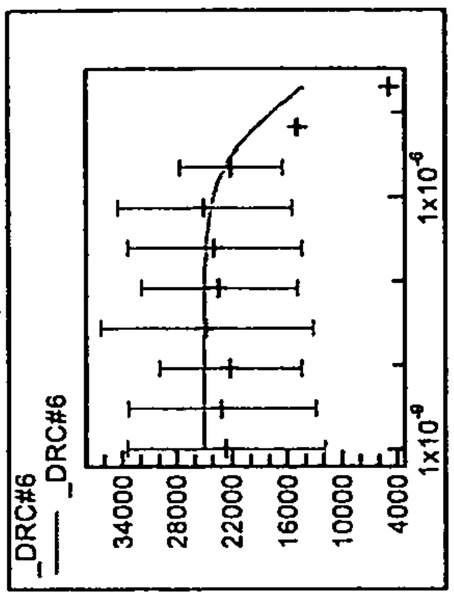
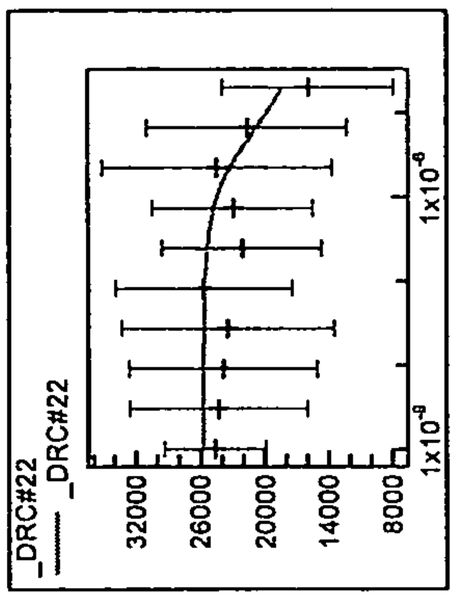
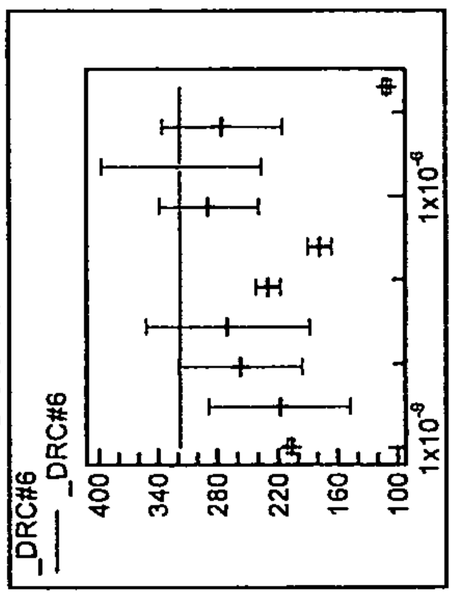
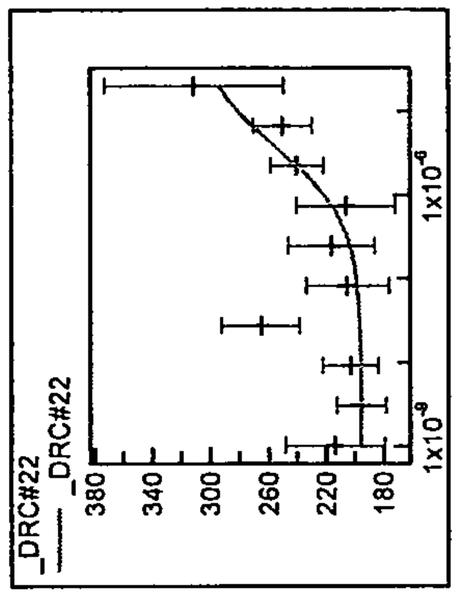
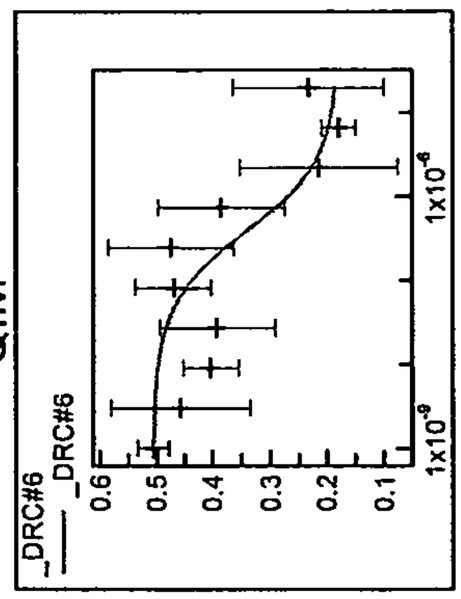
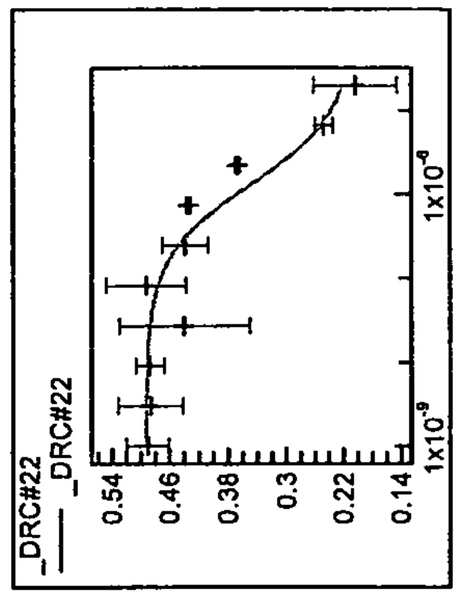
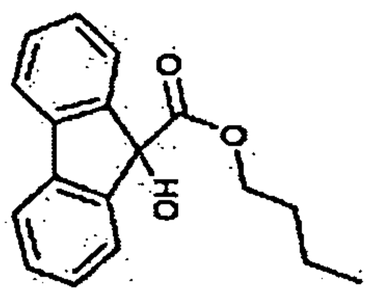
IPK00016362			
			
Scaffold III			

Table 2

Table 2

QUM		
Cell number		
QIM		
IPK00010556		
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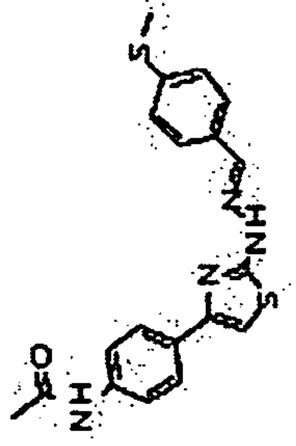
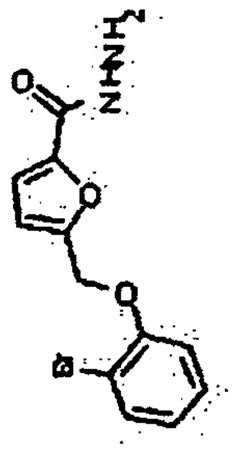
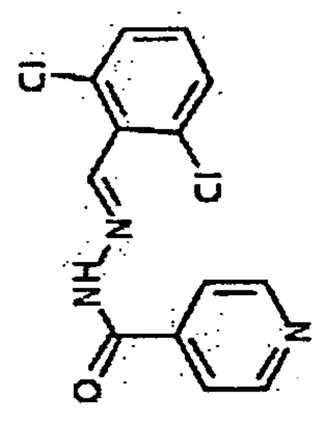
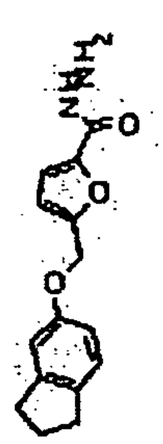
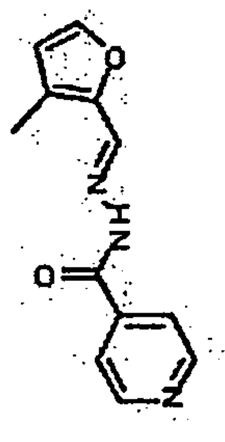
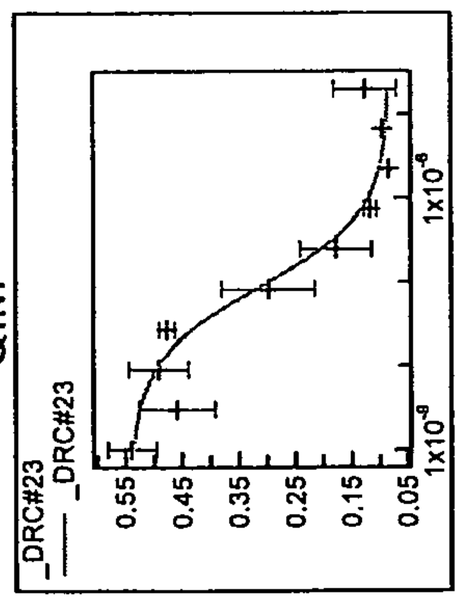
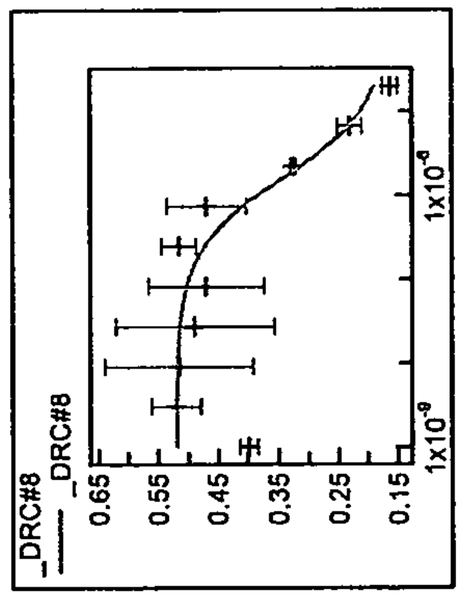
IPK00016367		
Scaffold III		
IPK00012464		
Scaffold V		

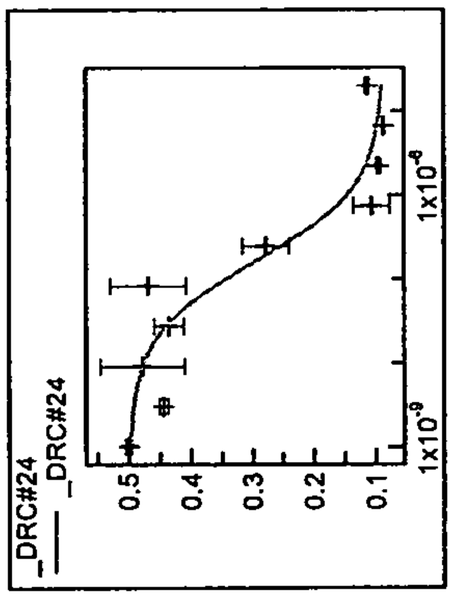
Table 2

IPK00017033		Scaffold I	
IPK00012330		Scaffold V	
IPK00019376		Scaffold I	

QIM







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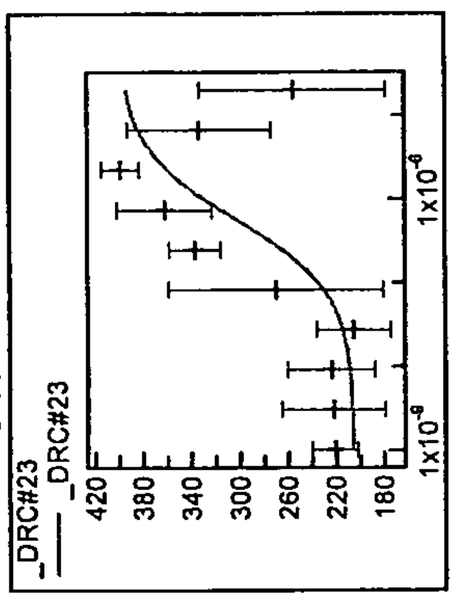
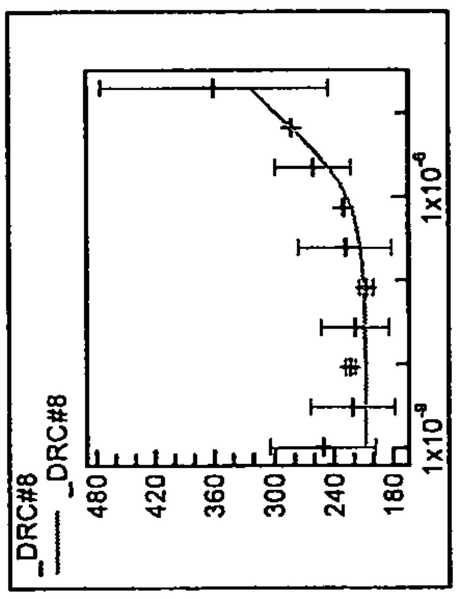
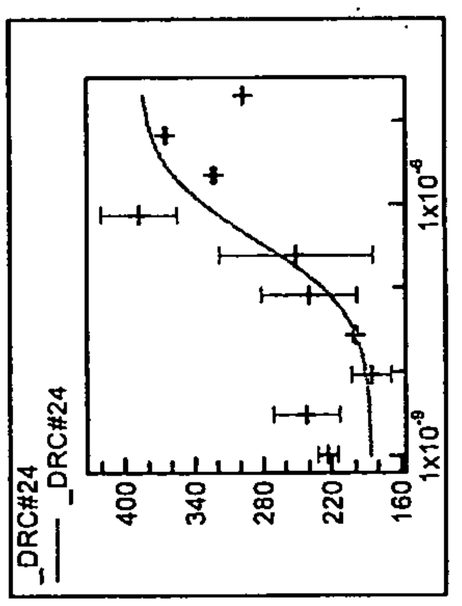
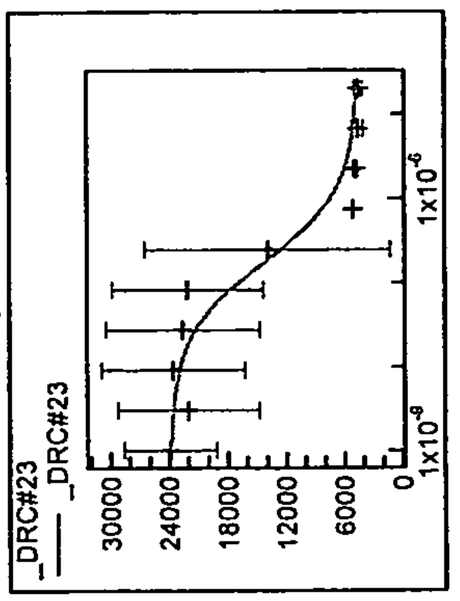
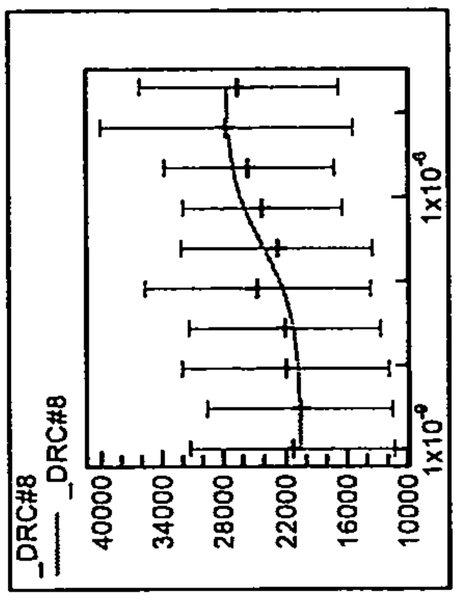


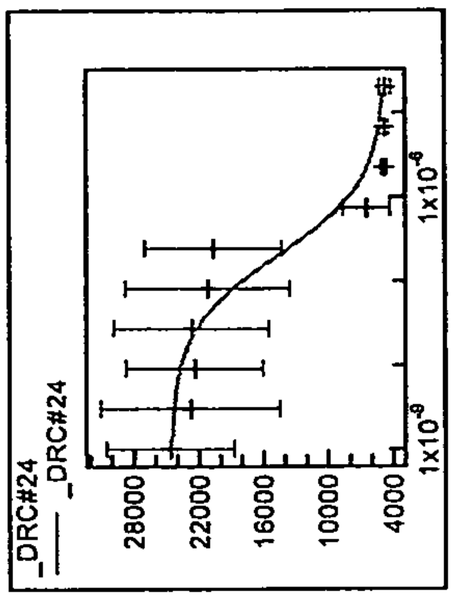




QUM







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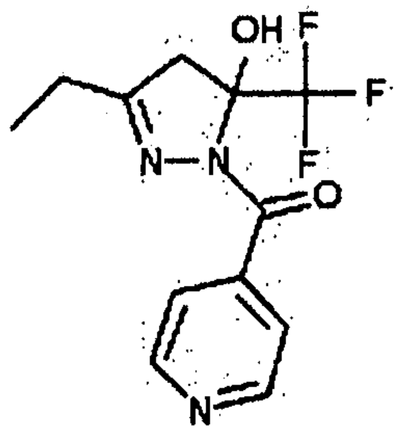
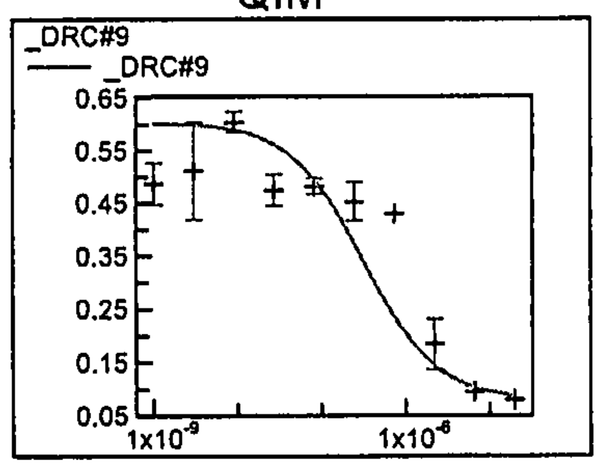
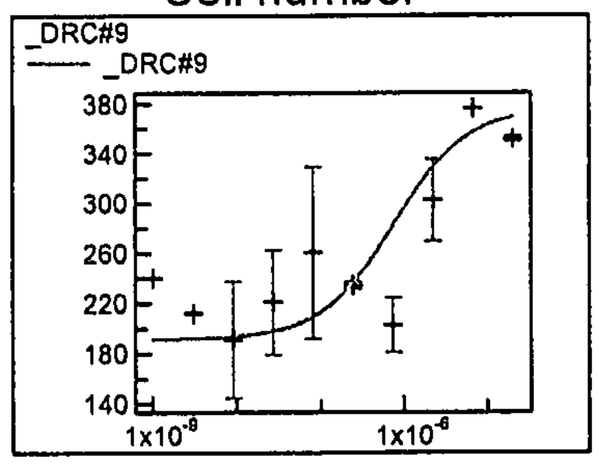
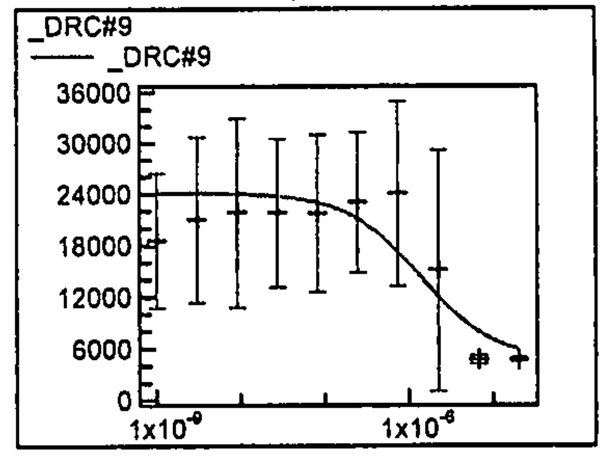
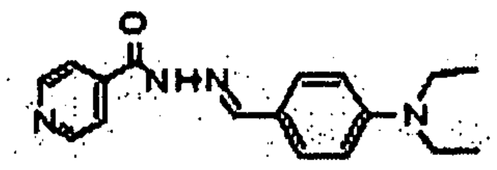
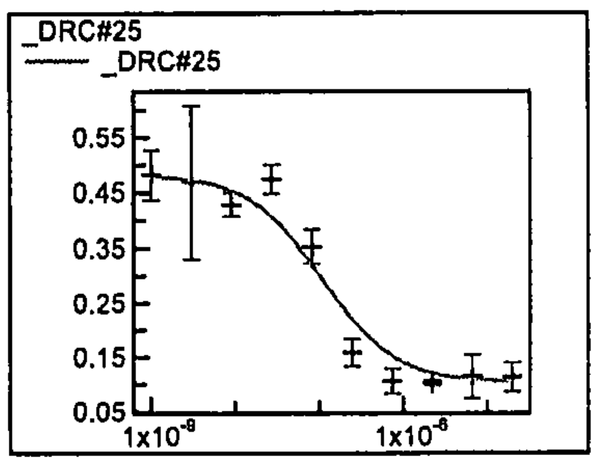
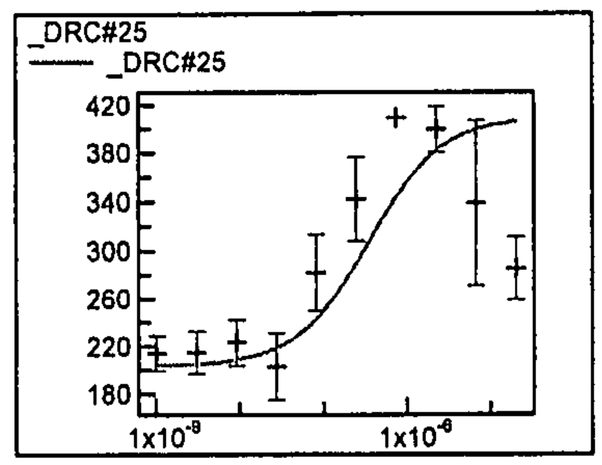
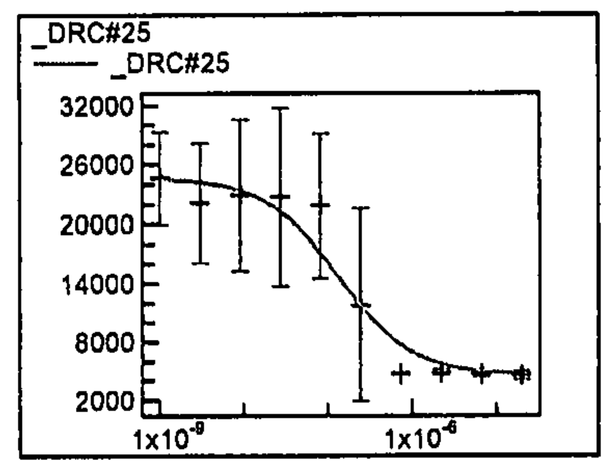
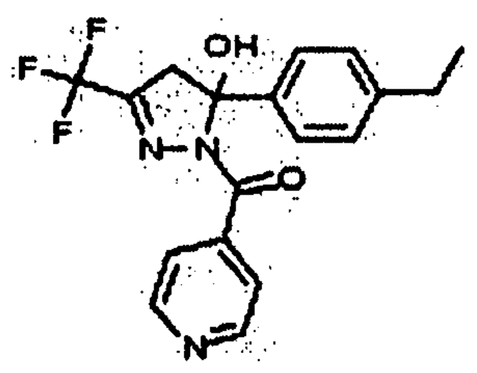
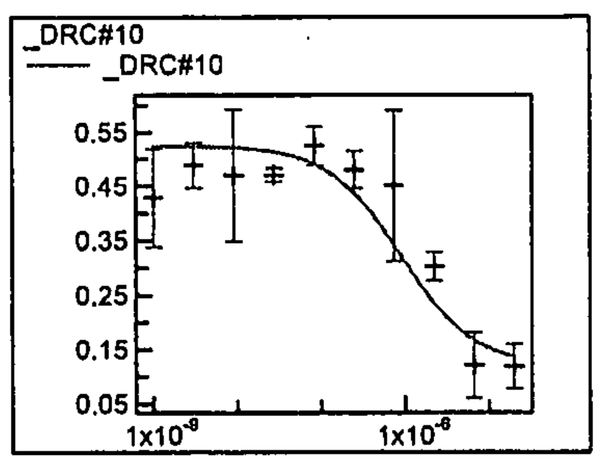
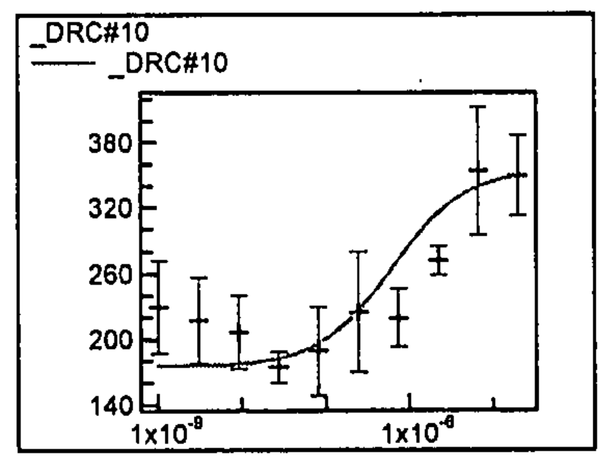
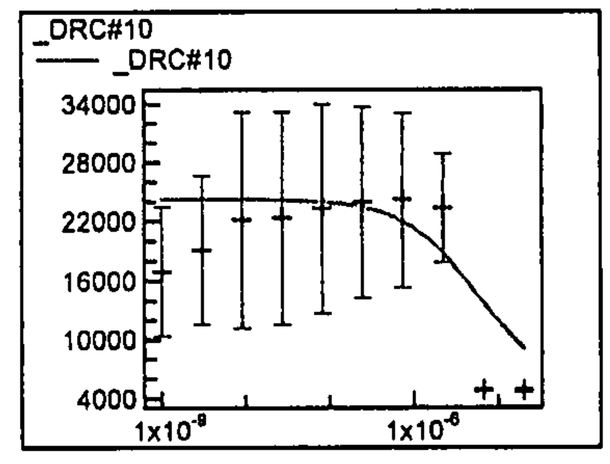
IPK00013450  Scaffold VII			
IPK00019599  Scaffold I			
IPK00013451  Scaffold VII			

Table 2

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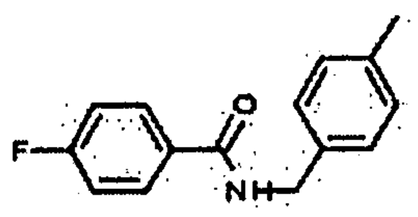
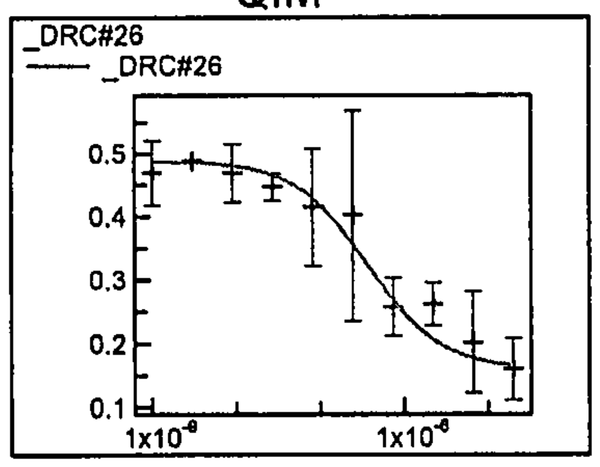
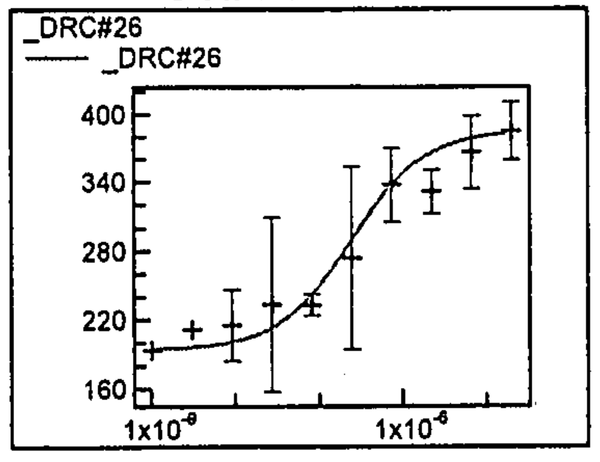
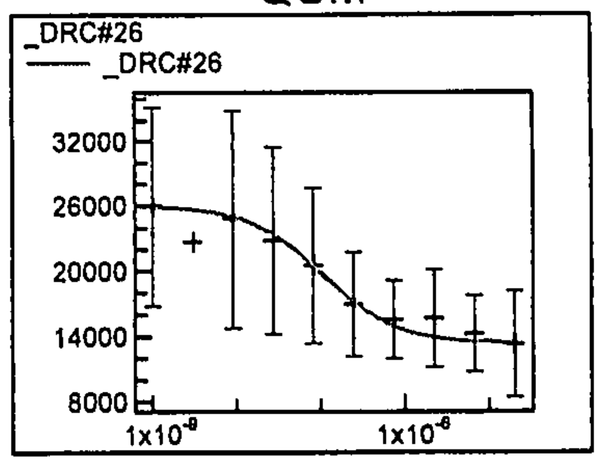
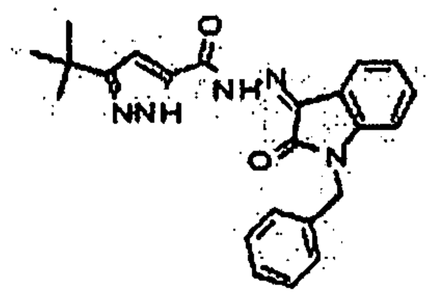
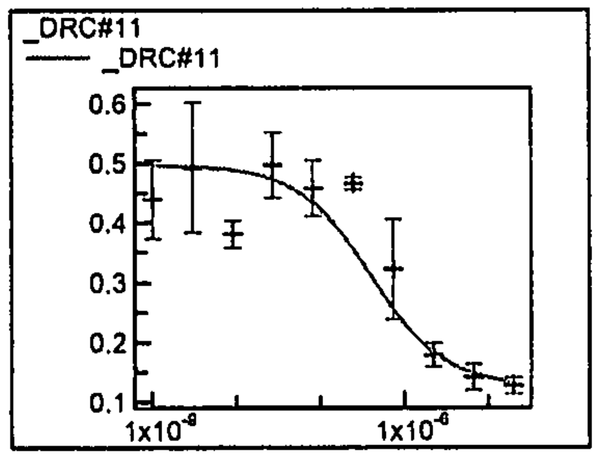
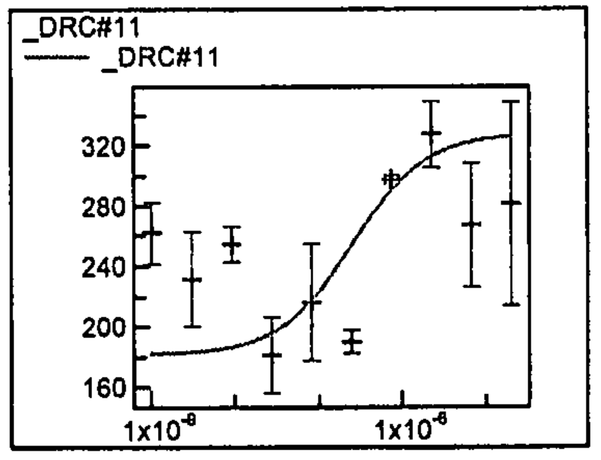
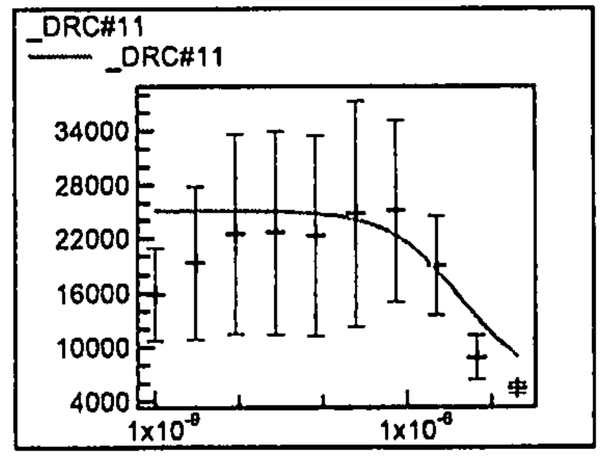
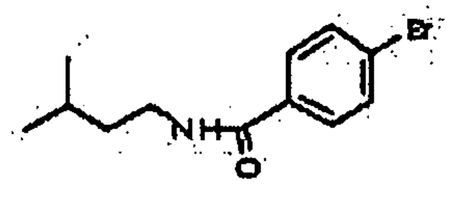
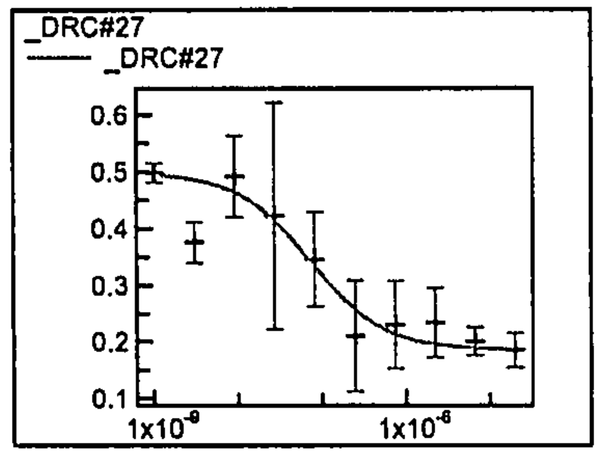
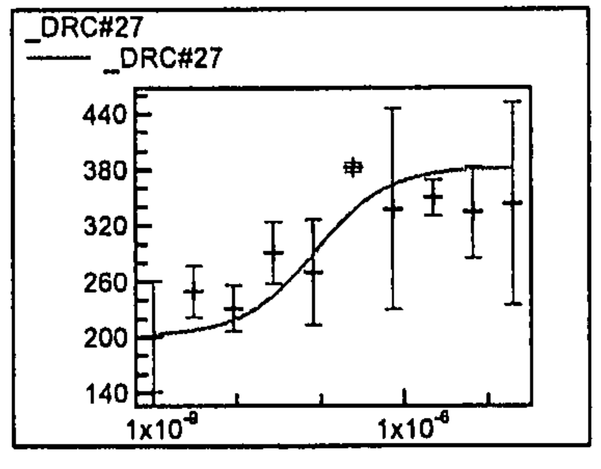
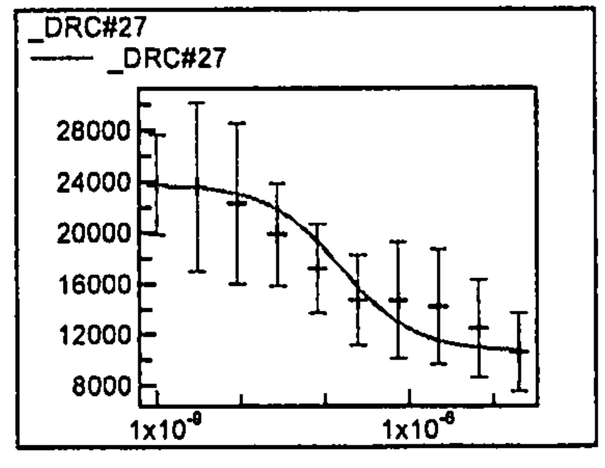
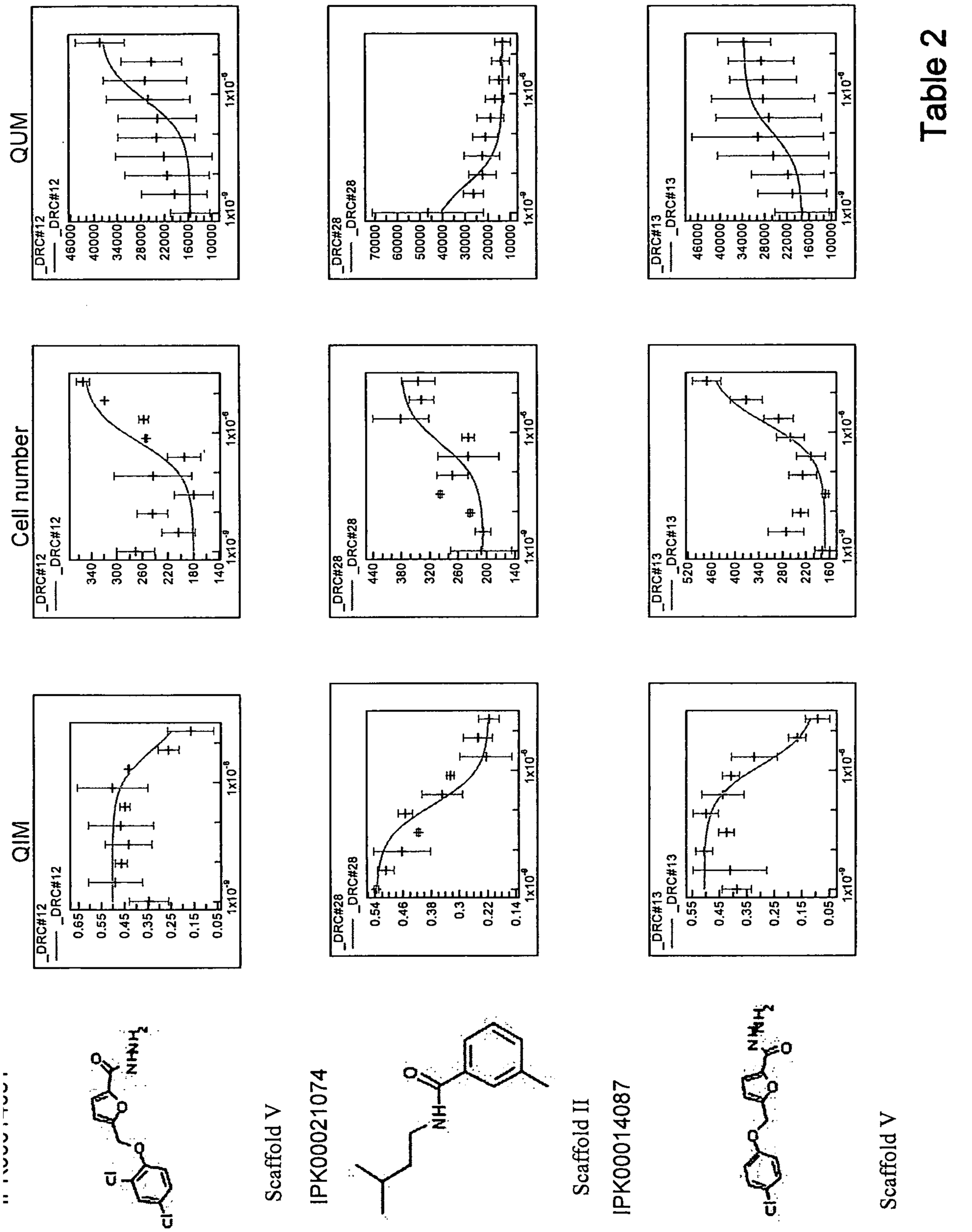
IPK00020542  Scaffold II			
IPK00013812  Scaffold XI			
IPK00021070  Scaffold II			

Table 2

Table 2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Cell number
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_DRC#28
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1x10⁻⁹
1x10⁻⁸
Scaffold V
IPK00021074
Scaffold II
IPK00014087
Scaffold V

Table 2

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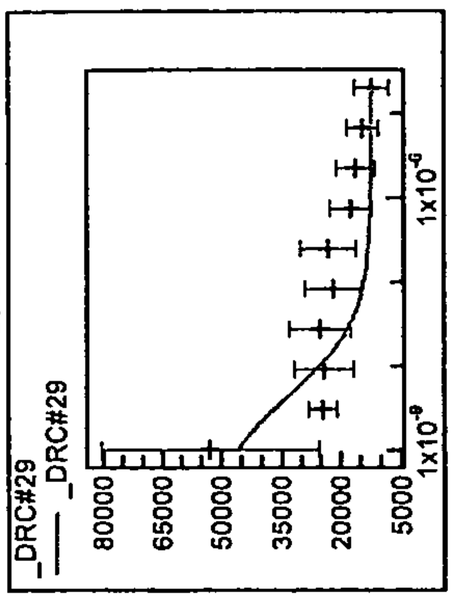
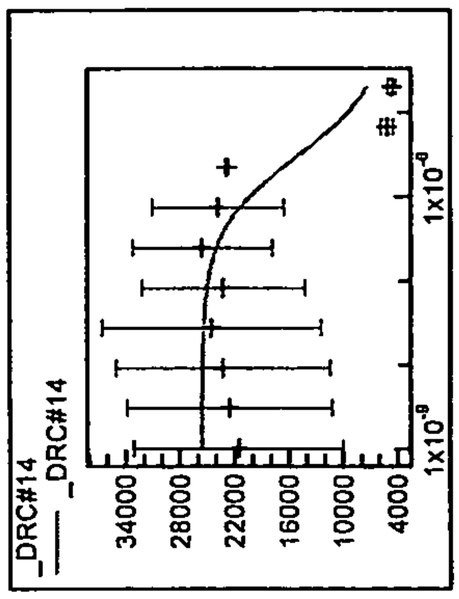
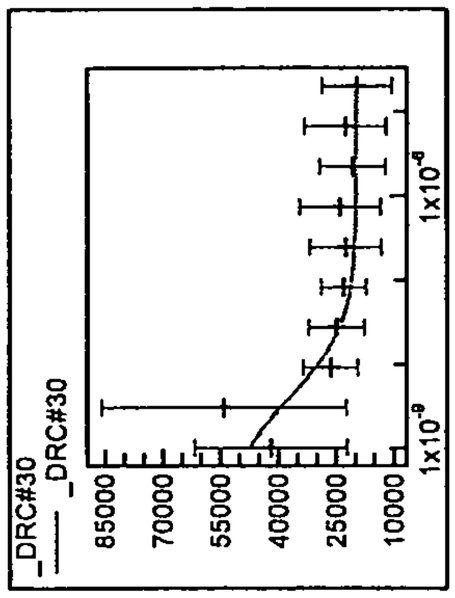
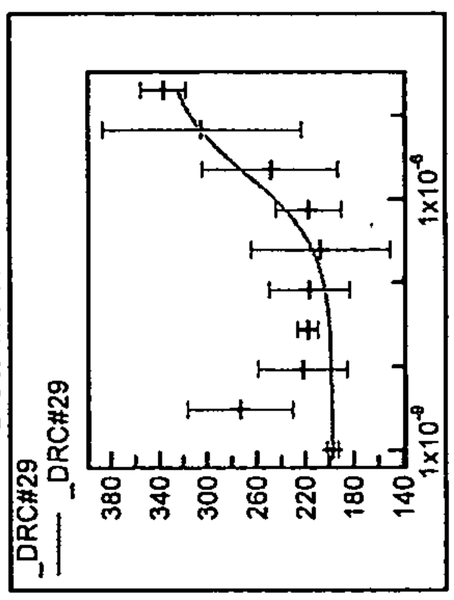
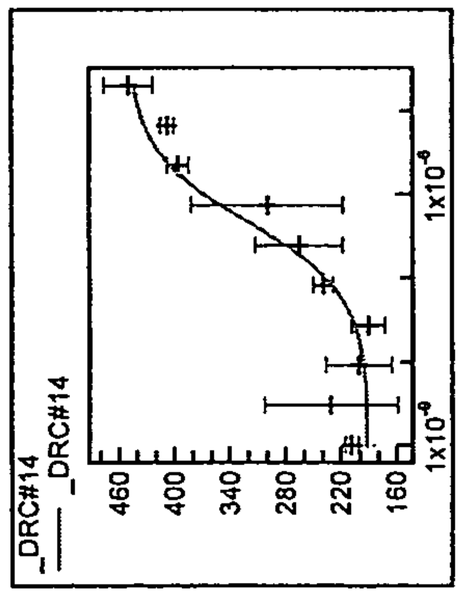
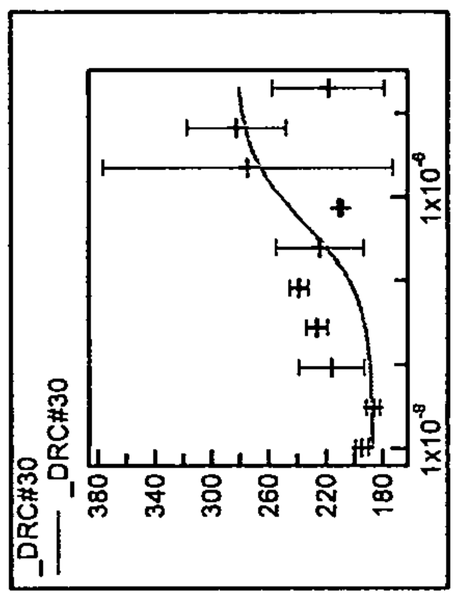
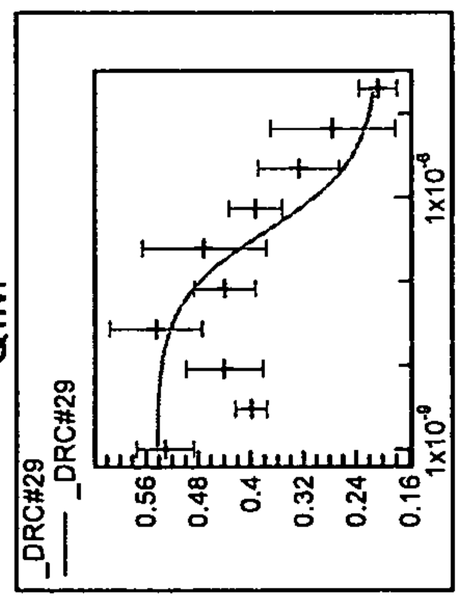
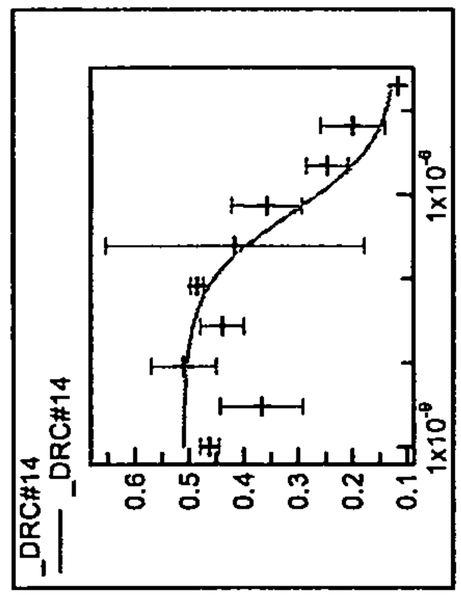
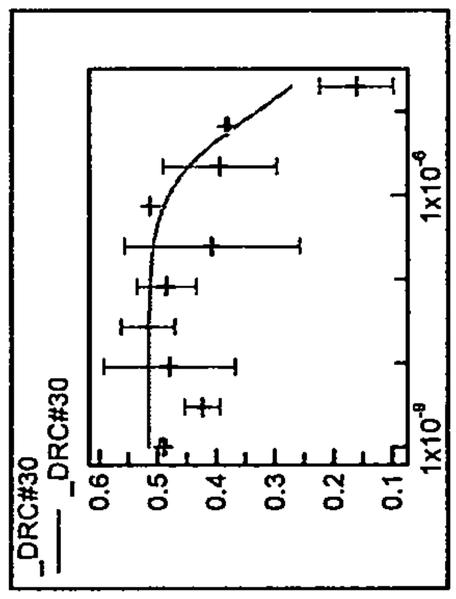
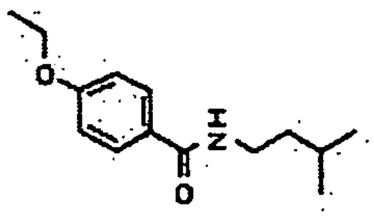
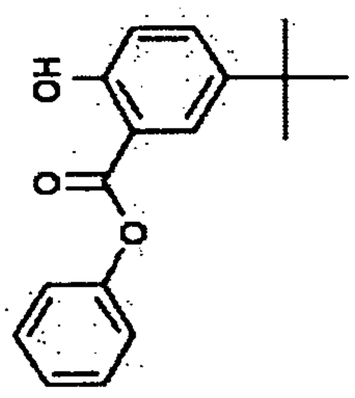
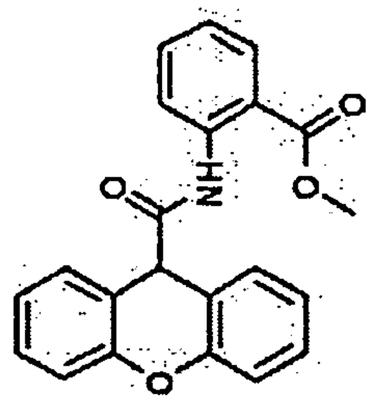
QUM			
Cell number			
QIM			
IPK00024103			

