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(54) **Title:** AMINE DERIVATIVE CONTAINING COMPOSITIONS AND METHODS FOR THE TREATMENT OF VIRAL INFECTIONS RELATED TO THE ETIOLOGY OF CANCER

(57) **Abstract:** Amine derivative containing compositions and methods of use thereof for the treatment or inhibition of viral infections are disclosed.

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AMINE DERIVATIVE CONTAINING COMPOSITIONS AND METHODS FOR THE TREATMENT OF VIRAL INFECTIONS RELATED TO THE ETIOLOGY OF CANCER

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FIELD OF THE INVENTION

The present invention relates to the fields of virology and cellular signaling. More specifically, the invention provides compositions and methods useful for the treatment of viral infections, particularly those caused by herpes viruses.

BACKGROUND OF THE INVENTION

Several publications and patent documents are cited throughout the specification in order to describe the state of the art to which this invention pertains. Each of these citations is incorporated herein by reference as though set forth in full.

HHV-8 is a recently identified virus that has been associated with Kaposi's sarcoma (KS) and possibly with a type of cancer called body cavity lymphoma (a tumor that arises from the lymph tissue) (1). KSHV is an important pathogen capable of causing disease that affects all age groups worldwide (2). Kaposi's sarcoma is an unusual skin tumor that is seen primarily in HIV-infected men. HHV-8 has also been isolated in the semen of HIV infected individuals. Because of these factors, it is believed that HHV-8 may cause a sexually transmitted infection.

From the beginning of the AIDS epidemic, it was suspected that there might be another infectious agent besides HIV that causes KS. In the 1980's, around 30-40% of homosexual men with AIDS developed KS at some point in their illness. In contrast, KS was a rare occurrence in women or hemophiliacs with HIV. This suggested that there was an additional factor among gay and bisexual men that increased their chances of developing KS. In 1994, researchers identified a previously unknown virus in KS biopsies (3). This virus was named human herpesvirus 8 (also known as Kaposi's sarcoma-associated herpesvirus-KSHV). It belongs to the important family of human

herpesviruses that includes varicella-zoster (chickenpox/shingles), Epstein-Barr virus (mononucleosis), and herpes simplex 1 and 2 (oral and genital herpes). After identification of HHV-8, researchers have been able to detect this virus in virtually all types of Kaposi's sarcoma tumors, including those seen before the AIDS epidemic.

Analysis of the complete HHV-8 genome sequence reveals similarities to other gammaherpesviruses, including herpesvirus saimiri (HVS), murine gammaherpesvirus 68 (MHV68) and Epstein-Barr Virus (HHV-4). The ~165 kb genome contains over 80 open reading frames arranged in a long unique region flanked by multiple 801bp terminal repeat units of high G+C content. The long unique region contains blocks of conserved genes found in most herpesviruses, interspersed with blocks of non-homologous genes that are specific for HHV-8 and related viruses.

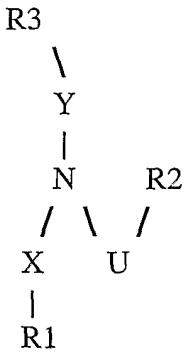
The pathogenic mechanism by which KSHV induces tumorigenicity is presently unknown. However, KSHV encodes a G-protein coupled receptor (GPCR) that acts as an oncogene, the expression of which causes malignant transformation of rodent fibroblast cells, and produces tumors in nude mice. Transgenic mice expressing the KSHV-GPCR develop highly vascular endothelial tumors (4-6). Such tumorigenicity relates to the ability of the KSHV-GPCR to constitutively activate the extracellular signal-regulated kinase (ERK) signal-transduction cascade (7),(8). One of the main activators of the ERK cascade is the Ras-Raf-MEK1/2-ERK signaling axis (9). Further, in some cases, GPCRs are known to signal through Ras (10-12). Considering that a number of viruses utilize this signaling axis to establish infection, agents which disrupt this pathway should prove efficacious against viruses which include, for example, Epstein Bar Virus and Hepatitis B virus.

Clearly a need exists for compositions and methods useful for treating viral infections, including KSHV. The present application provides such compositions and methods.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method for the treatment of viral infection in a patient in need thereof is provided. An exemplary method entails

administration to a patient of a therapeutically effective amount of a compound having the formula



(I)

wherein U is $(CH_2)_n$, CO, SO_2 or CONH;

n is 0, 1, 2, 3, 4 or 5;

X is CH_2, CO, SO_2 or CONH;

Y is CH_2, CO, SO_2 or CONH;

R1 is an optionally substituted aryl, aralkyl, heteroaryl or heteroarylalkyl;

R2 is an optionally substituted heteroalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkyl, heterocycloalkyl or heteroalkylcycloalkyl and

R3 is an optionally substituted alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, heteroarylalkyl or aralkyl;or a pharmacologically acceptable salt, solvate, hydrate or formulation thereof.

A preferred embodiment of the invention comprises administration of the compound disclosed above in combination with a MAPK pathway inhibitor and/or an antiproliferative agent. Suitable inhibitors and agents are provided in Table I hereinbelow. The methods of the invention should prove efficacious in the treatment of viral infection caused by HHV-8, HHV-4 and hepatitis B virus.

In yet another embodiment of the invention, a pharmaceutical composition for treating or inhibiting viral infection is provided. An exemplary composition comprises the above-mentioned compound and at least one agent or inhibitor selected from the group provided in Table I in a pharmaceutically acceptable carrier.

Another embodiment of the invention comprises a method for prophylaxis of viral infection in a host susceptible to said infection. One such method comprises administration of a therapeutically effective amount of the above mentioned compound and optionally at least one MAPK pathway inhibitor and/or an antiproliferative agent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Inhibition of anchorage-dependent growth of NIH3T3 cells transformed with KSHV-GPCR oncogene by MCP compounds. IC50 values in a WST-1 proliferation assay were established in KSHV-GPCR transformed NIH3T3 cells treated with MCP1, MCP110 or control compounds BAY43-9006 and U0126.

Figure 2. Inhibition of anchorage-independent growth of NIH3T3 cells transformed with KSHV-GPCR oncogene by MCP compounds. IC50 values in a colony formation assay were established in KSHV-GPCR transformed NIH3T3 cells treated with MCP1, MCP110 or control compounds BAY43-9006 and U0126.