Table 2

IPK00015751	IPK00021929	IPK00016327
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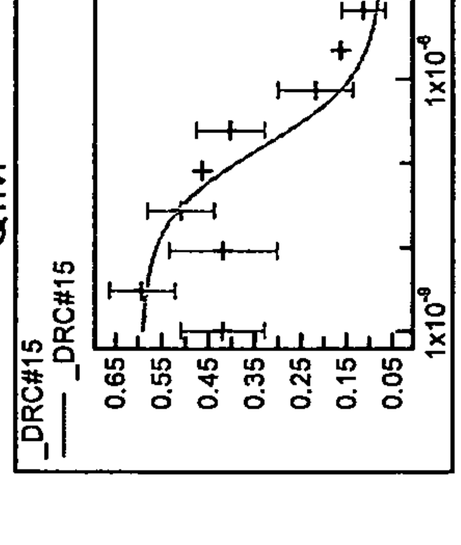
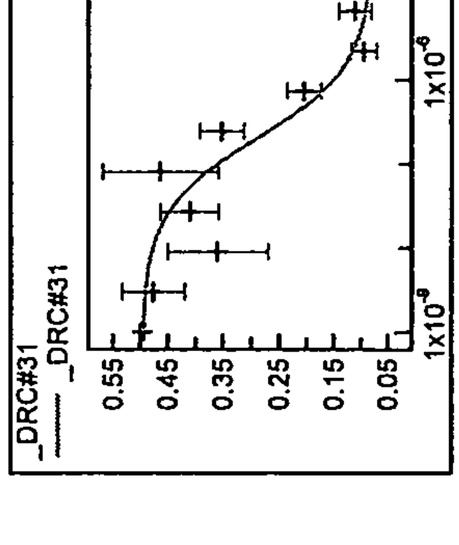
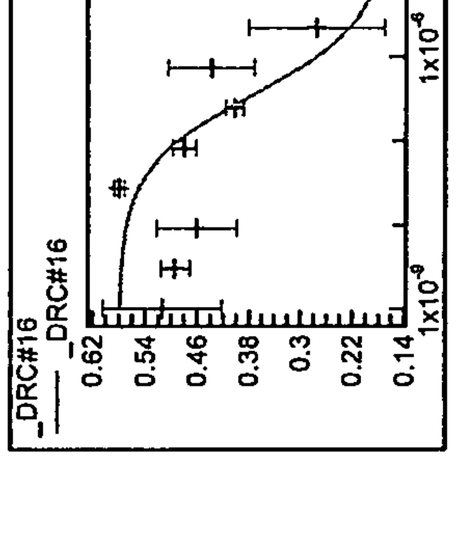
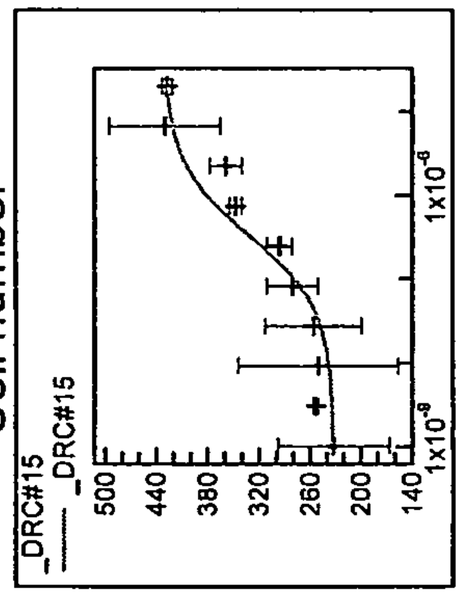
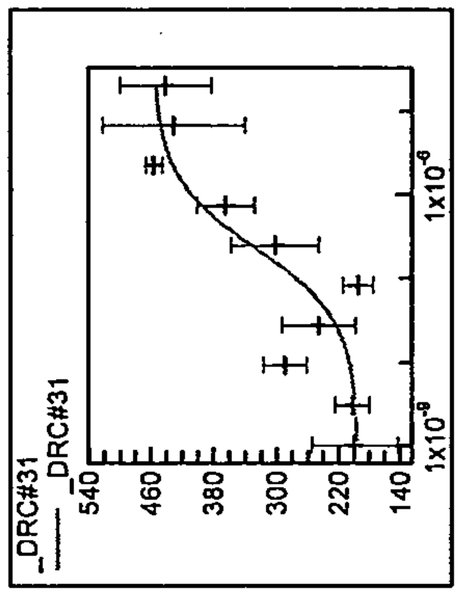
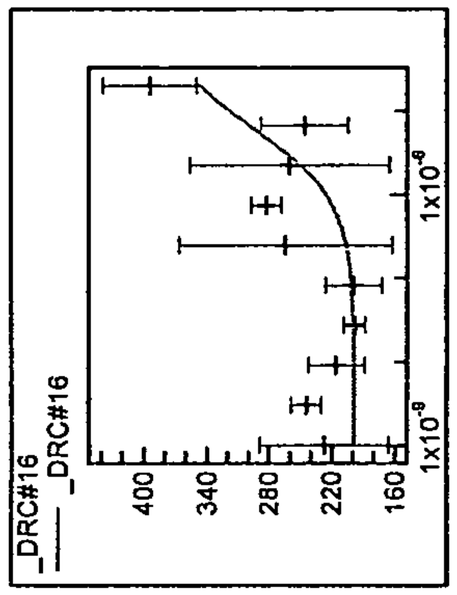
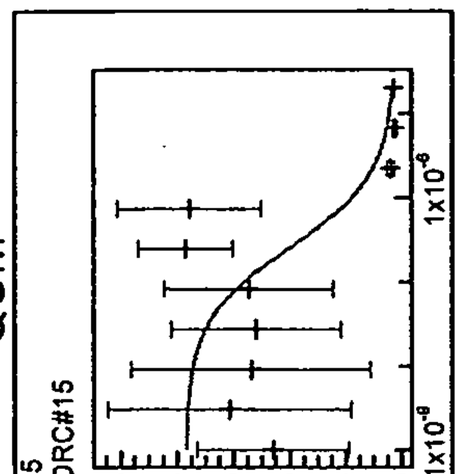
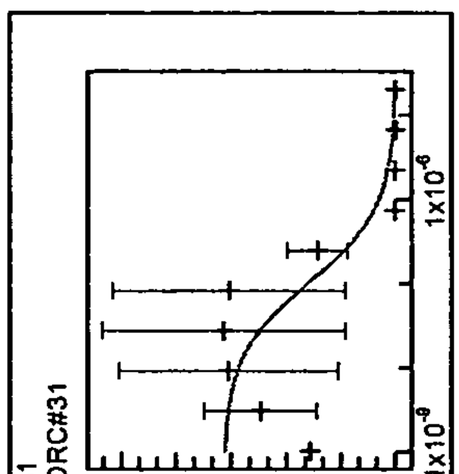
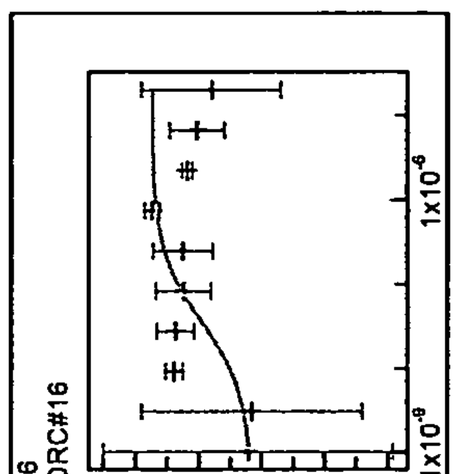
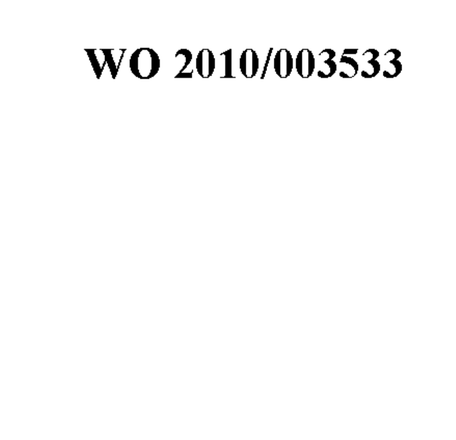
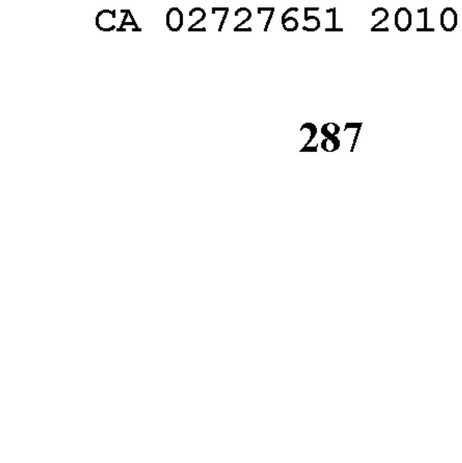
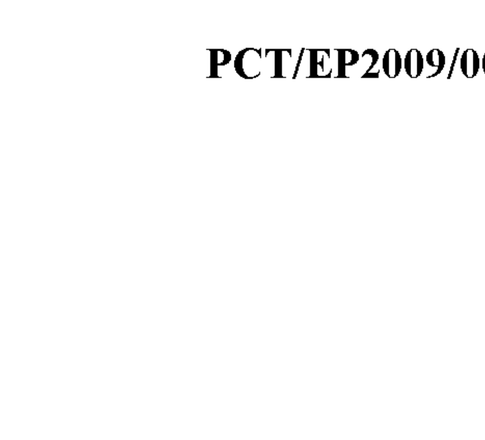
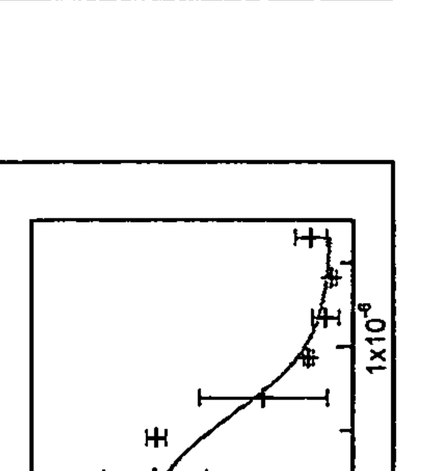
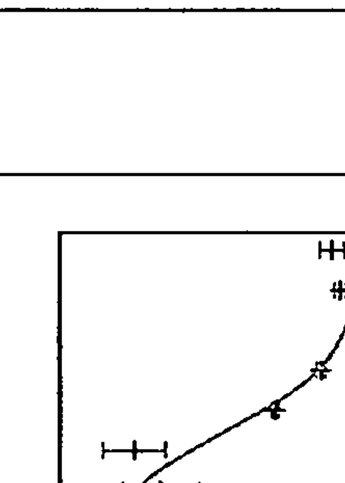
		
		
		
		

Table 2

IPK00021930	Scaffold X	
IPK00021926	Scaffold I	
IPK00021927	Scaffold I	

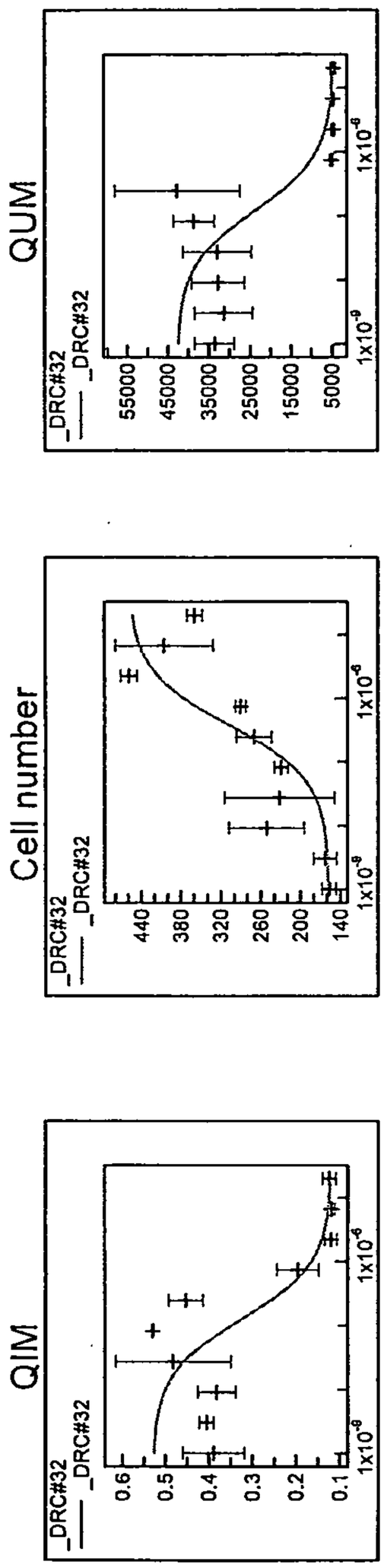
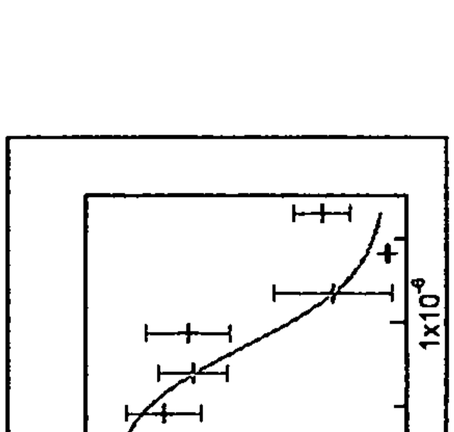
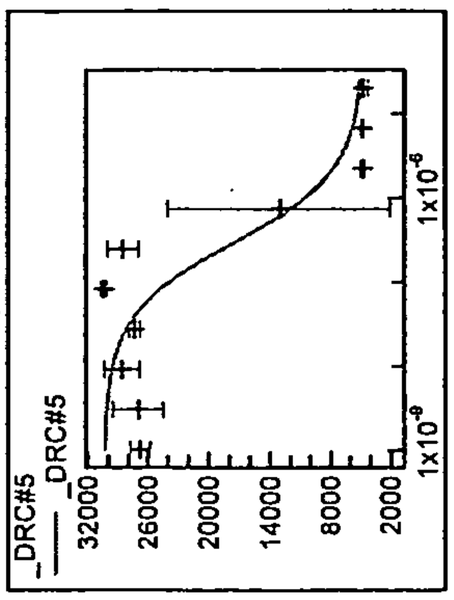
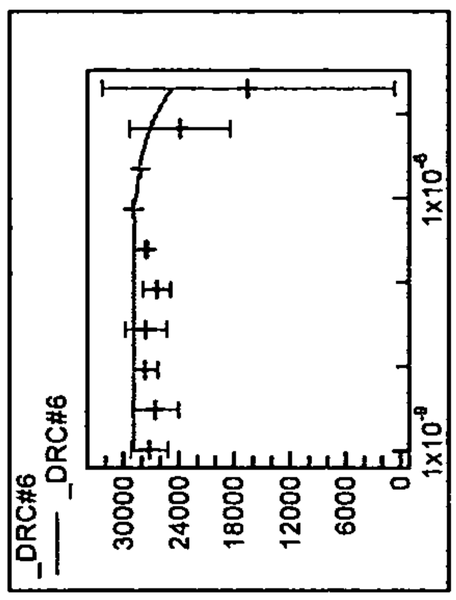
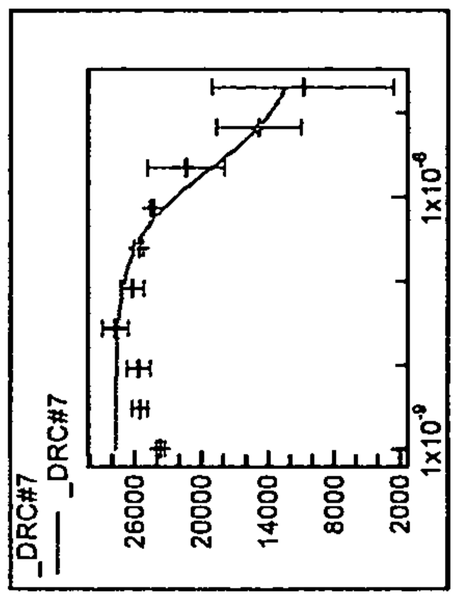
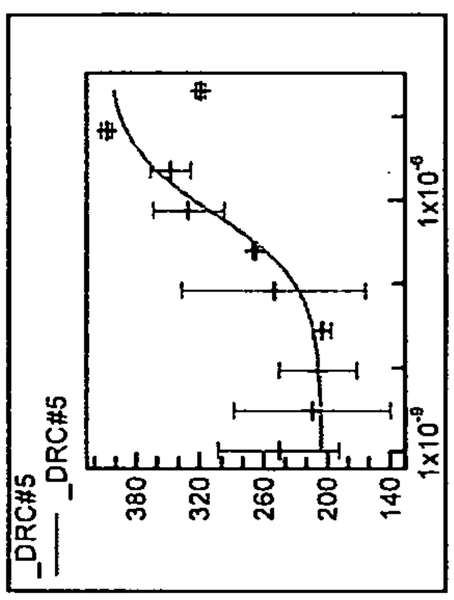
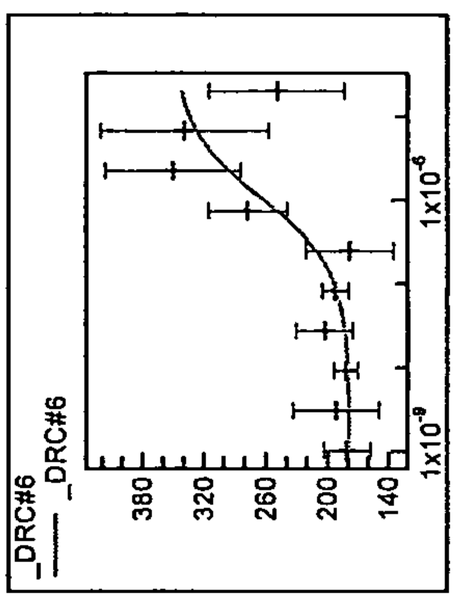
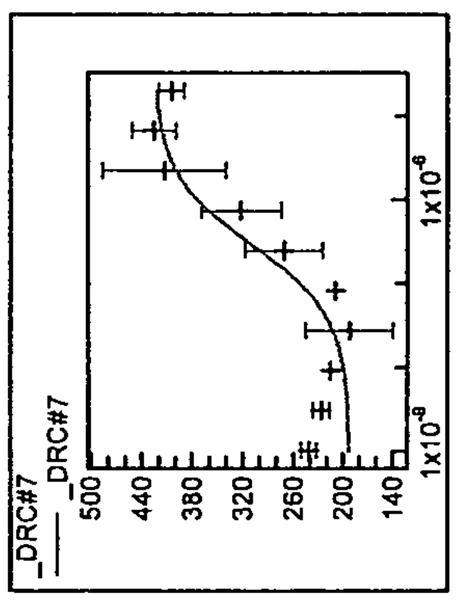
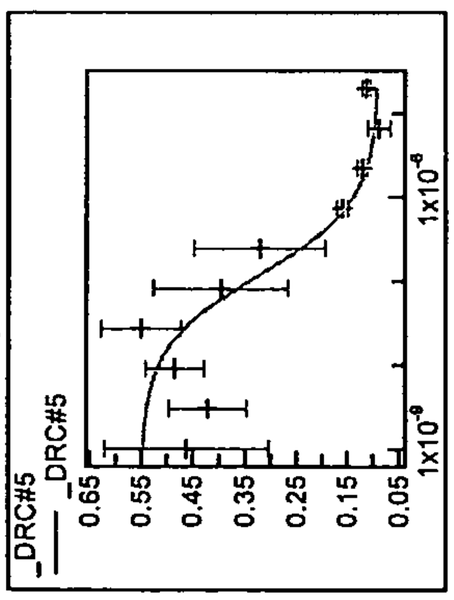
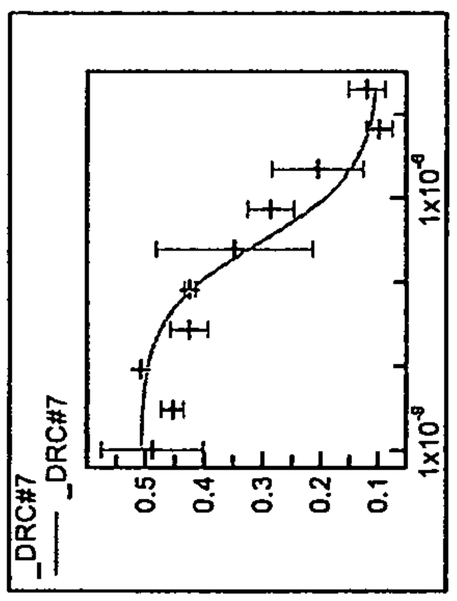
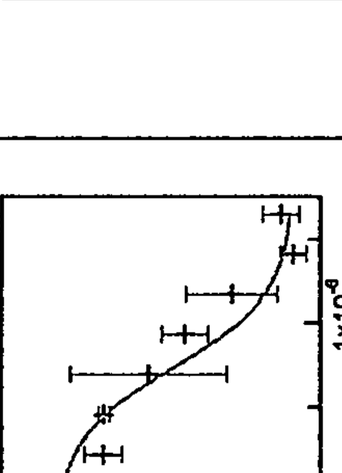


Table 2

	IPK00021928	Scaffold I	
QUM			
Cell number			
QIM			
	IPK00023002	Scaffold II	IPK00022972	Scaffold XV
				

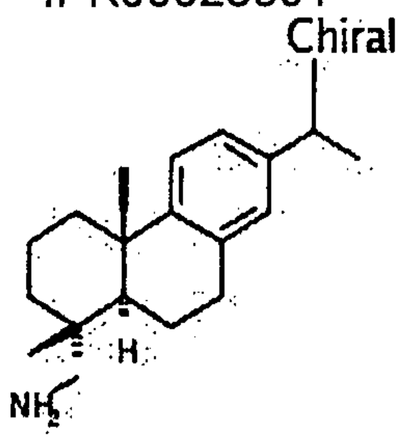
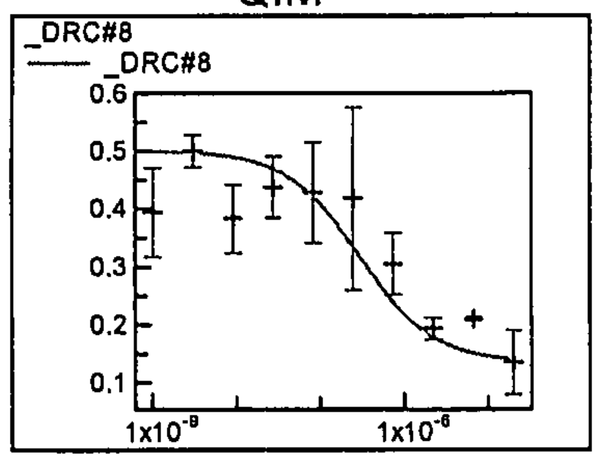
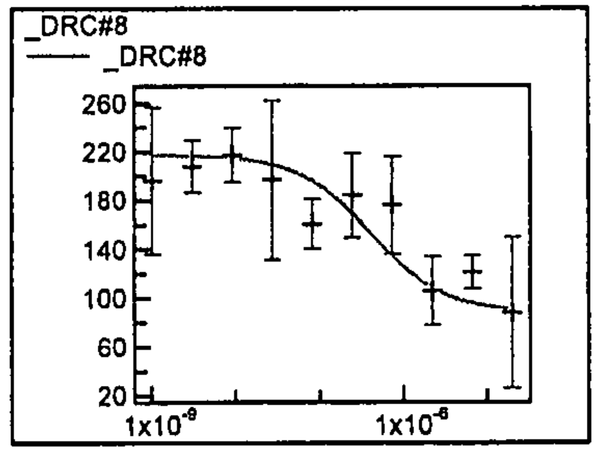
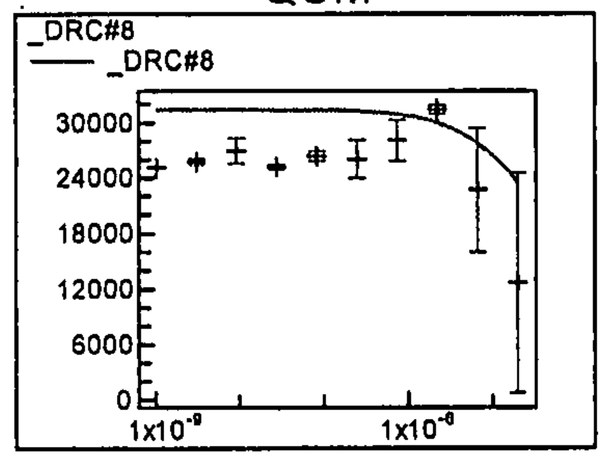
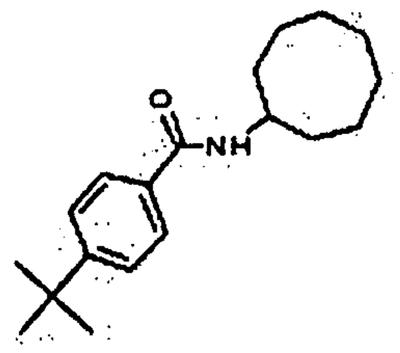
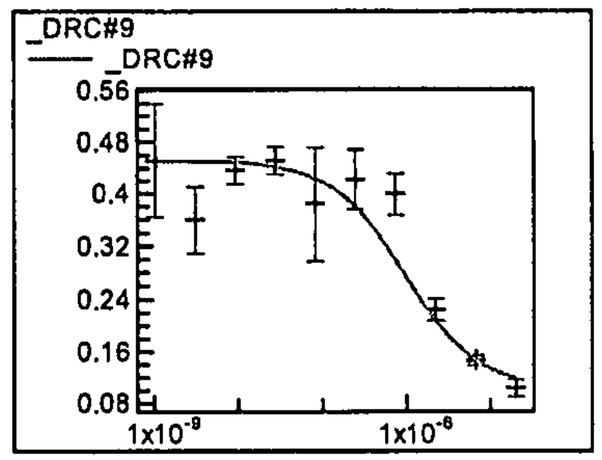
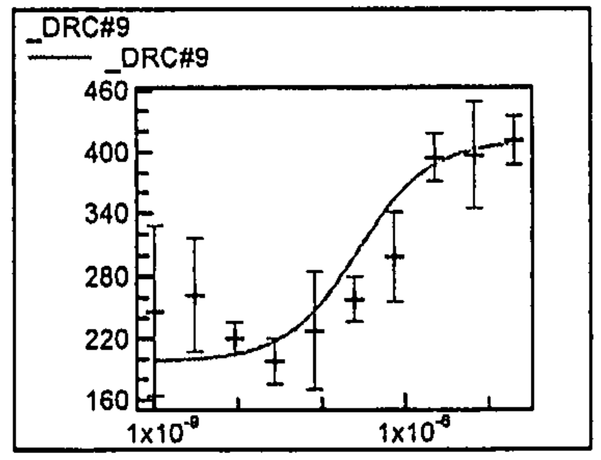
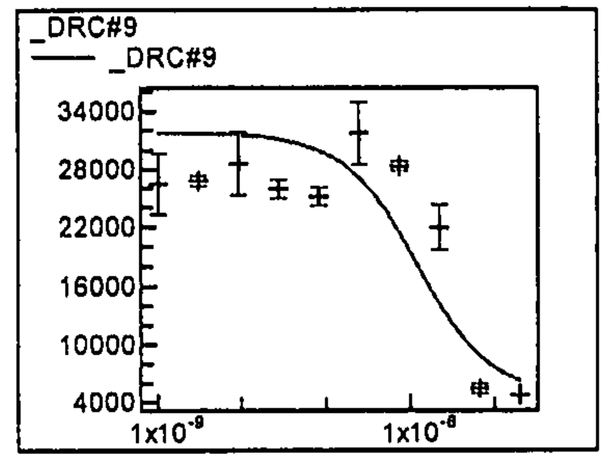
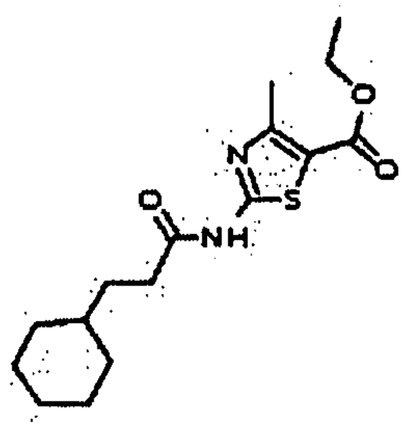
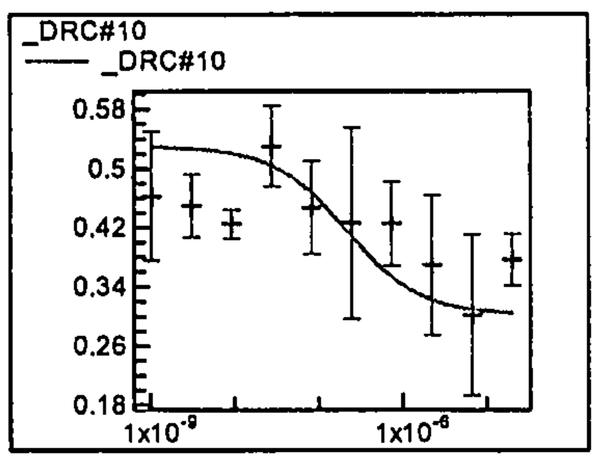
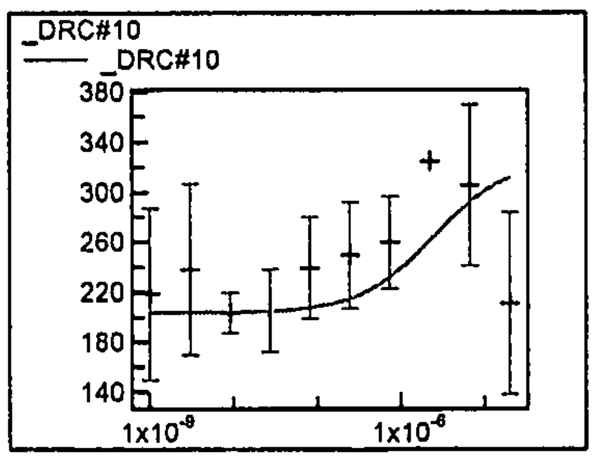
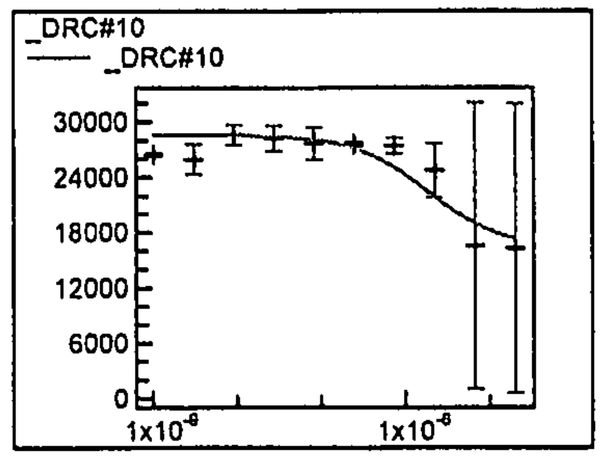
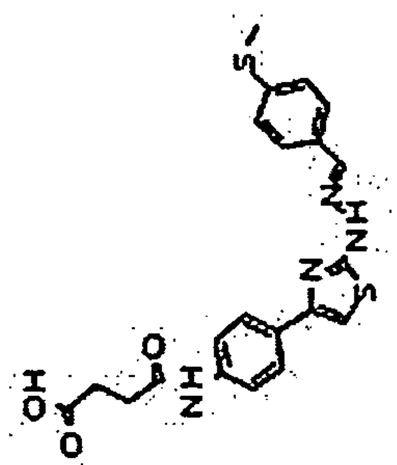
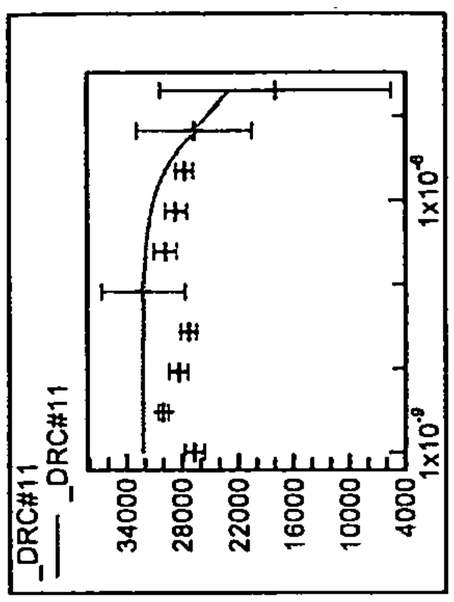
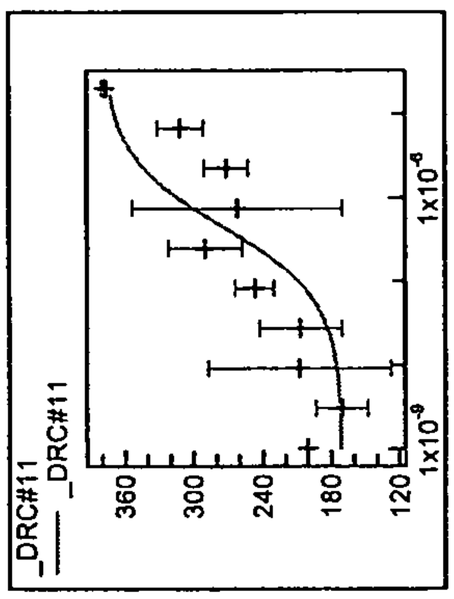
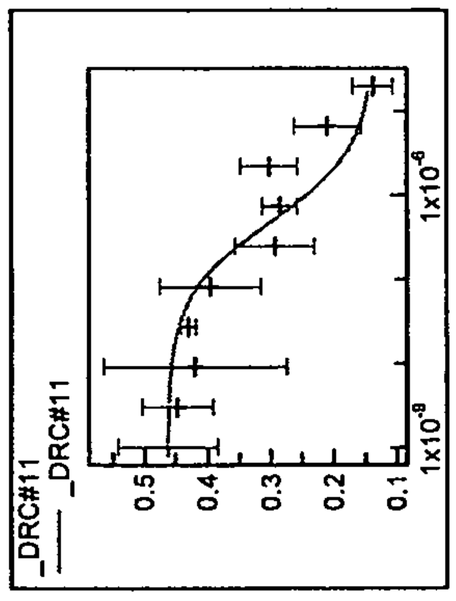
	QIM	Cell number	QUM
IPK00023891 Chiral  Scaffold XIII			
IPK00024871  Scaffold II			
IPK00025546  Scaffold XIV			

Table 2

Table 2

IPK00026207	
QUM	
Cell number	
QIM	

Scaffold III

Table 3

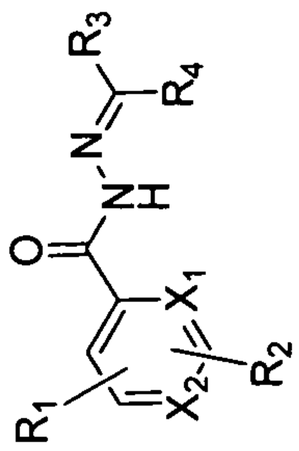
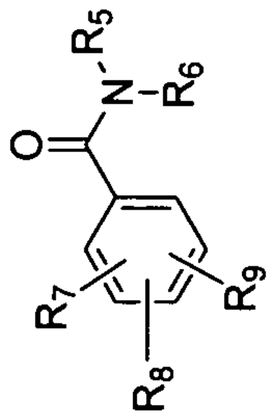
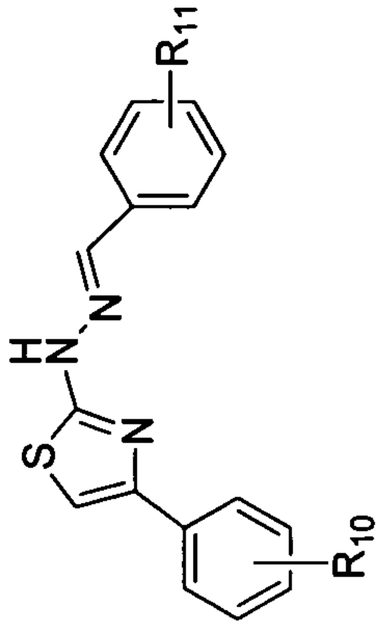
Scaffold Name	Scaffold Coding	Number of Compounds	Scaffold Structure
Isonicotinohydrazides	I	69	
Benzamides	II	19	
Thiazolhydrazides	III	6	

Table 3

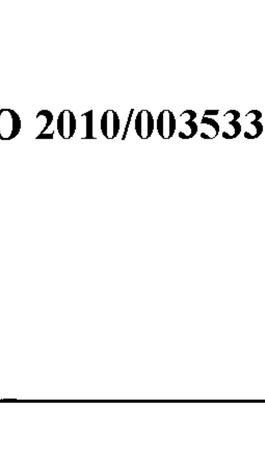
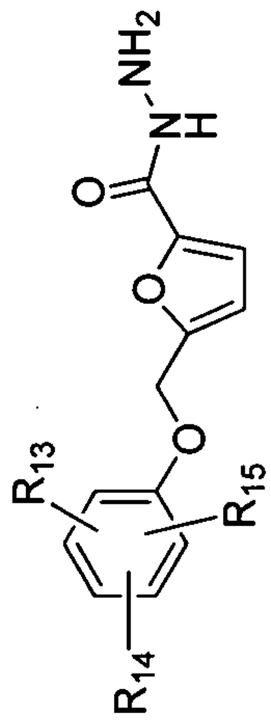
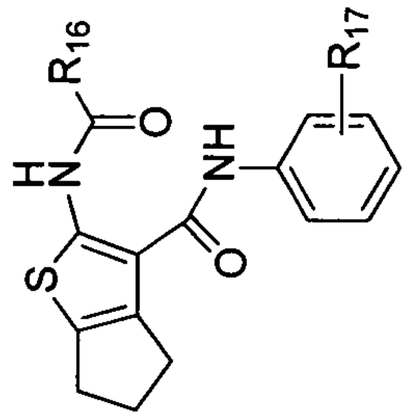
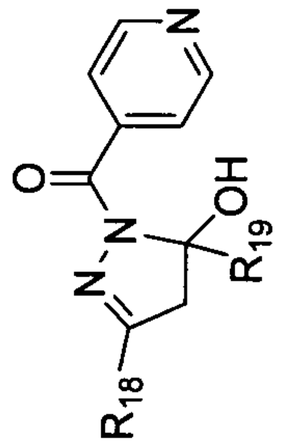
IV	V	VI	VII
5	4	3	2
			
Hydrazinecarbothioamides	Furancarbohydrazides	Thiophenes	Pyrazole-pyridines

Table 3

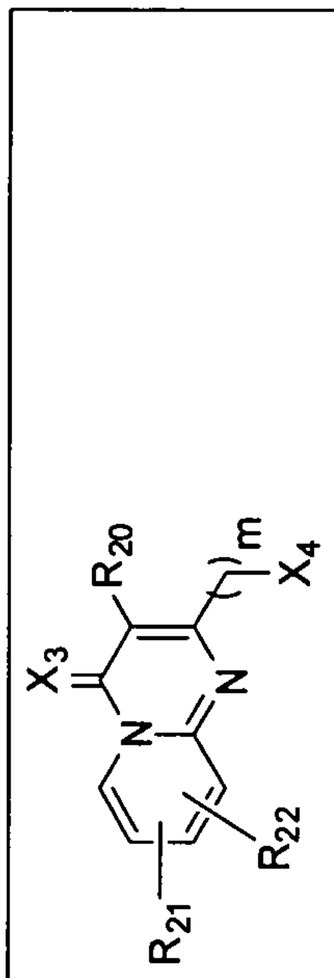
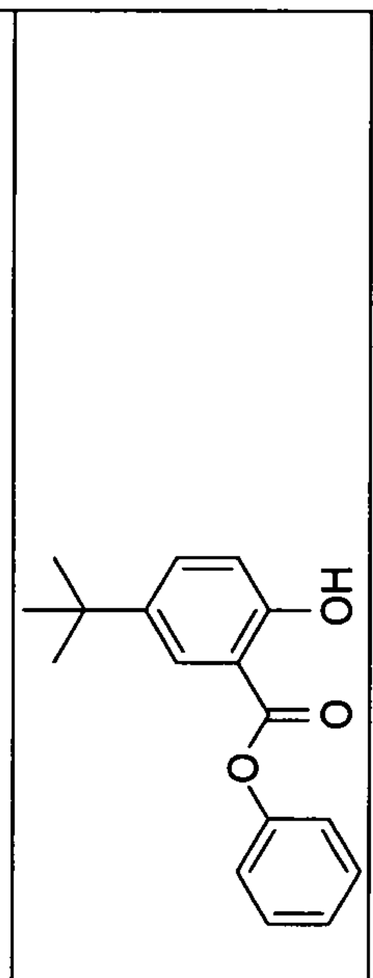
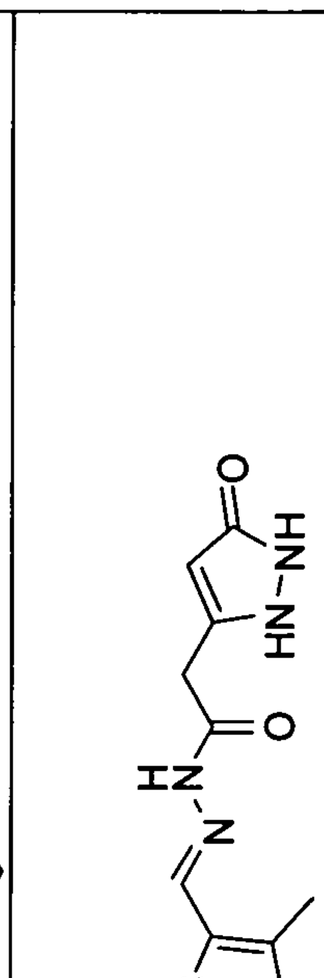
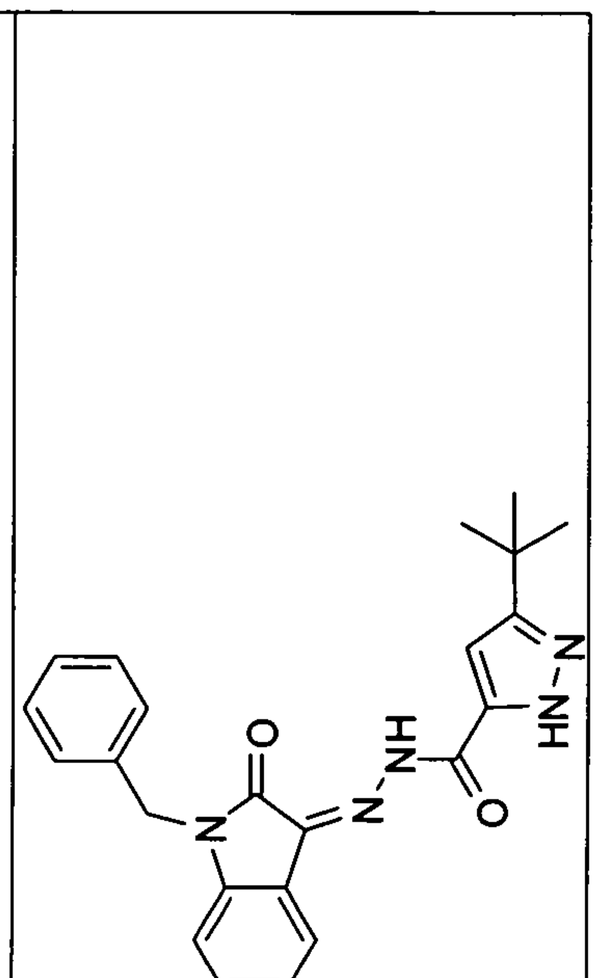
			
1	1	1	1
VIII	IX	X	XI
Pyridopyrimidinone	One hit compound	One hit compound	One hit compound

Table 3

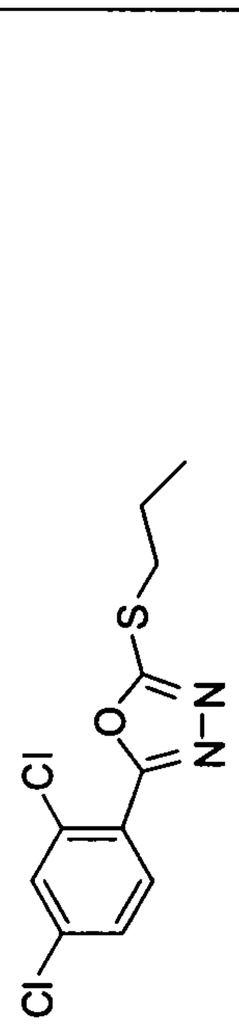
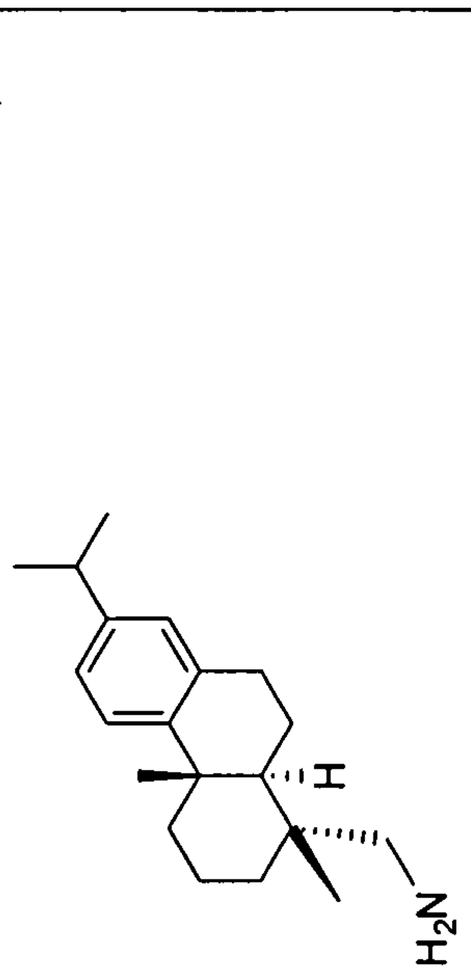
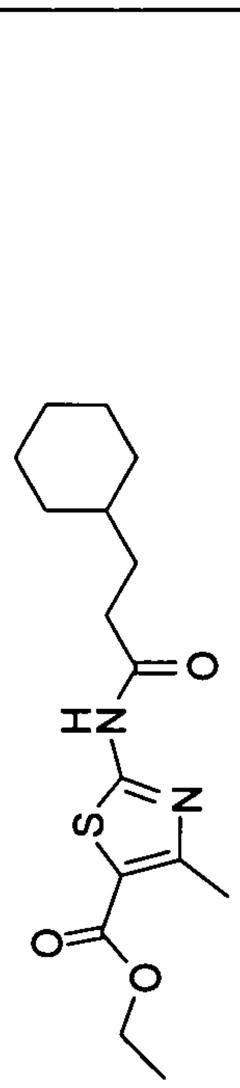
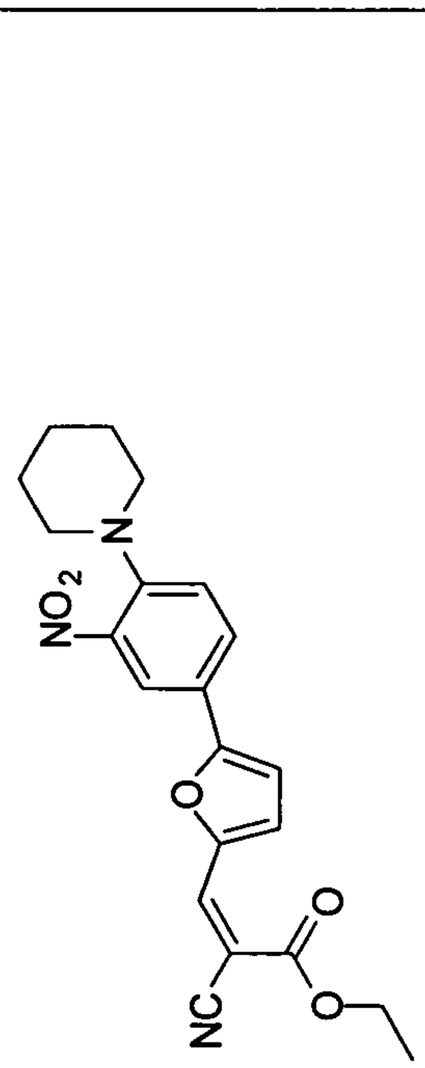
			
1	1	1	1
XII	XIII	XIV	XV
One hit compound	One hit compound	One hit compound	One hit compound

Table 3

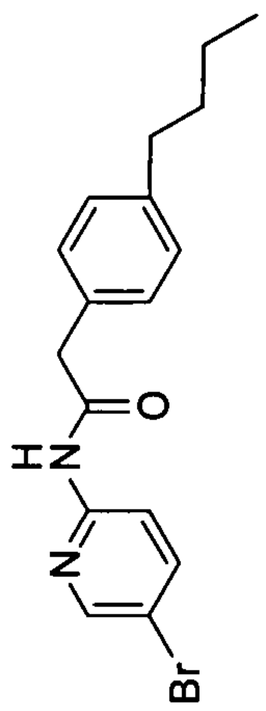
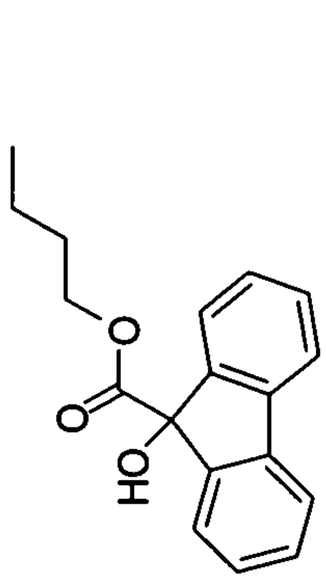
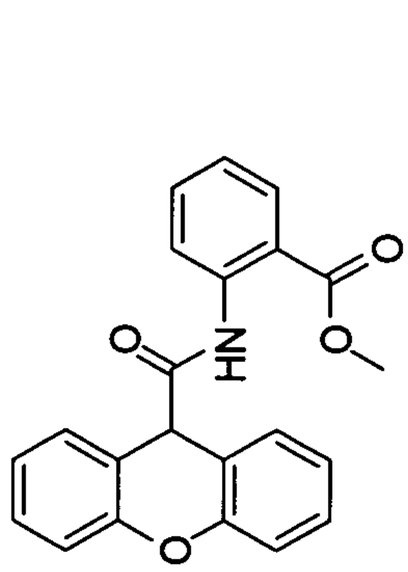
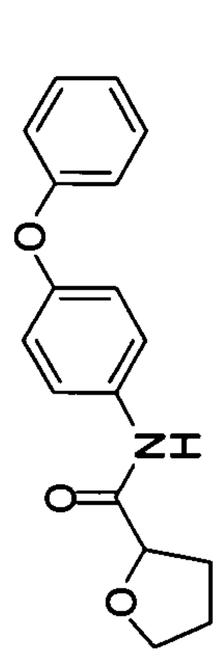
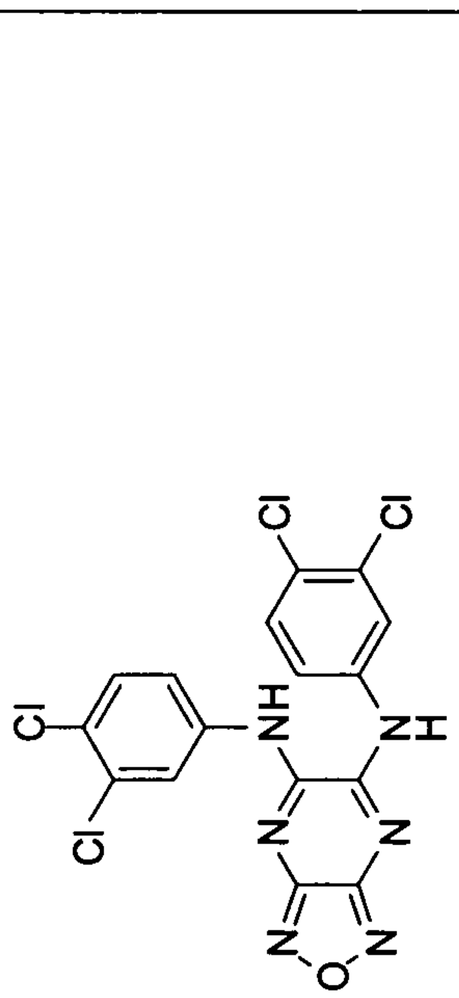
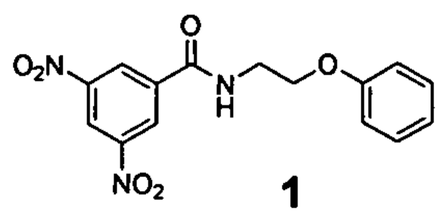
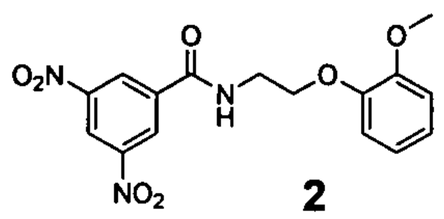
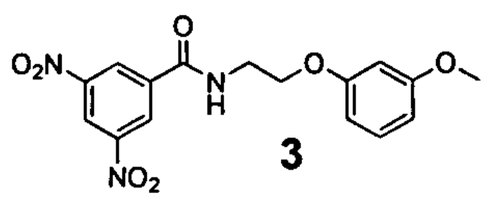
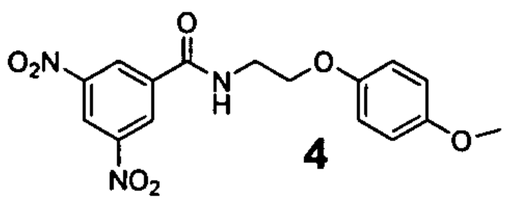
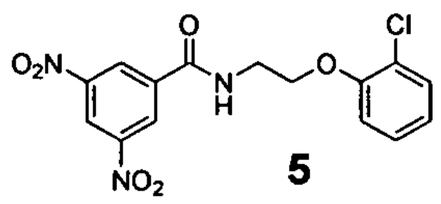
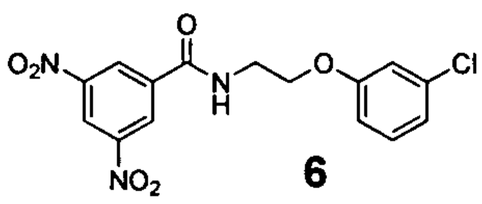
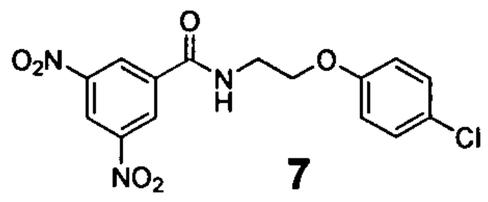
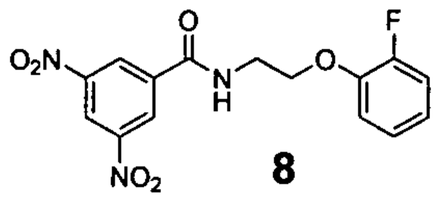
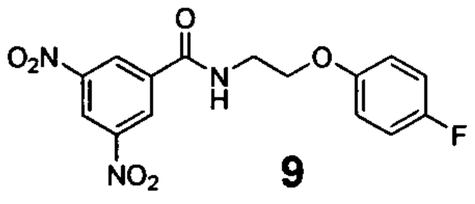
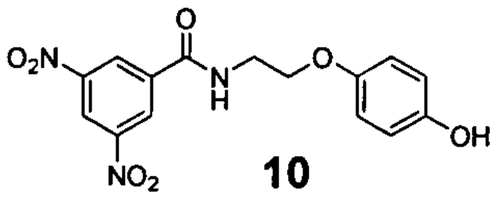
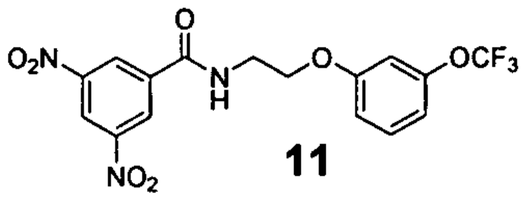
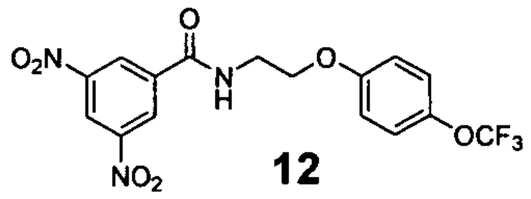
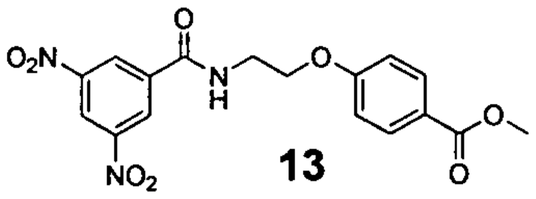
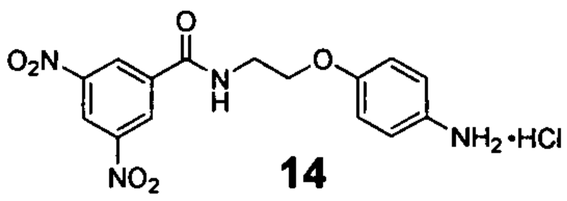
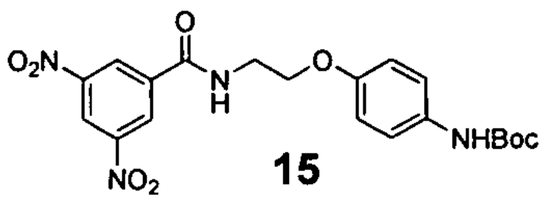
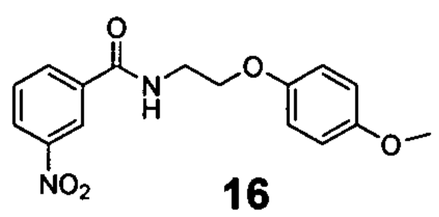
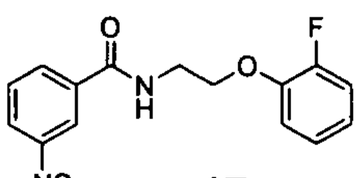
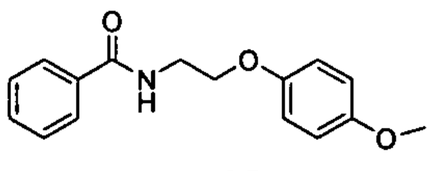
			
1	1	1	1
XVI	XVII	XVIII	XIX
One hit compound	One hit compound	One hit compound	One hit compound

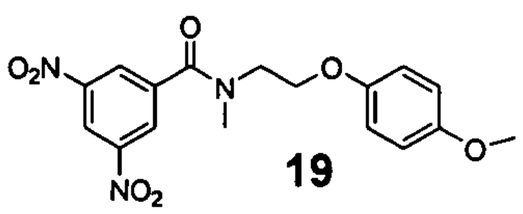
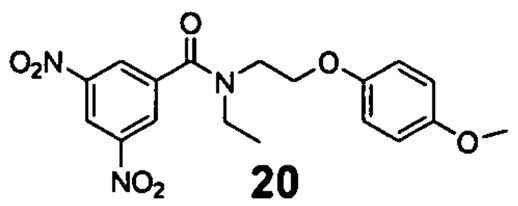
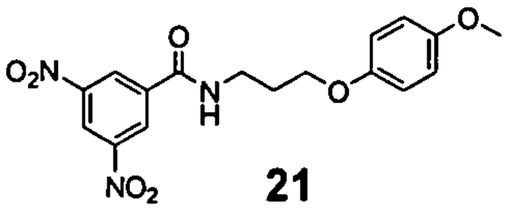
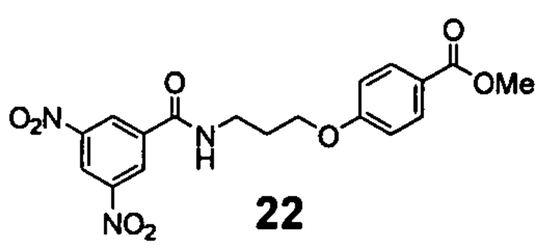
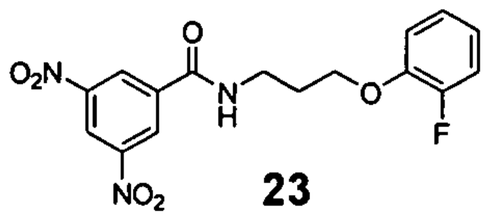
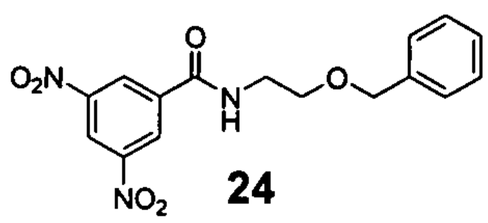
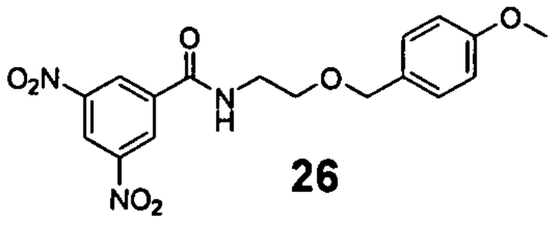
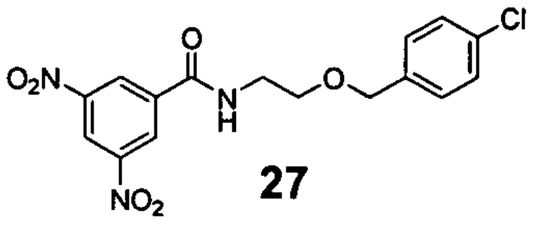
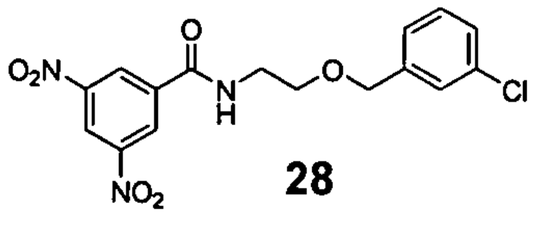
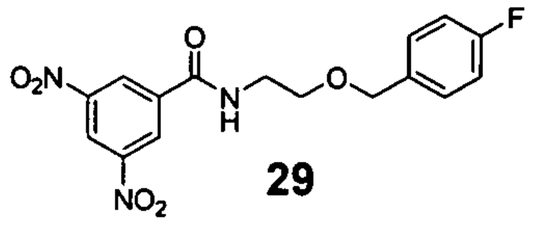
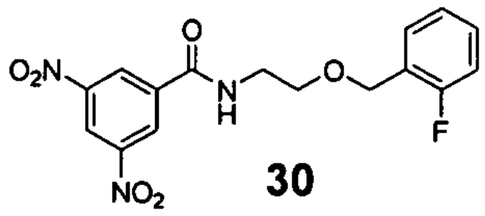
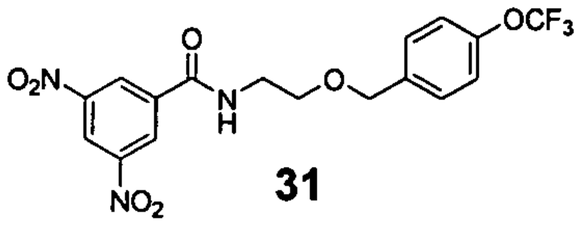
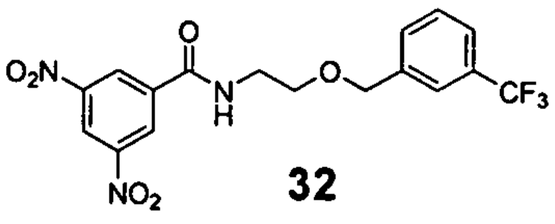
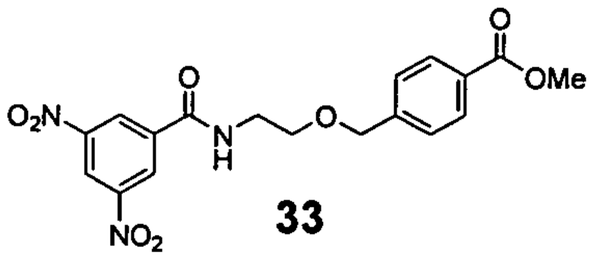
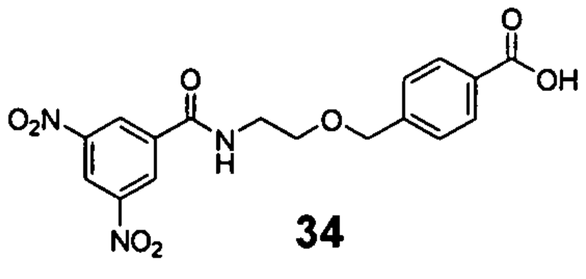
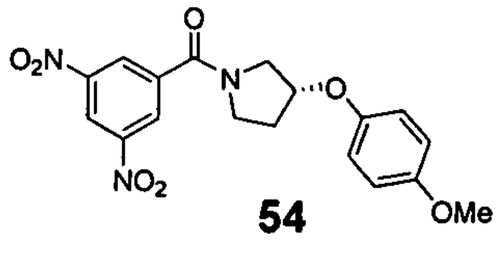
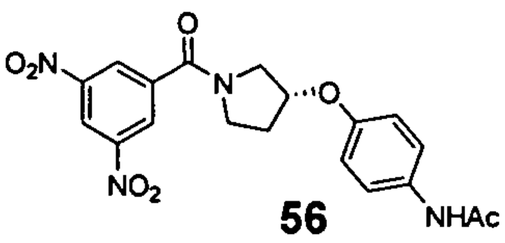
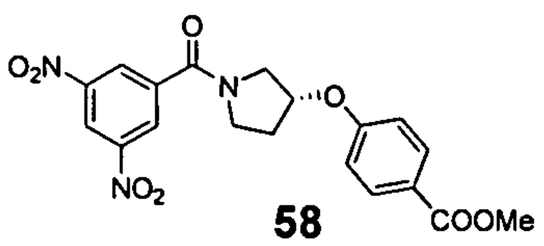
Table 3

One hit compound	XX	1
		

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
 1	+++	+++	 2	++	+++
 3	++	+++	 4	++	+++
 5	++	+++	 6	++	+++
 7	++	+++	 8	+++	+++
 9	++	+++	 10	++	+++
 11	+++	+++	 12	+++	+++
 13	++	+++	 14	++	++
 15	++	+++	 16	+	+
 17	+	+	 18	+	+

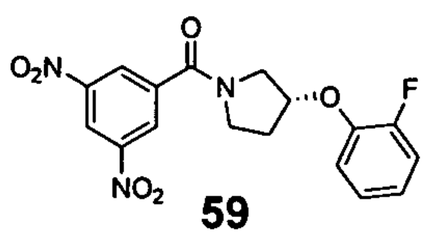
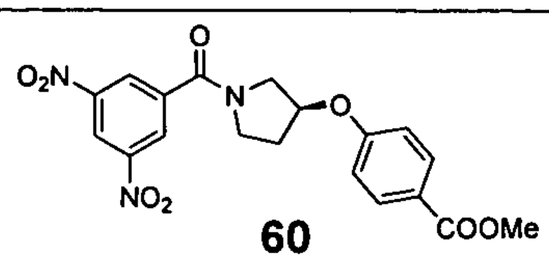
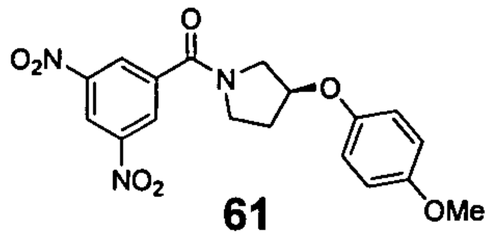
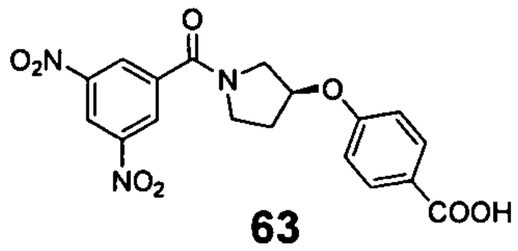
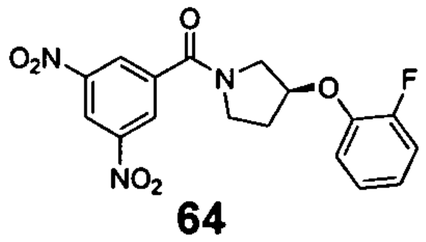
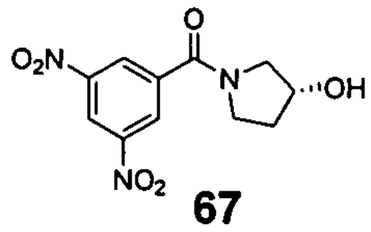
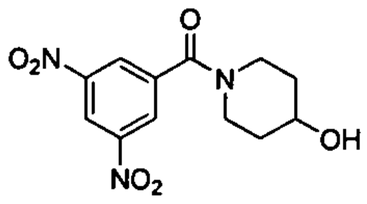
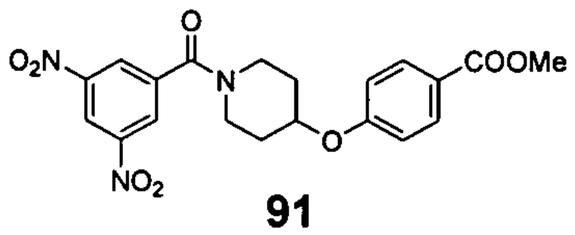
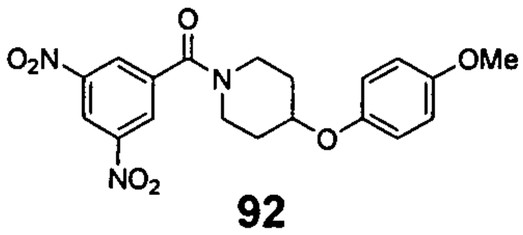
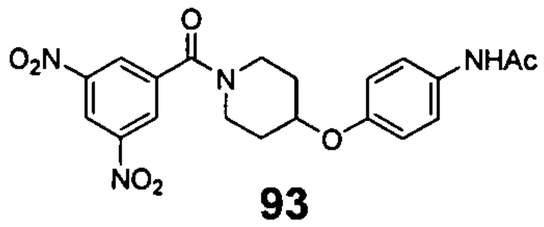
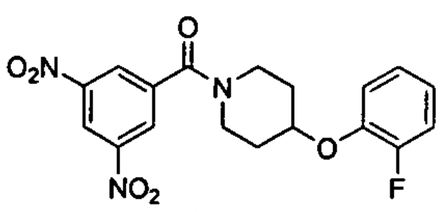
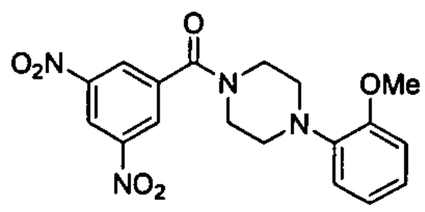
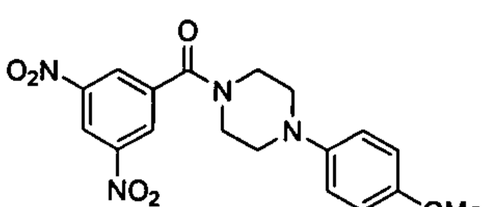
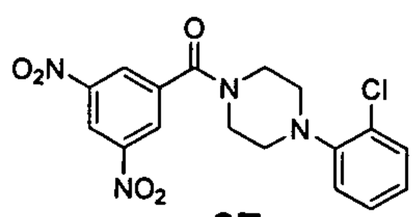
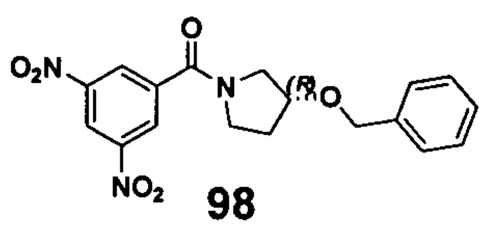
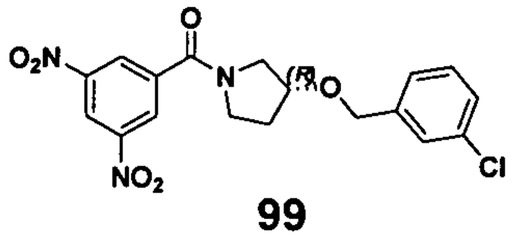
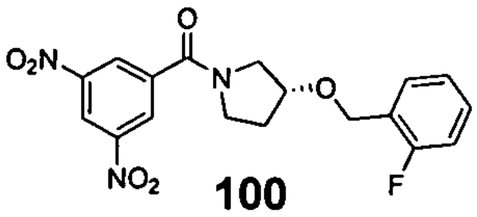
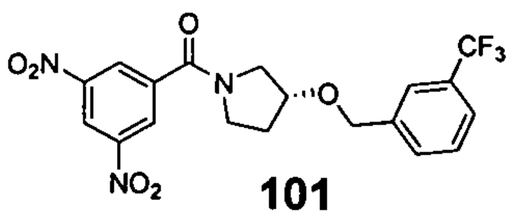
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
 19	+	++	 20	+	+
 21	++	+++	 22	++	+++
 23	+++	+++	 24	+++	+++
 26	++	+++	 27	+++	+++
 28	+++	+++	 29	+++	+++
 30	+++	+++	 31	+++	+++
 32	+++	+++	 33	++	+++
 34	+	+	 54	++	+++
 56	+	++	 58	+	+++

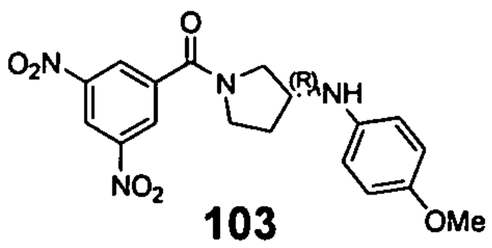
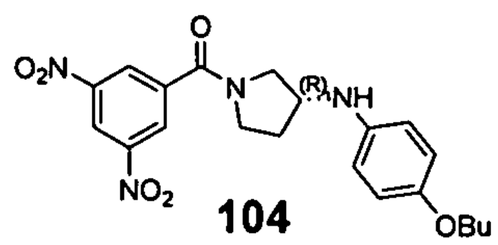
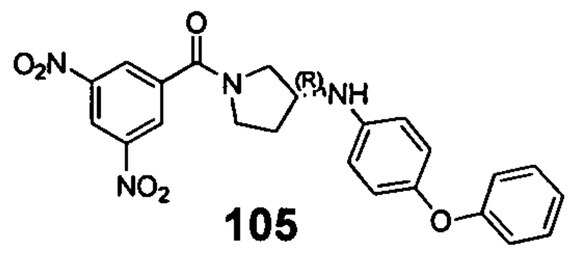
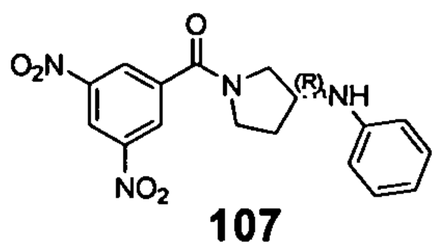
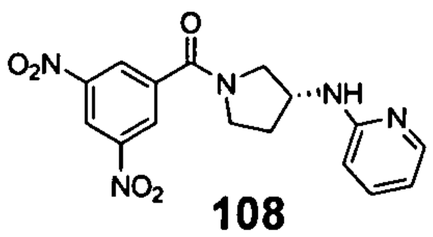
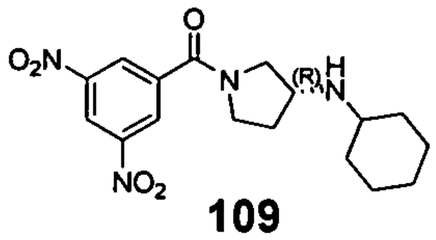
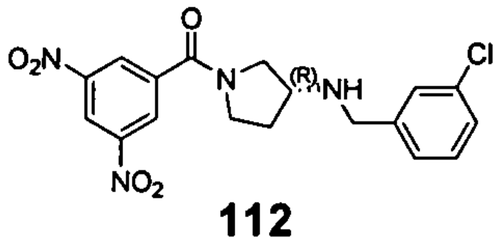
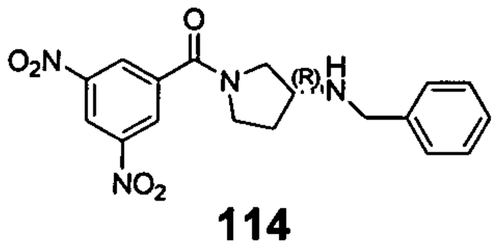
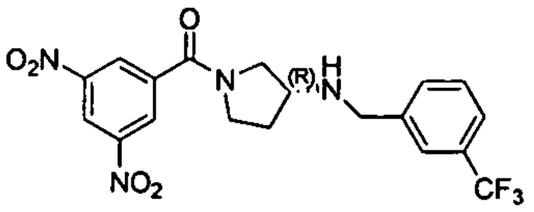
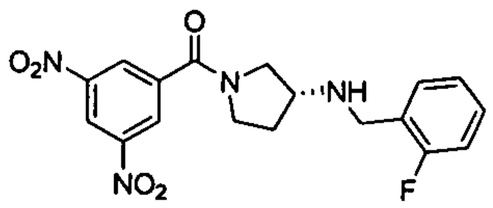
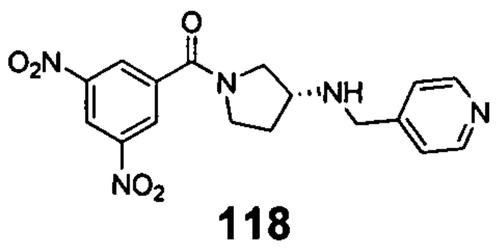
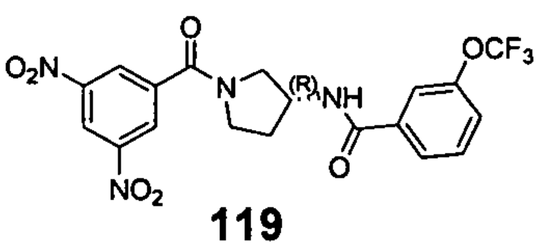
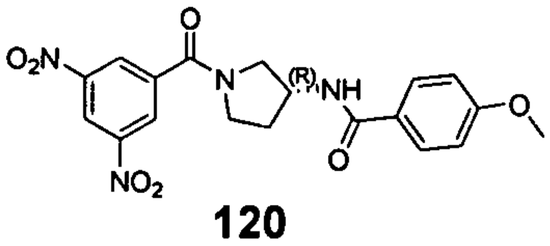
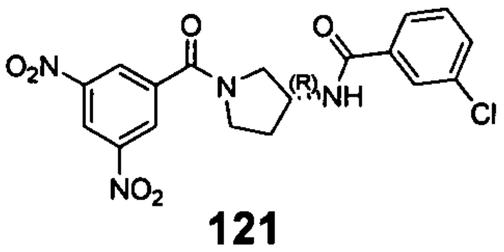
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
 59	++	+++	 60	+	+++
 61	+	+++	 63	+	++
 64	++	+++	 67	+	+
 90	+	+	 91	++	+++
 92	+	+++	 93	+	++
 94	++	+++	 95	+	++
 96	++	++	 97	+	+++
 98	+	++	 99	+	++
 100	+	+++	 101	+	++

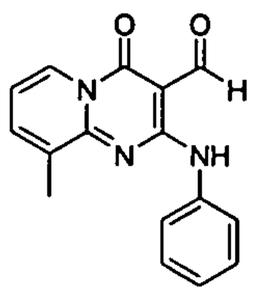
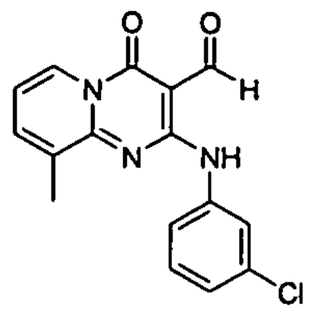
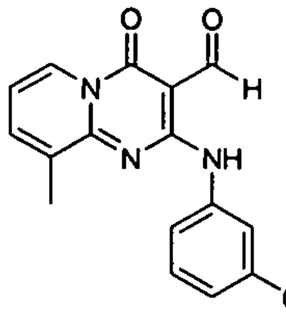
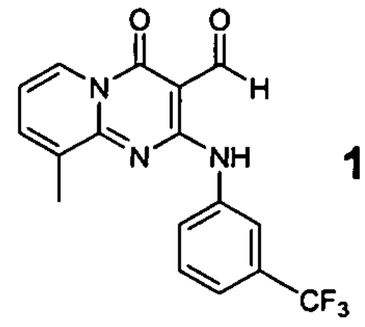
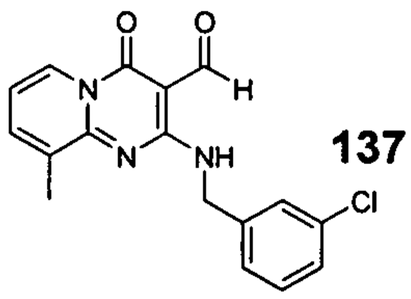
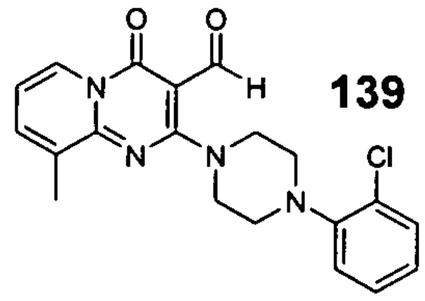
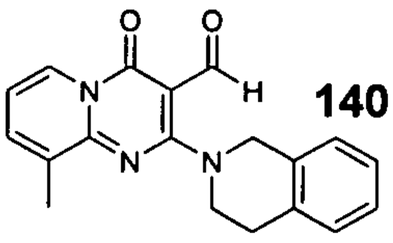
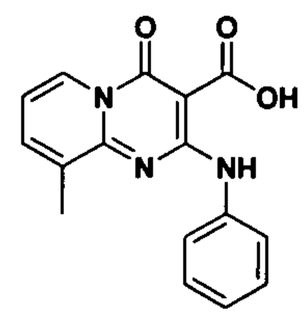
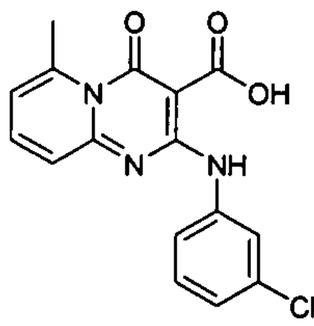
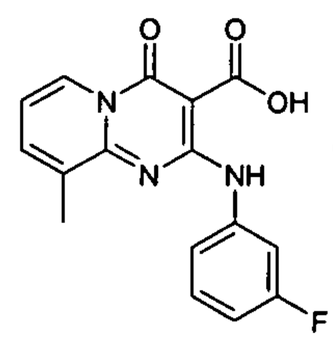
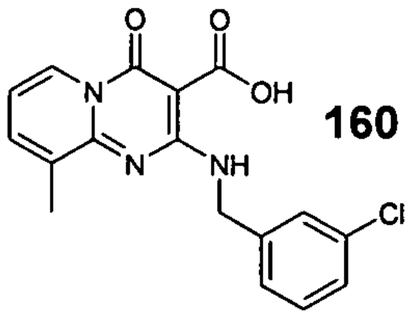
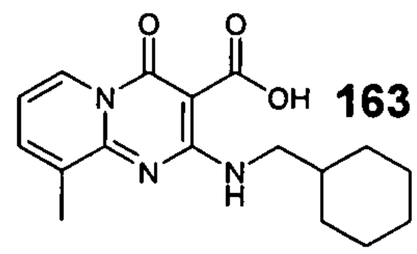
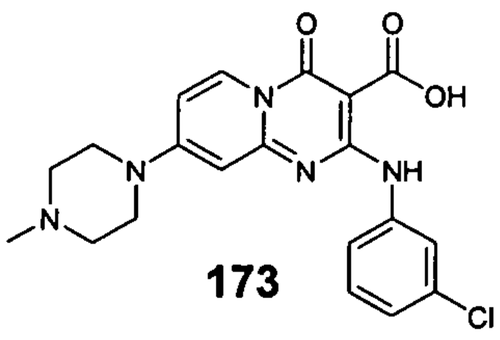
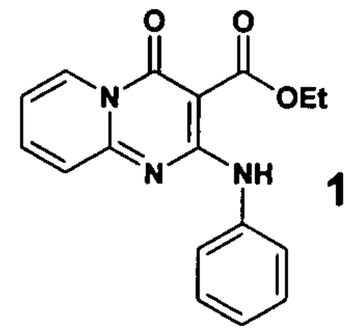
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
 103	++	+++	 104	++	+++
 105	+++	++	 107	++	+++
 108	++	+++	 109	+	+
 112	+	++	 114	+	++
 115	+	++	 116	+	++
 118	+	++	 119	+	+++
 120	+	+++	 121	+	++

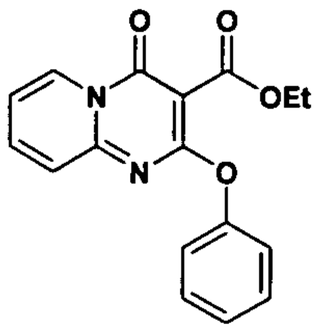
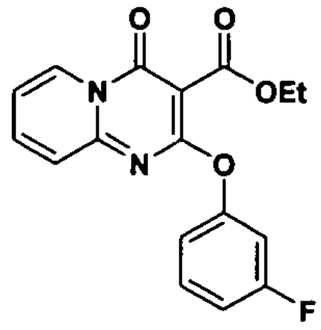
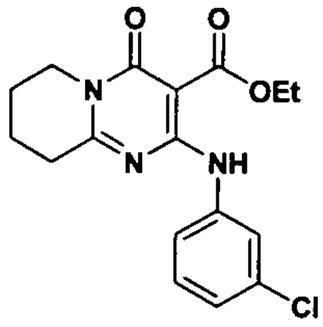
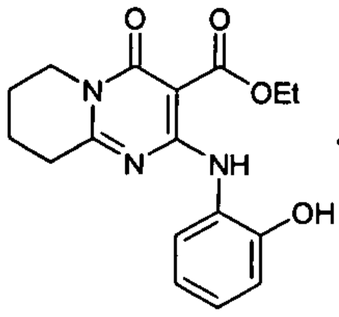
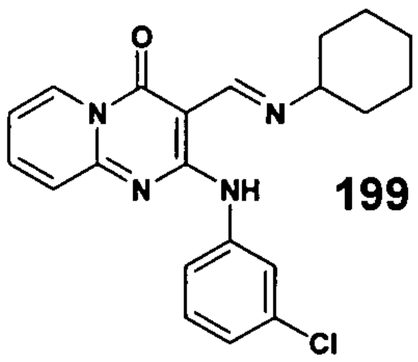
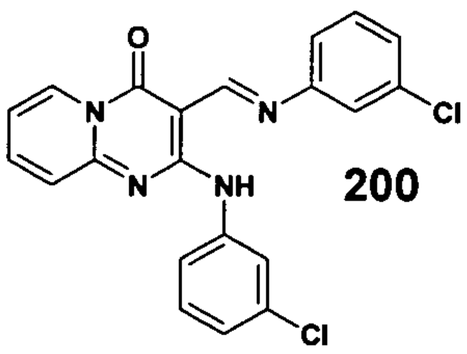
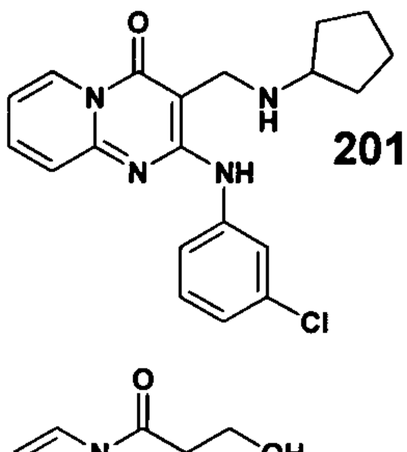
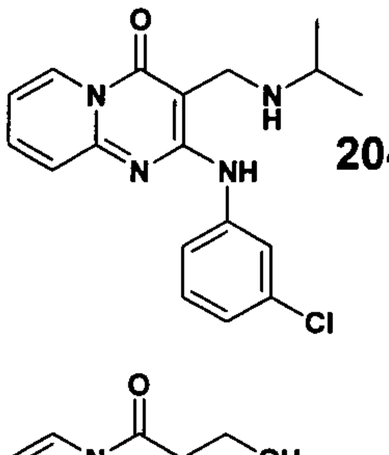
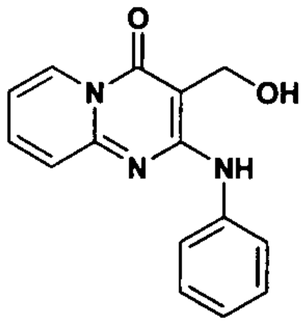
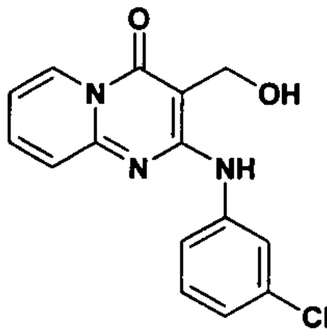
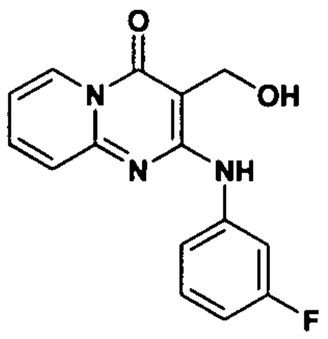
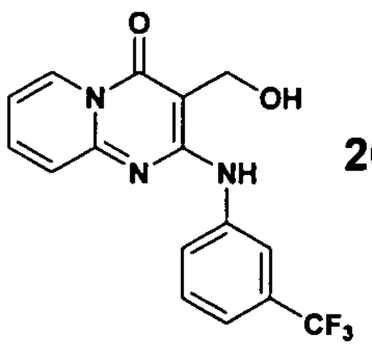
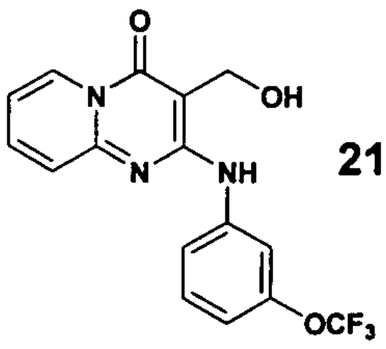
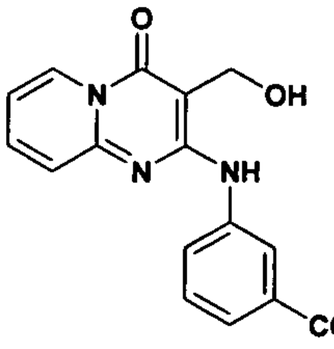
Activity range: +++ indicates < 5uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	132	+	++		133	++	+++
	134	+	++		135	+	++
	137	+	+		139	+	+
	140	+	+		147	+	+
	151	++	+		152	+	+
	160	+	+		163	+	+
	173	+	+		180	+	+

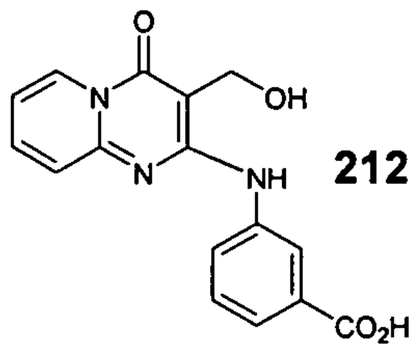
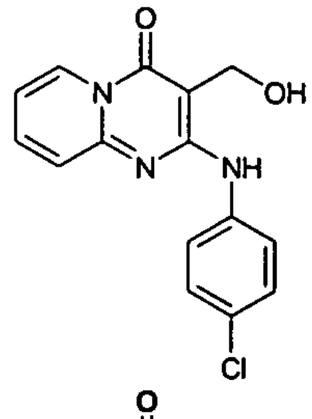
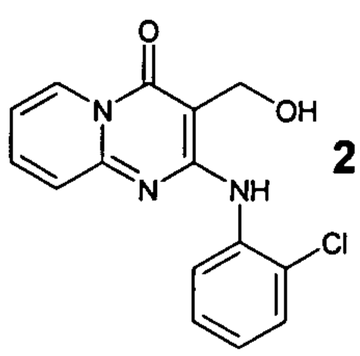
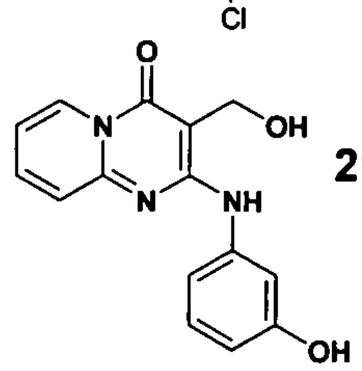
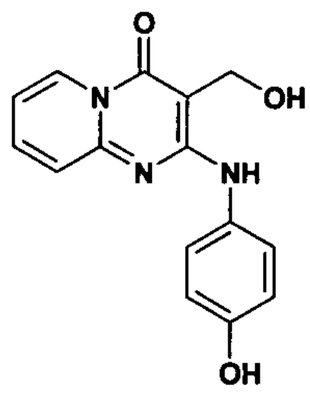
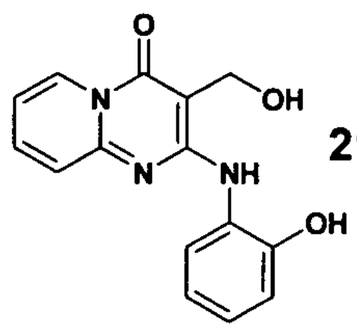
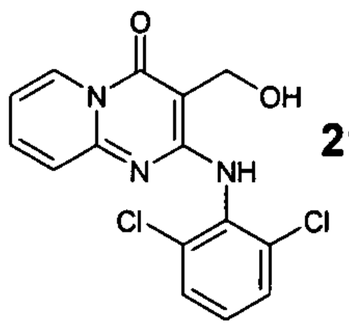
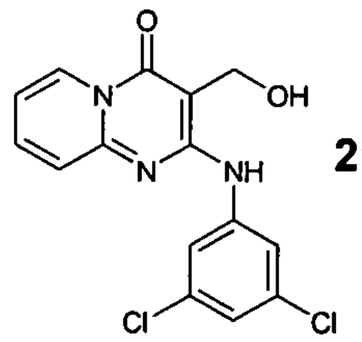
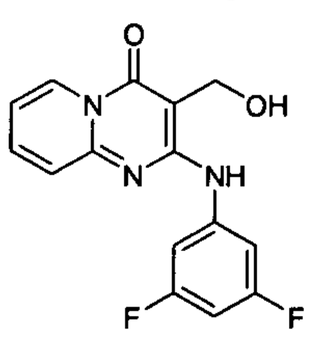
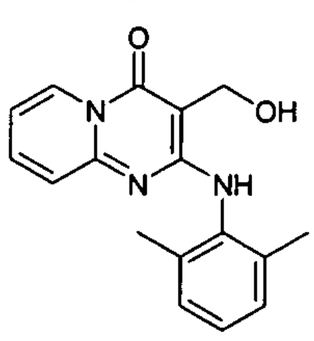
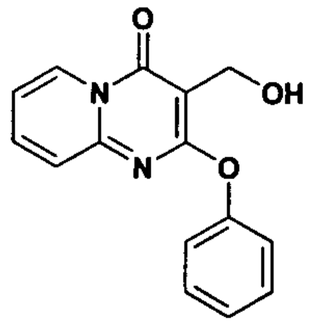
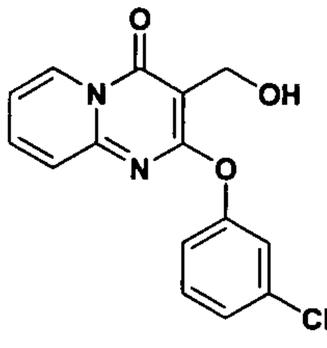
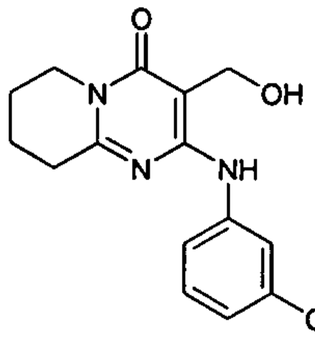
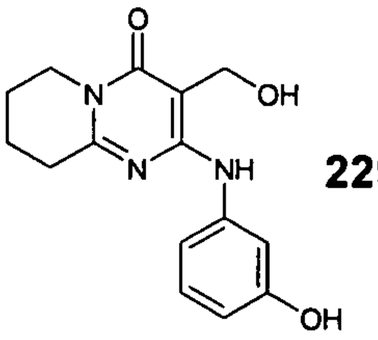
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
 184	+	+	 185	+	++
 193	+	+	 195	+	+
 199	+	++	 200	+	+
 201	+	+	 204	+	+
 206	+++	+++	 207	+++	+++
 208	+++	+++	 209	+++	+++
 210	+++	+++	 211	+	+++