Figure 3. Inhibition of anchorage-independent growth: combination effect of MCP110 at 10 μM with BAY43-9006, U0126 and staurosporine. A. NIH3T3 cells transformed with K-Ras(G12V) were treated with the DMSO control, 10 μM of MCP110, compounds at concentrations indicated, or combinations of MCP110 and compounds, and grown in soft agar for 21 days, then colonies scored. **B.** HLR-Elk1 cells were incubated with DMSO control, 5 μM of MCP110, compounds at concentrations indicated, or combinations of MCP110 and compounds for 6 hours (see Materials and Methods). Percent of residual luciferase activity was determined in comparison with DMSO treated cells. **C.** Experiment as in **A.**, except with staurosporine. Solid black bar – DMSO treated cells; solid white bars – cells treated only with inhibitors; black dotted bars – cells treated with combinations of MCP110 and the compounds. Results reflect the average of at least 3 independent experiments with 3 repetitions in each assay.

Figure 4. Inhibition of anchorage-independent and dependent growth: combination of MCP1 or MCP110 with taxol. **A.** IC50 values for taxol were established in Raf22W, K-Ras(V12) and KSHV-vGPCR-transformed NIH3T3 cells, for growth in soft agar, or by WST-1 proliferation assay. **B.** IC50 values for taxol inhibition of NIH3T3-K-Ras(V12) cell growth in soft agar were re-assessed in the presence of MCP1, MCP110, BAY43-9006, or U0126 at the concentrations as indicated. **C.** IC50 values for taxol inhibition of NIH3T3-K-Ras(V12) cell proliferation were re-assessed in the presence of MCP110, BAY43-9006, or U0126 at the concentrations as indicated. **D.** IC50 values for taxol inhibition of NIH3T3-KSHV-GPCR cell growth in soft agar were re-assessed in the presence of MCP1, MCP110, BAY43-9006, or U0126 at the concentrations as indicated. **E.** IC50 values for taxol inhibition of EC-vGPCR cell growth in WST-1 proliferation assay were determined in the presence of MCP110, BAY43-9006, or U0126 at the concentrations as indicated.

Figure 5. Cell cycle profile induced by MCP110 and taxol in K-Ras(V12) versus cRaf22W NIH3T3 transformed cells. Cells were treated with compounds at the concentrations shown for 72 hours then fixed, stained with propidium iodide and analyzed by FACS. The software program CellQuest was used to establish the frequency of G1 versus G2/M cells.

Figure 6. Induction of cell death by MCP110 and taxol in K-Ras(V12) versus cRaf22W NIH3T3 transformed cells. Cells were treated with compounds at the concentrations as indicated for 72 hours, then fixed and stained with annexin V and analyzed by FACS. The frequency of annexin V - positive cells is shown.

Figure 7. Schematic of the signaling cascade that leads to cellular transformation associated with KSHV infection.

Figure 8. Schematic of the signaling cascade that leads to cellular transformation associated with HHV-4/Epstein Barr virus infection.

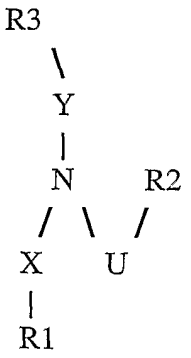
Figure 9. Schematic of the signaling cascade that leads to cellular transformation associated with Hepatitis B infection.

DETAILED DESCRIPTION OF THE INVENTION

Recently, a new class of protein-protein interaction inhibitors, represented by MCP1, *N*-(4-Benzyloxy-3-methoxybenzyl)-*N*-(2-pyridin-2-ylethyl)-2-chlorobenzamide, and MCP110, *N*-(4-Benzyloxy-3-methoxybenzyl)-*N*-(2-pyridin-2-ylethyl)-5-phenylpentanoic acid amide, were described that block interaction between the human Ras and Raf oncoproteins, and inhibit various oncogenic phenotypes associated with activated Ras (13, PCT/EP02/1222 (WO 03/037865)). Based on the implication of Ras in KSHV-GPCR cell transformation, we evaluated these compounds for efficacy against KSHV-GPCR-transformed cells. We determined that these compounds were able to inhibit anchorage dependent and independent growth of murine fibroblasts transformed with GPCR oncogene from HHV-8 (KSHV) virus, and produce strong additive effects in growth inhibition of transformed cells when they were combined with the MAPK pathway inhibitors U0126 and BAY43-9006, as well as the cytotoxic agent taxol.

The compounds described herein block the interaction between Ras and Raf and thus should have demonstrable efficacy as antiviral agents. Based on previously identified signaling cascades, such viruses include without limitation, HHV-8, HHV-4 and hepatitis B virus. Furthermore, the compounds used in the practice of this invention can be combined with other known anti-viral or anti-proliferative agents in methods of treating or preventing viral infection.

Compounds useful in the practice of the present invention include those of Formula (I):



(I)

wherein U is $(CH_2)_n$, CO, SO_2 or CONH;

n is 0, 1, 2, 3, 4 or 5;

X is CH_2, CO, SO_2 or CONH;

Y is CH_2, CO, SO_2 or CONH;

R1 is an optionally substituted aryl, aralkyl, heteroaryl or heteroarylalkyl;

R2 is an optionally substituted heteroalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkyl, heterocycloalkyl or heteroalkylcycloalkyl and

R3 is an optionally substituted alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, heteroarylalkyl or aralkyl; or a pharmacologically acceptable salt, solvate, hydrate or formulation thereof.

The term alkyl refers to a saturated or unsaturated (i. e. alkenyl and alkinyl) straight or branched chain alkyl group, containing from one or two to ten carbon atoms, preferably from one or two to six carbon atoms, e.g. 1 or 2 to 6 carbon atoms, for example methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert.-butyl, n-pentyl, n-hexyl, 2,2-dimethylbutyl, n-octyl, ethenyl (vinyl), propenyl, iso-propenyl, butenyl, isoprenyl or hexa-2-enyl; ethinyl, propinyl or butinyl groups.

The terms alkenyl and alkinyl refer to unsaturated straight or branched chain alkyl groups, containing from two to ten carbon atoms, preferably from two to six carbon atoms, e.g. 2 to 6 carbon atoms, for example ethenyl (vinyl), propenyl, iso-propenyl, butenyl, isoprenyl or hexa-2-enyl; ethinyl, propinyl or butinyl groups.