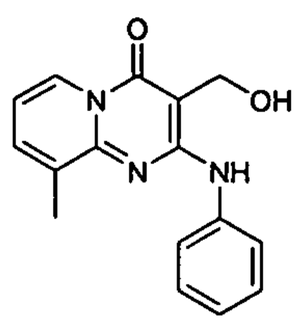
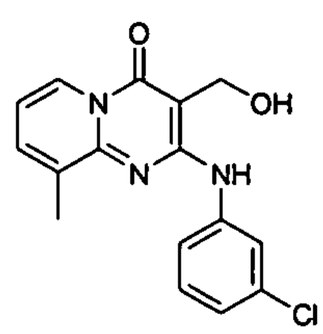
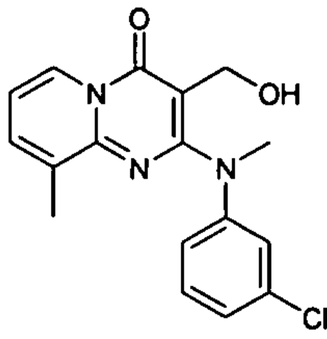
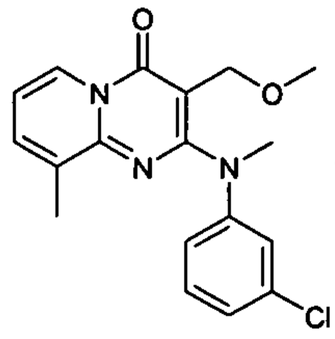
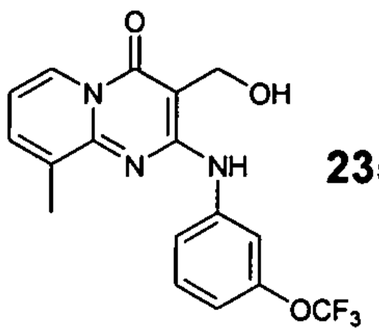
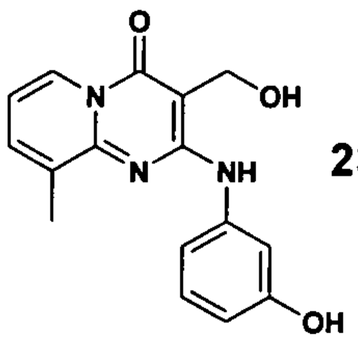
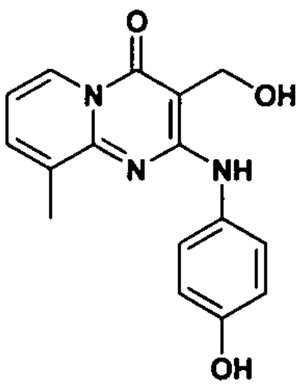
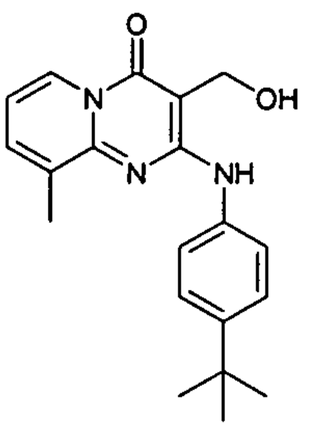
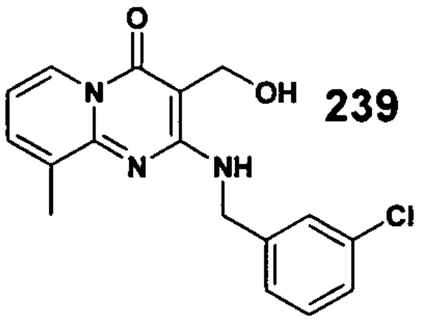
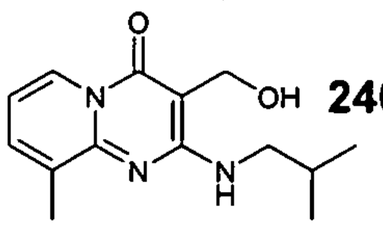
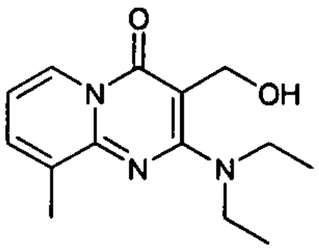
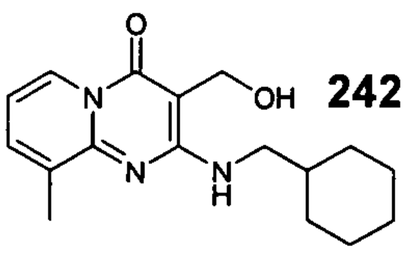
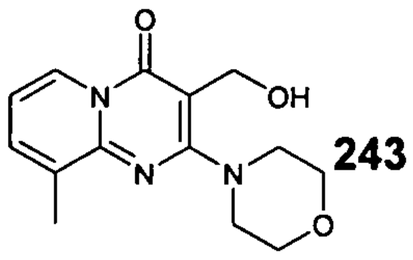
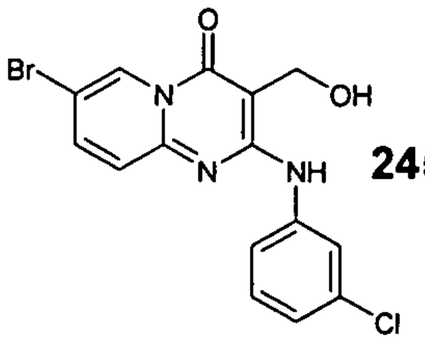
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	212	+	+		213	+	+++
	214	+	++		215	+	+
	216	+	+		217	+	+++
	218	+	+		219	+	+
	220	++	+++		221	+	+
	222	+	+		224	+	+
	226	+	+		229	+	+

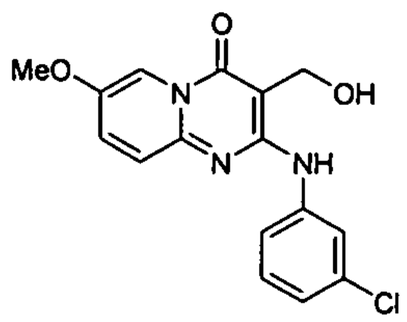
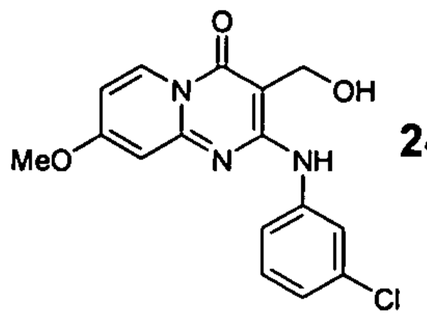
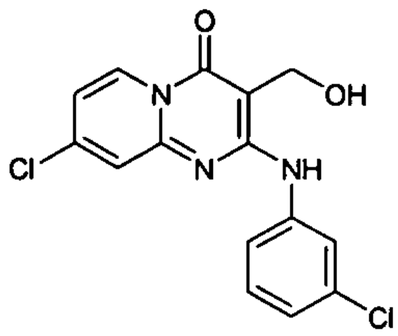
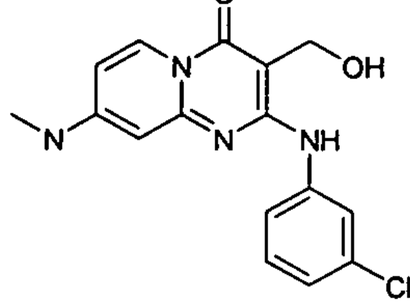
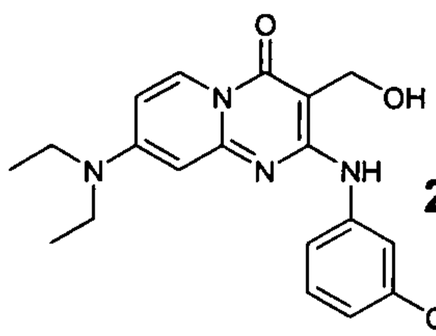
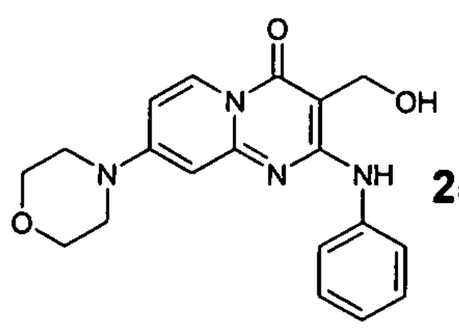
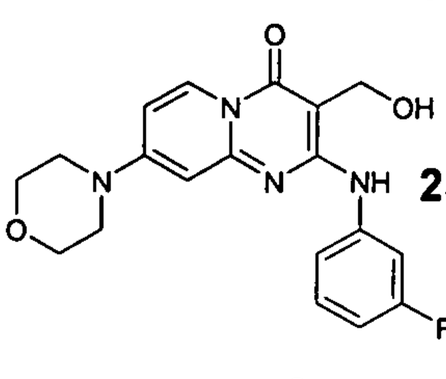
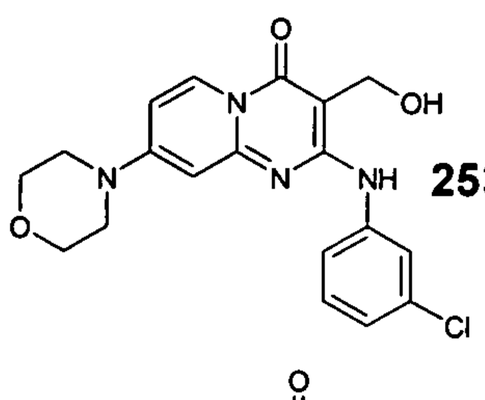
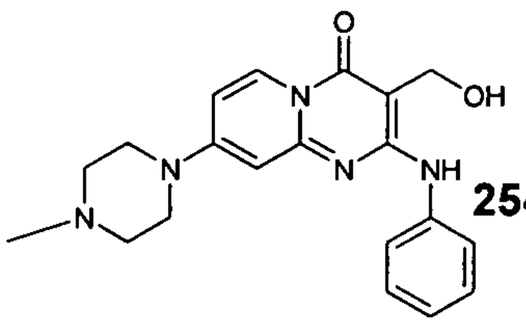
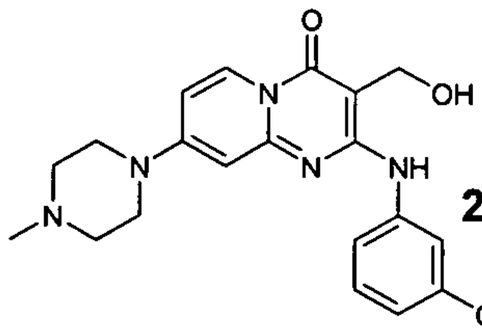
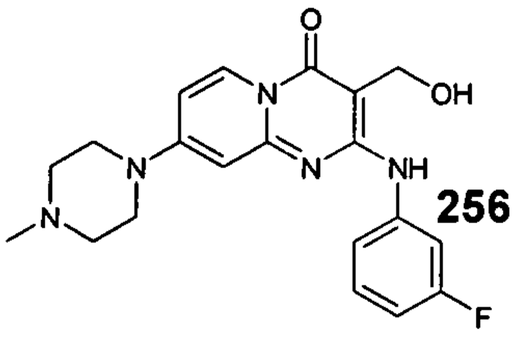
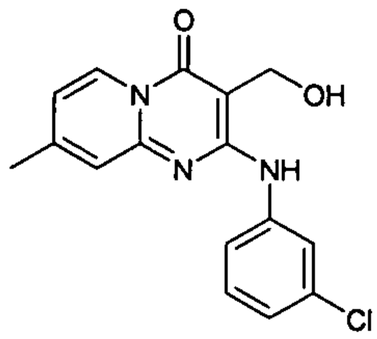
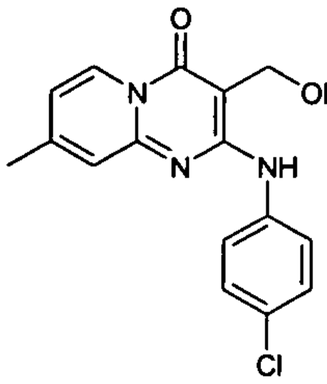
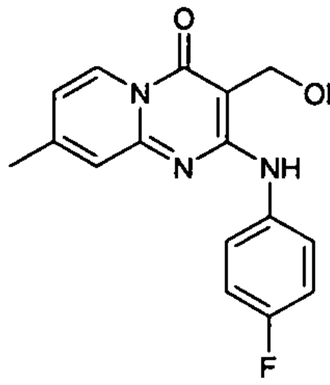
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	231	+++	+++		232	+++	++
	233	+	+		234	+	+
	235	+++	+++		236	++	+++
	237	+	+++		238	+	+
	239	+	+		240	++	++
	241	+	+		242	+	+
	243	+	+		245	+	++

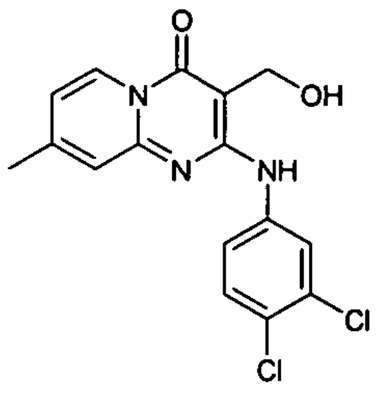
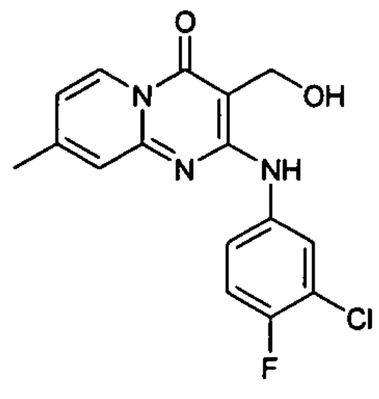
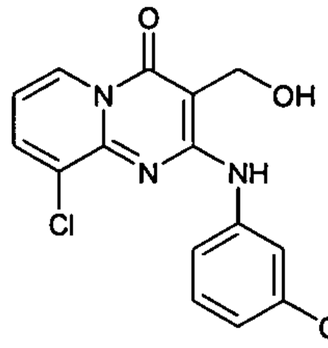
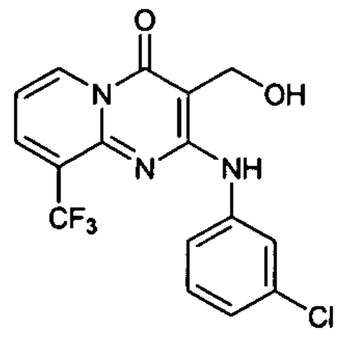
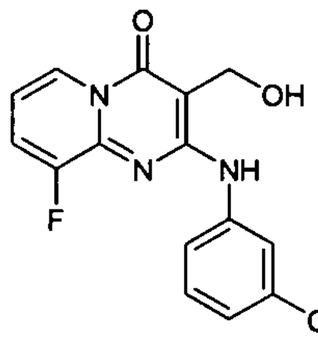
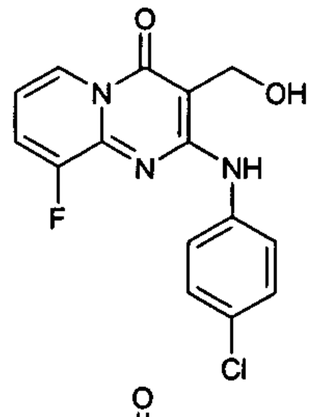
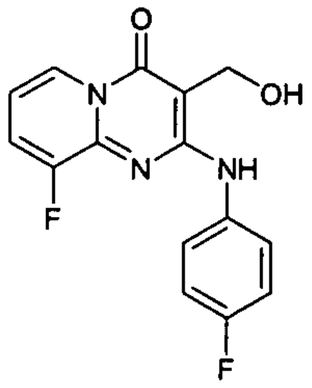
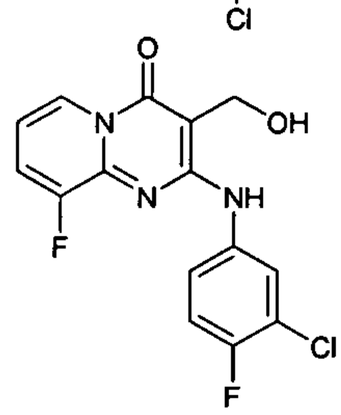
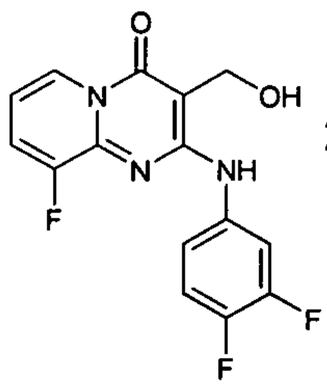
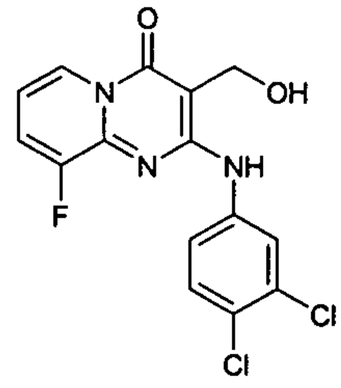
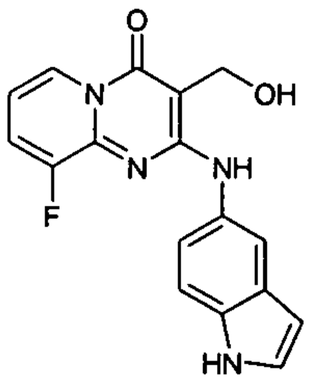
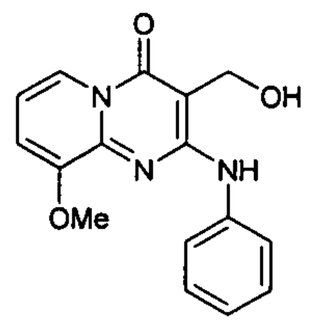
Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound	QIM (μM)	QUM (μM)	Compound	QIM (μM)	QUM (μM)
	246	+	+		247	+	+++
	248	+	+++		249	+	+
	250	+	+		251	+	+
	252	+	+		253	+	+
	254	+	+		255	+	+
	256	+	+		257	+++	+++
	258	+++	+++		259	++	+++

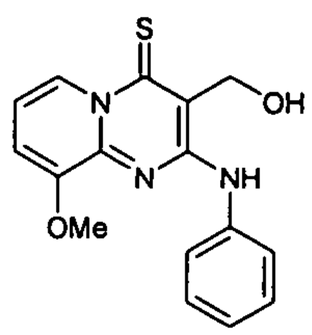
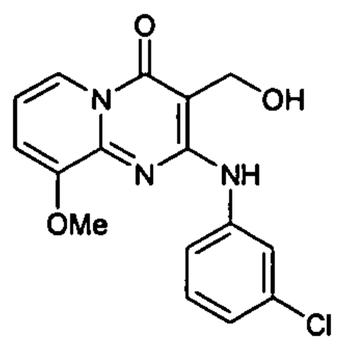
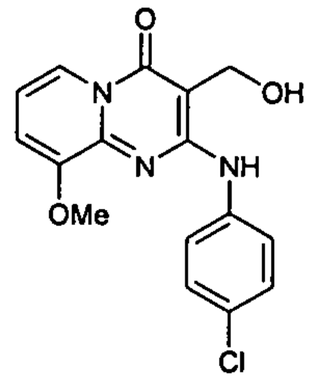
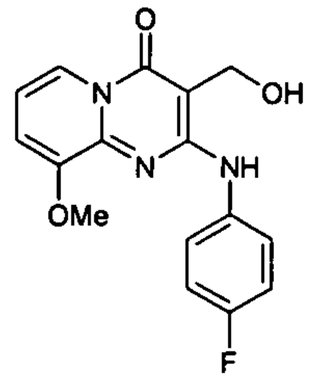
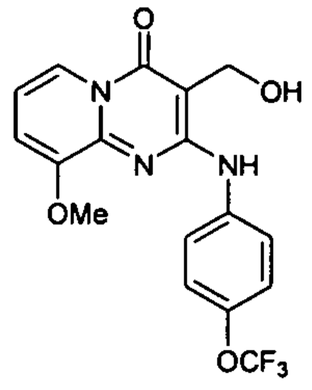
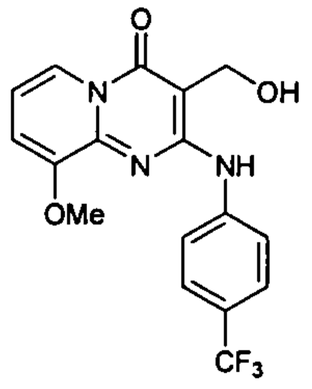
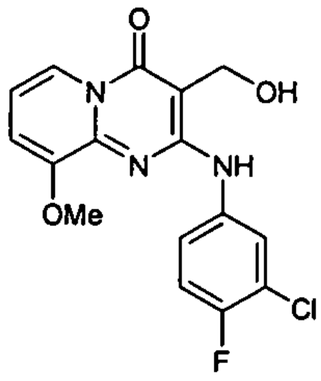
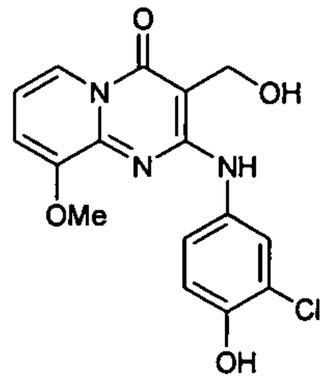
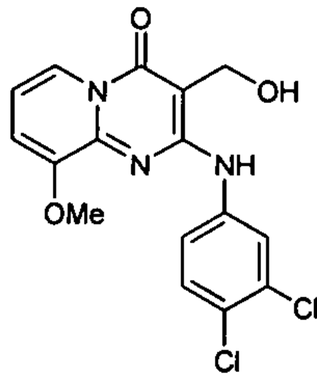
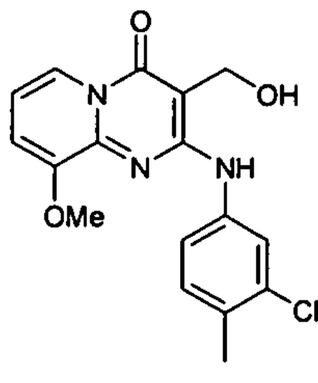
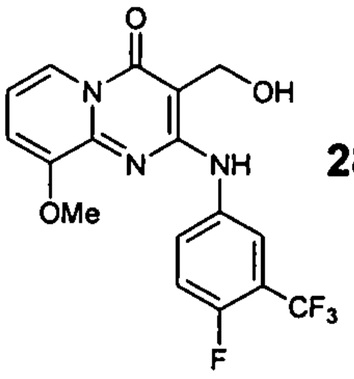
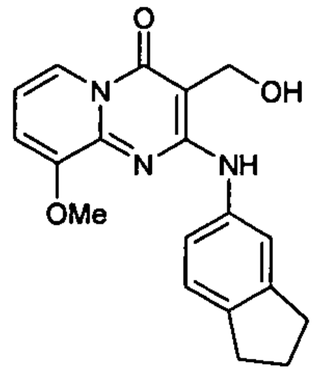
Activity range: +++ indicates <5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	260	+	++		261	+++	+++
	262	+	+		263	+	+
	264	++	+++		265	+++	+++
	266	+	+++		267	+++	+++
	268	+	++		269	++	++
	270	+++	+++		271	+	+++

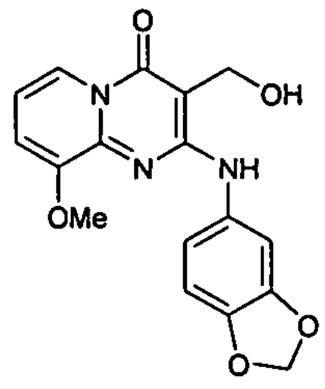
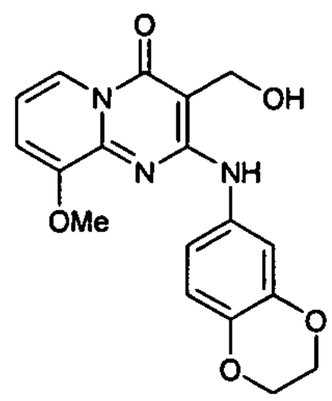
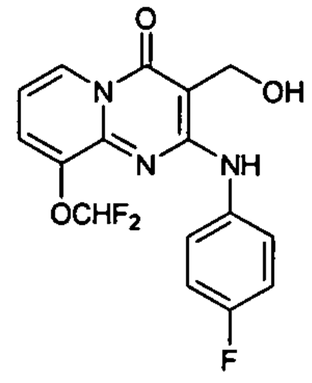
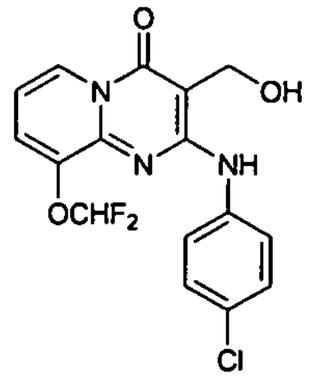
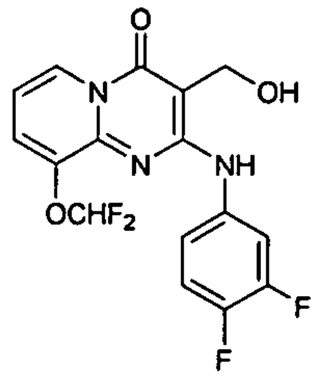
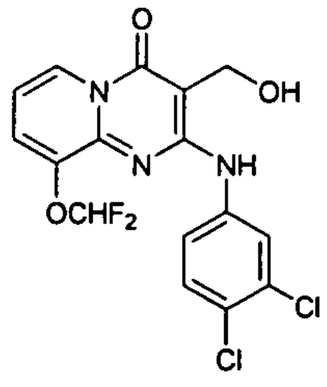
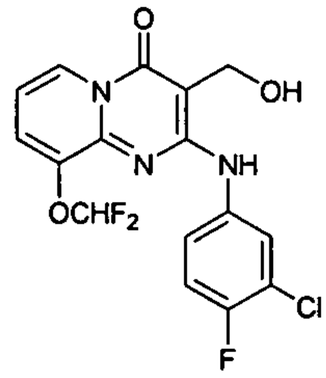
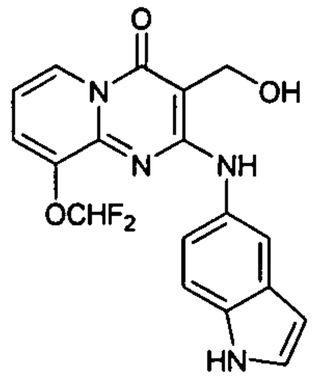
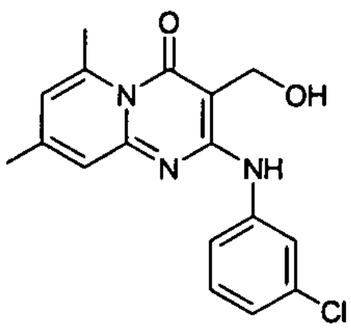
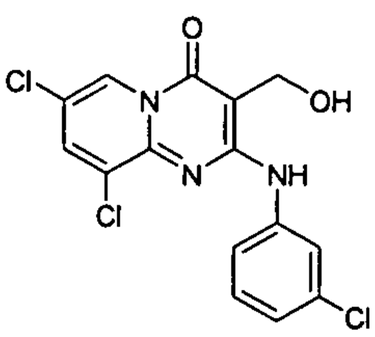
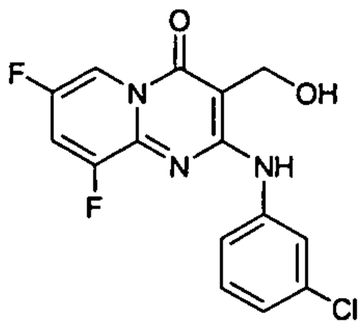
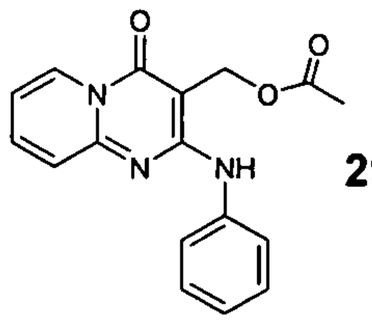
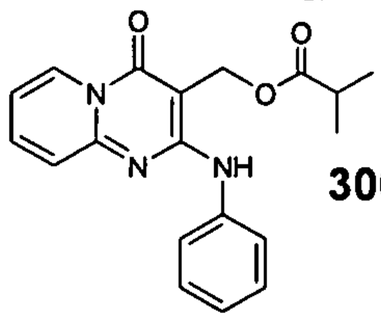
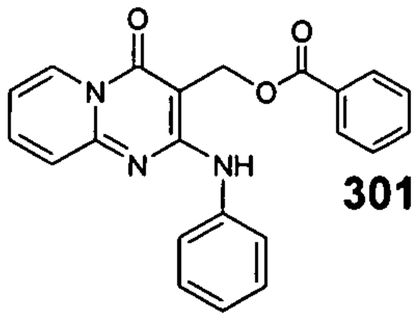
Activity range: +++ indicates <5 uM. ++ indicates between 5-20 uM. + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	**272**	+	+		**273**	+++	+++
	**274**	+	+++		**275**	+	+++
	**276**	+	++		**277**	+	++
	**278**	+++	+++		**280**	+	+++
	**281**	+	+++		**282**	+	+
	**283**	+	+		**284**	+	+++

Activity range: +++ indicates < 5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Compound		QIM (μM)	QUM (μM)	Compound		QIM (μM)	QUM (μM)
	**285**	+	+++		**286**	+	+++
	**290**	+	+		**291**	+	+
	**292**	+	+		**293**	+	+
	**294**	+	+		**295**	++	+++
	**296**	+	+		**297**	+	+
	**298**	+	+		**299**	++	+++
	**300**	++	+++		**301**	++	+++

Activity range: +++ indicates <5 uM, ++ indicates between 5-20 uM, + indicates > 20 uM

Table 4

Cytotoxicity					
Compounds				4	24
Host Cells				Range of MTC_{50} (μM)	
SK-N-SH -Brain				>100	>100
HepG2-Hepatocytes				>100	>100
MRC5- Lung				>100	>100
BJ- Skin				>100	>100
HEK293- Kidney				>100	>100
Antibacterial activity & Specificity					
Mycobacterium					
Strains/Isolates	Type		Origin	Number	Range of MICs for multiple strains (μM)
M. tuberculosis clinical isolates[1]	Drug Sensitive		Tissue	1	0.38 0.31
	RIF^R		Sputum	2	0.05 0.08
			Tissue	2	0.02-0.05 0.08
	INH^R RIF^R $Strep^R$		Sputum	1	0.1 0.08
			Tissue	3	0.05-0.1 0.04-0.08
	XDR		Sputum	5	0.02-0.05 0.04-0.08
			Tissue	0	0.05-0.1 0.08
	MDR		Sputum	3	0.05-0.1 0.04-0.08
			Tissue	5	0.05-0.1 0.04-0.08
M. tuberculosis laboratory strains	H37Rv				0.6 0.6
	H37Ra				1.2 1.3
	Beijing 1237				0.3 0.1
M. bovis BCG	BCG Tokyo				1.2 0.6
	BCG Pasteur				1.2 1.2
M. smegmatis	mc^2 155				1.2 0.6
Gram-negative					
Acinetobacter baumannii, Escherichia coli, Enterobacter cloacae, E. aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa, Salmonella enteridis, Vibrio mimicus					>250 >250
Gram-positive					
Staphylococcus aureus, S. epidermis, S. capitis, S. xylosus, Micrococcus luteus, Listeria innocua, Lactobacillus gallinarum, group G Streptococcus, Streptococcus agalactiae, S. pyogenes, Enterococcus faecalis, E. faecium, E. gallinarum, Bacillus pumilus					>250 >250
Corynebacterium					
C. striatum					27 27
C. jeikeium					2.7 2.7
Fungi					
Candida albicans, C. glabrata, C. parapsilosis					>250 >250

INH: Isoniazid, RIF: Rifampin, Strep: Streptomycin, ^{R}: resistant. [1]The clinical isolates were isolated either from resected lung tissue or sputum specimen, which were collected from active tuberculosis in-patients from the National Masan Tuberculosis Hospital during October 2003 to March 2007.

Table 5

Cytotoxicity				
Compounds				133
Host Cells				Range of MTC_{50} (μM)
SK-N-SH -Brain				>100
HepG2-Hepatocytes				>100
MRC5- Lung				>100
BJ- Skin				>100
HEK293- Kidney				>100
Jurkat -T-cell				>100
THP-1 - Monocytes				>100
Primary BMDM				>100
Primary human macrophages				>100
Antibacterial activity & Specificity				
Mycobacterium				
Strains/Isolates	Type	Origin	Number	Range of MICs for multiple strains (μM)
M. tuberculosis clinical isolates[1]	Drug Sensitive	Sputum	2	5->20
		Tissue	2	2.5-5
	RIF^R	Sputum	1	2.5
		Tissue	1	1.2
	INH^R RIF^R $Strep^R$	Sputum	3	0.3-1.2
		Tissue	1	1.2
	XDR	Sputum	4	0.6-2.5
		Tissue	5	0.3-5
	MDR	Sputum	3	0.3-1.2
		Tissue	1	1.2
M. tuberculosis laboratory strains	H37Rv			2
	H37Ra			2
	BCG Pasteur-Tokyo			2
M. smegmatis	mc^2 155			>100
Gram-negative				
Acinetobacter baumannii, Escherichia coli, Enterobacter cloacae, E. aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa, Salmonella enteridis, Vibrio mimicus				NE
Gram-positive				
Staphylococcus aureus, S. epidermis, S. capitis, S. xylosus, Micrococcus luteus, Listeria innocua, Lactobacillus gallinarum, group G Streptococcus, Streptococcus agalactiae, S. pyogenes, Enterococcus faecalis, E. faecium, E. gallinarum, Bacillus pumilus				NE
Corynebacterium				
C. striatum				
C. jeikeium				NE
Fungi				
Candida albicans, C. glabrata, C. parapsilosis				NE

INH: Isoniazid, RIF: Rifampin, Strep: Streptomycin, ^{R}: resistant. [1]The clinical isolates were isolated either from resected lung tissue or sputum specimen, which were collected from active tuberculosis in-patients from the National Masan Tuberculosis Hospital during October 2003 to March 2007. NE: No effect up to 100 μg/mL equivalent to 320 μM. The antimicrobial spectrum was performed on clinical isolates from CHU d'Angers, France.

Table 6

Compound	Concentration (µg/ml)	Bacteria inoculum (CFU) 10^5	10^6	10^7	10^8	Frequency of resistance
4	0.2	-	-	12	>100	1×10^{-6}
	0.8	-	-	-	<100	
	1.6	-	-	-	<100	
	3.2	-	-	-	1	1×10^{-8}
24	0.2	-	-	7	>100	7×10^{-7}
	0.8	-	-	-	<100	
	1.6	-	-	-	>100	
	3.2	-	-	-	1	1×10^{-8}
INH-control	10	ND	ND	33	ND	3×10^{-6}

ND: not done; -: no colonies

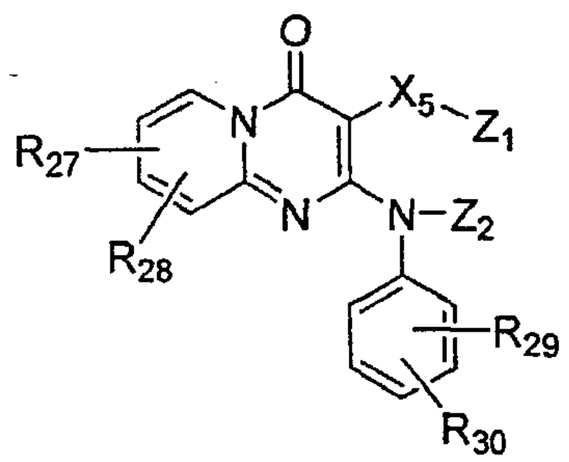
Compound	Concentration (µg/ml)	Bacteria inoculum (CFU) 10^6	10^7	10^8	Frequency of resistance
264	0.4	-	37	306	3.4×10^{-6}
	0.8	-	5	117	8×10^{-6}
	1.6	-	-	22	2×10^{-8}
	3.2	-	-	2	2×10^{-8}
INH-control	10	-	4	18	2.9×10^{-6}

-: no colonies

Table 7

What is claimed is:

1. A compound having the general formula VIIIa:



VIIIa

wherein

X_5 is CH_2 or C=O;

Z_1 is alkoxy, alkylamino, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heterarylamino, heterocycloalkyl, or hydroxyl, any of which is optionally substituted;

Z_2 is alkoxy, alkyl, alkylamino, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, or hydrogen, any of which is optionally substituted;

R_{27} and R_{28} are each independently alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heterarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thiol, sulfonyl, sulfonate or sulfonylamino, any of which is optionally substituted; and

R_{29} and R_{30} are each independently alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio,

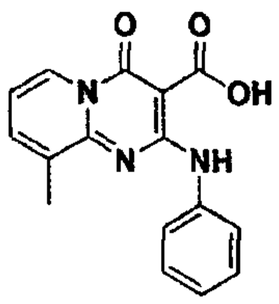
carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thiol, sulfonate, sulfonyl or sulfonylamino, or

R_{29} and R_{30} are connected with each other to make five or six membered cyclic, heterocyclic, aryl, or heteroaryl rings, any of which is optionally substituted,

wherein Z_1 and Z_2 may be connected with each other to make five or six membered cyclic, hetercyclic, aryl, or heteroaryl rings, any of which is optionally substituted.

2. A compound having a formula of 147-159,173, 180-183, 188, 189, 198, 201-221, 231-238, 245-271, 273-300, or 301:

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (147)



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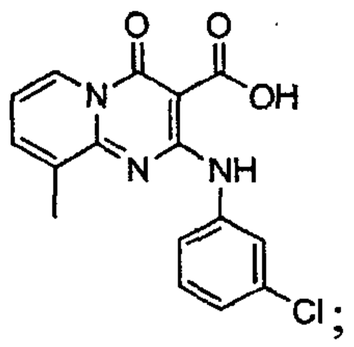
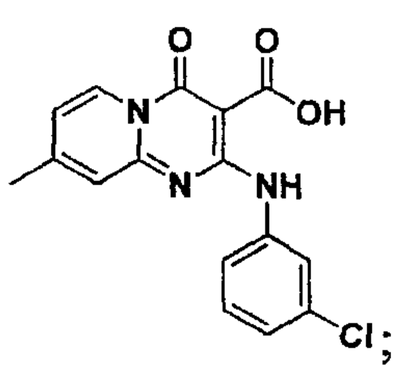
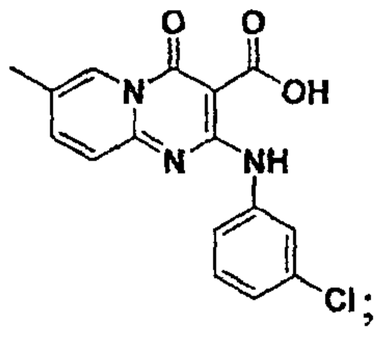
2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (148)



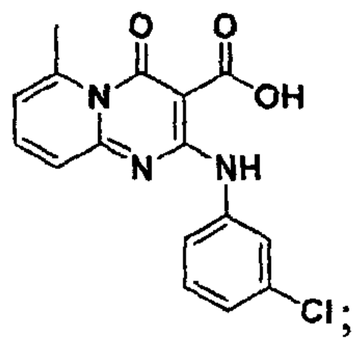
2-(3-Chlorophenylamino)-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (149)



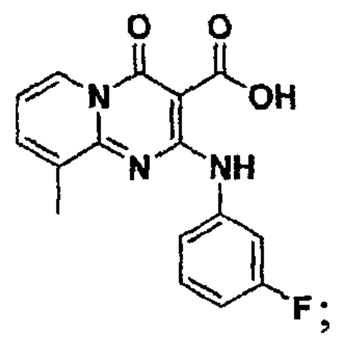
2-(3-Chlorophenylamino)-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (150)



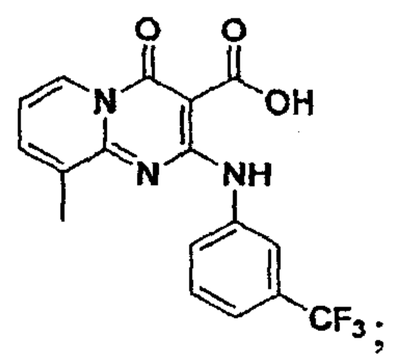
2-(3-Chlorophenylamino)-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (151)



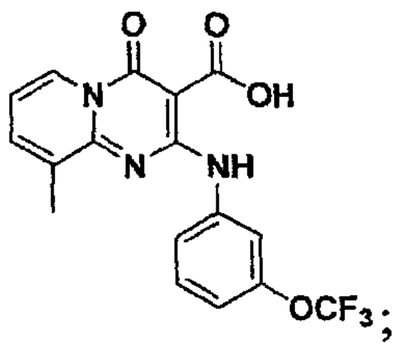
2-(3-Fluorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (152)



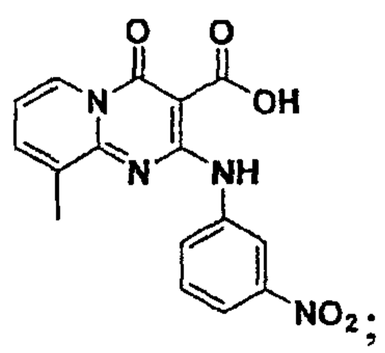
9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (153)



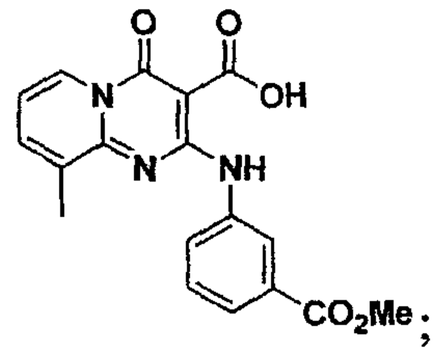
9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (154)



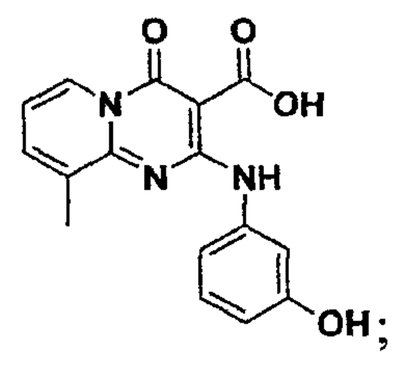
9-Methyl-2-(3-nitrophenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (155)



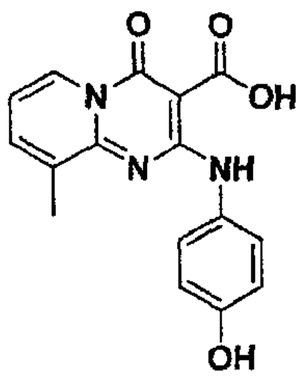
2-(3-(Methoxycarbonyl)phenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (156)



2-(3-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (157)



2-(4-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (158)



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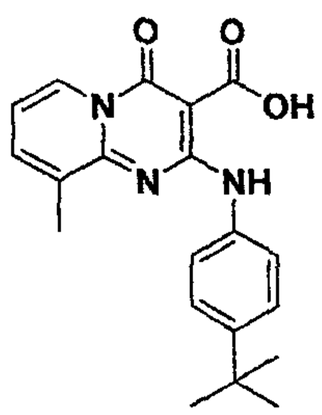
2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (159)



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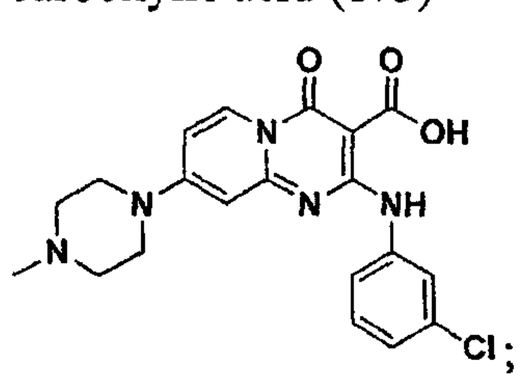
2-(3-Chlorophenylamino)-8-(4-methylpiperazin-1-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (173)



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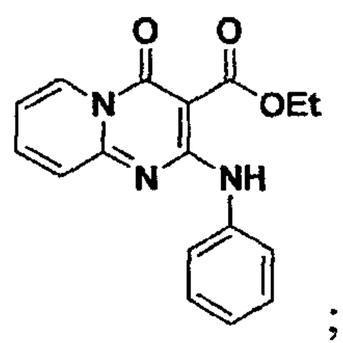
Ethyl 4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (180)



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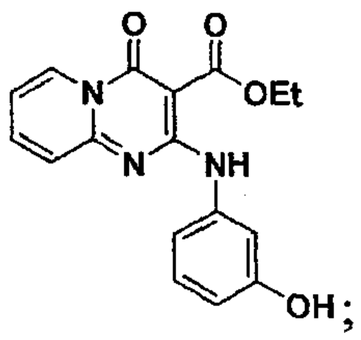
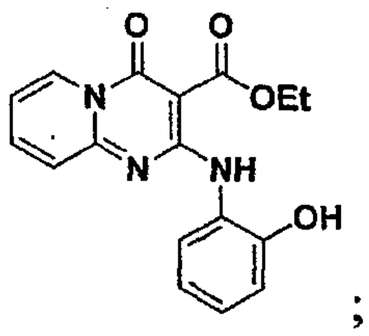
Ethyl 2-(3-hydroxyphenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (181)



Ethyl 2-(2-hydroxyphenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (182)



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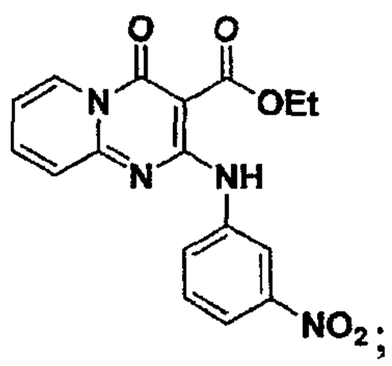
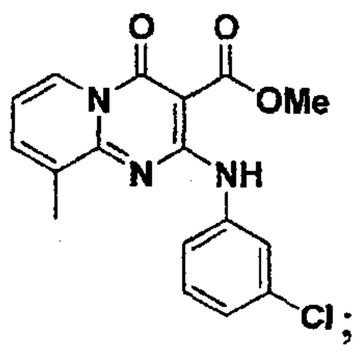
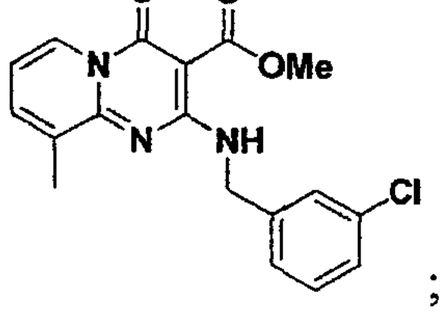
Ethyl 2-(3-nitrophenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (183)



Methyl 2-(3-chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (188)



Methyl 2-(3-chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (189)



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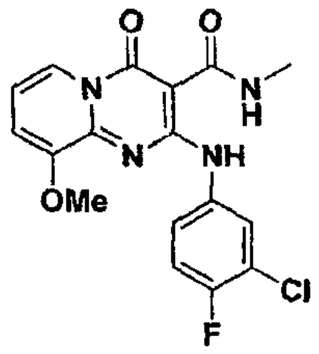
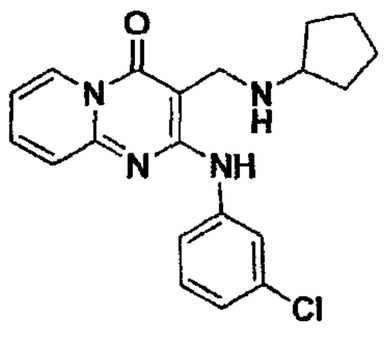
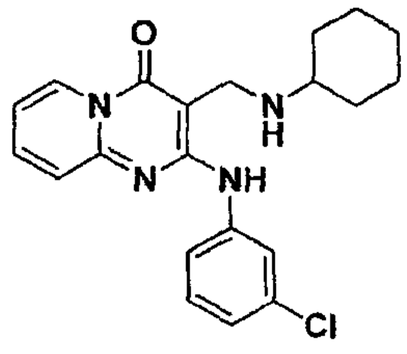
2-(3-Chloro-4-fluorophenylamino)-9-methoxy-N-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (198)



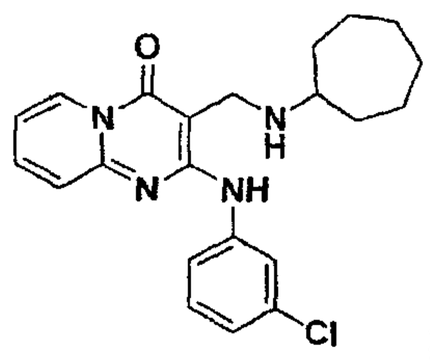
2-(3-Chlorophenylamino)-3-((cyclopentylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (201)



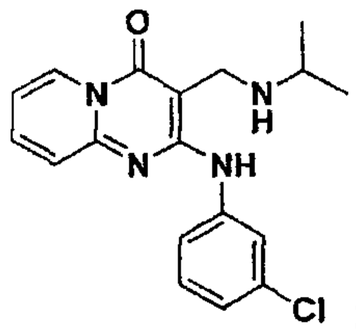
2-(3-Chlorophenylamino)-3-((cyclohexylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (202)



2-(3-Chlorophenylamino)-3-((cycloheptylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (203)



2-(3-Chlorophenylamino)-3-((isopropylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (204)



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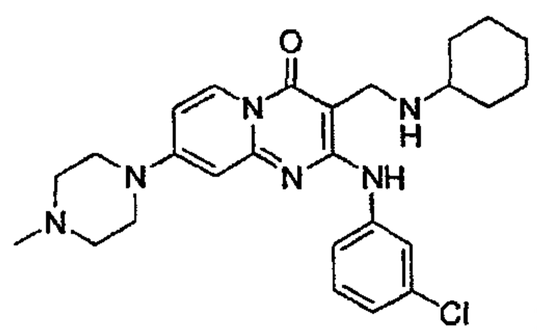
2-(3-Chlorophenylamino)-3-((cyclohexylamino)methyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (205)



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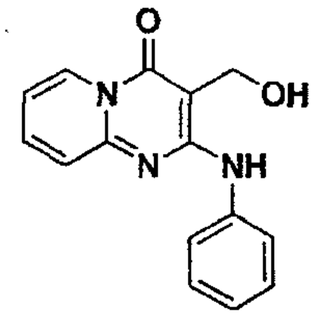
3-(Hydroxymethyl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (206)



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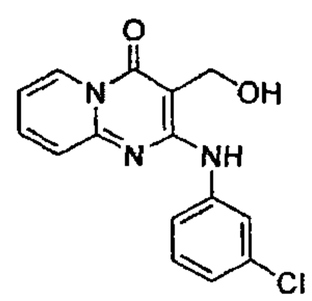
2-(3-Chlorophenylamino)-3-(hydroxy methyl)-4H-pyrido-[1,2-a] pyrimidin-4-one (207)



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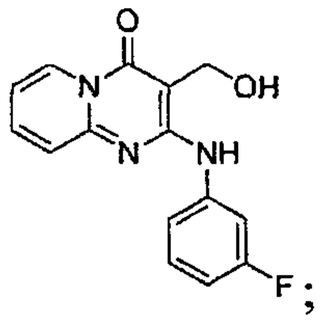
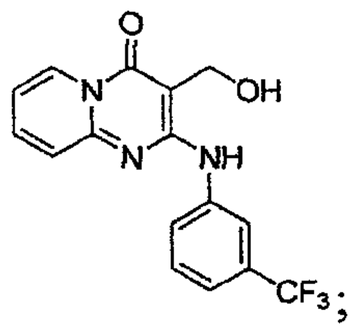
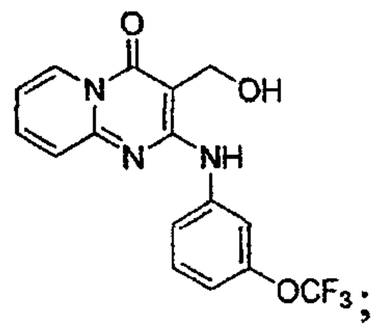
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido [1,2-a] pyrimidine-3-carbaldehyde (208)



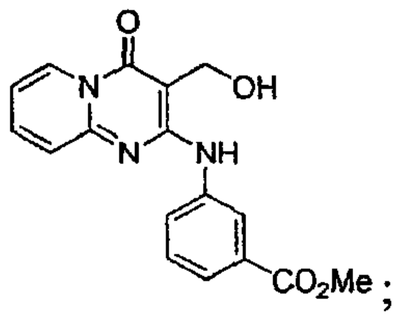
3-(Hydroxymethyl)-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (209)



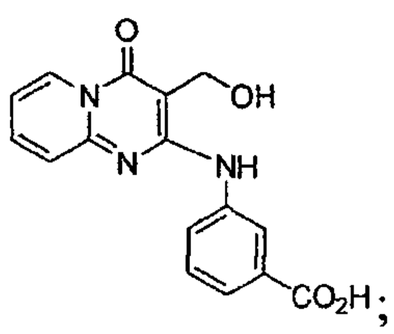
3-(Hydroxymethyl)-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (210)



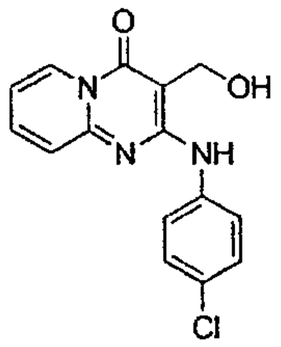
Methyl 3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino)benzoate (211)



3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino) benzoic acid (212)



2-(4-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (213)



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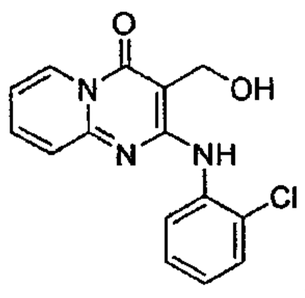
2-(2-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (214)



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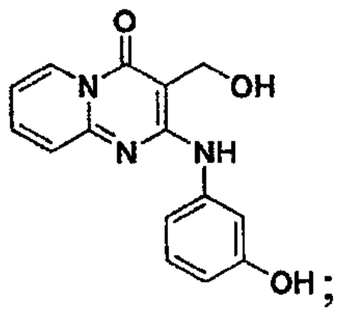
3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (215)



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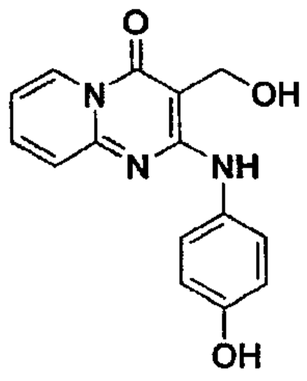
3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (216)



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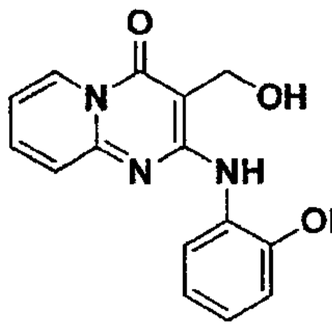
3-(Hydroxymethyl)-2-(2-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (217)



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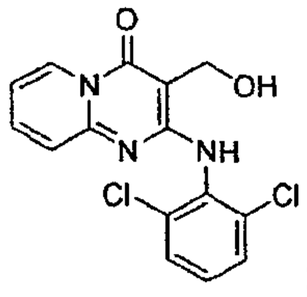
2-(2,6-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (218)



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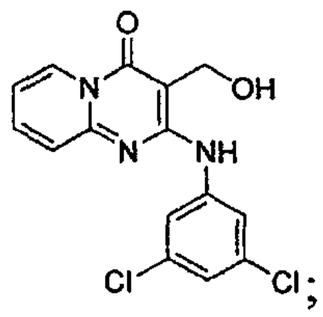
2-(3,5-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (219)



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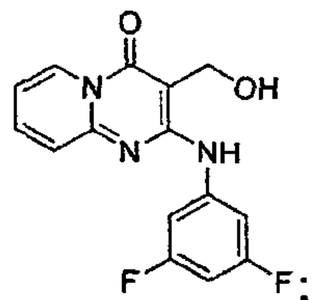
2-(3,5-Difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (220)



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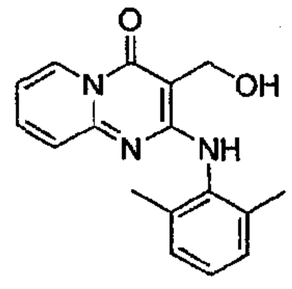
2-(2,6-Dimethylphenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (221)



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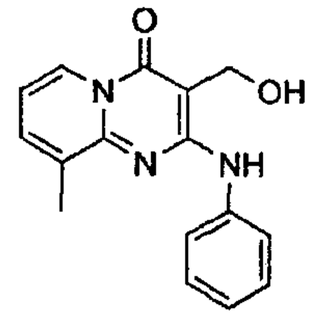
3-(Hydroxymethyl)-9-methyl-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (231)



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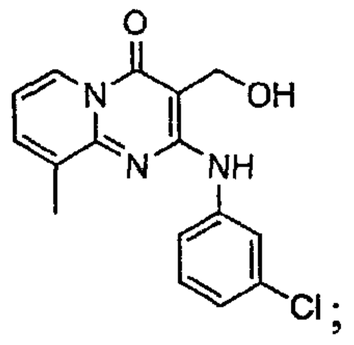
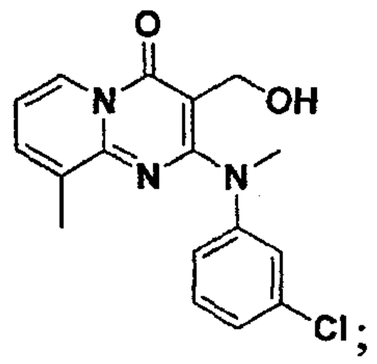
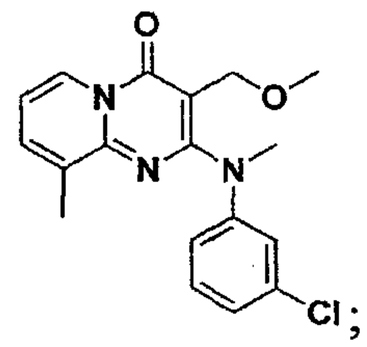
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (232)



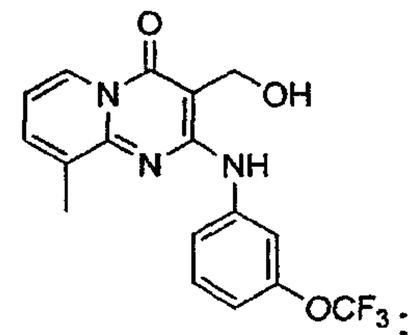
2-((3-Chlorophenyl)(methyl)amino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (233)



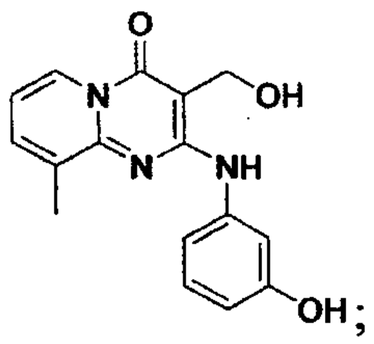
2-((3-Chlorophenyl)(methyl)amino)-3-(methoxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (234)



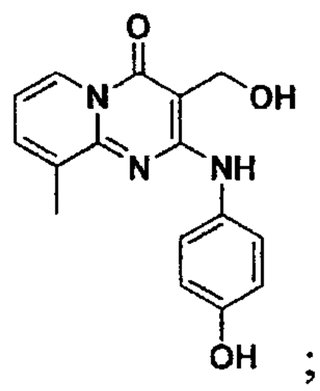
3-(Hydroxymethyl)-9-methyl-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (235)



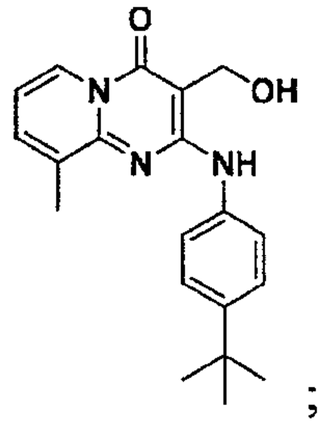
3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (236)



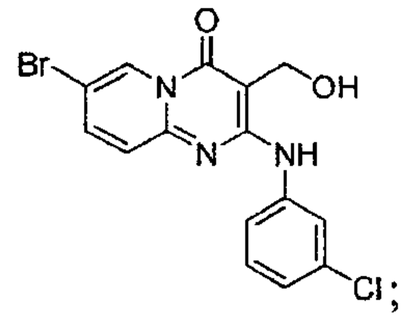
3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (237)



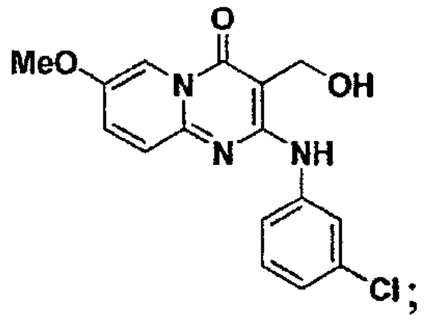
2-(4-tert-Butylphenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (238)



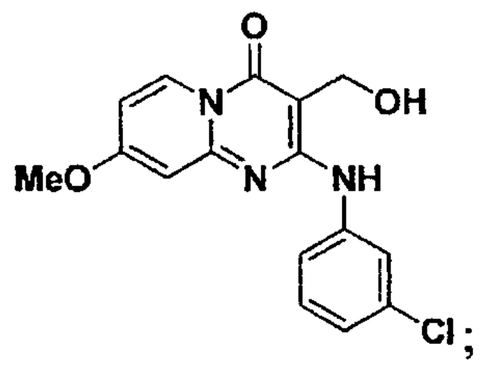
7-Bromo-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (245)



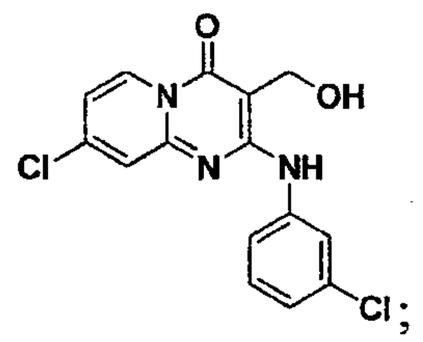
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-7-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (246)



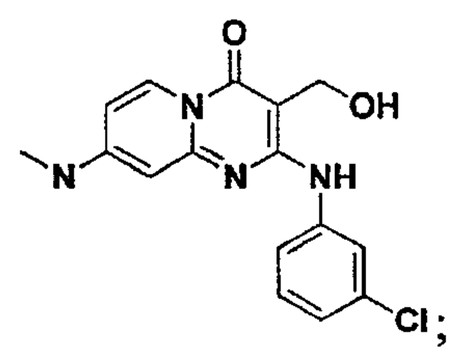
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (247)



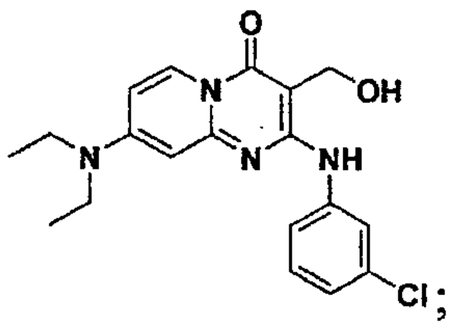
8-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (248)



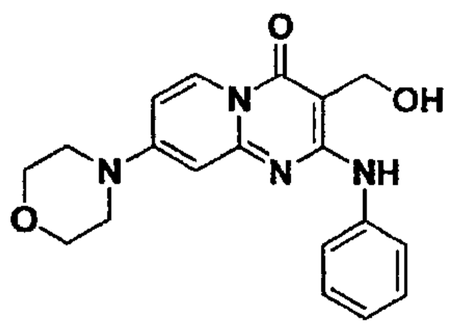
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(methylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (249)



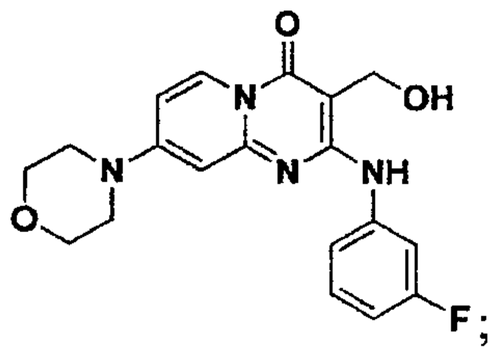
2-(3-Chlorophenylamino)-8-(diethylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (250)



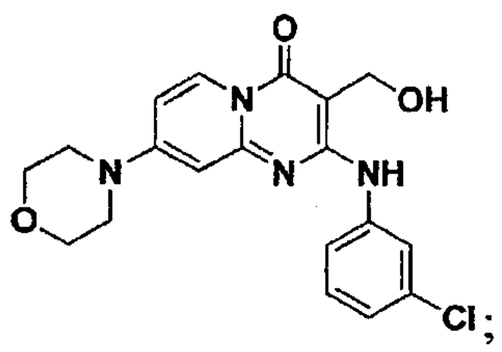
3-(Hydroxymethyl)-8-morpholino-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (251)



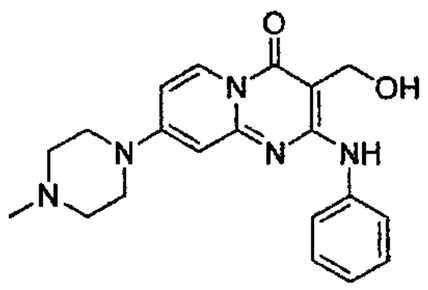
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (252)



2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (253)



3-(Hydroxymethyl)-8-(4-methylpiperazin-1-yl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (254)



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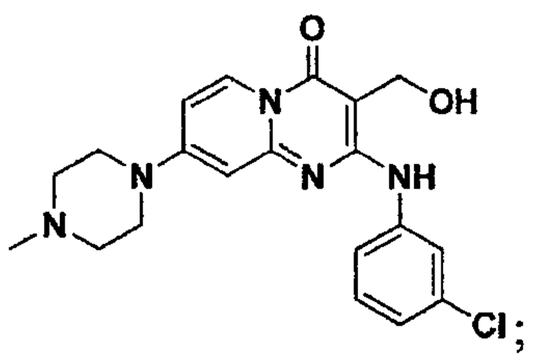
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (255)



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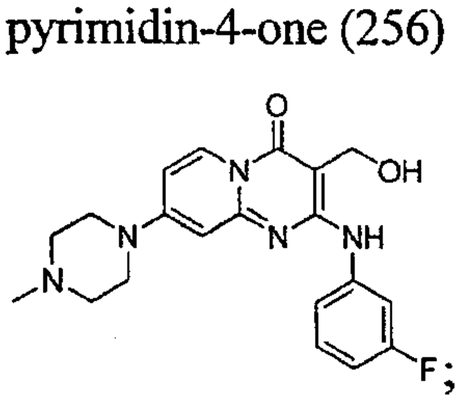
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a] pyrimidin-4-one (256)



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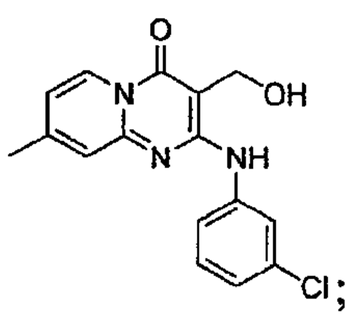
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (257)



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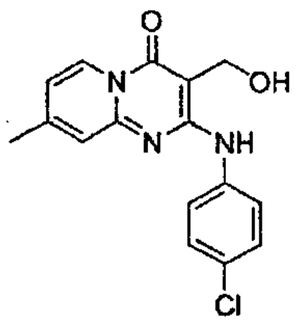
2-(4-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (258)



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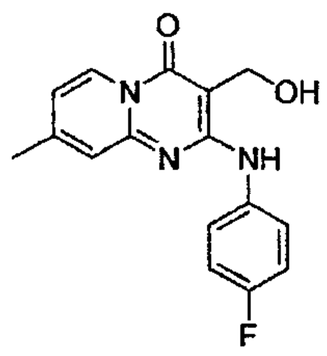
2-(4-Fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (259)



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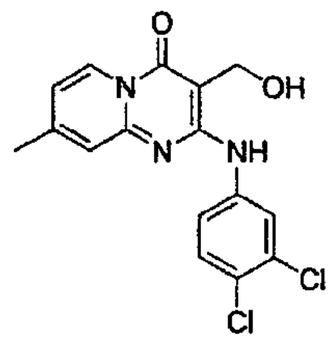
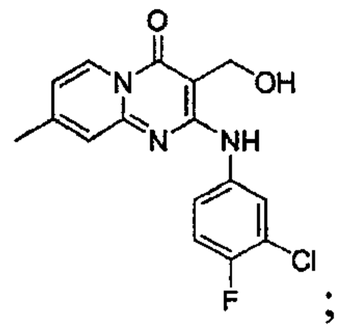
2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (260)



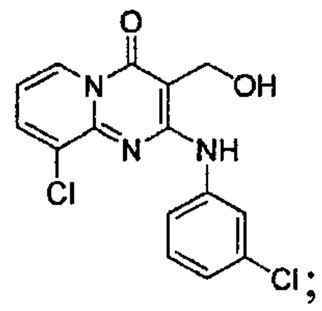
;

2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (261)



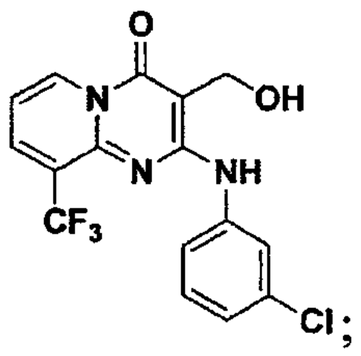
;

9-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (262)

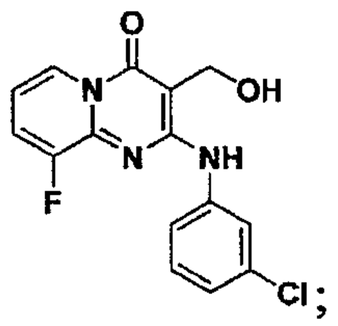


;

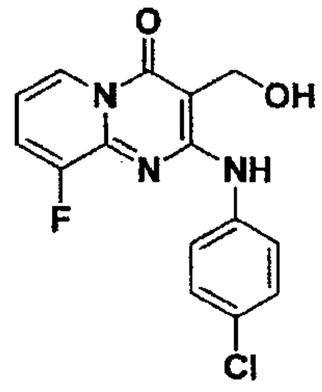
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (263)



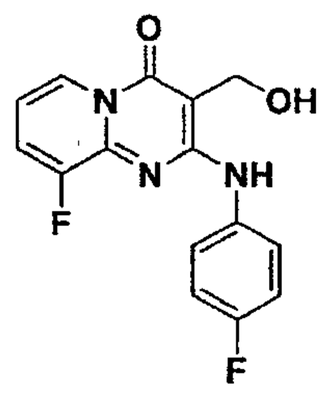
2-(3-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (264)



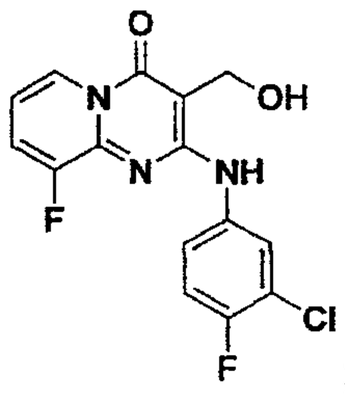
2-(4-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (265)



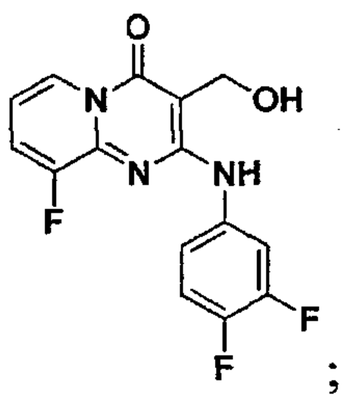
9-Fluoro-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (266)



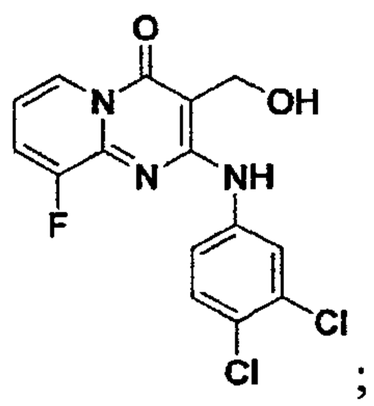
2-(3-Chloro-4-fluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (267)



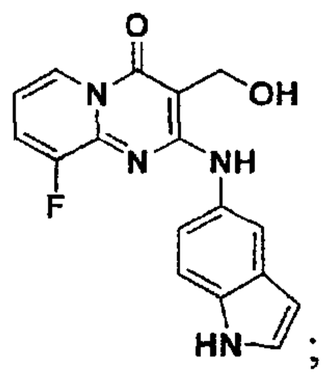
2-(3,4-Difluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (268)



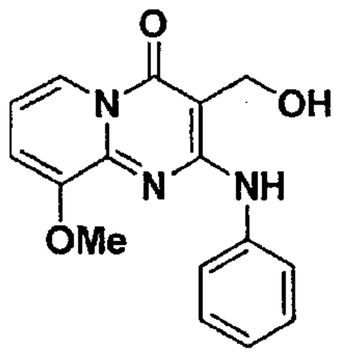
2-(3,4-Dichlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (269)



2-(1H-Indol-5-ylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (270)



3-(Hydroxymethyl)-9-methoxy-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (271)



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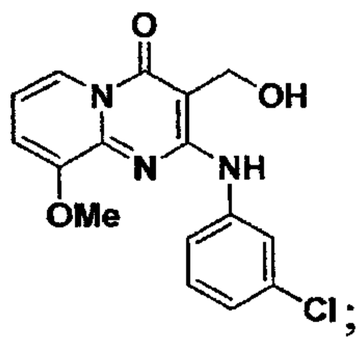
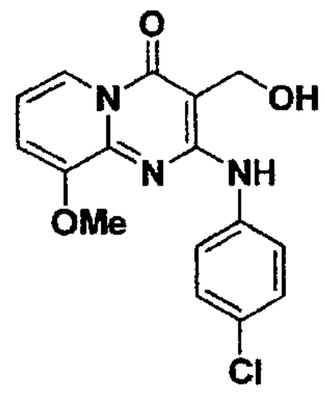
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (273)



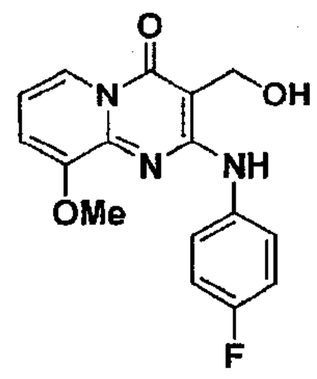
;

2-(4-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (274)



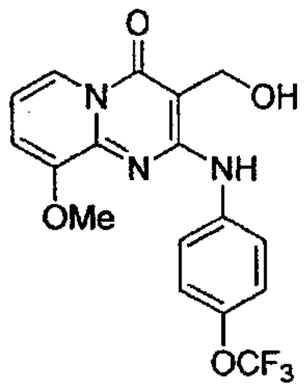
;

2-(4-Fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (275)



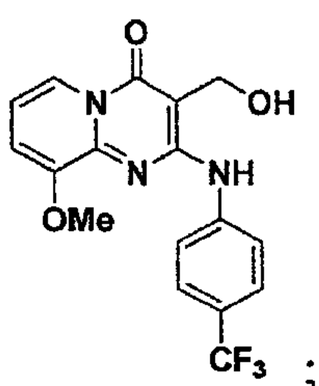
;

3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (276)



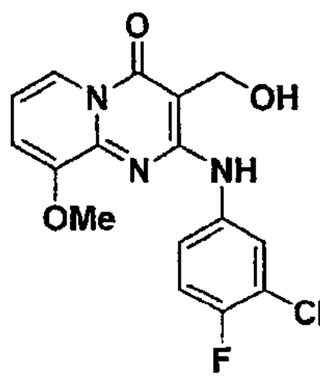
;

3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (277)



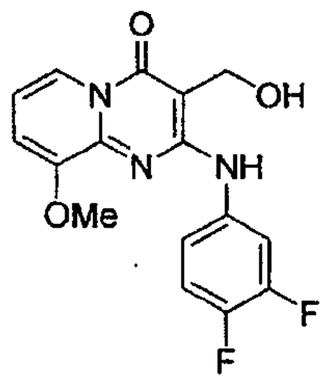
;

2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (278)



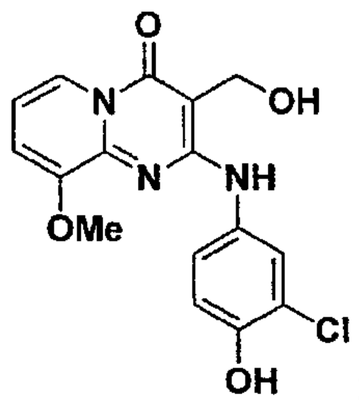
;

2-(3,4-Difluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (279)

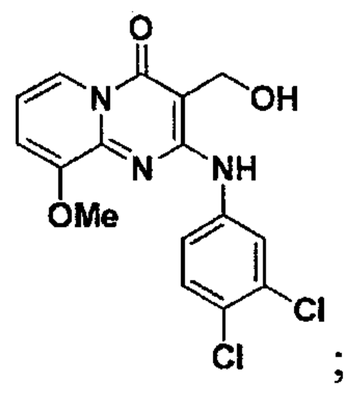


;

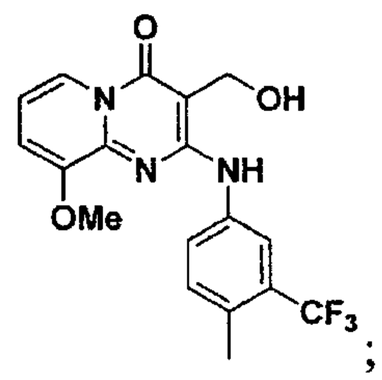
2-(3-Chloro-4-hydroxyphenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (280)



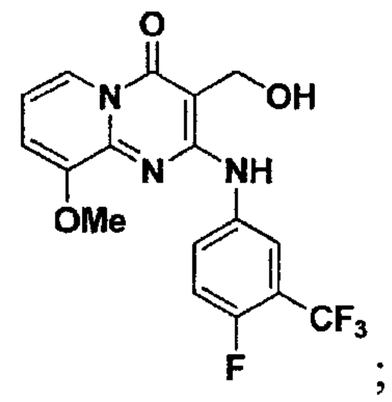
2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (281)



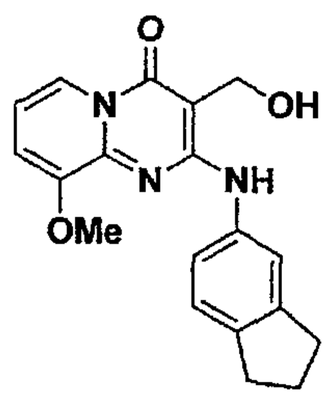
3-(Hydroxymethyl)-9-methoxy-2-(4-methyl-3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (282)



2-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (283)

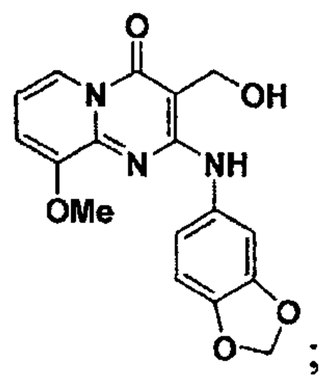


2-(2,3-Dihydro-1H-inden-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (284)



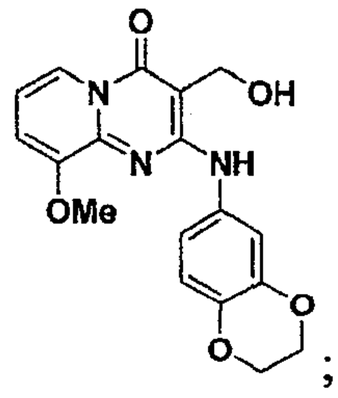
;

2-(Benzo[d][1,3]dioxol-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (285)



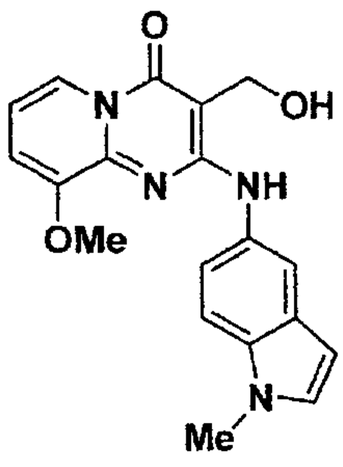
;

2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (286)



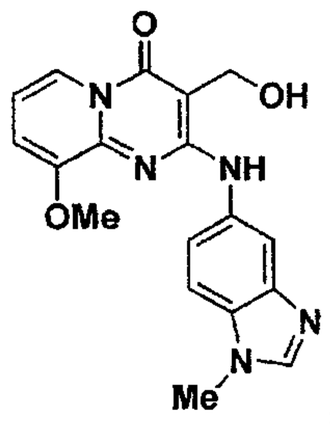
;

3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (287)



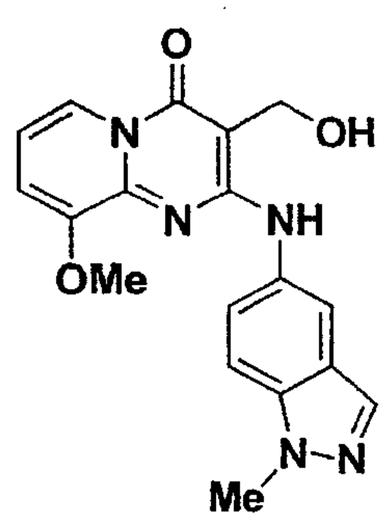
;

3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-benzo[d]imidazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (288)



;

3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (289)

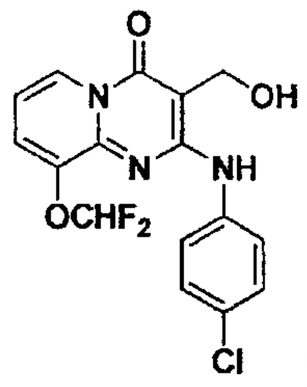


;

9-(Difluoromethoxy)-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (290)

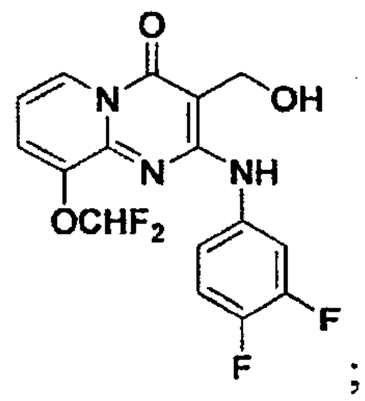
;

2-(4-Chlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (291)



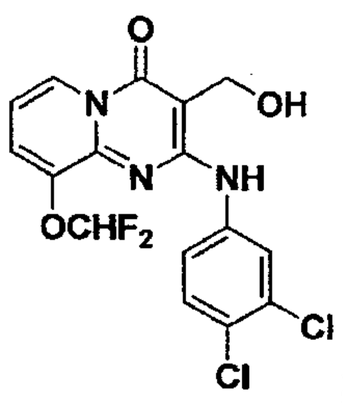
;

9-(Difluoromethoxy)-2-(3,4-difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (292)

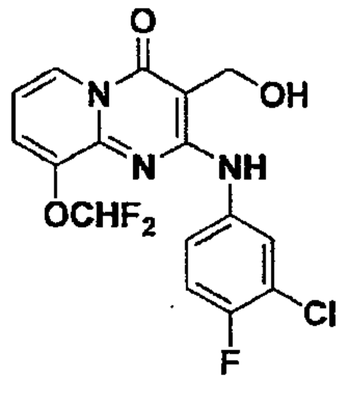


;

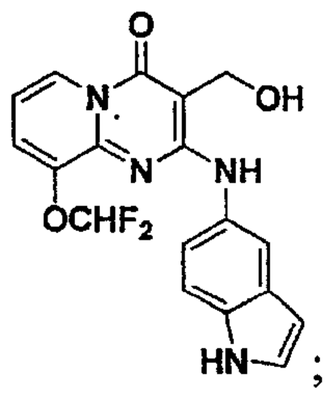
2-(3,4-Dichlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (293)



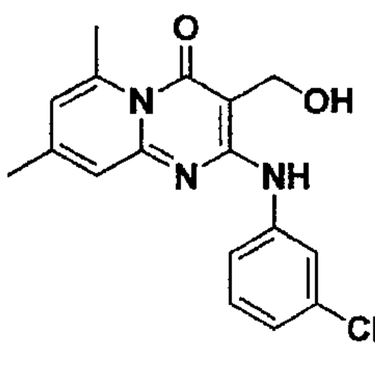
2-(3-Chloro-4-fluorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (294)



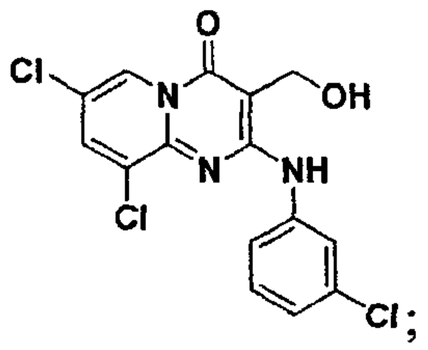
2-(1H-Indol-5-ylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (295)



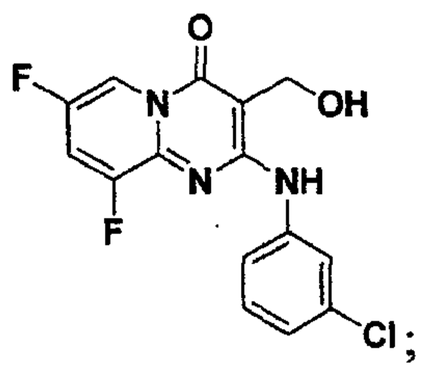
2-(3-chlorophenylamino)-3-(hydroxymethyl)-6,8-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (296)



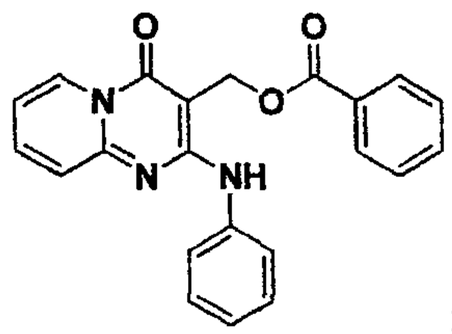
7,9-Dichloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (297)



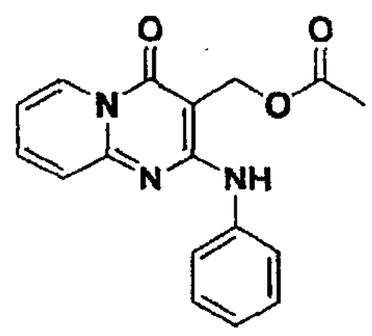
2-(3-Chlorophenylamino)-7,9-difluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (298)



(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl benzoate (299)

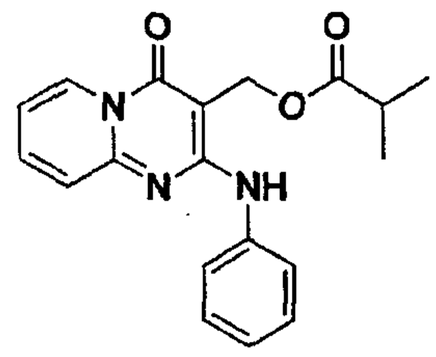


(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl acetate (300)



; or

(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl isobutyrate (301)

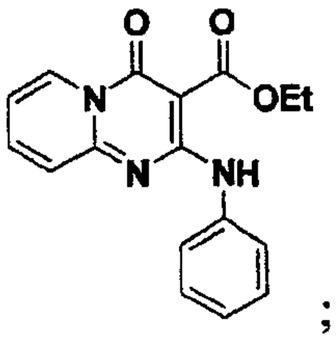


3. A pharmaceutical composition comprising a compound of claim 1 and a carrier.

4. A pharmaceutical composition comprising a compound of claim 2 and a carrier.

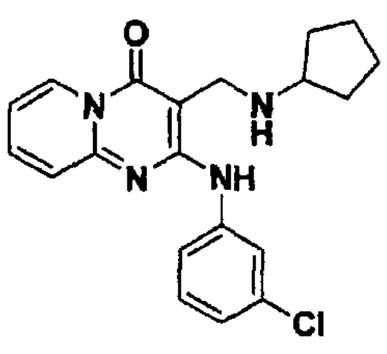
5. A compound having a formula of 180, 201, 204, 206-221, 231-238, 245-271, 273-278, 280-286, 290-300, or 301:

Ethyl 4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (180)



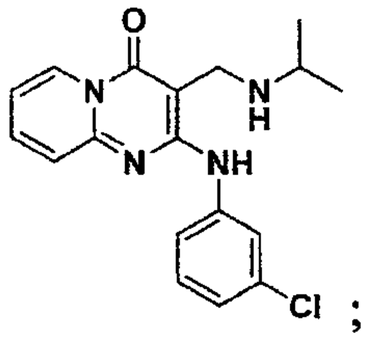
;

2-(3-Chlorophenylamino)-3-((cyclopentylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (201)



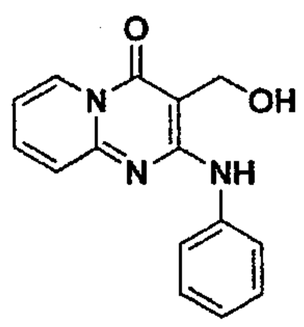
;

2-(3-Chlorophenylamino)-3-((isopropylamino)methyl)-4H-pyrido[1,2-a]pyrimidin-4-one (204)



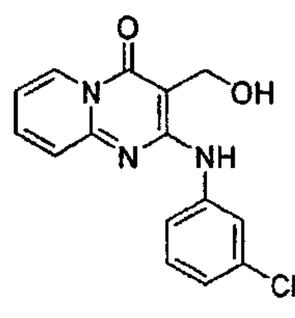
;

3-(Hydroxymethyl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (206)



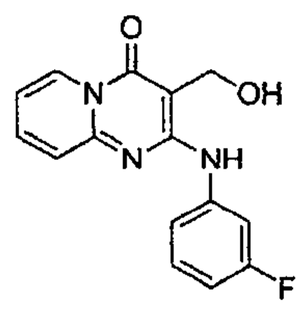
;

2-(3-Chlorophenylamino)-3-(hydroxy methyl)-4H-pyrido-[1,2-a] pyrimidin-4-one (207)



;

2-(3-Fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido [1,2-a] pyrimidine-3-carbaldehyde (208)



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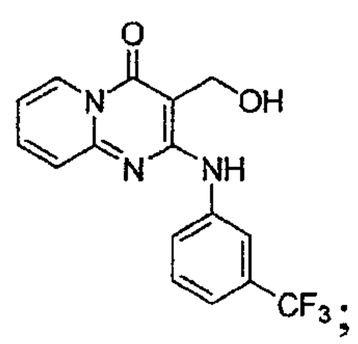
3-(Hydroxymethyl)-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (209)



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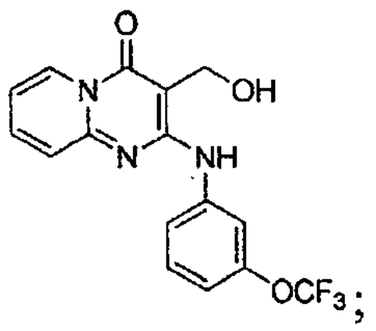
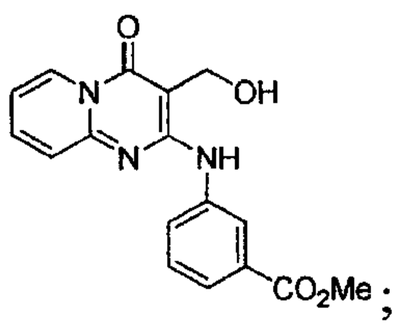
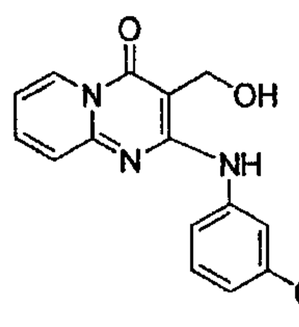
3-(Hydroxymethyl)-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido [1,2-a] pyrimidin-4-one (210)



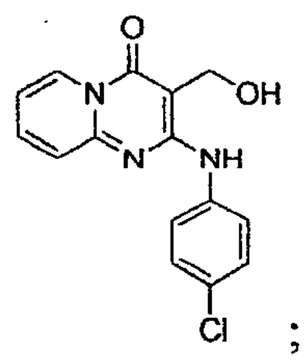
Methyl 3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino)benzoate (211)



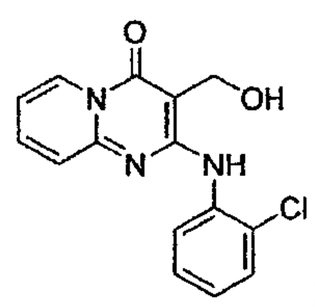
3-(3-(hydroxymethyl)-4-oxo-4H-pyrido [1,2-a] pyrimidin-2-ylamino) benzoic acid (212)



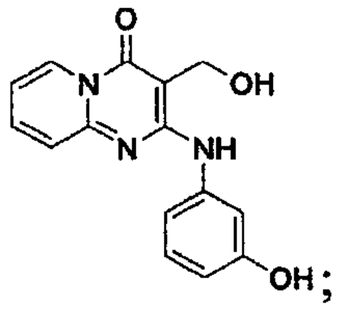
2-(4-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (213)



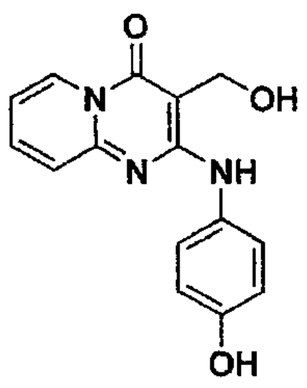
2-(2-Chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (214)