The term heteroalkyl refers to an alkyl, alkenyl or alkinyl group as defined herein where one or more carbon atoms are replaced by an oxygen, nitrogen, phosphorous or sulphur atom, for example an alkoxy group containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms, such as methoxy, ethoxy, propoxy, iso-propoxy, butoxy or tert.-butoxy, a (1-4C)alkoxy(1-4C)alkyl group such as methoxymethyl, ethoxymethyl, 1-methoxyethyl, 1-ethoxyethyl, 2-methoxyethyl or 2- ethoxyethyl; or a cyano group; or a 2,3-dioxyethyl group. The term heteroalkyl furthermore refers to a group derived from a carboxylic acid or carboxylic acid amide containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms, and may, for example, be acyl containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms, such as acetyl, propionyl, butyryl or pivaloyl; acyloxy containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms such as acetyloxy, propionyloxy, butyrlyoxy or pivaloyloxy; carboxyalkyl containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms such as carboxymethyl, carboxyethyl, carboxypropyl, carboxybutyl, carboxyalkyl ester containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms, such as carboxyalkyl methyl ester, carboxyalkyl ethyl ester, carboxyalkyl propyl ester, carboxyalkyl isopropyl ester, carboxyalkyl butyl ester or carboxyalkyl tert.-butyl ester, carboxyalklyl aminde or alkylcarbamoyl such as N-(1-4C)alkylcarbamoyl or N, N'-(1-4C)dialkylcarbamoyl) containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms such as N-methylcarbamoyl, N-ethylcarbamoyl, N-propylcarbamoyl, N, N'-dimethylcarbamoyl, N-ethyl-N-methylcarbamoyl or N, N'-dipropylcarbamoyl, alkoxycarbonyl containing from one to ten carbon atoms, preferably from one to six carbon atoms, e. g. 1 to 4 carbon atoms, such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxy- or tert.-butoxycarbonyl or alkoxycarbonyloxy containing from one to ten carbon atoms, preferably from one to six carbon atoms, e.g. 1 to 4 carbon atoms such as methoxycarbonyloxy, ethoxycarbonyloxy, propoxycarbonyloxy, isopropoxycarbonyloxy, butoxycarbonyloxy, tert.-butoxycarbonyloxy.

The term cycloalkyl refers to a saturated or partially unsaturated cyclic group, having one or more rings, formed by a skeleton that contains from three to 14 carbon atoms, preferably from three, four, five or six to nine or ten carbon atoms, for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, tetralin, cyclopentenyl or cyclohex-2-enyl groups.

The term heterocycloalkyl refers to a cycloalkyl group as defined herein where one or more carbon atoms are replaced by one or more oxygen, nitrogen, phosphorous or sulphur atoms. Specific examples for heterocyclalkyl are piperidino, morpholino, N-methyl-piperazino or N-phenyl-piperazino groups.

The term aryl refers to an aromatic cyclic group, having one or more rings, formed by a skeleton that contains from five to 14 carbon atoms preferably from five or six to nine or ten carbon atoms, for example phenyl, inden or naphthyl groups. Specific examples are a benzyl, tolyl, phenethyl, biphenyl, xylyl, cumyl, 2-, 3-or 4-methoxyphenyl, 2-, 3- or 4- ethoxyphenyl, 4-carboxyphenyl alkyl or a 4-hydroxyphenyl group.

The term heteroaryl refers to an aryl group as defined herein where one or more carbon atoms are replaced by an oxygen, nitrogen, phosphorous or sulphur atom, for example 4- pyridyl, 2-imidazolyl, 3-pyrazolyl, quinolinyl, isoquinolinyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, 1,2, 3-triazolyl, 1,2, 4-triazolyl, oxadiazolyl, thiadia- zolyl, indolyl, indazolyl, tetrazolyl, pyrazinyl, pyridinyl, pyrimidinyl and pyridazinyl groups.

The terms aralkyl and heteroarylalkyl refer to groups that comprise both aryl or, respectively, heteroaryl as well as alkyl, alkenyl, alkinyl and/or heteroalkyl (for example alkoxy groups in case of aralkyloxy) and/or cycloalkyl and/or heterocycloalkyl ring systems as defined herein. Examples of such groups are arylalkyl-, arylalkenyl-, arylalkinyl-, arylheteroalkyl-, arylheteroalkenyl-, arylheteroalkinyl-, heteroarylheteroalkyl-, heteroarylheteroalkenyl-, heteroarylheteroalkinyl-, arylcycloalkyl, heteroaryl- cycloalkyl-, arylheterocycloalkyl-, heteroarylheterocycloalkyl-, arylcycloalkenyl-, heteroarylcycloalkenyl-, arylcycloalkinyl-, heteroarylcycloalkinyl-, arylheteroalkenyl-, heteroarylheteroalkenyl-, arylheteroalkinyl-, heteroarylheteroalkinyl-, heteroarylalkyl-, heteroalkenyl- and heteroarylakinyl-groups, wherein the cyclic groups

can be saturated or once, twice or three-times unsaturated. Examples are the tetrahydroisoquinolinyl, benzyl, benzyloxy, 2-or 3-ethyl-indolyl or 4-methylpyridino groups.

The terms alkylcycloalkyl and heteroalkylcycloalkyl refer to groups that comprise both cycloalkyl or, respectively, heterocycloalkyl as well as alkyl, alkenyl, alkinyl and/or heteroalkyl (for example alkoxy groups in case of aralkyloxy) groups as defined herein. Examples of such groups are alkylcycloalkyl, alkenylcycloalkyl, alkinylcycloalkyl, alkylheterocycloalkyl, alkenylheterocycloalkyl, alkinylheterocycloalkyl, heteroalkylcycloalkyl, heteroalkenylcycloalkyl, heteroalkinylcycloalkyl, heteroalkylheterocycloalkyl, heteroalkenylheterocylcloalkyl, heteroalkinylheterocycloalkyl, which cyclic groups can be saturated or once, twice or three-times unsaturated.

Any alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl aralkyl or heteroarylalkyl groups as defined herein may be substituted with one or more halogen atoms, NH_2, SH, NO_2 or OH groups or unsubstituted alkyl, heteroalkyl, aryl, aralkyl, aralkyloxy, heteroaryl, cycloalkyl or heterocycloalkyl groups as defined herein.

The term "optionally substituted" refers to groups wherein one or more hydrogen atoms may be replaced by a halogen atom, a NH_2, SH, NO_2 or OH group or by an unsubstituted alkyl, heteroalkyl, aryl, aralkyl, aralkyloxy, heteroaryl, cycloalkyl or heterocycloalkyl group as defined herein.

Preferred are compounds of Formula (I), wherein U is $(CH2)_n$ and n is 0, 1 or 2.

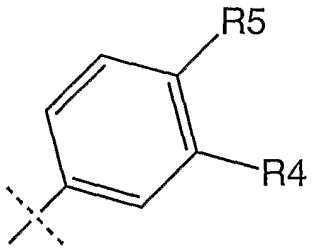
Further preferred are compounds of Formula (I), wherein R1 is an optionally substituted phenyl ring; moreover preferred the phenyl ring is substituted by a benzyloxy group.

Also preferred are compounds of Formula (I), wherein R2 is heterocycloalkyl or heteroaryl (especially preferred nitrogen containing heterocycloalkyl or heteroaryl groups).