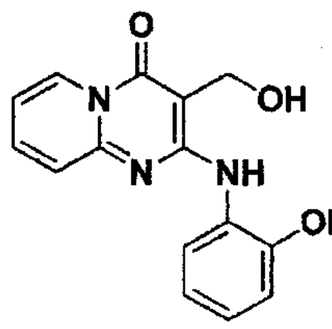
3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (215)



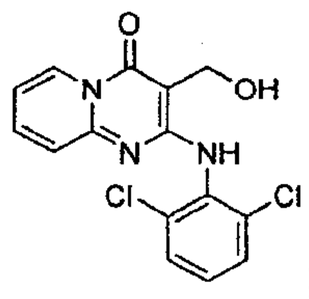
3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (216)



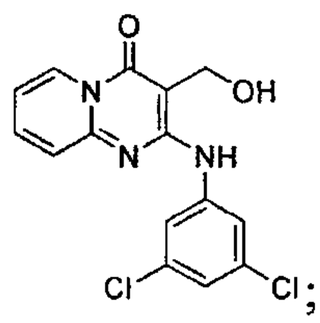
3-(Hydroxymethyl)-2-(2-hydroxyphenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (217)



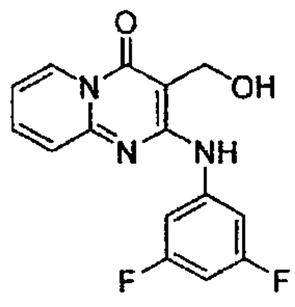
2-(2,6-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (218)



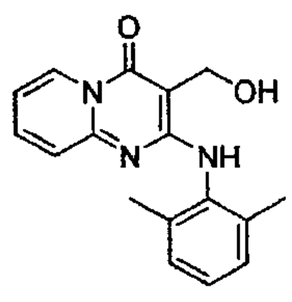
2-(3,5-Dichlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (219)



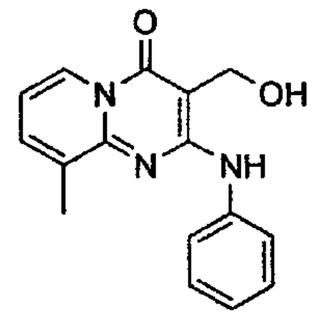
2-(3,5-Difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (220)



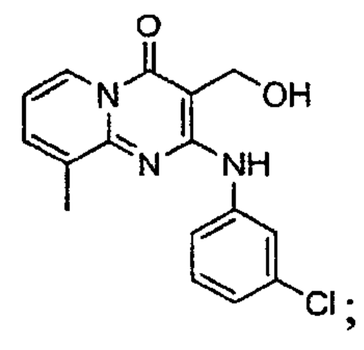
2-(2,6-Dimethylphenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (221)



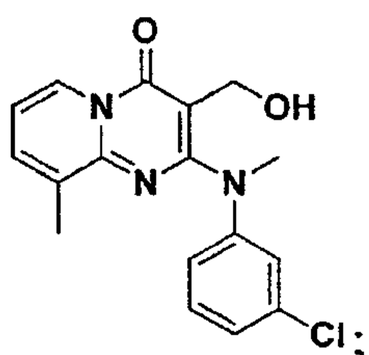
3-(Hydroxymethyl)-9-methyl-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (231)



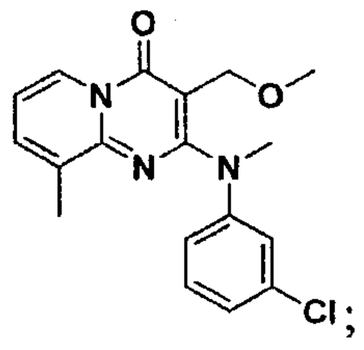
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (232)



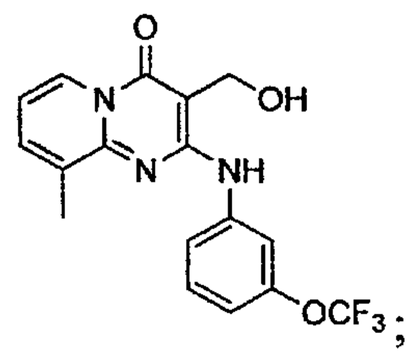
2-((3-Chlorophenyl)(methyl)amino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (233)



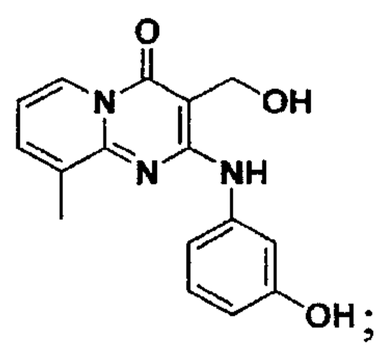
2-((3-Chlorophenyl)(methyl)amino)-3-(methoxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (234)



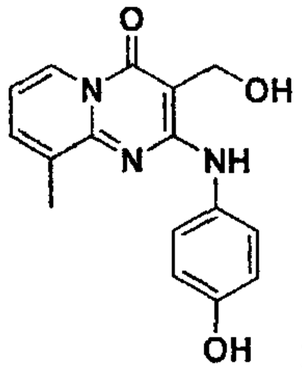
3-(Hydroxymethyl)-9-methyl-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (235)



3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (236)



3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (237)



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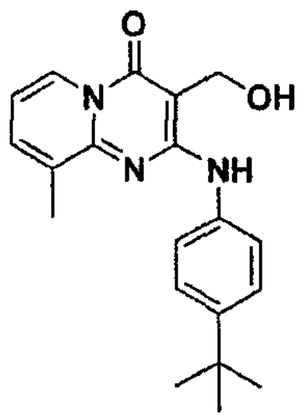
2-(4-tert-Butylphenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (238)



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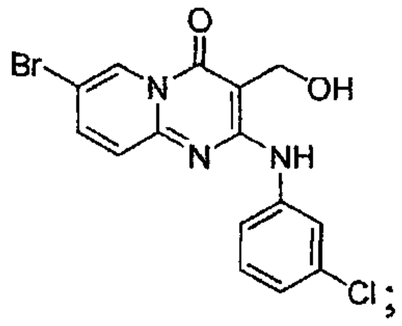
7-Bromo-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (245)



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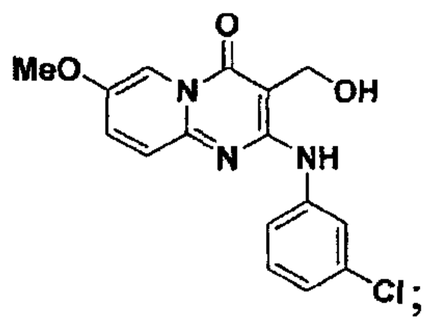
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-7-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (246)



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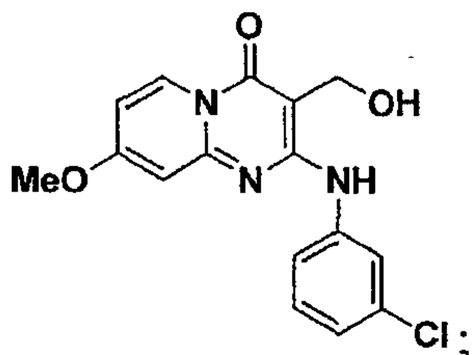
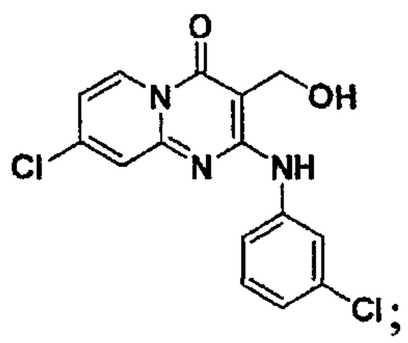
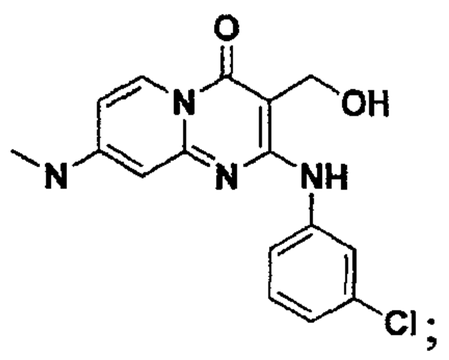
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (247)



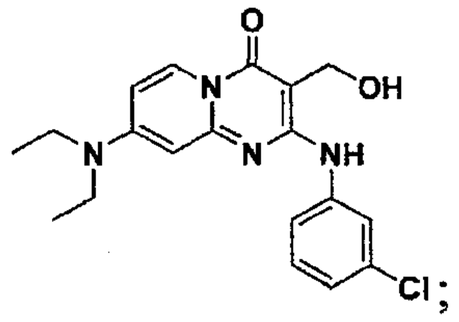
8-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (248)



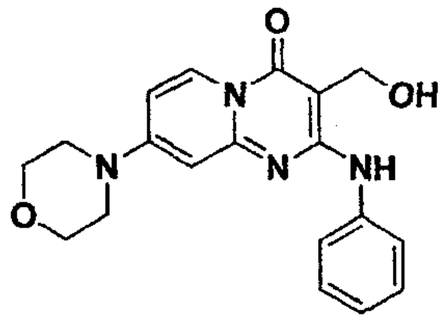
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(methylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (249)



2-(3-Chlorophenylamino)-8-(diethylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (250)



3-(Hydroxymethyl)-8-morpholino-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (251)



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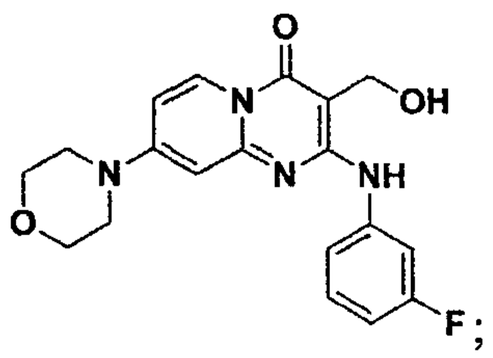
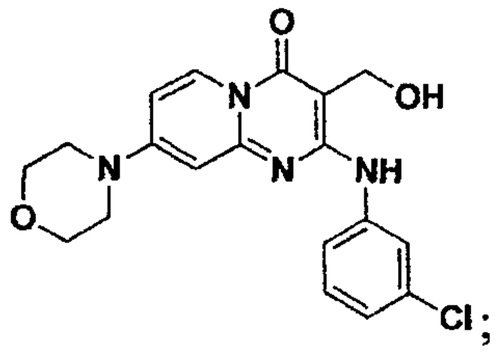
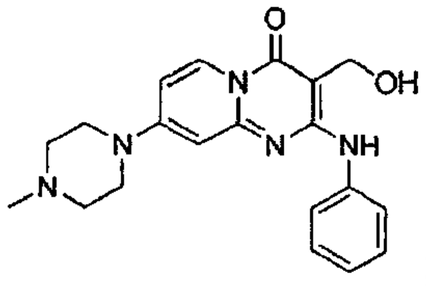
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (252)



2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (253)



3-(Hydroxymethyl)-8-(4-methylpiperazin-1-yl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (254)



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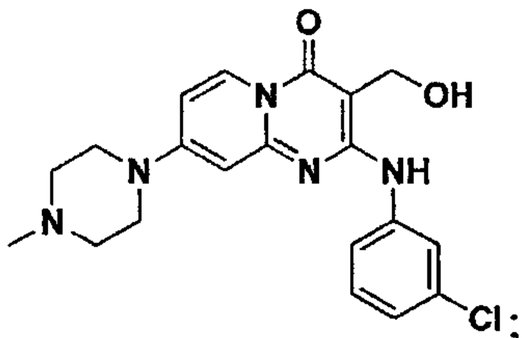
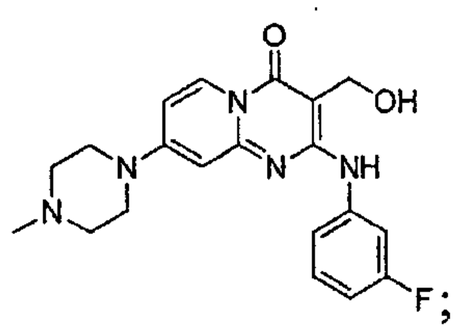
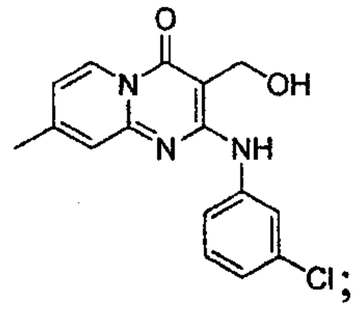
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (255)



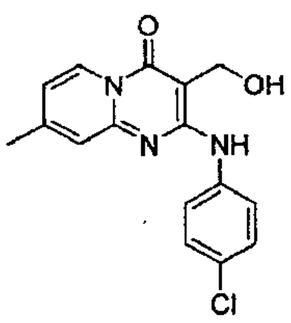
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (256)



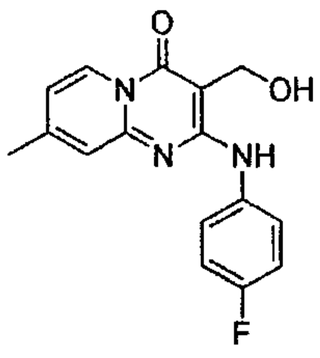
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (257)



2-(4-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (258)



2-(4-Fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (259)



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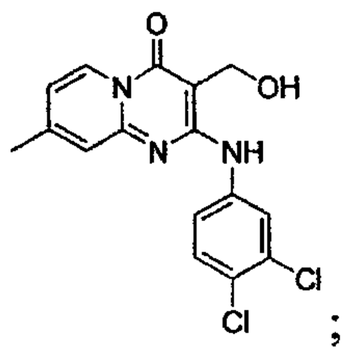
2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (260)



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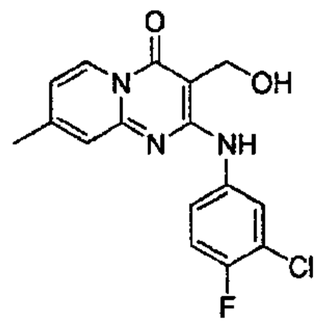
2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (261)



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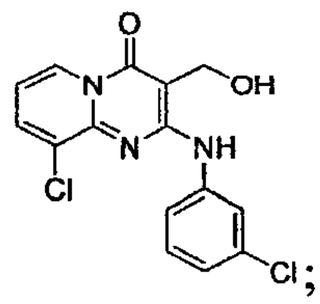
9-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (262)



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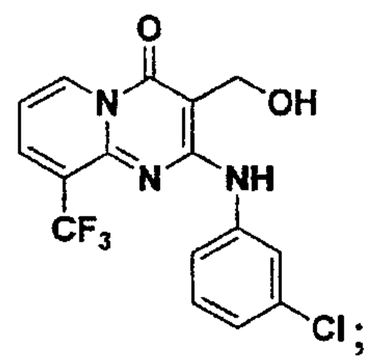
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (263)



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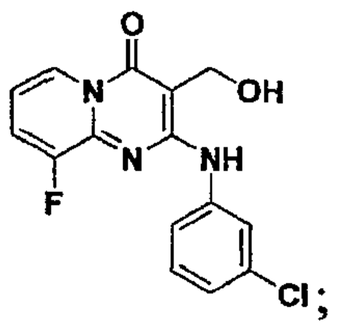
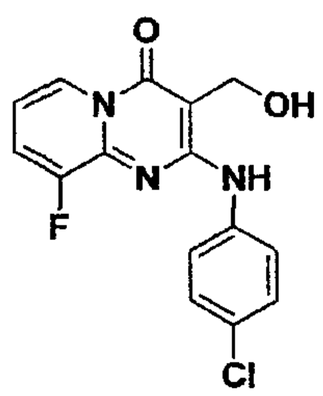
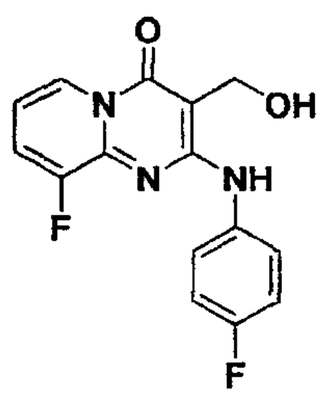
2-(3-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (264)



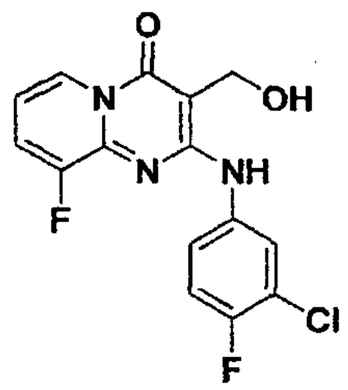
2-(4-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (265)



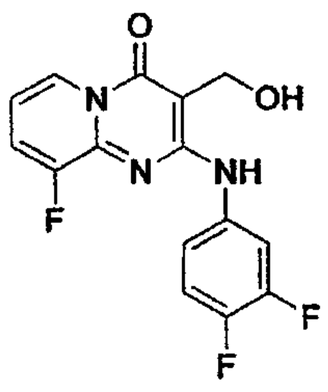
9-Fluoro-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (266)



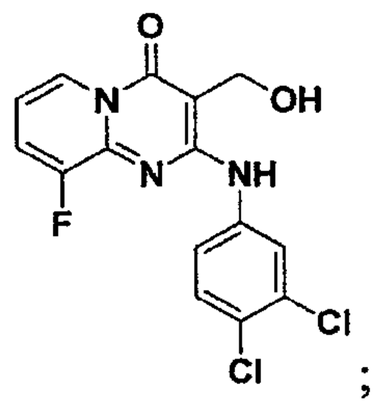
2-(3-Chloro-4-fluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (267)



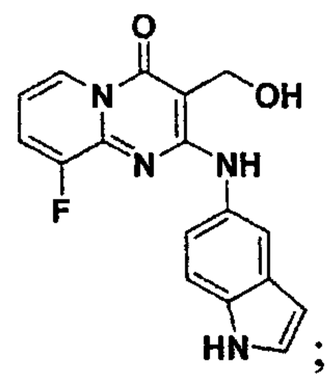
2-(3,4-Difluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (268)



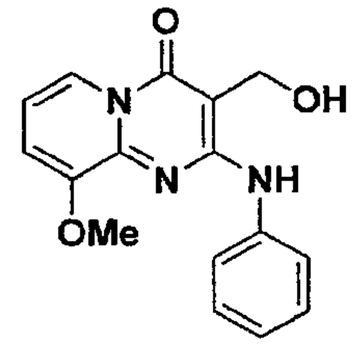
2-(3,4-Dichlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (269)



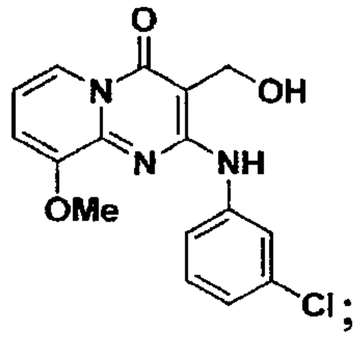
2-(1H-Indol-5-ylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (270)



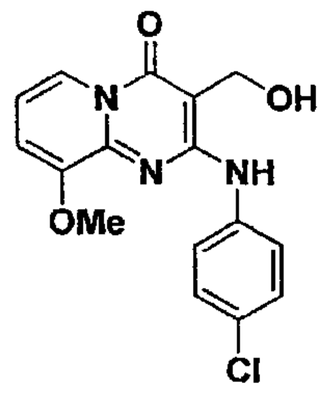
3-(Hydroxymethyl)-9-methoxy-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (271)



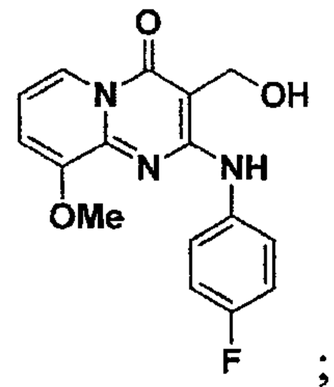
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (273)



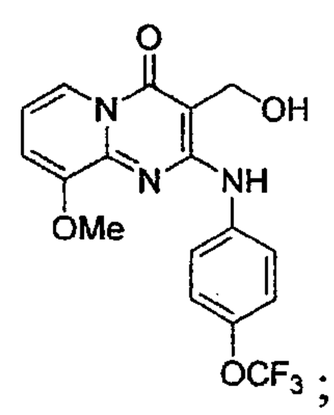
2-(4-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (274)



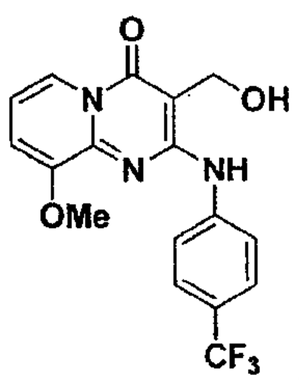
2-(4-Fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (275)



3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (276)



3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (277)



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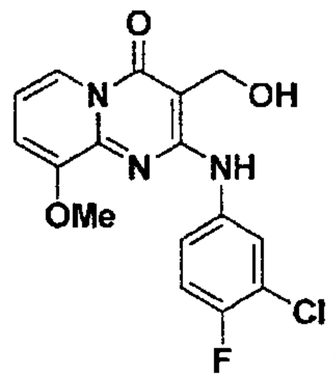
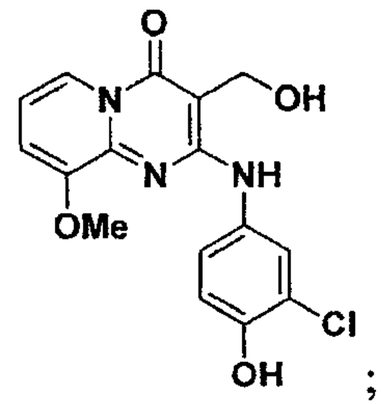
2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (278)



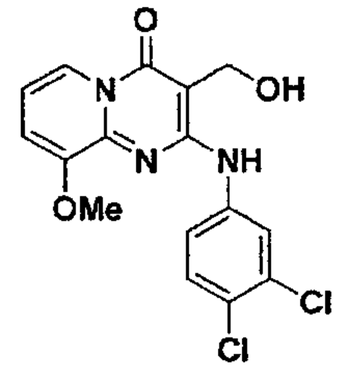
;

2-(3-Chloro-4-hydroxyphenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (280)



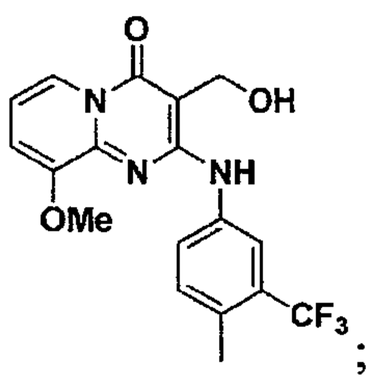
;

2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (281)

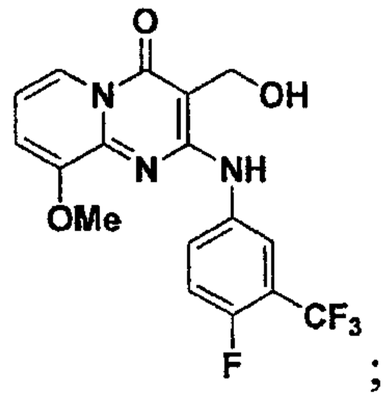


;

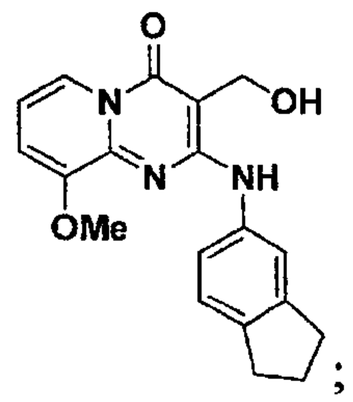
3-(Hydroxymethyl)-9-methoxy-2-(4-methyl-3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (282)



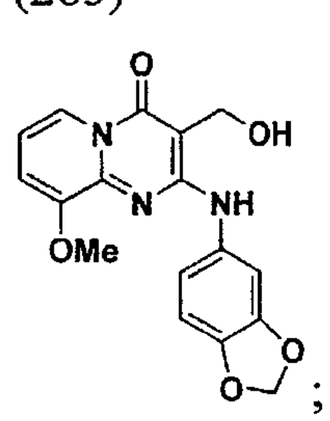
2-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (283)



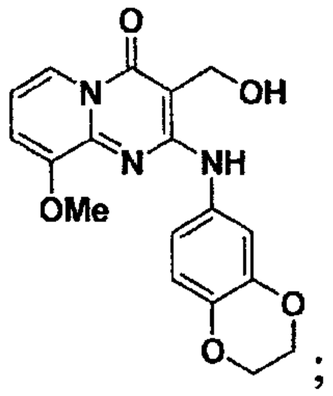
2-(2,3-Dihydro-1H-inden-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (284)



2-(Benzo[d][1,3]dioxol-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (285)

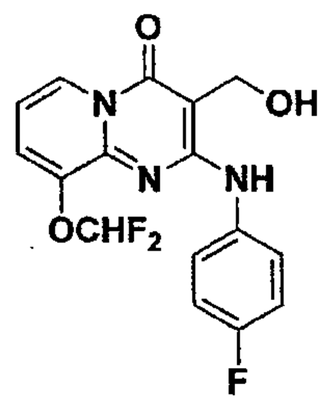


2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (286)



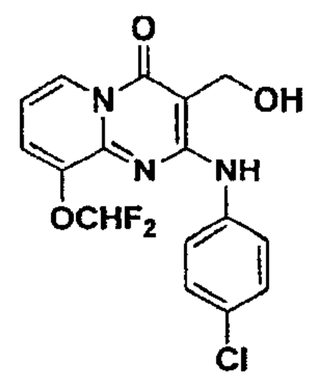
;

9-(Difluoromethoxy)-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (290)



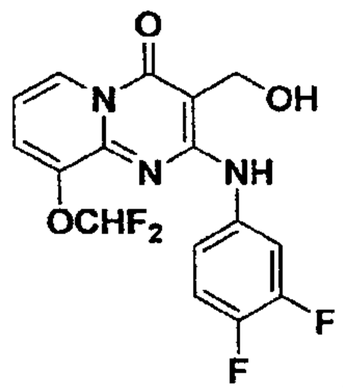
;

2-(4-Chlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (291)



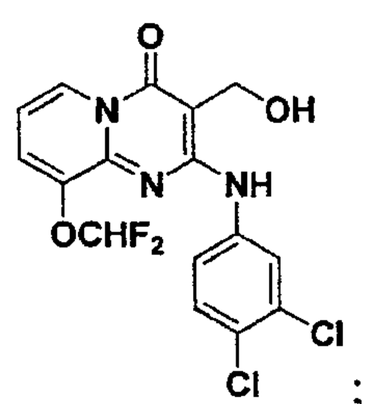
;

9-(Difluoromethoxy)-2-(3,4-difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (292)



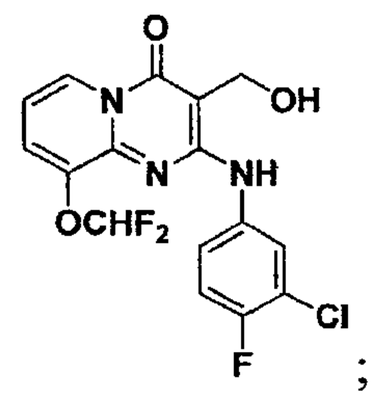
;

2-(3,4-Dichlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (293)



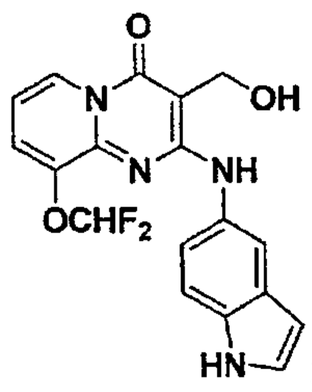
;

2-(3-Chloro-4-fluorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (294)

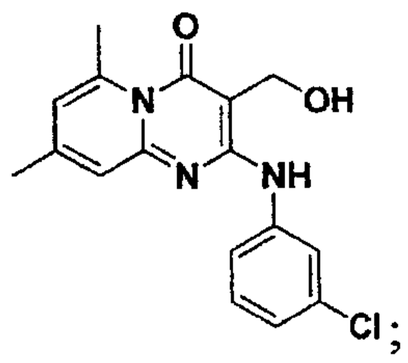


;

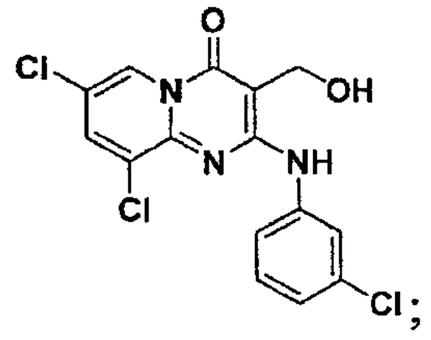
2-(1H-Indol-5-ylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (295)



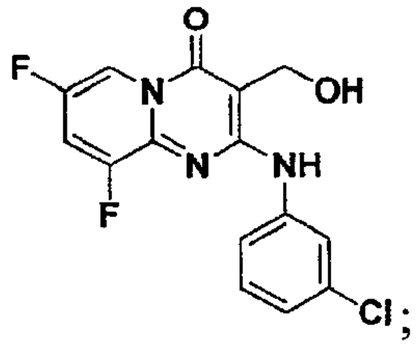
2-(3-chlorophenylamino)-3-(hydroxymethyl)-6,8-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (296)



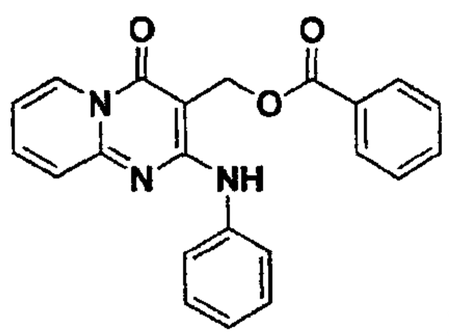
7,9-Dichloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (297)



2-(3-Chlorophenylamino)-7,9-difluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (298)

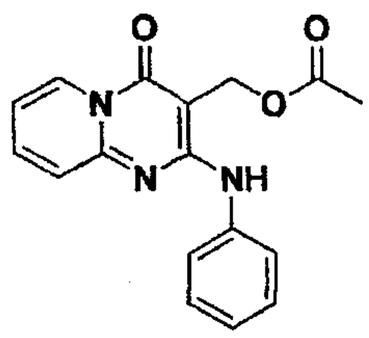


(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl benzoate (299)



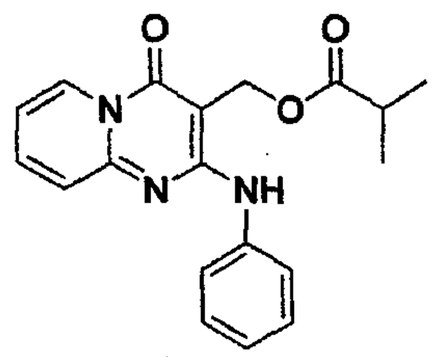
;

(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl acetate (300)



; or

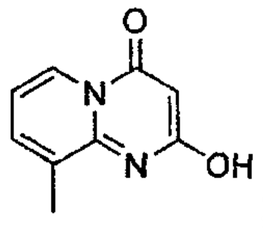
(4-Oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-3-yl)methyl isobutyrate (301)



6. A pharmaceutical composition comprising a compound of claim 5 and a carrier.

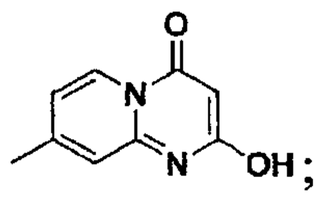
7. A compound having a formula of 125, 126, 132-142, or 143:

2-Hydroxy-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (125)



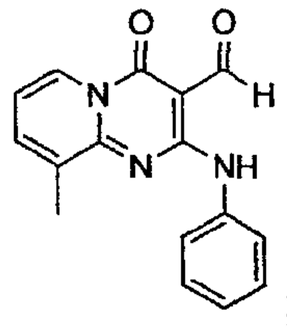
;

2-Hydroxy-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (126)



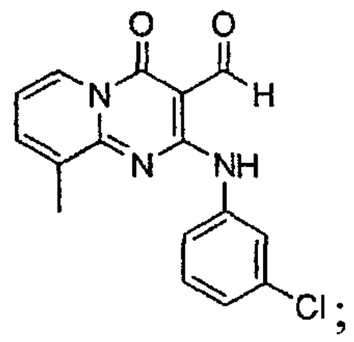
;

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (132)



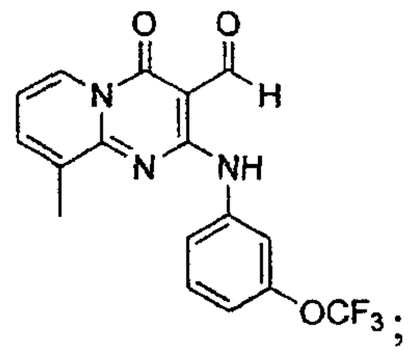
;

2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (133)



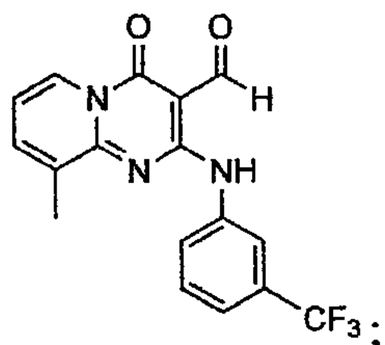
;

9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (134)



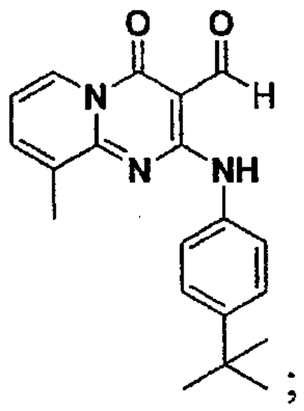
;

9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (135)



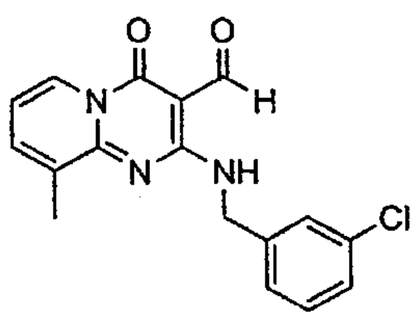
;

2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (136)



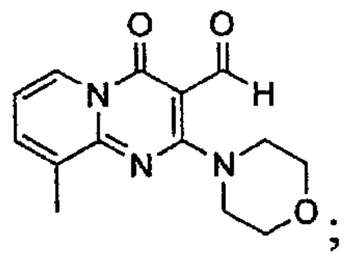
;

2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (137)



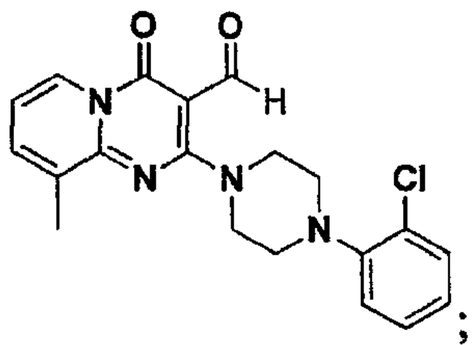
;

9-Methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (138)



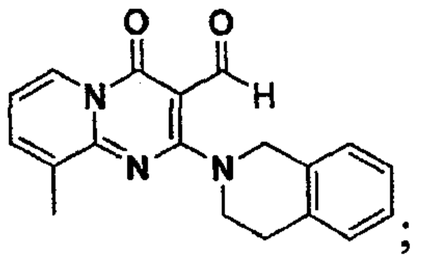
;

2-(4-(2-Chlorophenyl)piperazin-1-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (139)



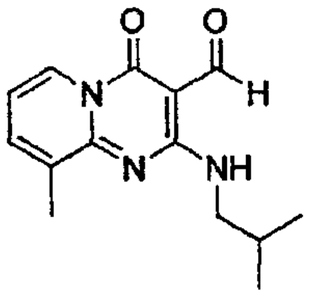
;

2-(3,4-Dihydroisoquinolin-2(1H)-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (140)



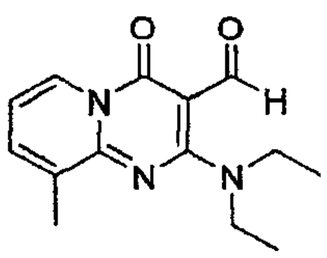
;

2-(Isobutylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (141)



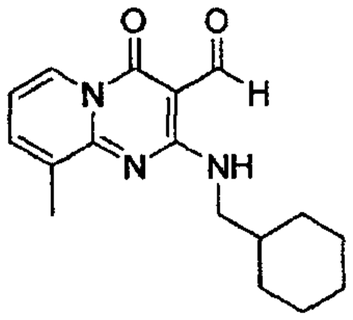
;

2-(Diethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (142)



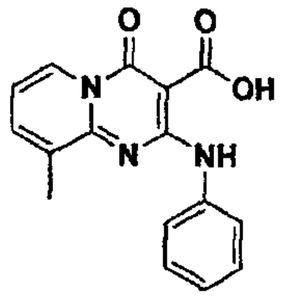
; or

2-(Cyclohexylmethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (143)



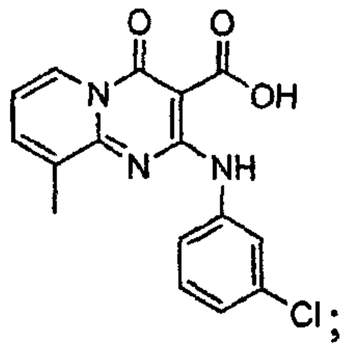
8. A compound having a formula of 147-173, 188-189, 198, 205, 231-271, 273-297, or 298:

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (147)



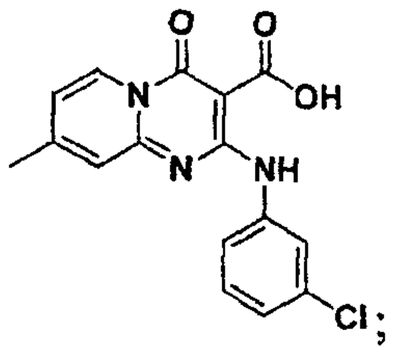
;

2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (148)



;

2-(3-Chlorophenylamino)-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (149)



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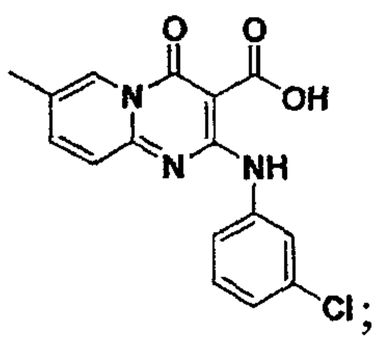
2-(3-Chlorophenylamino)-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (150)



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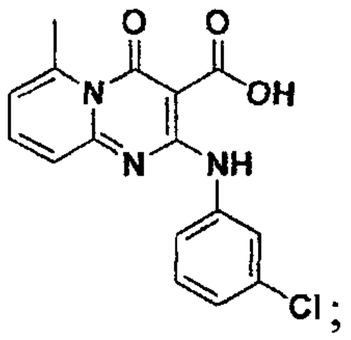
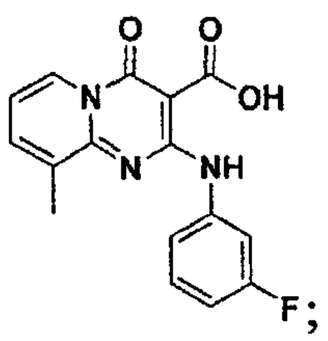
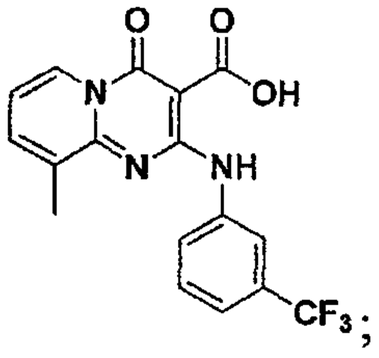
2-(3-Chlorophenylamino)-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (151)



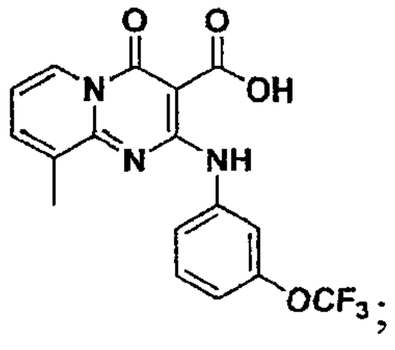
2-(3-Fluorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (152)



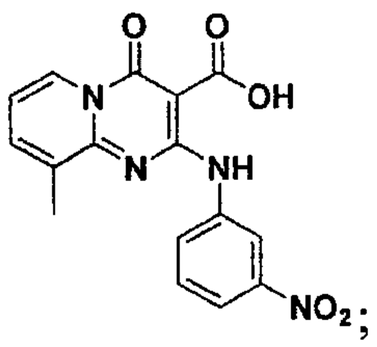
9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (153)



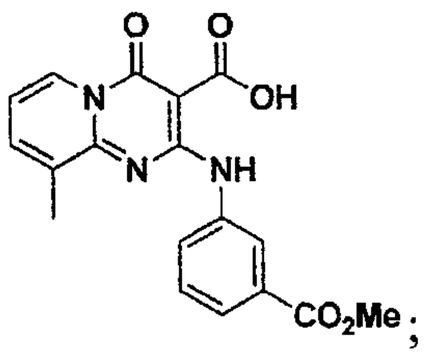
9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (154)



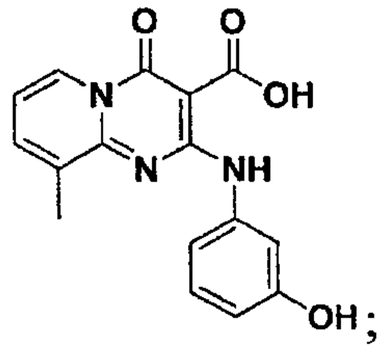
9-Methyl-2-(3-nitrophenylamino)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (155)



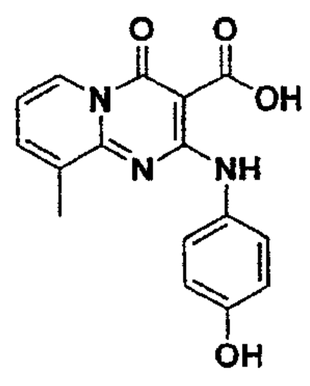
2-(3-(Methoxycarbonyl)phenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (156)



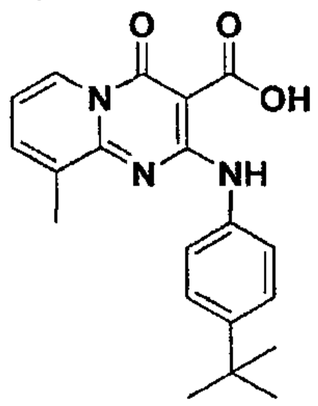
2-(3-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (157)



2-(4-Hydroxyphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (158)



2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (159)



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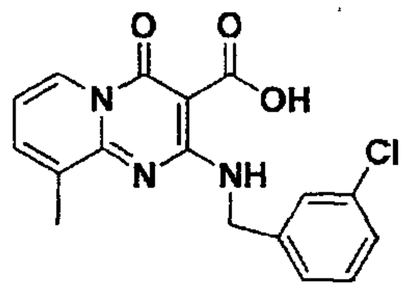
2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (160)



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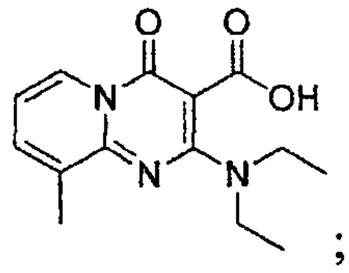
2-(Diethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (161)



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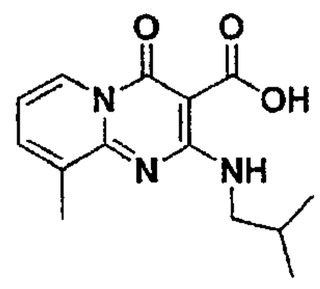
2-(Isobutylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (162)



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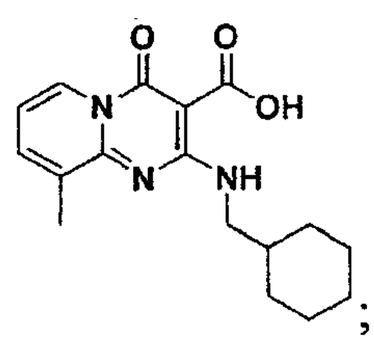
2-(Cyclohexylmethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (163)



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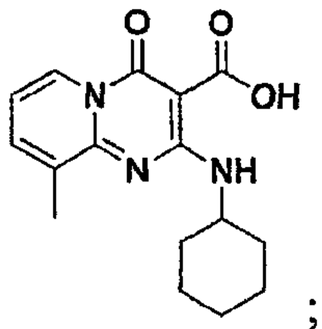
2-(Cyclohexylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (164)



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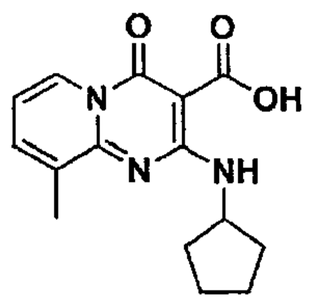
2-(Cyclopentylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (165)



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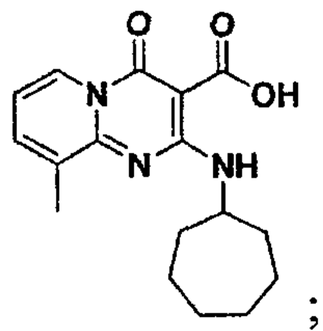
2-(Cycloheptylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (166)



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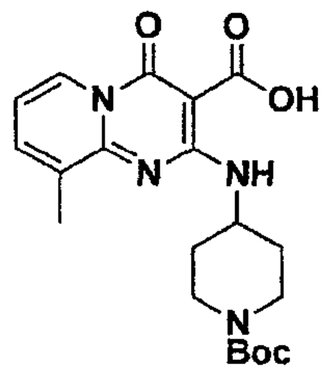
2-(1-(tert-Butoxycarbonyl)piperidin-4-ylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (167)



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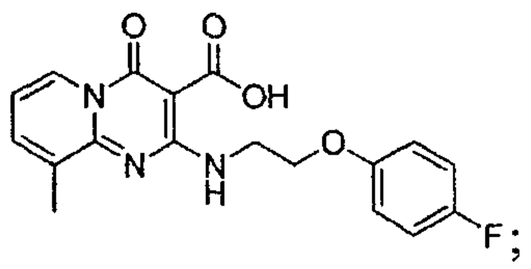
2-(2-(4-Fluorophenoxy)ethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (168)



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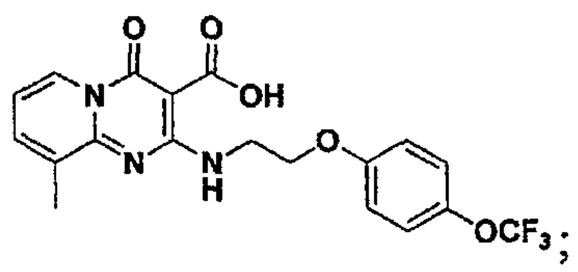
9-Methyl-4-oxo-2-(2-(4-(trifluoromethoxy)phenoxy)ethylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (169)



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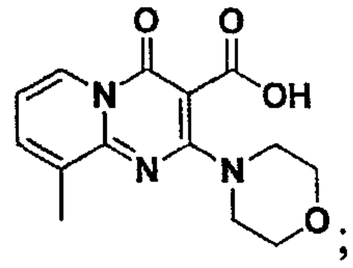
9-Methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (170)