Further preferred are compounds of Formula (I), wherein R2 is a pyridyl or a piperidyl group.

Further preferred are compounds of Formula (I), wherein R3 is aryl or aralkyl.

Also preferred are compounds of formula (I), wherein R1 is a group of the formula



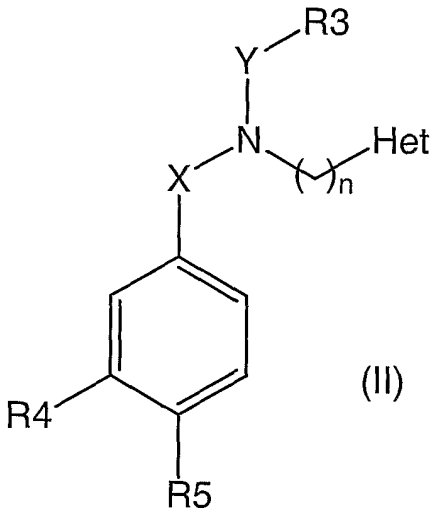
wherein R4 is H, alkyloxy or aralkyloxy (more preferred H, methoxy or benzyloxy) and R5 is F, Cl, alkyl, heteroalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl (preferred Cl, methoxy or benzyloxy; more preferred benzyloxy).

Further preferred are compounds of Formula (I) wherein R3 is a C_1-C_6 alkyl group (especially an isopropyl group).

Moreover preferred are compounds of Formula (I), wherein X is CO or SO_2 and Y is CH_2.

Further preferred are compounds of Formula (I), wherein X is CH_2 and Y is CO or SO_2.

Also preferred are compounds of Formula (II)



(II)

wherein Het is a pyridyl group; n is 0,1 or 2; X is CH_2 ; Y is CO or SO_2 ; R3 is aryl or aralkyl ; R4 is H, alkyloxy or aralkyloxy (more preferred H, methoxy or benzyloxy) and R5 is F, Cl, alkyl, heteroalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl (preferred Cl, methoxy or benzyloxy; more preferred benzyloxy).

Further preferred are compounds of Formula (II) wherein Het is a pyridyl group; n is 0,1 or 2; X is CO or SO_2 ; Y is CH_2 ; R3 is aryl or aralkyl ; R4 is H, alkyloxy or aralkyloxy (preferred H, methoxy or benzyloxy) and R5 is F, Cl, alkyl, heteroalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl (preferred Cl, methoxy or benzyloxy; more preferred benzyloxy).

Also preferred are compounds of Formula (II), wherein Het is a piperidyl group; n is 0,1 or 2; X is CH_2 ; Y is CO or SO_2 ; R3 is aryl or aralkyl ; R4 is H, alkyloxy or aralkyloxy (more preferred H, methoxy or benzyloxy) and R5 is F, Cl, alkyl, heteroalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl (preferred Cl, methoxy or benzyloxy; more preferred benzyloxy).

Further preferred are compounds of Formula (II) wherein Het is a piperidyl group; n is 0, 1 or 2; X is CO or SO_2 ; Y is CH_2 ; R3 is aryl or aralkyl ; R4 is H, alkyloxy or aralkyloxy (preferred H, methoxy or benzyloxy) and R5 is F, Cl, alkyl, heteroalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl (preferred Cl, methoxy or benzyloxy; more preferred benzyloxy).

Further preferred are compounds of Formulas (I) or (II) wherein R3 is a group of the Formula $(CH_2)_mPh$ wherein m is 0,1, 2,3, 4 or 5 (more preferred m is 2,3 or 4) and wherein the phenyl group may be optionally substituted.

The compounds of Formula (I) or (II) do not include *N*-(4-Benzyloxy-3-methoxy-benzyl)-*N*-(2-pyridin-2-yl-ethyl)-2-chloro-benzamide.

Especially preferred are the following compounds:

- *N*-(4-Benzyloxy-3-methoxy-benzyl)-*N*-(2-pyridin-4-yl-ethyl)-benzenesulfonamide,
- *N*-(2-Chloro-benzyl)-*N*-(2-pyridin-2-yl-ethyl)-(4-Benzyloxy-3-methoxy)-benzamide,
- *N*-(4-Benzyloxy-3-methoxy-benzyl)-*N*-(2-piperidin-2-yl-ethyl)-4-phenyl-butyric acid amide,

- *N*-(4-Benzyloxy-3-methoxy-benzyl)-*N*-(2-pyridin-2-yl-ethyl)-5-phenyl-pentanoic acid amide.

It should be appreciated that certain compounds of Formula (I) or (II) may have tautomeric forms from which only one might be specifically mentioned or depicted in the following description, different geometrical isomers (which are usually denoted as cis/trans isomers or more generally as (E) and (Z) isomers) or different optical isomers as a result of one or more asymmetric or chiral carbon atoms (which are usually nomenclatured under the Cahn-Ingold-Prelog or R/S system). Further, some compounds may display polymorphism. Unless expressly excluded, all these tautomeric forms, geometrical or optical isomers (as well as racemates and diastereomers) and polymorphous forms are included in the invention.

The present invention also relates to pharmacologically acceptable salts, or solvates and hydrates, respectively, and to compositions and formulations of compounds of Formula (1) or (II). The pharmaceutical compositions according to the present invention contain at least one compound of Formula (I) or (II) as the active agent and optionally carriers and/or diluents and/or adjuvants. Examples of such pharmacologically acceptable salts of sufficiently basic compounds of Formula (I) or (II) are salts of physiologically acceptable mineral acids like hydrochloric, hydrobromic, sulfuric and phosphoric acid; or salts of organic acids like methanesulfonic, p-toluenesulfonic, lactic, acetic, trifluoroacetic, citric, succinic, fumaric, maleinic and salicylic acid. Further, a sufficiently acid compound of Formula (I) or (II) may form alkali or earth alkaline metal salts, for example sodium, potassium, lithium, calcium or magnesium salts; ammonium salts; or organic base salts, for example methylamine, dimethylamine, trimethylamine, triethylamine, ethylenediamine, ethanolamine, choline hydroxide, N-methyl-D-aminomethane (meglumin), piperidine, morpholine, tris-(2-hydroxyethyl) amine, lysine or arginine salts. Compounds of Formula (I) or (II) may be solvated, especially hydrated. The hydration can occur during the process of production or as a consequence of the hygroscopic nature of the initially water free compounds of Formula (1) or (II). The compounds of Formula (I) or (II) contain asymmetric C-atoms and may be present either as achiral compounds, mixtures of diastereomers, mixtures of enantiomers or as optically pure compounds.