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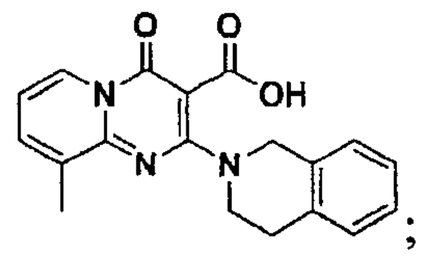
2-(3,4-Dihydroisoquinolin-2(1H)-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (171)



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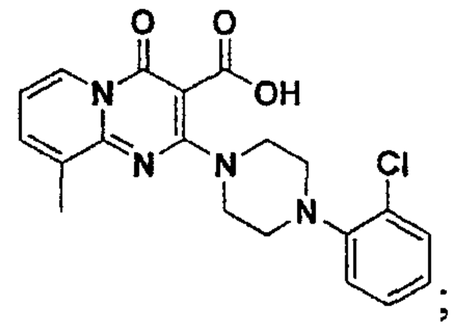
2-(4-(2-Chlorophenyl)piperazin-1-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (172)



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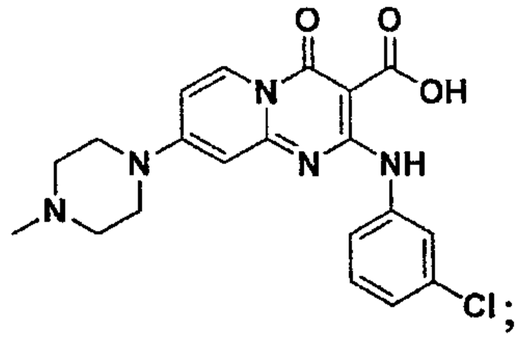
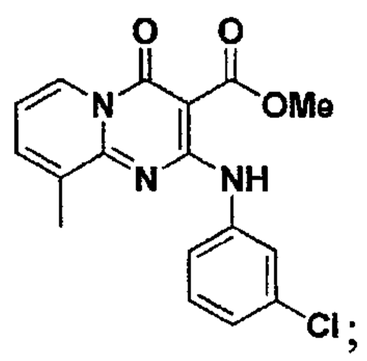
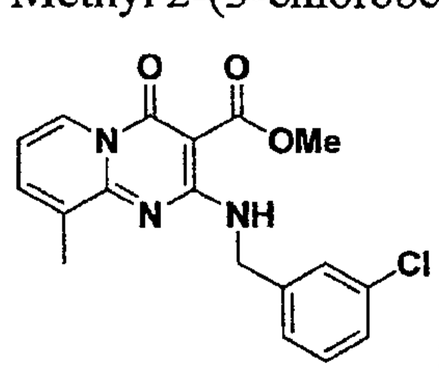
2-(3-Chlorophenylamino)-8-(4-methylpiperazin-1-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (173)



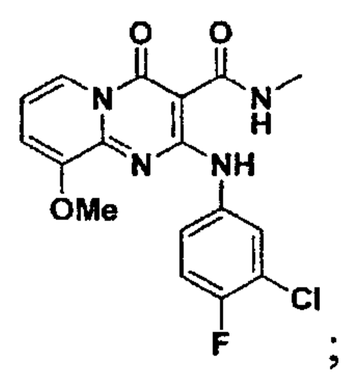
Methyl 2-(3-chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (188)



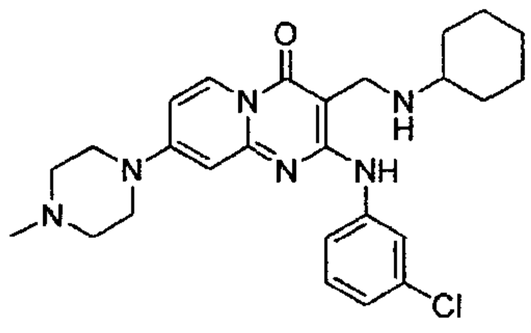
Methyl 2-(3-chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (189)



2-(3-Chloro-4-fluorophenylamino)-9-methoxy-N-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxamide (198)



2-(3-Chlorophenylamino)-3-((cyclohexylamino)methyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (205)



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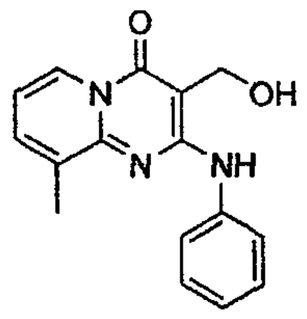
3-(Hydroxymethyl)-9-methyl-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (231)



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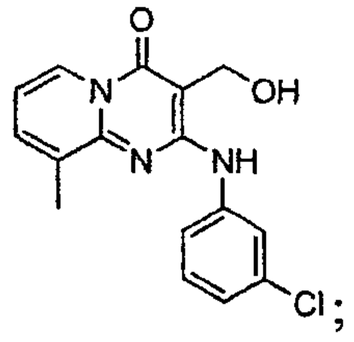
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (232)



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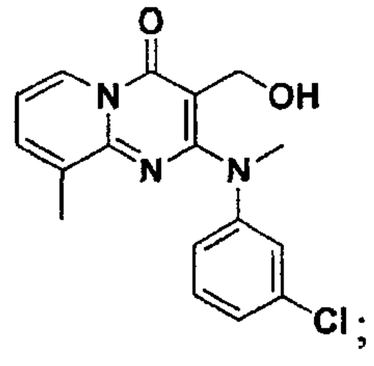
2-((3-Chlorophenyl)(methyl)amino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (233)



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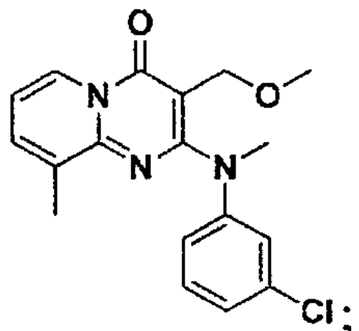
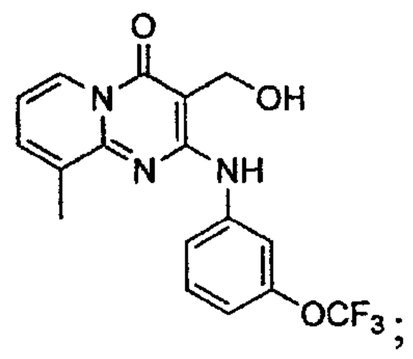
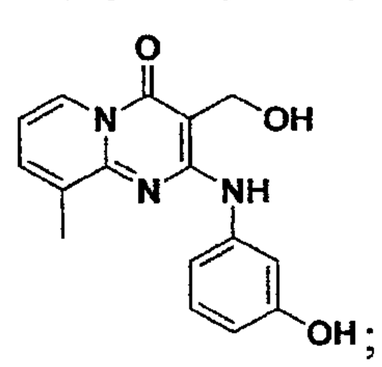
2-((3-Chlorophenyl)(methyl)amino)-3-(methoxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (234)



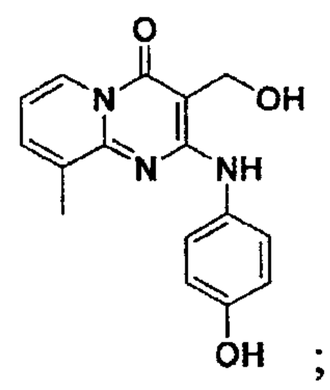
3-(Hydroxymethyl)-9-methyl-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (235)



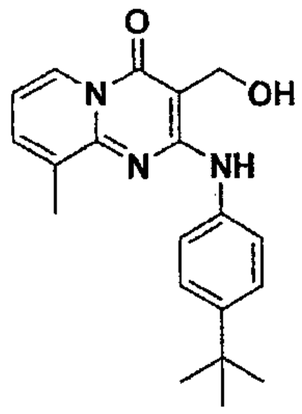
3-(Hydroxymethyl)-2-(3-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (236)



3-(Hydroxymethyl)-2-(4-hydroxyphenylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (237)



2-(4-tert-Butylphenylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (238)



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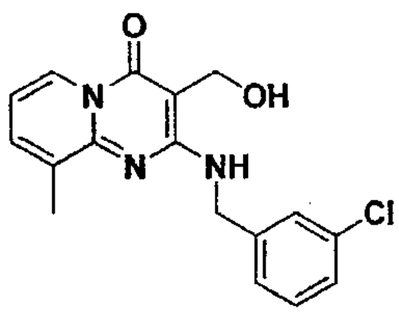
2-(3-Chlorobenzylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (239)



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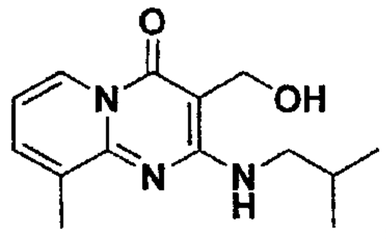
3-(Hydroxymethyl)-2-(isobutylamino)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (240)



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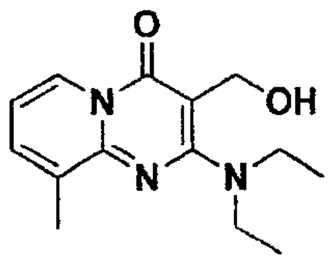
2-(Diethylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (241)



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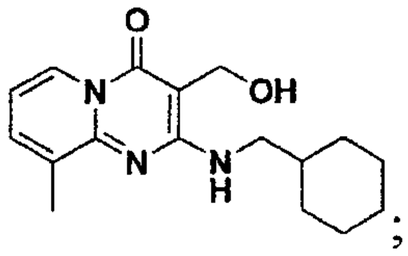
2-(Cyclohexylmethylamino)-3-(hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (242)



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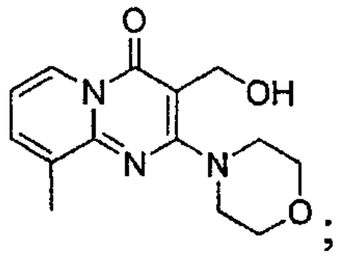
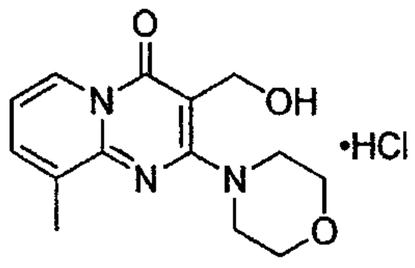
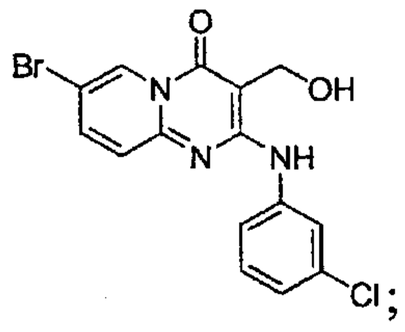
3-(Hydroxymethyl)-9-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (243)



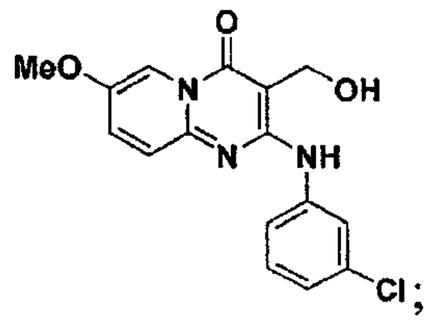
3-(Hydroxymethyl)-9-methyl-2-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one hydrochloride (244)



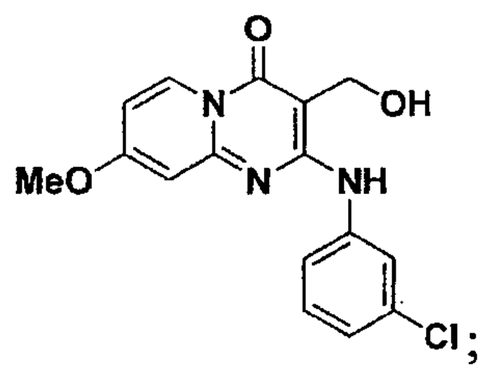
7-Bromo-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (245)



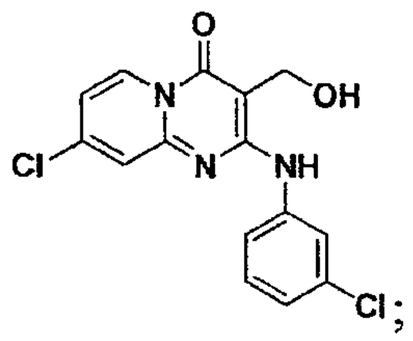
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-7-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (246)



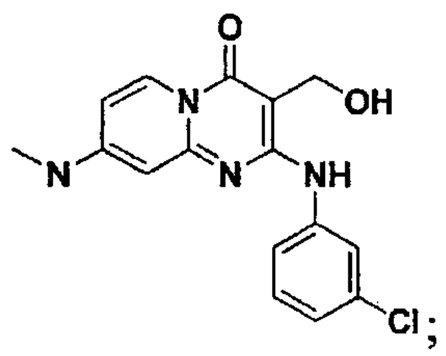
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (247)



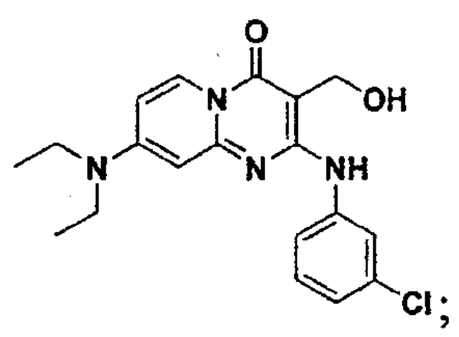
8-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (248)



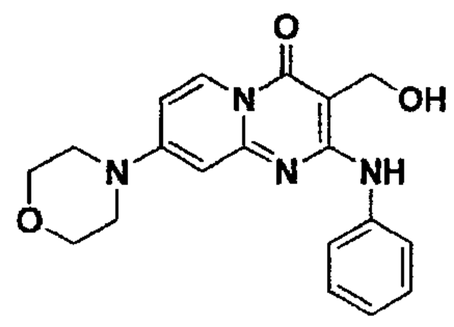
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(methylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (249)



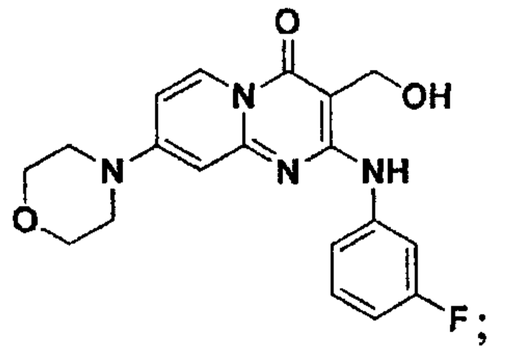
2-(3-Chlorophenylamino)-8-(diethylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (250)



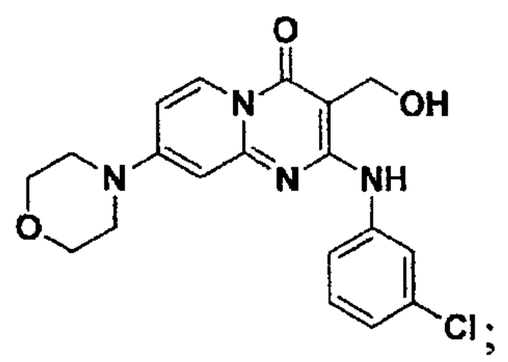
3-(Hydroxymethyl)-8-morpholino-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (251)



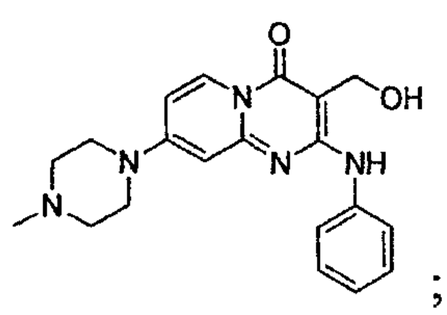
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (252)



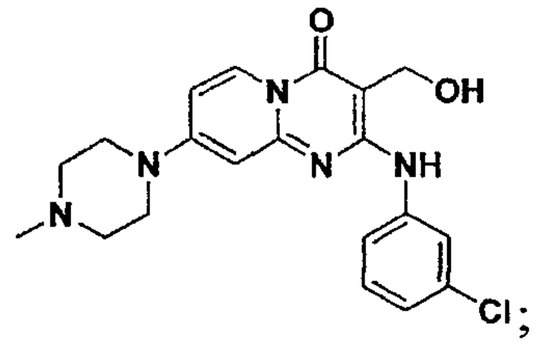
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-morpholino-4H-pyrido[1,2-a]pyrimidin-4-one (253)



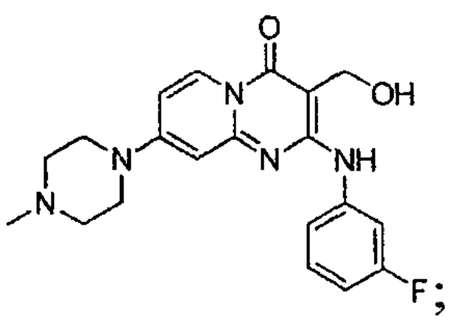
3-(Hydroxymethyl)-8-(4-methylpiperazin-1-yl)-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (254)



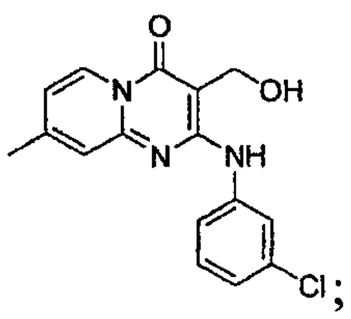
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (255)



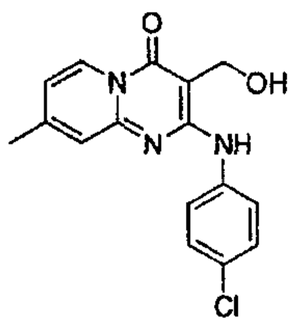
2-(3-Fluorophenylamino)-3-(hydroxymethyl)-8-(4-methylpiperazin-1-yl)-4H-pyrido[1,2-a] pyrimidin-4-one (256)



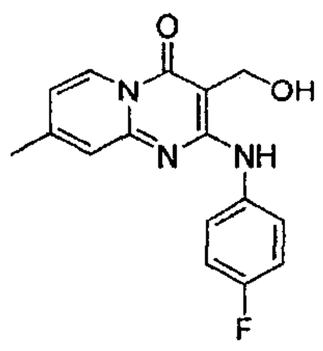
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (257)



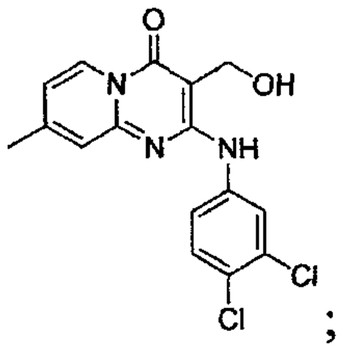
2-(4-Chlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (258)



2-(4-Fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (259)



2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (260)



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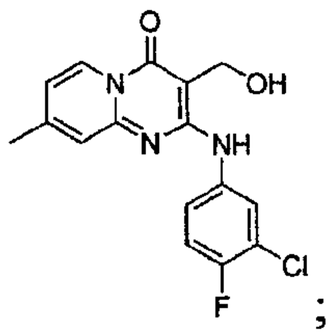
2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-8-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (261)



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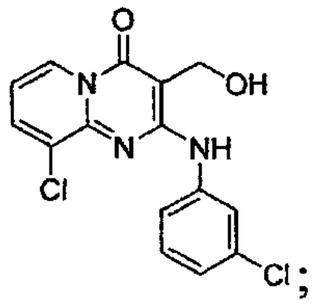
9-Chloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (262)



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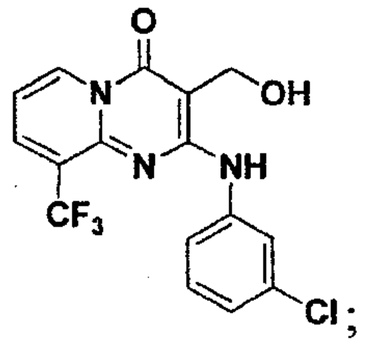
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-(trifluoromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (263)



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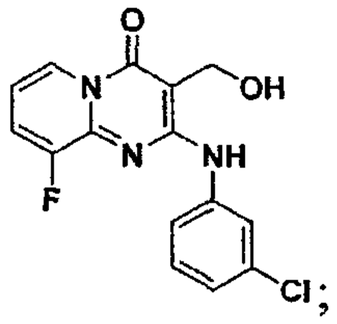
2-(3-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (264)



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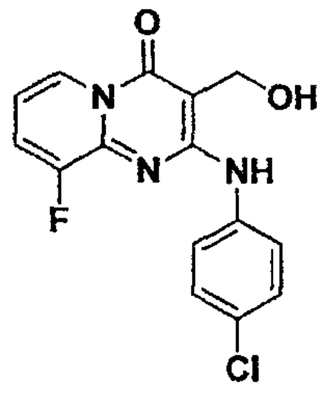
2-(4-Chlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (265)



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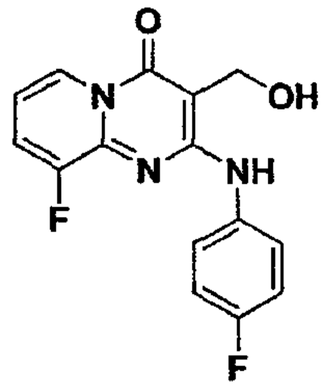
9-Fluoro-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (266)



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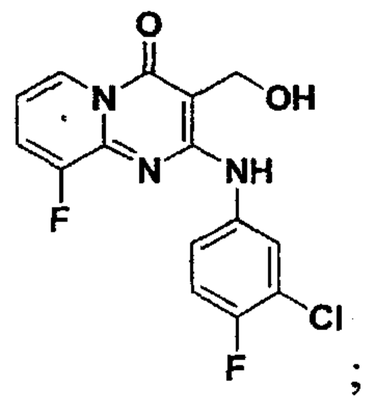
2-(3-Chloro-4-fluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (267)



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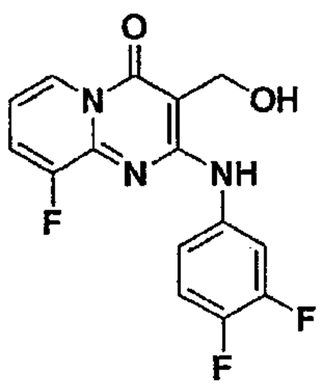
2-(3,4-Difluorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (268)



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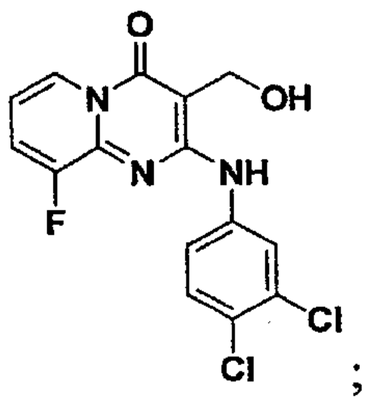
2-(3,4-Dichlorophenylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (269)



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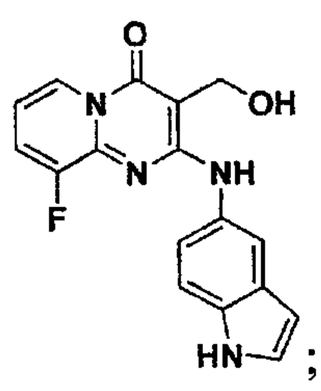
2-(1H-Indol-5-ylamino)-9-fluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (270)



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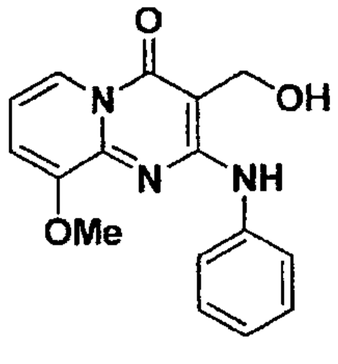
3-(Hydroxymethyl)-9-methoxy-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (271)



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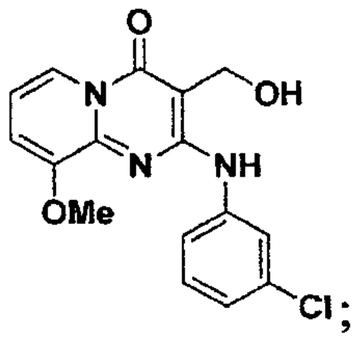
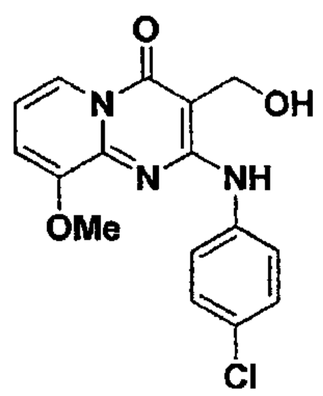
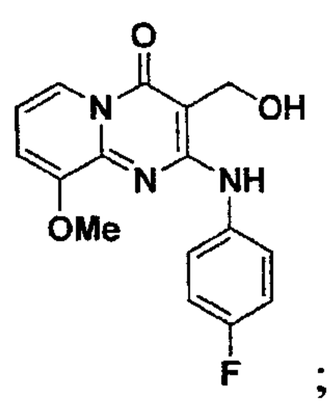
2-(3-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (273)



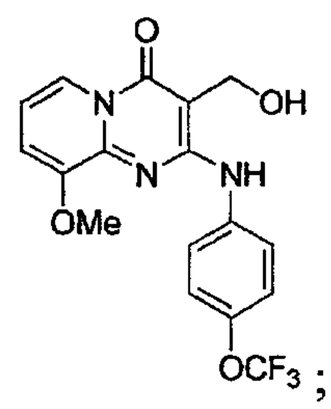
2-(4-Chlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (274)



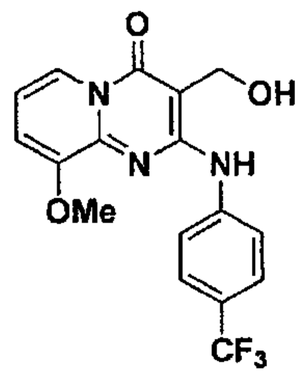
2-(4-Fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (275)



3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (276)



3-(Hydroxymethyl)-9-methoxy-2-(4-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (277)



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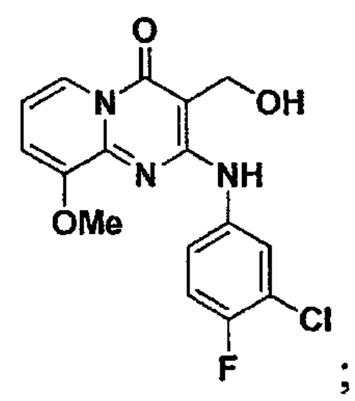
2-(3-Chloro-4-fluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (278)



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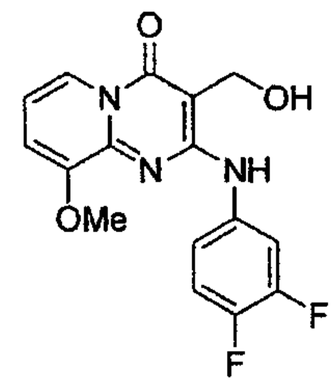
2-(3,4-Difluorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (279)



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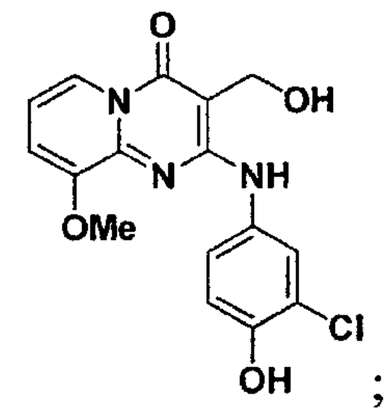
2-(3-Chloro-4-hydroxyphenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (280)



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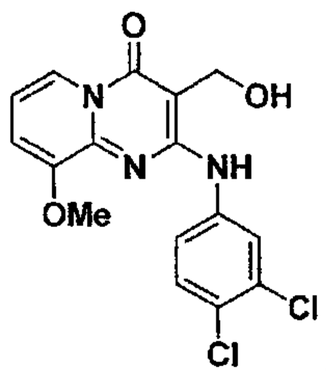
2-(3,4-Dichlorophenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (281)



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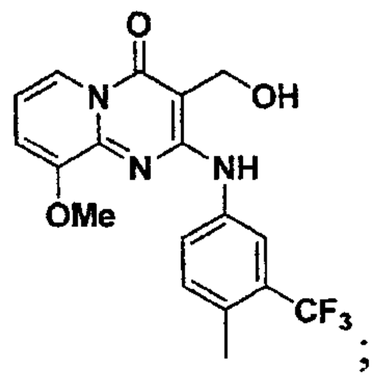
3-(Hydroxymethyl)-9-methoxy-2-(4-methyl-3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (282)



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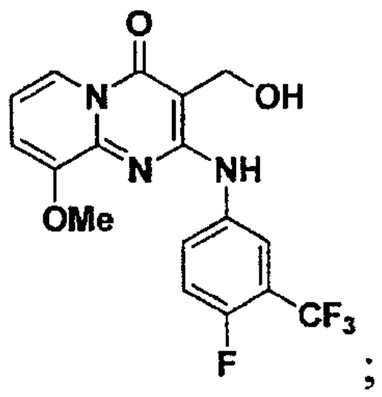
2-(4-Fluoro-3-(trifluoromethyl)phenylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (283)



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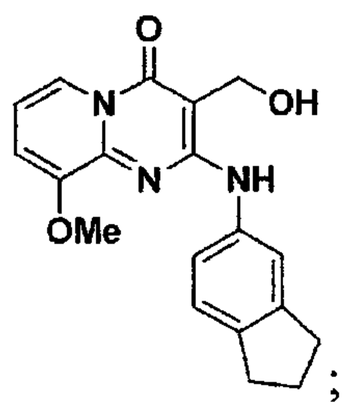
2-(2,3-Dihydro-1H-inden-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (284)



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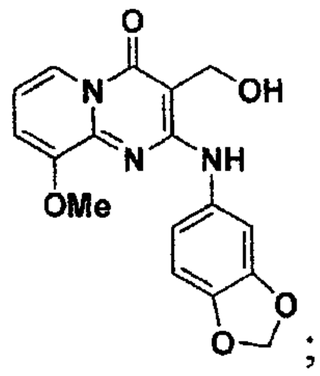
2-(Benzo[d][1,3]dioxol-5-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (285)



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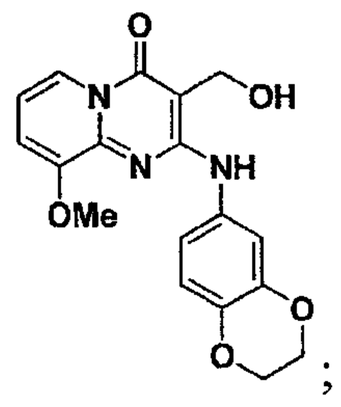
2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-ylamino)-3-(hydroxymethyl)-9-methoxy-4H-pyrido[1,2-a]pyrimidin-4-one (286)



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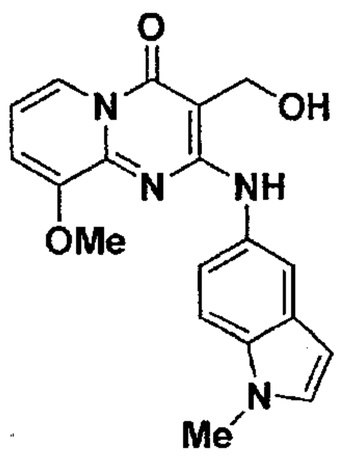
3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (287)



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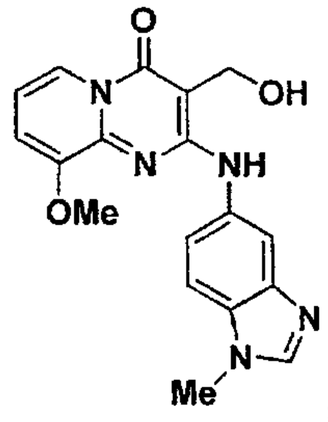
3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-benzo[d]imidazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (288)



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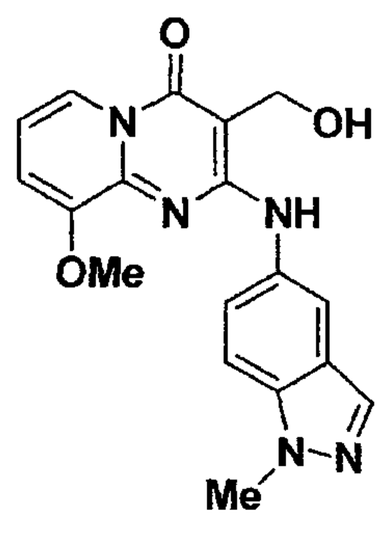
3-(Hydroxymethyl)-9-methoxy-2-(1-methyl-1H-indazol-5-ylamino)-4H-pyrido[1,2-a]pyrimidin-4-one (289)



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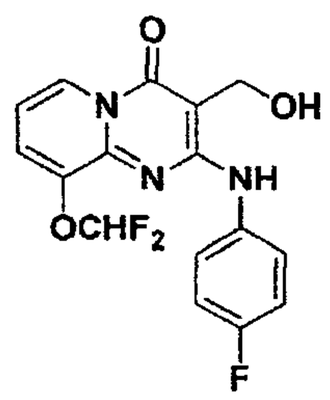
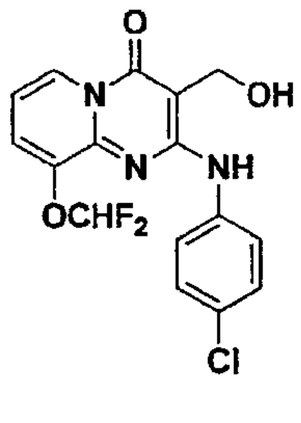
9-(Difluoromethoxy)-2-(4-fluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (290)



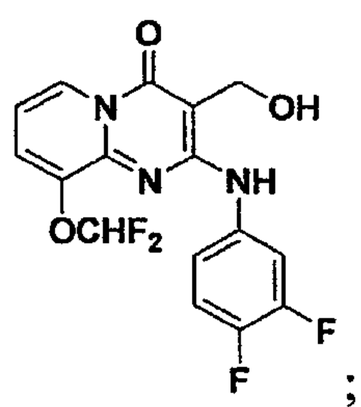
;

2-(4-Chlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (291)



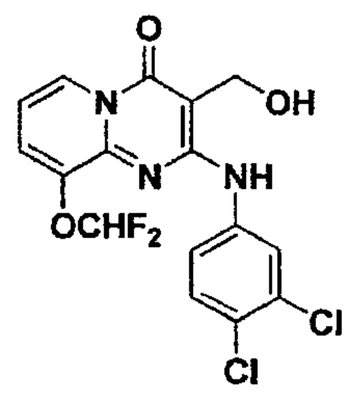
;

9-(Difluoromethoxy)-2-(3,4-difluorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (292)



;

2-(3,4-Dichlorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (293)

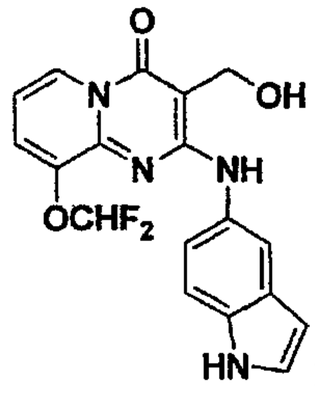


;

2-(3-Chloro-4-fluorophenylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (294)

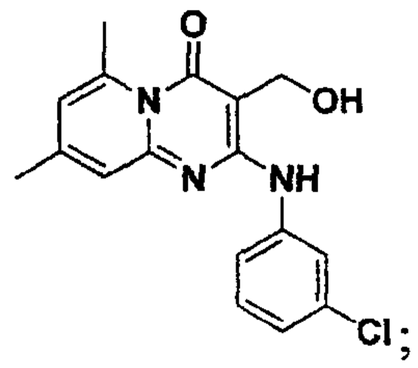
;

2-(1H-Indol-5-ylamino)-9-(difluoromethoxy)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (295)



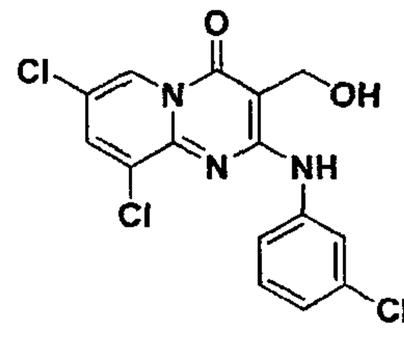
;

2-(3-chlorophenylamino)-3-(hydroxymethyl)-6,8-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one (296)



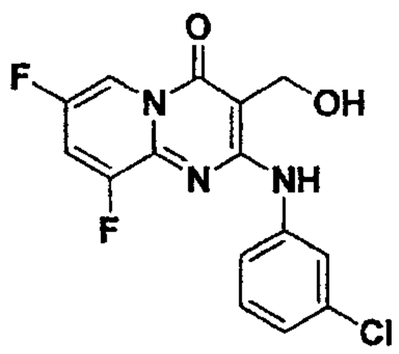
;

7,9-Dichloro-2-(3-chlorophenylamino)-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (297)



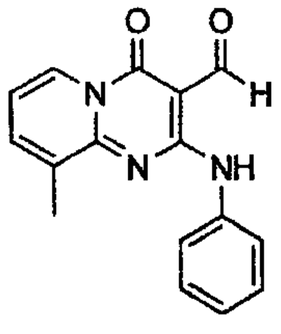
; or

2-(3-Chlorophenylamino)-7,9-difluoro-3-(hydroxymethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (298)



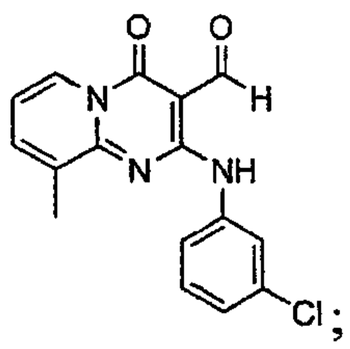
9. A compound having a formula of 132-135, 137, 139-140, 147, 151-152, 160, 163, or 173:

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (132)



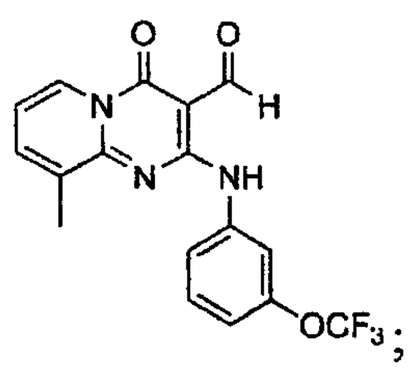
;

2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (133)



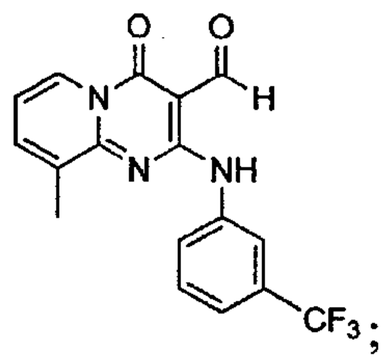
;

9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (134)

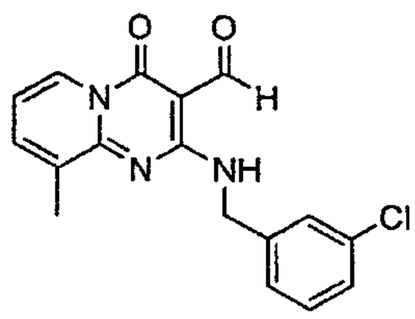


;

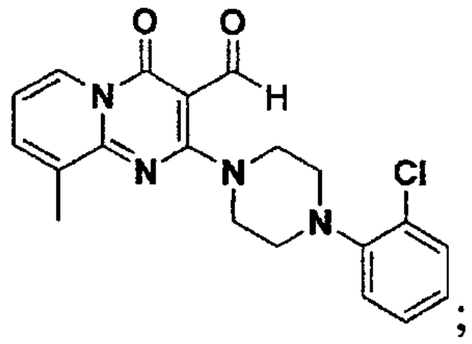
9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (135)



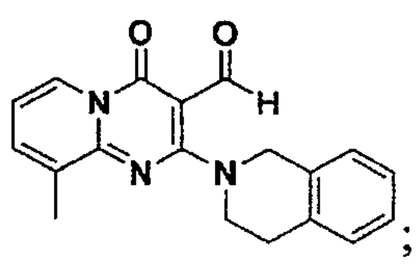
2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (137)



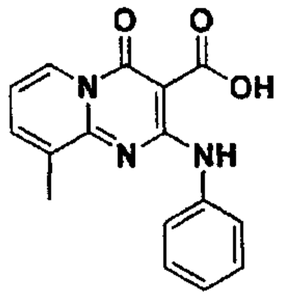
2-(4-(2-Chlorophenyl)piperazin-1-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (139)



2-(3,4-Dihydroisoquinolin-2(1H)-yl)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (140)

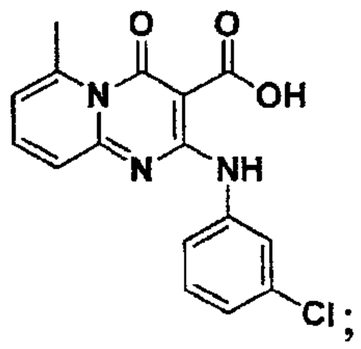


9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (147)

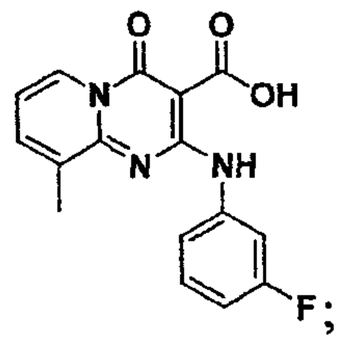


;

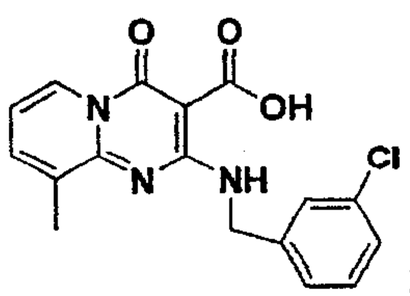
2-(3-Chlorophenylamino)-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (151)



2-(3-Fluorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (152)

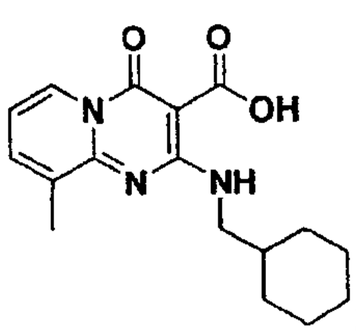


2-(3-Chlorobenzylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (160)



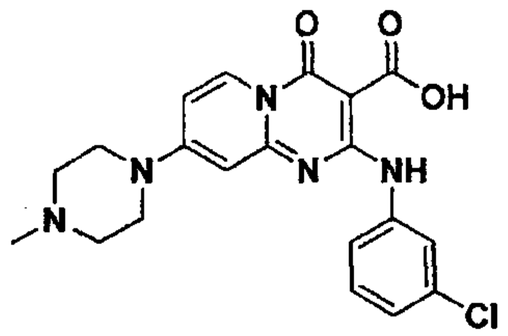
;

2-(Cyclohexylmethylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (163)



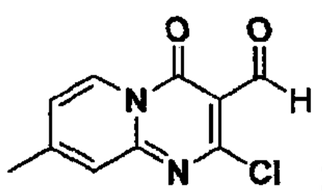
; or

2-(3-Chlorophenylamino)-8-(4-methylpiperazin-1-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (173)



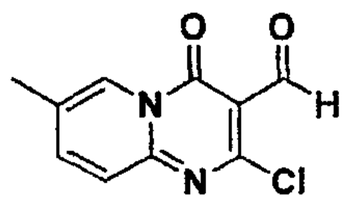
10. A compound having a formula of 129-131, 144-146, 174-177, or 187:

2-Chloro-8-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (129)



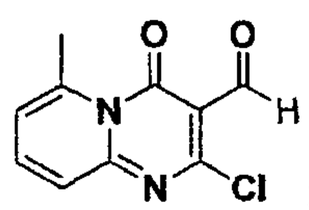
;

2-Chloro-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (130)



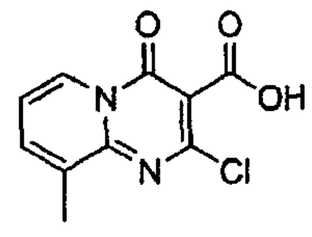
;

2-Chloro-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (131)



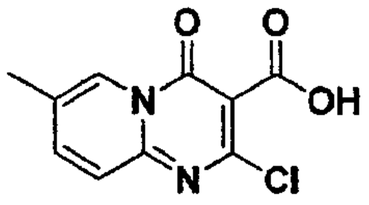
;

2-Chloro-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (144)



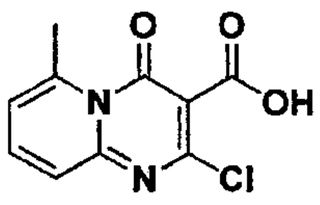
;

2-Chloro-7-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (145)



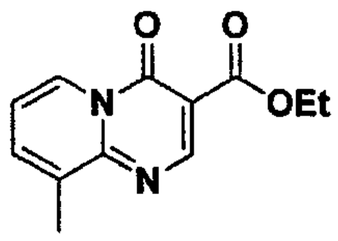
;

2-Chloro-6-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (146)



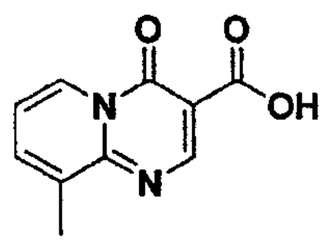
;

Ethyl 9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (174)



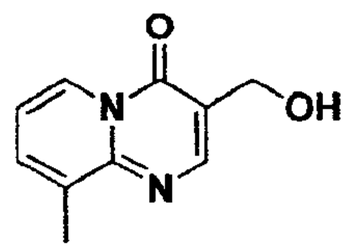
;

9-Methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylic acid (175)



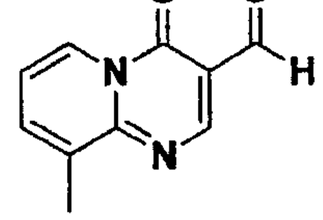
;

3-(Hydroxymethyl)-9-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (176)



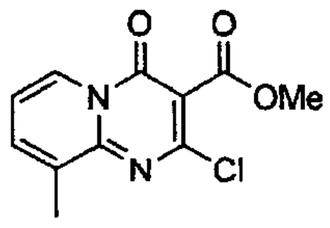
;

9-Methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (177)

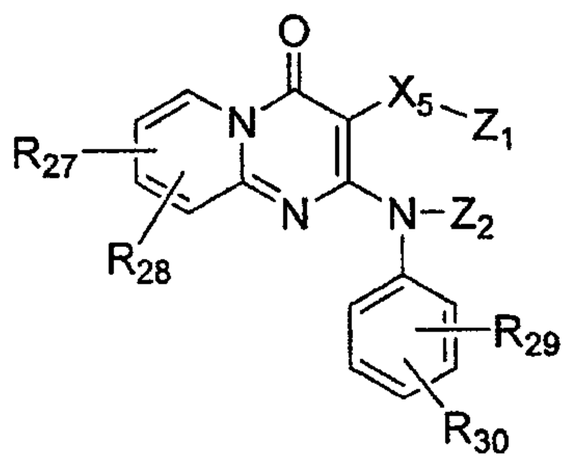


; or

Methyl 2-chloro-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (187)



11. A compound having the general formula VIIIa:



VIIIa

wherein

X_5 is C=O;

Z_1 and Z_2 are each independently alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, or hydrogen, or Z_1 and Z_2 are connected with each other to make five or six membered cyclic, heterocyclic, aryl, or heteroaryl rings, any of which is optionally substituted;

R_{27} is alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thiol, sulfonate, sulfonyl or sulfonylamino, any of which is optionally substituted;

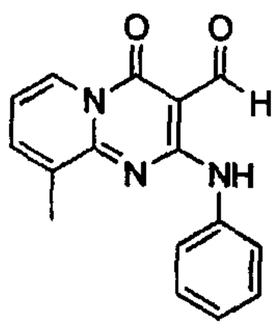
R_{28} is alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, nitro, thiol, sulfonate, sulfonyl or sulfonylamino, any of which is optionally substituted; and

R_{29} and R_{30} are each independently alkoxy, alkyl, alkylamino, alkenyl, alkylthio, alkynyl, amino, aryl, arylalkoxy, arylamino, arylthio, carboxy, cyano, cycloalkyl, halo, haloalkoxy, haloalkyl, heteroaryl, heteroarylamino, heterocycloalkyl, hydroxyl, hydrogen, nitro, thiol, sulfonate, sulfonyl or sulfonylamino, or

R_{29} and R_{30} are connected with each other to make five or six membered cyclic, heterocyclic, aryl, or heteroaryl rings, any of which is optionally substituted.

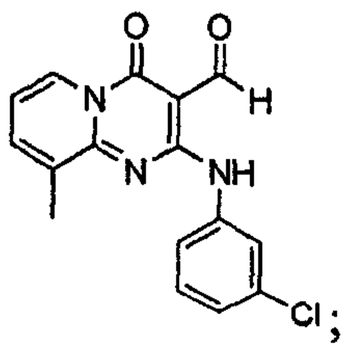
12. A compound having a formula of 132-135 or 136:

9-Methyl-4-oxo-2-(phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (132)

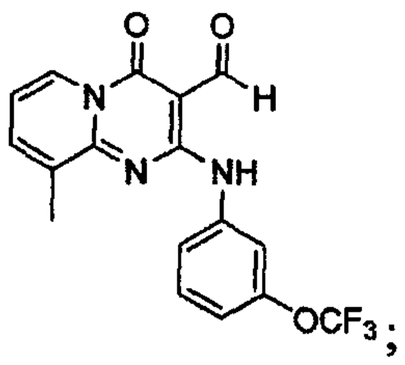


;

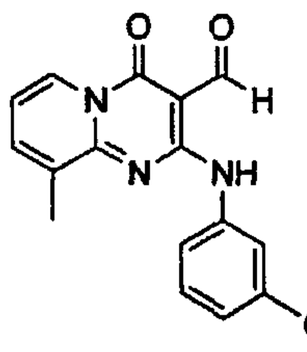
2-(3-Chlorophenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (133)



9-Methyl-4-oxo-2-(3-(trifluoromethoxy)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (134)

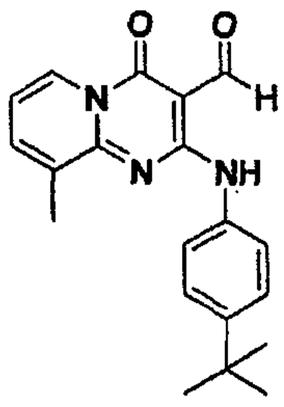


9-Methyl-4-oxo-2-(3-(trifluoromethyl)phenylamino)-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (135)



; or

2-(4-tert-Butylphenylamino)-9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbaldehyde (136)



.

13. A pharmaceutical composition comprising a compound of claim 11 and a carrier.

14. A pharmaceutical composition comprising a compound of claim 8 and a carrier.

15. A pharmaceutical composition comprising a compound of claim 9 and a carrier.

16. A pharmaceutical composition comprising a compound of claim 10 and a carrier.

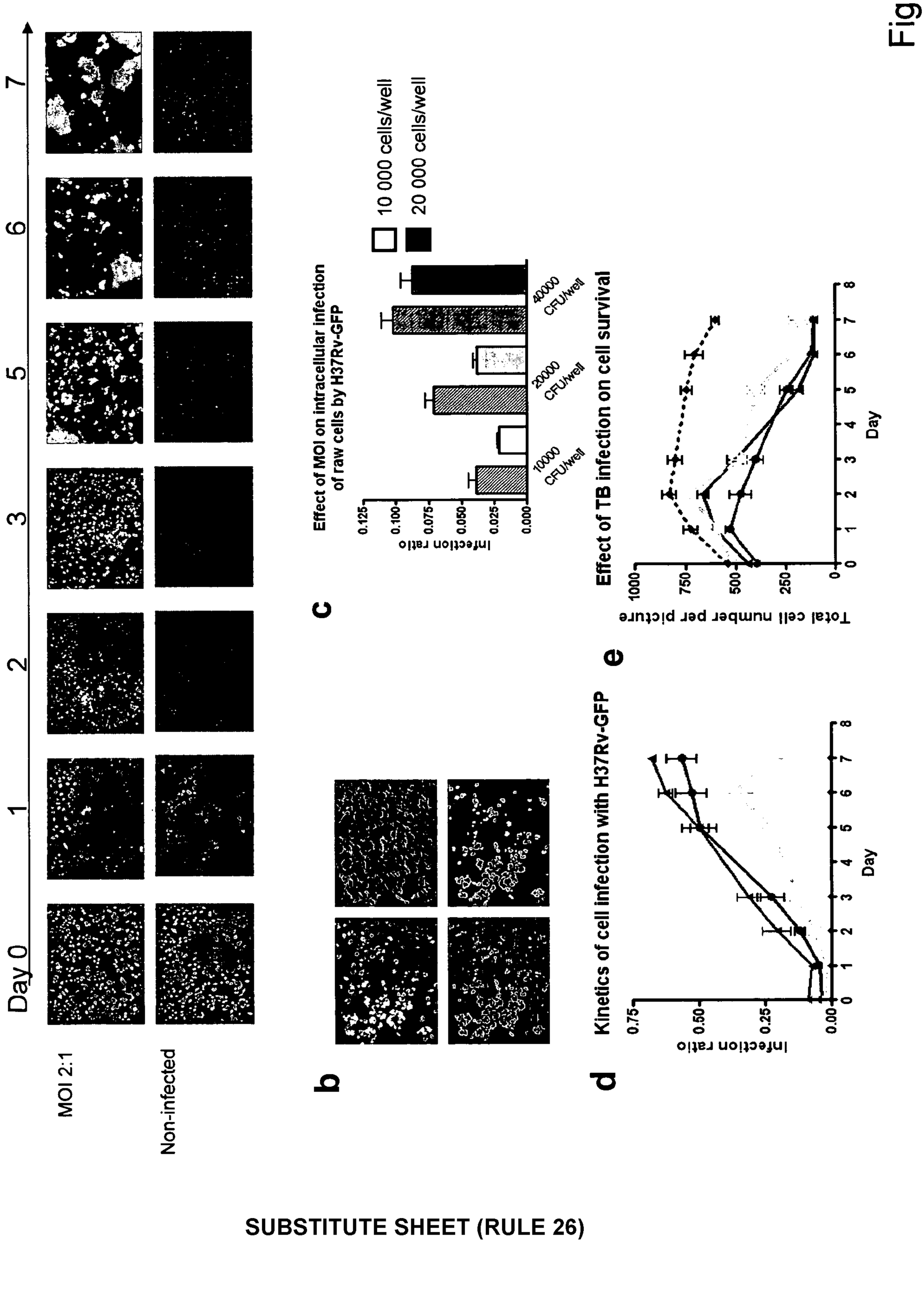
17. Use of a compound of claim 1, 2, 5 or 7 to 12 for treating a bacterial infection, wherein the bacterial infection is tuberculosis.

18. Use of a compound of any one of claims 1, 2, 5 or 7 to 12, for the manufacture of a medicament for treating a bacterial infection, wherein the bacterial infection is tuberculosis.

19. A pharmaceutical composition comprising a compound of claim 5 and a carrier.

20. A pharmaceutical composition comprising a compound of claim 12 and a carrier.

Figure 1


Day 0
1
2
3
4
5
6
7
MOI 2:1
Non-infected
a
b
c
d
e
Effect of MOI on intracellular infection of raw cells by H37Rv-GFP
Infection ratio
0.125
0.100
0.075
0.050
0.025
0.000
10 000 cells/well
20 000 cells/well
10000 CFU/well
20000 CFU/well
40000 CFU/well
Kinetics of cell infection with H37Rv-GFP
Effect of TB infection on cell survival
Total cell number per picture
1000
750
500
250
0
Day
0
1
2
3
4
5
6
7
8

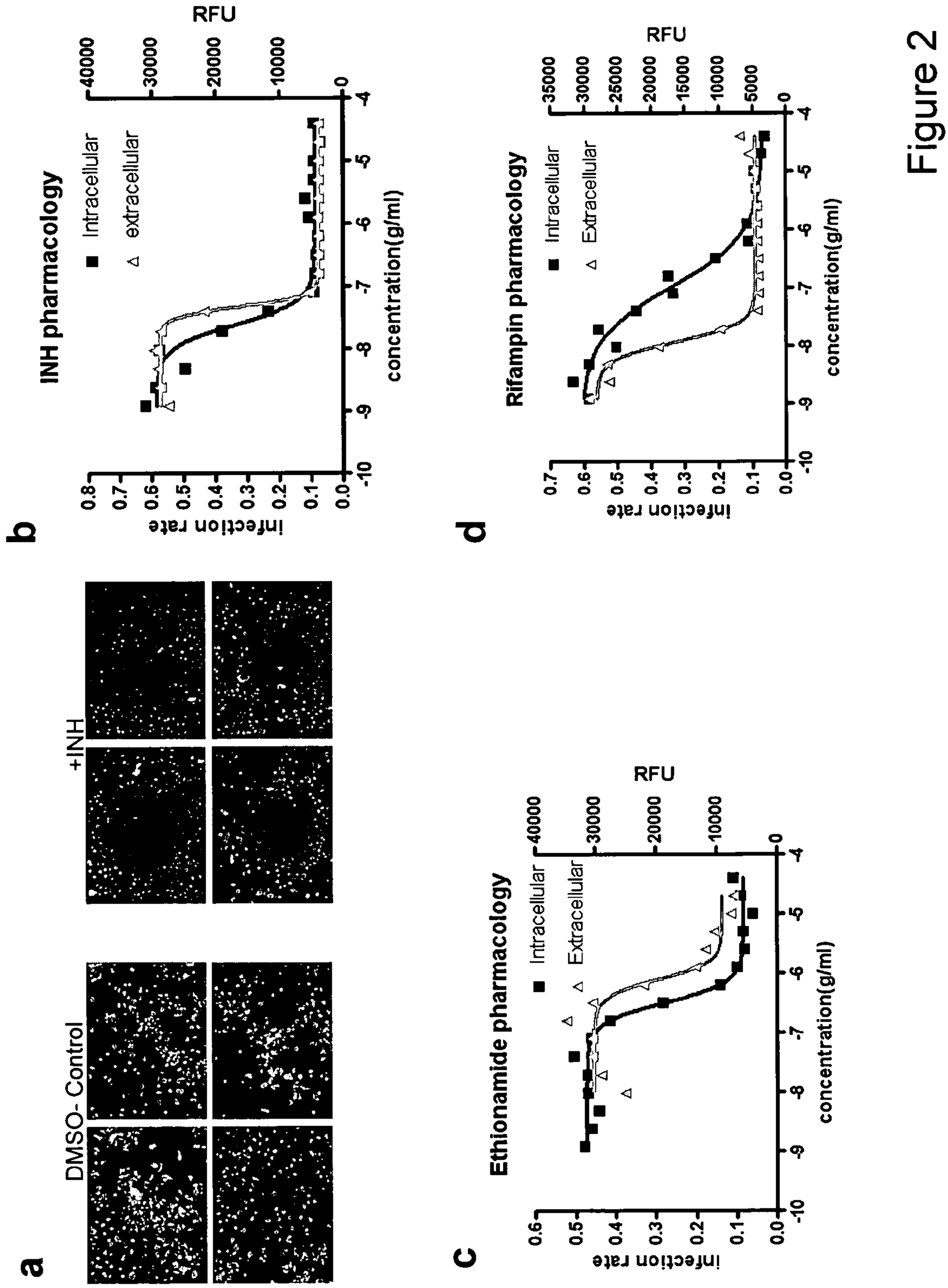

a
DMSO- Control
+INH
b
INH pharmacology
Intracellular
extracellular
infection rate
concentration(g/ml)
RFU
c
Ethionamide pharmacology
Intracellular
Extracellular
infection rate
concentration(g/ml)
RFU
d
Rifampin pharmacology
Intracellular
Extracellular
infection rate
concentration(g/ml)
RFU

Figure 2

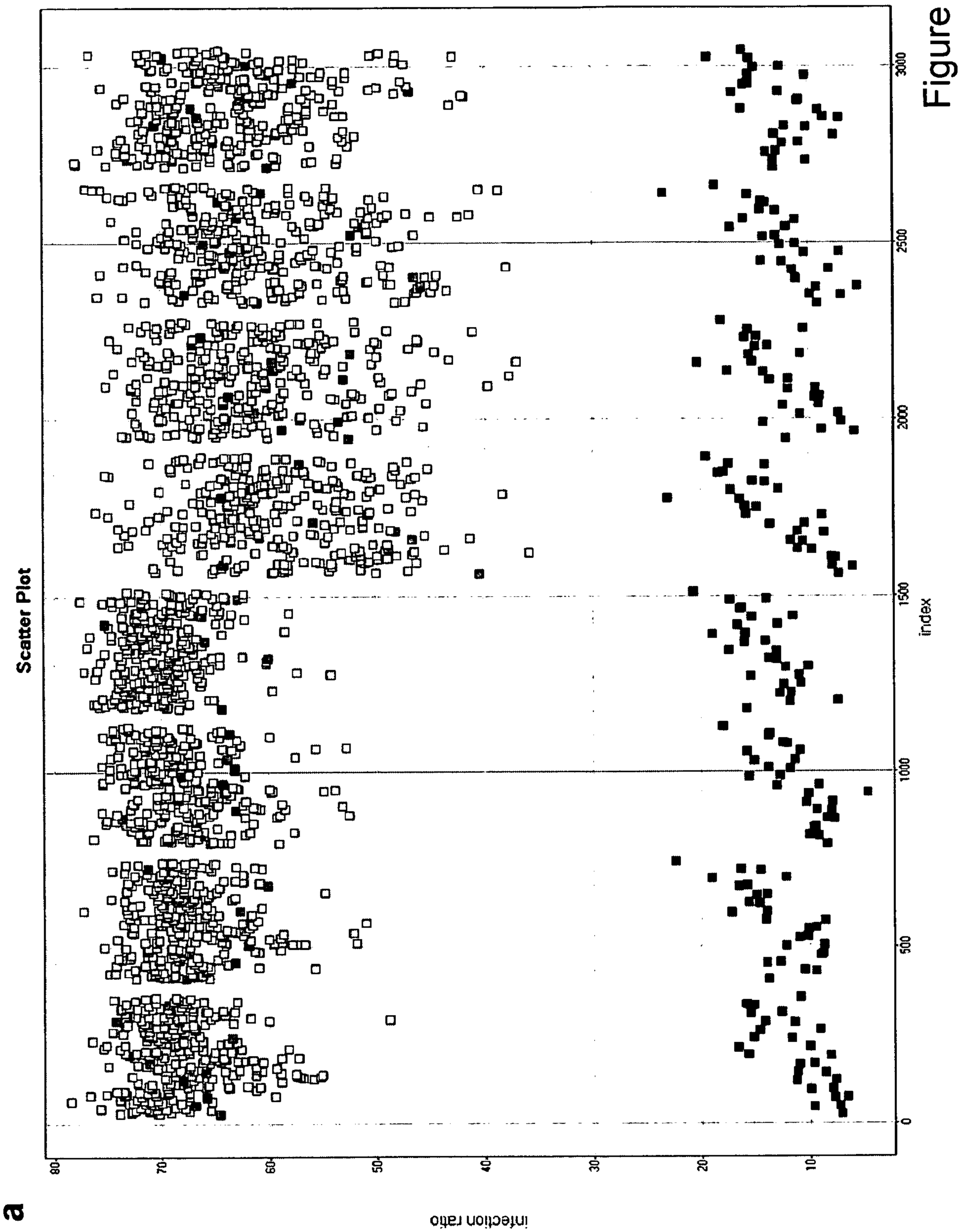
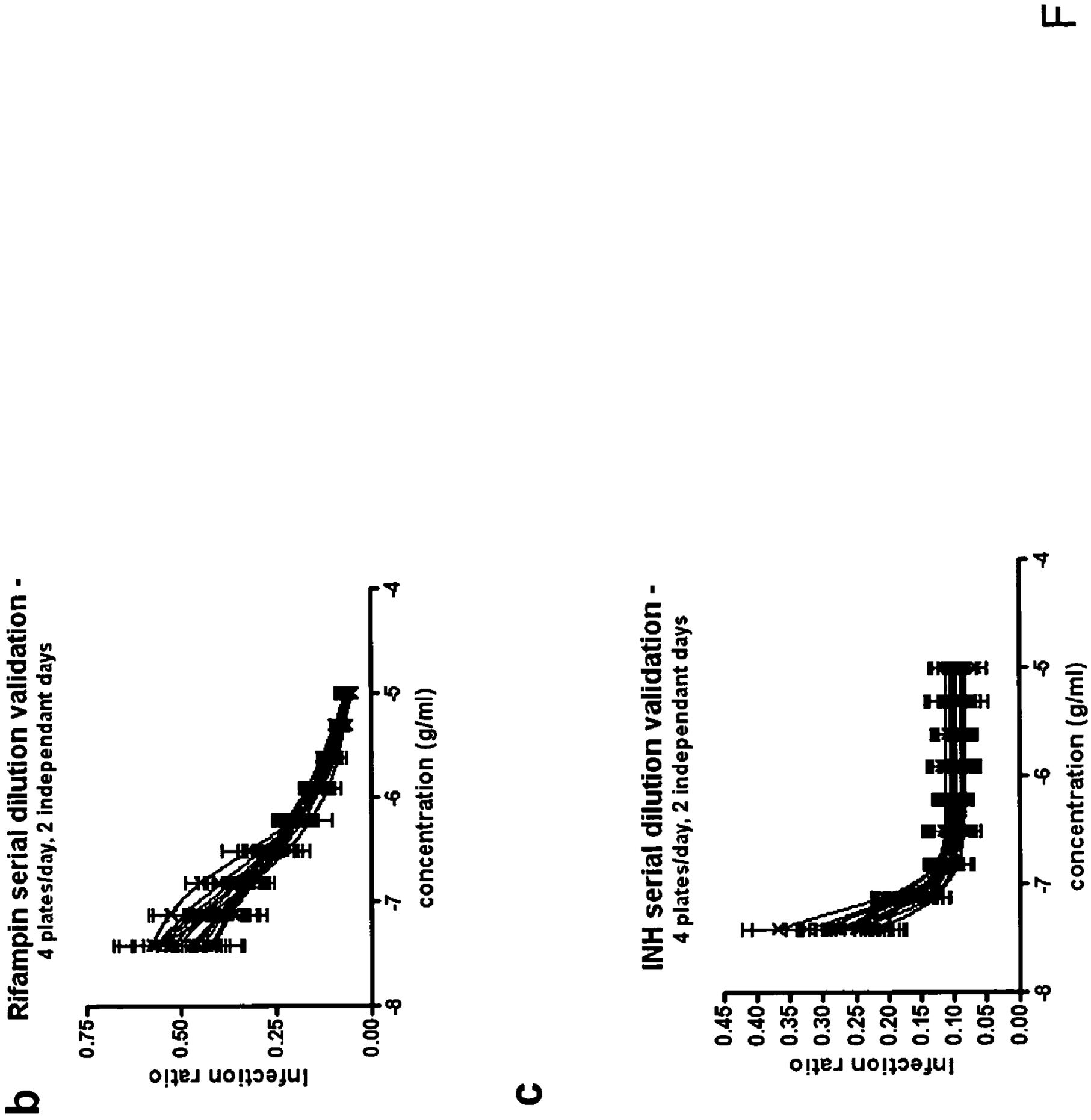

Scatter Plot
a
index
3000
2500
2000
1500
1000
500
0
80
70
60
50
40
30
20
10
interaction ratio

Figure 3

Figure 3


b
Rifampin serial dilution validation -
4 plates/day, 2 independant days
Infection ratio
0.75
0.50
0.25
0.00
-8
-7
-6
-5
-4
concentration (g/ml)
c
INH serial dilution validation -
4 plates/day, 2 independant days
Infection ratio
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-8
-7
-6
-5
-4
concentration (g/ml)

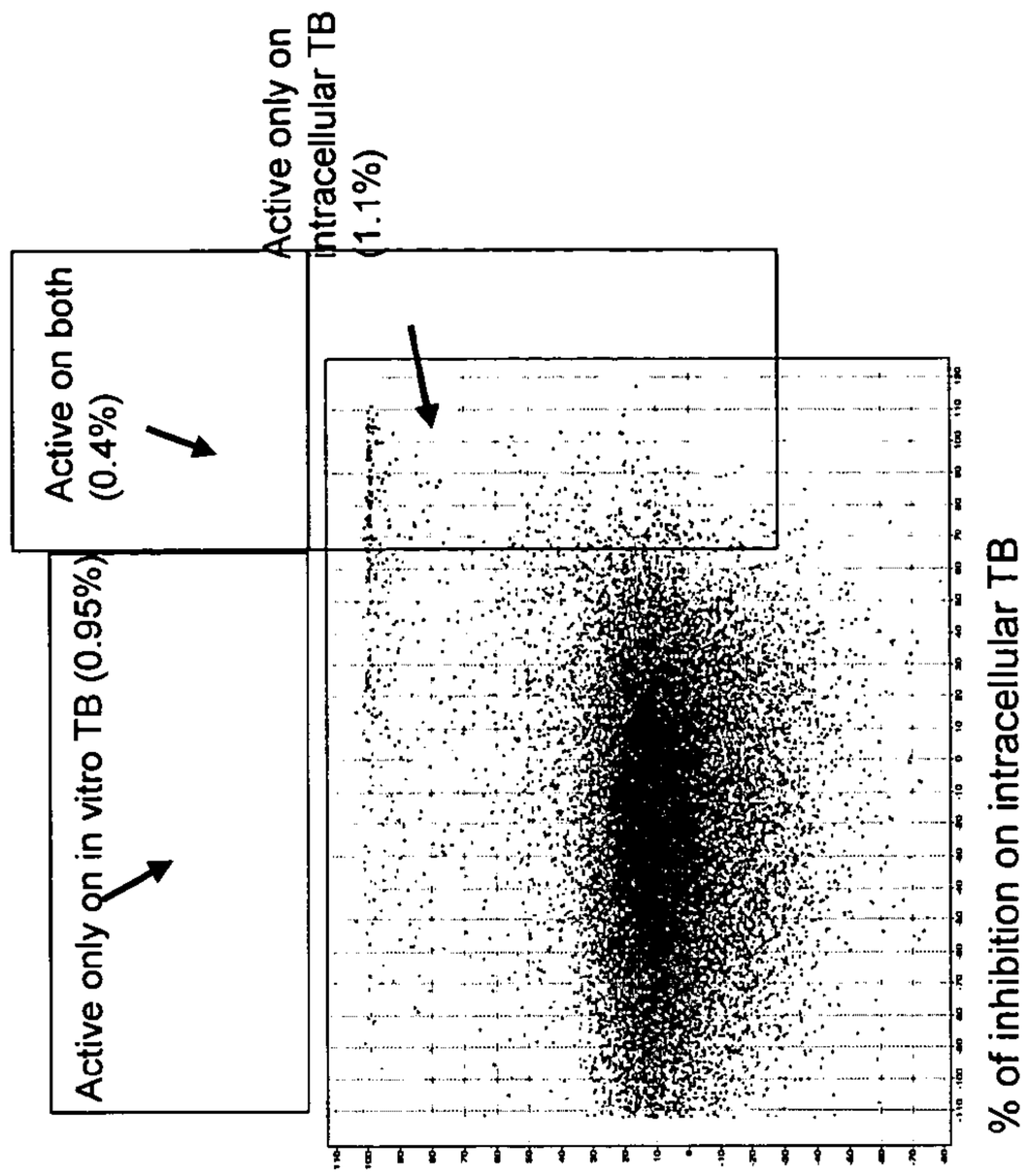
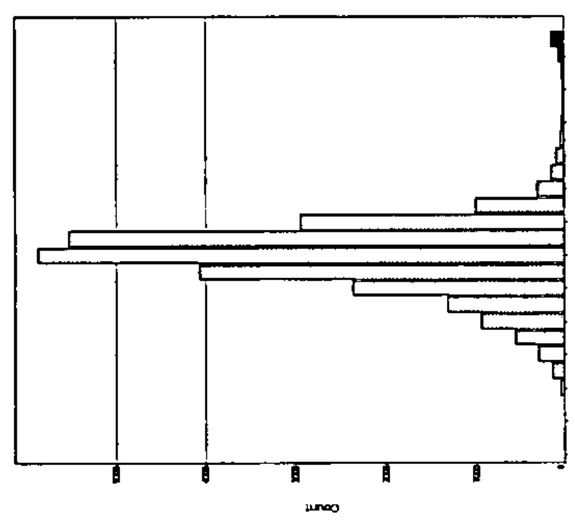
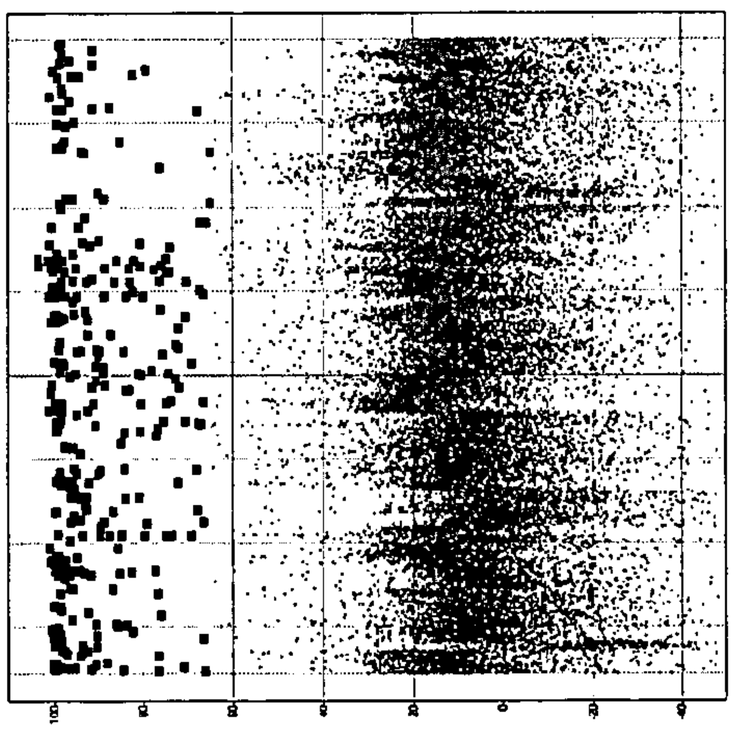
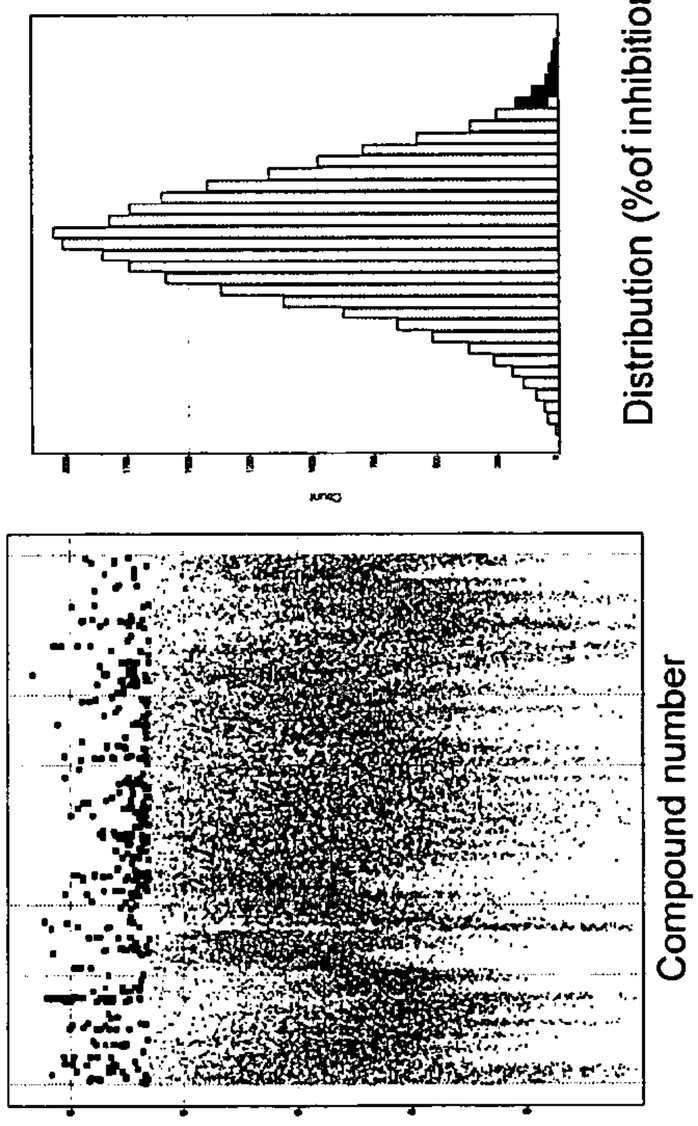

Active only on
Intracellular TB
(1.1%)
Active on both
(0.4%)
Active only on in vitro TB (0.95%)
% of inhibition on intracellular TB
% of inhibition on in
vitro TB
c

Figure 4


Count
Distribution (%
of inhibition)


Compound number
% of inhibition (RFU based)
b


Count
Distribution (%of inhibition)
Compound number
%inhibition of Infection ratio
a

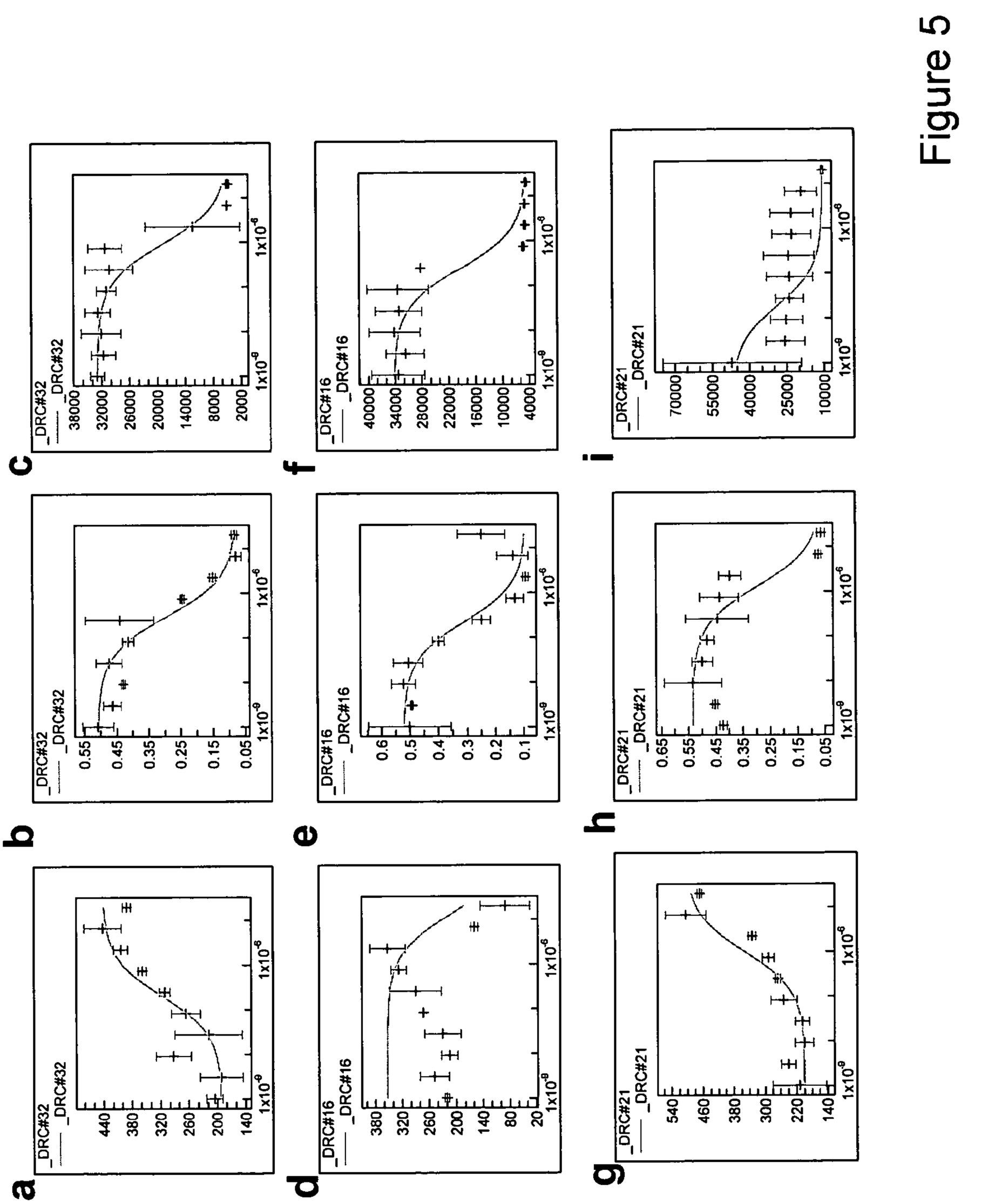


Figure 5

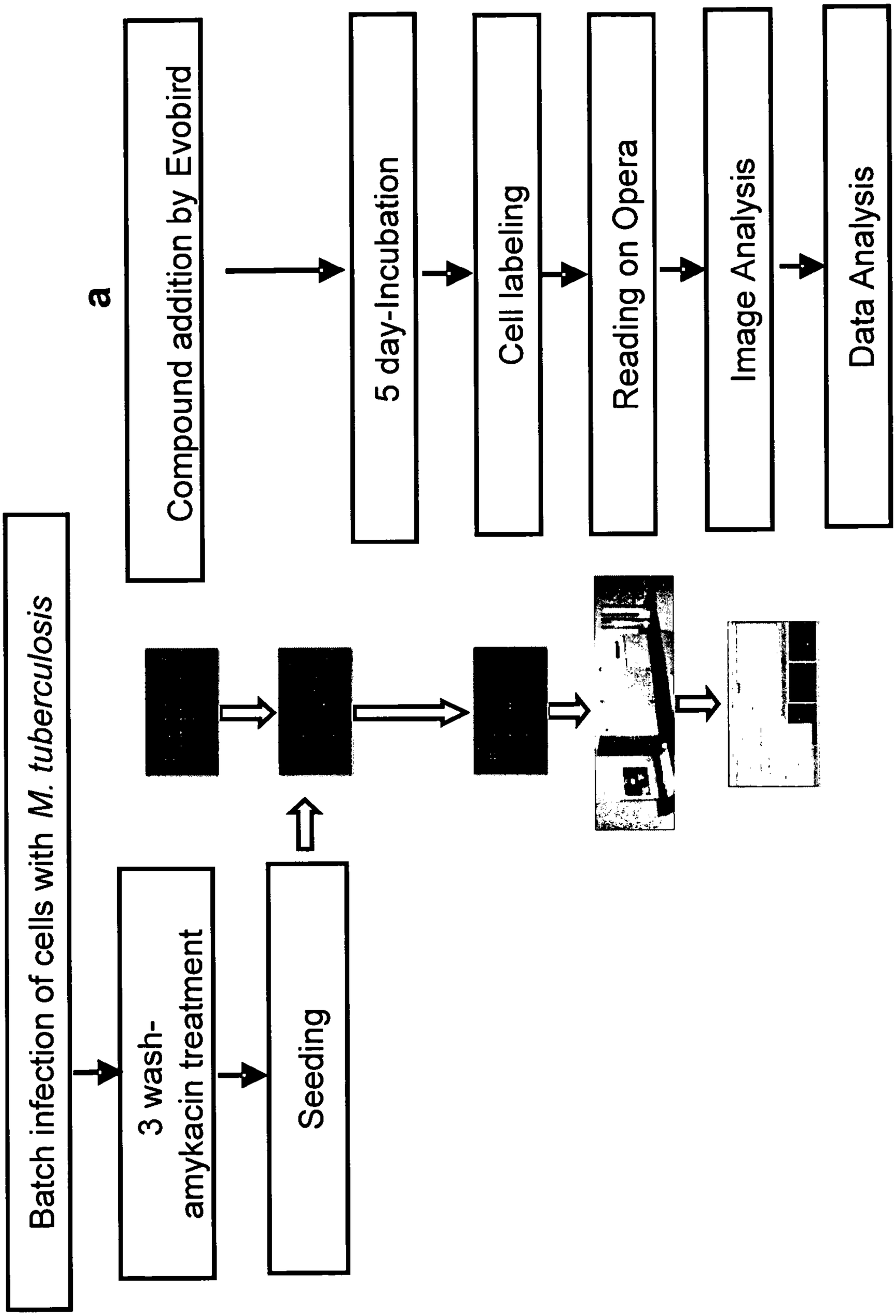

a
Compound addition by Evobird
5 day-Incubation
Cell labeling
Reading on Opera
Image Analysis
Data Analysis
Batch infection of cells with M. tuberculosis
3 wash-amykacin treatment
Seeding

Figure 6

b

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
B	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
C	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
D	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
E	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
F	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
G	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
H	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
I	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
J	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
K	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
L	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
M	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
N	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
O	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]
P	RIF	DMSO	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	Cpd	INH100	[illegible]

Figure 6

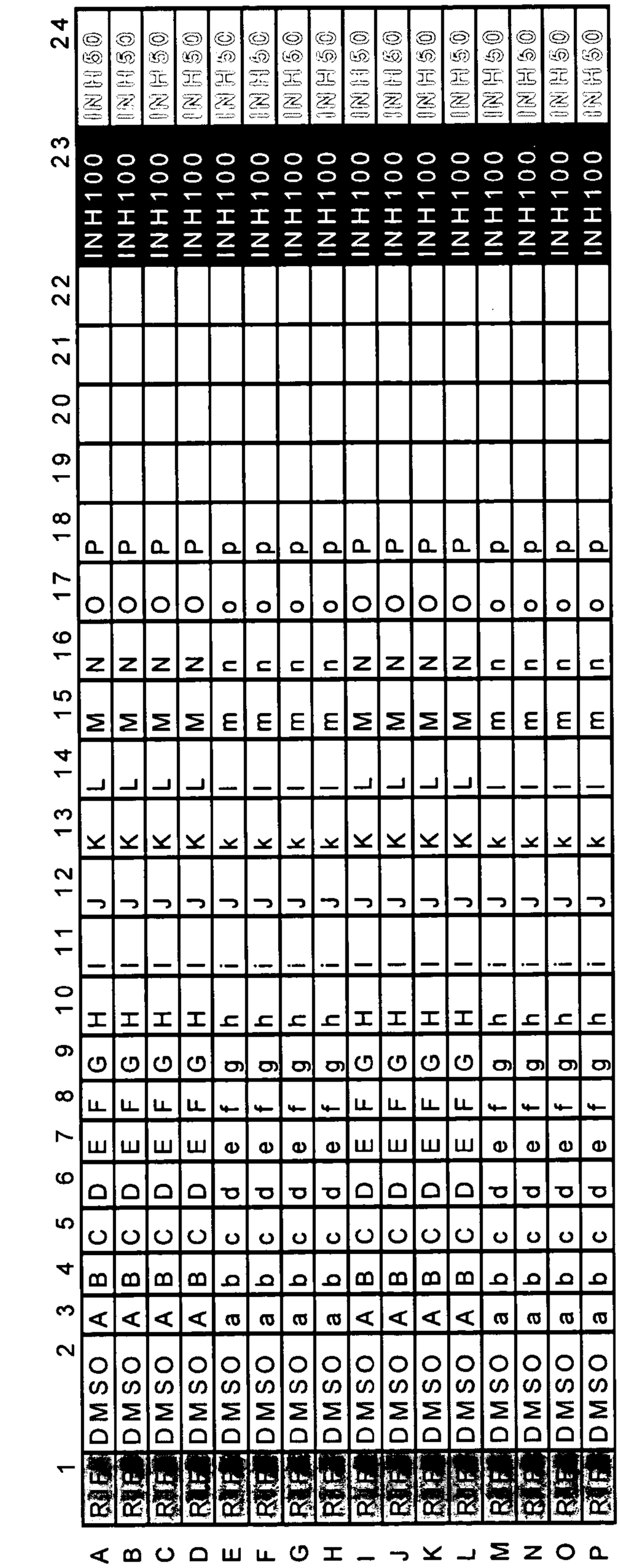

C
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A B C D E F G H I J K L M N O P
RIF DMSO A B C D E F G H I J K L M N O P INH100 INH50

Figure 6

Figure 7

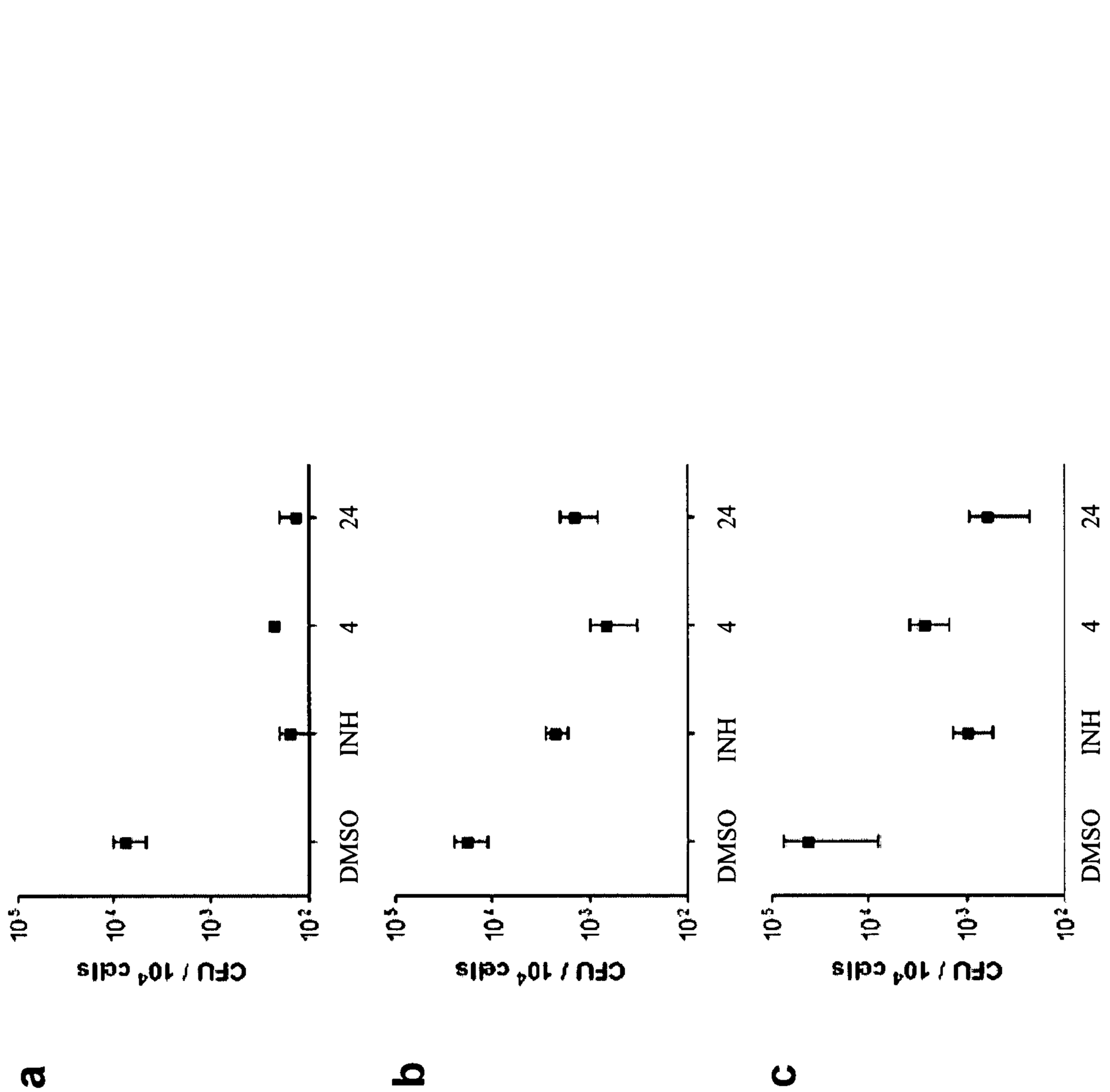
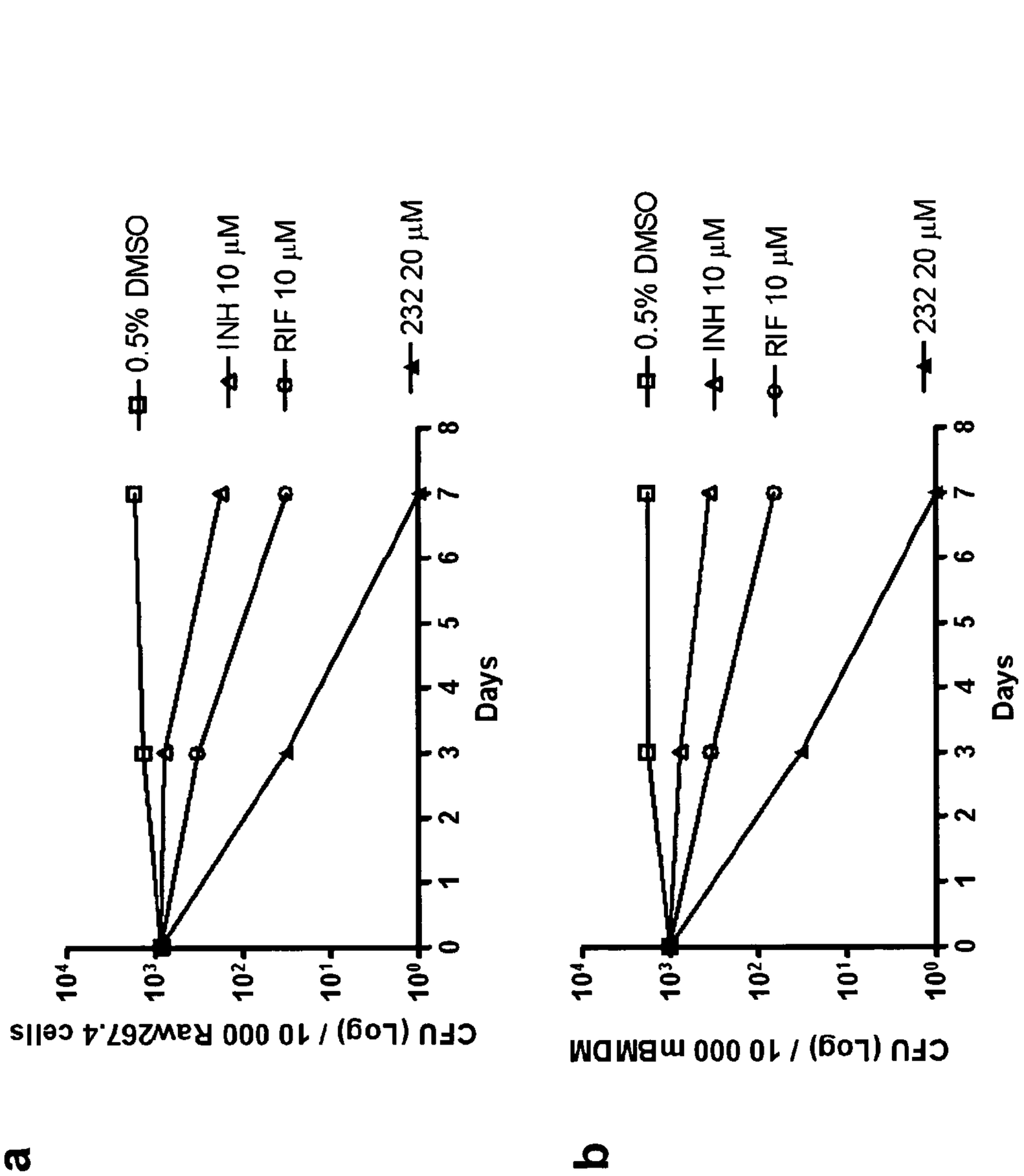


Figure 8


a
b
CFU (Log) / 10 000 Raw267.4 cells
CFU (Log) / 10 000 mBMDM
0.5% DMSO
INH 10 µM
RIF 10 µM
232 20 µM
Days
0
1
2
3
4
5
6
7
8
10^0
10^1
10^2
10^3
10^4