The present invention also relates to pro-drugs which are composed of a compound of Formula (I) or (II) and at least one pharmacologically acceptable protective group which will be cleaved off under physiological conditions, such as an alkoxy-, aralkyloxy-, acyl- or acyloxy group as defined herein, e. g. ethoxy, benzyloxy, acetyl or acetyloxy.

As mentioned above, therapeutically useful agents that contain compounds of Formula (I) or (II), their solvates, salts and formulations are also comprised in the scope of the present invention. In general, compounds of Formula (I) or (II) will be administered by using the known and acceptable modes known in the art, either alone or in combination with any other therapeutic agent. Such therapeutically useful agents can be administered by one of the following routes: oral, e. g. as tablets, dragees, coated tablets, pills, semisolids, soft or hard capsules, for example soft and hard gelatine capsules, aqueous or oily solutions, emulsions, suspensions or syrups, parenteral including intravenous, intramuscular and subcutaneous injection, e. g. as an injectable solution or suspension, rectal as suppositories, by inhalation or insufflation, e. g. as a powder formulation, as microcrystals or as a spray (e. g. liquid aerosol), transdermal, for example via an transdermal delivery system (TDS) such as a plaster containing the active ingredient or intranasal. For the production of such tablets, pills, semisolids, coated tablets, dragees and hard, e. g. gelatine, capsules the therapeutically useful product may be mixed with pharmaceutically inert, inorganic or organic excipients e. g., lactose, sucrose, glucose, gelatin, malt, silica gel, starch or derivatives thereof, talc, stearinic acid or their salts, dried skim milk, and the like. For the production of soft capsules one may use excipients e. g., vegetable, petroleum, animal or synthetic oils, wax, fat, polyols. For the production of liquid solutions, emulsions or suspensions or syrups one may use excipients e. g., water, alcohols, aqueous saline, aqueous dextrose, polyols, glycerin, vegetable, petroleum, animal or synthetic oils. For suppositories one may use excipients e.g., vegetable, petroleum, animal or synthetic oils, wax, fat and polyols. For aerosol formula tions one may use compressed gases suitable for this purpose, e.g., oxygen, nitrogen and carbon dioxide. The pharmaceutically useful agents may also contain additives for conservation, stabilisation, e.g., UV stabilizers, emulsifiers, sweetener,

aromatisers, salts to change the osmotic pressure, buffers, coating additives and antioxidants.

Combinations with other therapeutic agents may include other therapeutically useful agents, e.g., those used to prevent or treat cancer (see Table I.).

A detailed description of methods used for the synthesis of compounds of Formula (I) and (II), above, is provided in PCT/EP02/12222, published May 8, 2003 (WO 03/037865 A1), the entire disclosure of which is incorporated by reference herein.

The following examples are provided to illustrate various embodiments of the invention. They are not intended to limit the invention in any way.

EXAMPLE I

The following materials and methods are provided to facilitate the practice of Example I.

Cell lines. NIH3T3 cells stably transfected with K-Ras(G12V), N-terminally truncated, constitutively active Raf-1 (Raf22w) (13), (kindly provided by Drs. Channing Der and Janiel M. Shields), or KSHV-GPCR (7), EC-vGPCR endothelial cells (80) (kindly provided by Dr. Silvio Gutkind) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) calf serum and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml puromycin or 500 μg/ml G-418) in 5% CO_2 at 37°C. HeLa cells stably transfected with a yeast Gal4 binding site-luciferase transcriptional reporter gene and GAL4-DNA binding domain fusions with Elk1 (HLR-Elk1, Stratagene) were maintained according to supplier recommendations.

Compounds. The amine derivatives *N*-(4-Benzyloxy-3-methoxybenzyl)-*N*-(2-pyridin-2-ylethyl)-2-chlorobenzamide] (MCP1) and *N*-(4-Benzyloxy-3-methoxybenzyl)-*N*-(2-pyridin-2-ylethyl)-5-phenylpentanoic acid amide (MCP110) were synthesized by a protocol described in (14). *Bay43-9006* *N*-(4-chloro-3-(trifluoromethyl)phenyl)-*N'*-(4-(2-

(*N*-methylcarbamoyl))-4-pyridyloxy)phenyl)urea and *U0126* 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)-butadiene were purchased from Calbiochem and Promega, respectively. Taxol and staurosporine were obtained from Biomol.

Proliferation assays. 5000 cells were plated in each well of 96-well flat bottom plates, and incubated overnight at 37^0C in 5% CO_2. The growth of plated cells was determined by adding 7.5 μl of a prepared WST-1 reagent (Roche Applied Sciences, Germany) to 3 control wells and measuring OD_{650} and OD_{450} absorbances with a SpectraMax250 plate reader. If the OD_{650}-OD_{450} values were above 0.5, the remainder of the plate was used for incubation with the above mentioned amine derivatives, other pharmacological agents or solvent control for 48 hours. After this incubation, WST-1 reagent was added to the wells and OD_{650}-OD_{450} values were calculated as before. Triplicate wells were assayed for each conditions and standard deviation was determined: all experiments were performed independently at least three times.

Anchorage-independent growth assays. Anchorage-independent growth assays were performed as described in (15) with minor modifications. Cells were resuspended in DMEM media supplemented with 10% calf serum and antibiotics, and mixed with 0.5% agar to a final concentration of 6,000 to 25,000 cells/ml in 0.33% agar. 0.3 ml of this cell suspension was layered over a 0.5% agar base in 24 well plates and incubated for 2 to 4 hours at 37^0C. After solidification of the top agar layer, compounds in DMSO, or DMSO diluted to 1% final concentration, were added, followed by incubation for 12 – 21 days. To visualize colonies, 40 μl of MTT (5mg/ml thiazolyl blue tetrazolium bromide) and 60 μl of PBS was added to each well, followed by overnight incubation under normal culture conditions. Stained colonies with sizes above 600 μm were scored using a Nikon SMZ1500 dissecting microscope coupled with a Roper Scientific Inc. Cool Snap color cooled charge coupled device (CCD) camera driven by Image Pro-Plus software (Media Cybernetics; Silver Spring, MD). For each compound, the survival curve was based on a minimum of six concentration points. Each concentration was assayed by at least three

separate experiments, with each assay performed in triplicate. IC50 values were determined by using XLfit software from Activity Base suite package (ID Business Solutions, Inc., NJ, USA).

Elk1-Luciferase transcription assay. HLR-Elk1 cells were plated (30,000 cells per well) in a 96 well plate. After 20 hours medium was replaced with 90 μl per well of starvation medium containing no serum. After 72 hours, compound or DMSO solvent was added. After one more hour, the cells were induced with 10 ng/ml EGF. As a negative control (minimal reporter assay), reference cells were not induced with EGF. After an additional five hours of incubation, the cells were lysed and luciferase activity was measured by using the Bright-Glo Luciferase Assay kit (Promega Co., Madison, WI).

Cell cycle analysis. Cell cycle compartmentalization was examined by flow cytometry. Cells were trypsinized, collected by centrifugation, washed twice and resuspended in sterile PBS, and fixed with 70% ethanol at -20^0C for 2–12 hours. Cells were again centrifuged, washed twice with cold PBS and resuspended in 100 μl of PBS. Cell suspensions were mixed with 0.5 ml of 30 mM sodium citrate, 20 μg/ml propidium iodide, and 1 μl of 10 mg/ml DNase-free RNase A. The cells were incubated for 1 hour at 37^0C in the dark, then DNA content from 10,000 cells was determined using the Becton Dickinson FACScan, equipped with argon-ion laser tuned to 488 nm wave length, and CellQuest program (Immunochemistry Systems, San Jose, CA). . Results were expressed as a histogram representing the relative cell number with a given DNA content.

Apoptosis assays. To estimate apoptosis of stably transfected cell lines treated with the above mentioned amine derivatives and/or taxol, 500,000 cells were seeded in six-well plates. Cells were treated for 72h with various concentrations of the amine derivatives and/or taxol and collected for flow cytometry or used to prepare lysates. Cells were collected by trypsinization and, labeled with annexin V-FITC and propidium iodide according to the manufacturer's recommendations (Clontech) for fluorescence-activated cell sorting analysis (Becton Dickinson).

RESULTS AND DISCUSSION

MCP1 and MCP110 strongly inhibit proliferation and soft-agar colony formation in KSHV-GPCR-transformed NIH3T3 fibroblasts. MCP1 and MCP110 were tested for their ability to inhibit anchorage dependent and independent growth of the NIH3T3 murine fibroblast cell line stably transformed with the KSHV-GPCR. Both MCP1 and MCP110 efficiently inhibited growth of NIH3T3-KSHV-GPCR cells in a WST-1 assay of cell proliferation, with IC50 of 24 and 28 μM, respectively (Figure 1). The MCP1 and MCP110 compounds also effectively inhibited soft-agar colony formation by the same cells, with IC50 values of 16 and 14 μM, respectively (Figure 2). To further test the idea that KSHV-GPCR relays signals through Ras/Raf/MEK/ERK signaling module, we also evaluated whether other relevant pathway inhibitors, including the Raf kinase catalytic inhibitor BAY43-9006, and the Mek kinase inhibitor U0126, would also be able to inhibit the proliferation of NIH3T3-KSHV-GPCR cells. Both BAY43-9006 and U0126 also inhibited growth of NIH3T3-KSHV-GPCR cells, with IC50 values comparable to the MCP compounds (Figures 1, 2).

Combination effect of MCP110 with BAY43-9006 and U0126. We have shown that MCP1 and MCP110 reduce signaling through the ERK signal transduction pathway by modulating Ras and Raf interaction, but do not result in 100% inhibition of ERK signaling, as judged by phosphorylation of endogenous ERK, or transcriptional activation of ERK-dependent promoters such as ELK1(16). We considered the possibility that combination of two different inhibitors of this Ras-dependent signaling axis might have an additive or synergistic effect in inhibiting ERK and on cell growth. As a first step, we evaluated the degree to which MCP compounds in combination with BAY43-9006 Raf kinase and U0126 MEK kinase inhibitors would inhibit the soft agar growth and proliferation of a NIH3T3 model cell line transformed with the constitutively activated K-Ras(G12V) oncogene. A potent synergistic effect was observed following the combination of MCP110 with each of these inhibitors in soft-agar colony formation assay (Figure 3A). These results were well supported by the observation that the combination

effect was also determined between MCP110 and BAY43-9006 and to a lesser extent with U0126 in an Elk1-luciferase reporter assay in HeLa cells (Figure 3B). Since all these compounds were shown to modulate signaling through MAPK signal transduction pathway and thus regulate expression of the Elk1-luciferase reporter, the possible mechanism of synergistic potentiation in soft-agar assay could be explained by more complete inhibition of MAPK signaling. In contrast, combination of MCP110 with staurosporine (a protein kinase C (PKC) inhibitor) did not result in synergistic inhibition of growth (Figure 3C), demonstrating that MCP110 is not promiscuously cytotoxic in combined application.

Combination effect of MCP110 with taxol: synergy in inhibition of anchorage-independent growth. Taxol (paclitaxel) is widely used in the treatment of lung, ovarian, and breast carcinomas (17). Recently, the FDA approved taxol as a second line treatment of Kaposi's sarcoma in AIDS patients (18). Although a primary mode of action of taxol is as a cytotoxic agent that disrupts microtubule dynamics, and taxol does not directly affect signaling through the MAPK pathway, taxol has also been implicated as a compound that both modulates Raf-1 activation, and has differing activity dependent on Raf-1 status (19-25), making it of interest to analyze the interaction of MCP compounds with taxol. We first established IC50 for inhibition of anchorage dependent or independent-growth by taxol in NIH3T3 cells transformed with K-Ras(V12), cRaf22W (constitutively activated Raf, independent of interaction with Ras), and KSHV-GPCR cells. In a WST-1 proliferation assay, the IC50 value of taxol was determined as 0.9 μM in K-Ras(V12) transformed cells, 0.2 μM in Raf22W transformed cells, and 0.75 μM in KSHV-GPCR transformed cells (Figure 4A). In a soft agar colony formation assay, the IC50 for taxol was established at 49.7, 8.7 and 63.3 nM, in K-Ras(V12), Raf22W and KSHV-GPCR, respectively (Figure 4A). We then determined the taxol IC50 values in the presence of U0126, BAY43-9006, or MCP110 at concentrations of 1 or 10 μM in a soft agar colony formation assay performed with NIH3T3-K-Ras(V12) cells. Strikingly, in the presence of 1 μM MCP1 or MCP110, the taxol IC50 was reduced from 49.7 nM to 10.7 nM; with 10 μM MCP1 or MCP110, the taxol IC50 values were reduced to 1-1.5 nM (Figure 4B). This reduction in IC50 values was specific for the MCP compounds, as only a minimal

effect was obtained following combination of U0126 or BAY43-9006 with taxol (Figure 4B). Similar results were obtained between the MCP110 and taxol combinations in inhibiting the anchorage-dependent proliferation of NIH3T3-K-Ras(V12) cells, although BAY43-9006 has some efficacy in these cells (Figure 4C).

We next examined the effect of combining the above-mentioned amine derivatives, BAY43-9006, and U0126 compounds in KSHV-GPCR transformed NIH3T3 cells. In this case, strong enhancement of inhibition of anchorage-independent growth was seen, similar to the results with Ras-transformed cells (Figure 4D). Together, these data suggest that the synergistic effect of the above-mentioned amine derivatives with taxol may be beneficial for the treatment of HHV-8 related diseases, and the mechanism of such effect may be related to that observed in K-Ras(V12) transformed cells.

MCP110 induces the G2/M arrest and apoptosis of taxol-treated NIH3T3-K-Ras(V12) cells. Since a strong dose dependent combination effect was established for the above-mentioned amine derivatives and taxol in K-Ras(V12) and KSHV-GPCR, the possible mechanism of the observed synergism was investigated. Previously, we have shown that MCP110 used at its IC50 concentration promotes cell cycle arrest in G1 (16). Dependent on the DNA damage and spindle checkpoint status of cells, taxol causes mitotic arrest leading to cell death (25, 26).

We examined the cell cycle compartmentalization of K-Ras(V12) and cRaf22W transformed NIH3T3 cells treated with low concentrations of MCP110 or taxol, alone or in combination, as well as a DMSO control (Figure 5). We first established the critical concentration necessary for taxol-dependent arrest of each cell line in G2/M (1 μM in each case). We next established that the cell cycle profiles of cells treated with 1 μM MCP110 were identical to those of cells grown with a DMSO control. Similarly, cells treated with an IC5 dose of taxol (100 nM in K-Ras(V12) cells, 10 nM in cRaf22W cells) did not differ from controls. However, the combination of 1 μM MCP110 with IC5 concentrations of taxol resulted in a substantial shift of cells from the G1 to the G2/M compartment in K-Ras(V12) cells (from 18-19% cells in G2/M to 86% G2/M), while no such effect was observed in cRaf22W cells (Figure 5). To determine whether this cell cycle shift was coupled with increased cellular apoptosis, we used annexin V and

propidium iodide co-staining in FACS analysis to identify apoptotic or necrotic cells. As shown (Figure 6), the combination of 1 μM MCP110 and taxol at IC5 doses led to a significant increase in the level of annexin V-positive cells in K-Ras(V12), but not cRaf22W-transformed cells. Moreover, in control experiments, U0126 at concentrations up to 20 μM had no effect on induction of apoptosis or cell cycle arrest in either of the cell lines tested (results not shown), arguing that the pro-apoptotic effect was induced at a level upstream of the Mek1,2 kinases in response to treatment of cells with taxol and MCP110.

The taxol combination results, together with the demonstrated ability of amine derivatives described herein, to inhibit proliferation and anchorage-independent growth properties of KSHV-GPCR transformed NIH3T3 cells, provide logical support for the use of the MCP compound class as anti-KSHV agents. In addition, the results suggest that the combination of the above-mentioned amine derivatives (or Raf-catalytic inhibitors such as BAY43-9006) with taxol, or combination of MCP compounds with BAY43-9006, may be a particularly potent means of inhibiting KSHV- or Ras-transformed cells.

Indeed, the following Table I lists a variety of anti-proliferative and cytotoxic agents that, when used in combination with the amine derivatives described herein, should have therapeutic efficacy for the treatment of viral infection.

TABLE I

Compound	Target	Phenotype/Assay	Reference	Source
FTI277	Farnesyl transferase p-MAPK1,2 level	Proliferation (WST-1)	(27)	Calbiochem
Bay43-9006	Raf-1, B-Raf kinases	Proliferation p-MAPK1,2 level	(28)	Calbiochem
UO126	Mek1,2 kinases	Proliferation p-MAPK1,2 level	(29)	Promega
CI-1040	Mek1,2 kinases	Proliferation p-MAPK1,2 level	(30)	Pfizer
AA-COCF3	cPLA2	Proliferation (WST-1) p-MAPK1,2 level	(31)	Biomol
Bryostatin	PKC	Proliferation (WST-1) p-MAPK1,2 level	(32,33)	Biomol
UCN-01	PKC, Chk1	Proliferation (WST-1) p-MAPK1,2 level	(34)	NCI
Iressa	EGFR	Proliferation (WST-1) p-MAPK1,2 level	(35)	FCCC

	Glivec	BCR/ABL, c-Kit, PDGFR	Proliferation (WST-1)	(36)	Novartis
	LY294002	PY3K	Proliferation (WST-1) p-AKT1,2 level	(37)	Cell Signaling
	Herceptin	Her-2	Proliferation (WST-1)	(38)	FCCC
	Sirolimus (CCI-779)	mTor	Proliferation (WST-1)	(39)	FCCC
	SP600125	JNK	Proliferation (WST-1 p-c-JUN level	(40)	Tocris Cookson Bristol, UK
	Gemcitabine	DNA synthesis	Proliferation (WST-1)	(38)	Eli Lilly
	Paclitaxel	Anti-mitotic, Tubulin polymerization	Proliferation (WST-1)	(41)	Biomol

While the previous example is directed to inhibition of HHV-8 infection, it is known that a number of viruses co-opt cellular signaling pathways to promote their replication, and in some cases may also induce tumorigenesis. A description of some of these viruses (e.g., HHV-8, HHV-4 and hepatitis B virus) follows.

1) As mentioned in the example above, Kaposi's sarcoma, caused by HHV-8, is a multifocal angioproliferative neoplasm induced following long-term infection with Kaposi's sarcoma herpesvirus/human herpesvisrus 8 (KSHV/HHV-8). Development of this neoplasm strictly depends upon the availability of multiple angiogenic growth factors and cytokines, which act in combination from virally encoded oncogenic signals provided by such proteins as the KSHV-encoded viral G-protein coupled receptor (vGPCR). As shown in Figure 7, vGPCR induction of transformation of KSHV-infected cells involves direct and indirect autocrine/paracrine mechanisms, which requires enhanced expression and secretion of number of angiogenic factors and cytokines. These factors include VEGF, IL-8, IL-6 , Gro α(42-44), and potentiate vGPCR signaling by enhancing vGPCR direct transformation effect in autocrine fashion (45,46). Recently, the KSHV-vGPCR was implicated in immortalization of human endothelial HUVEC cells via activation of their VEGF receptor-2/KDR protein (47). Finally, vGPCR induces expression of the cytokines and growth factors by activation of key transcription factors, including AP-1, NF-κB and NF-AT (48,7), through activation of p21-activated kinase-1 (Pak1) that forwards the signaling on Raf-1 and IKK kinases (7). Since MCP compounds regulate

activation of AP-1 and NF-κB transcription factors induced by oncogenic Ras growth factors and TNF (50), and because Raf and Ras signaling has been shown to be relevant to signal transduction induced by cellular GPCRs (51,52), it is reasonable to believe that interruption of the Ras-Raf interaction will interrupt KSHV-vGPCR-dependent functions. The preliminary data provided herein support this proposed mechanism for KSHV-vGPCR-dependent cell transformation.

HHV-4/Epstein-Barr virus (EBV) belongs to the same group of gammaherpes viruses as KSHV. Epstein Barr virus is associated with a number of malignancies of lymphoid and epithelial origin, including endemic Burkitt's lymphoma, T-cell lymphomas, Hodgkin's disease, undifferentiated nasopharyngeal carcinoma and several other carcinomas (53-56). Like KSHV, the EBV genome is frequently found in the lymphomas and lymphoproliferative disorders of immunocompromised transplant patients and individuals with AIDS (57,58). LMP1, an integral membrane protein expressed by EBV during type 2 and type 3 latent infections, is the only EBV protein that produces a classic oncogenic effect in Rat-1 and NIH3T3 cells, and in B cells (56, 59). An animal model for LMP1 exists, as LMP1 transgenic mice develop lymphomas at increased frequency (60). Significantly, LMP1 produces its effect through activation of two major signaling cascades, the NF-κB pathway and the Ras-MAPK signaling pathway (61-63) (see Figure 7). The induction of these signaling cascades occurs through interaction of cytoplasmic domain of LMP1 with TRAF and TRADD proteins (64,65) which subsequently signal through the NIK and IKK kinases towards IκB repressor (66). Significantly, the Raf kinase inhibitor protein (RKIP) has been found to also function as a negative regulator of the NFκB pathway (67), and may provide a physical bridge between the two complexes: in any case, this and other data from non-viral systems (68) indicate that the MAPK and NFκB signaling cascades are engaged in significant cross-talk that may be important in specifying the course of EBV infection.

Hepatitis B (HBV) virus is small hepatotropic pararetrovirus that causes persistent liver infection and cirrhosis, and is strongly associated with development of primary liver cancer (hepatocellular carcinoma, HCC) (69). HCC is one of the most prevalent forms of human cancer worldwide, with extremely limited treatment options (70). In this regard, many studies have focused on identification of potential viral oncogenes. HBV encodes

two transcriptional activators, HBx (71) and the pre-S2/S region of LHB/MHB proteins (72), which activate cellular targets and promote the transformation of infected cells(73). See Figure 9. The HBx protein of HBV is essential for infection and is also thought to be an essential cofactor in HCC development. The mechanism mediating HBx-dependent activator function includes the Ras-dependent activation of c-Raf-1/MEK/Erk2, PI3K/Akt and MEKK1/JNK cascades, causing induction of several major transcription factors, including AP-1 and NF-κB (74, 75). The HBx protein activates Ras-Raf-MAPK signaling pathway, most likely through activation of the Pyk2 (76), c-Src and Fyn kinases (77). Moreover, the activity and cellular localization of HBx protein are tightly regulated through phosphorylation by ERK1/2 kinase, causing the HBx protein to shuttle to the nucleus, where it induces the transcription of genes critical for HBV replication and cell transformation. Thus, the phosphorylation of HBx protein creates a possible feedback regulatory circuit between HBX and MAPK signaling pathway (78), indicating that the MAPK pathway may be a suitable target for developing novel anti-HBV therapeutic agents.

Combination effect of MCP110 with taxol: synergy in inhibition of anchorage-dependent growth in EC-vGPCR endothelial cells.

As KSHV-induced cancers are marked by action of the vGPCR in promotion of vascular endothelial growth factor (VEGF)-driven angiogenesis, this endothelial model has a higher physiological relevance than a fibroblast model. While EC-vGPCR cells do not form colonies in soft-agar, MCP110 was as effective in these cells as it was in NIH3T3-KSHV-GPCR cells in inhibiting proliferation. The proliferation was measured by WST-1 assay with IC50 value of 22 μM. Similar to NIH-3T3-KSHV-GPCR cells, MCP110 produced a strong synergistic effect with taxol in the endothelial cells. The IC50 of taxol in EC-vGPCR cells was markedly reduced by addition of 1-10 μM MCP110. Moreover, similar synergies were observed in combination of the BAY43-9006 Raf kinase inhibitor with taxol, and to a lesser degree with the MEK1 kinase inhibitor U0126 (Figure 4E). This observation is compatible with reports in the scientific literature indicating Raf activity is required for pro-survival signals involving Akt and NF-kappaB (79-81), and

indicating that KSHV transformation is associated with and requires activation of Akt signaling (82).

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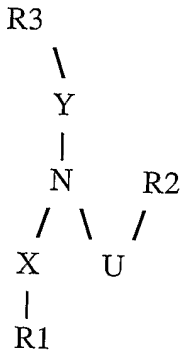
While certain preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

WHAT IS CLAIMED IS:

1. A method for the treatment of viral infection in a patient in need thereof, comprising administration of a therapeutically effective amount of a compound having the formula



(I)

wherein U is $(CH_2)_n$, CO, SO_2 or CONH;

n is 0, 1, 2, 3, 4 or 5;

X is CH_2, CO, SO_2 or CONH;

Y is CH_2, CO, SO_2 or CONH;

R1 is an optionally substituted aryl, aralkyl, heteroaryl or heteroarylalkyl;

R2 is an optionally substituted heteroalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkyl, heterocycloalkyl or heteroalkylcycloalkyl and

R3 is an optionally substituted alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, heteroarylalkyl or aralkyl;

or a pharmacologically acceptable salt, solvate, hydrate or formulation thereof.

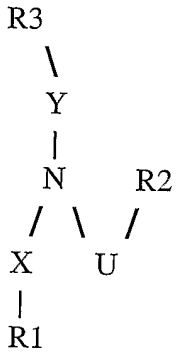
2. The method of claim 1, further comprising the administration of a MAPK pathway inhibitor and optionally an antiproliferative agent.

3. The method of claim 1, wherein said virus is selected from the group consisting of HHV-8, HHV-4 and hepatitis B virus.

4. The method of claim 2, wherein said MAPK pathway inhibitor is selected from the group of inhibitors provided in Table I.

5. The method of claim 2, wherein said anti-proliferative agent is selected from the group of agents provided in Table I.

6. A pharmaceutical composition for treating or inhibiting viral infection, said composition comprising in a pharmaceutically acceptable carrier, a compound having the formula



(I)

wherein U is $(CH_2)_n$, CO, SO_2 or CONH;

n is 0, 1, 2, 3, 4 or 5;

X is CH_2, CO, SO_2 or CONH;

Y is CH_2, CO, SO_2 or CONH;

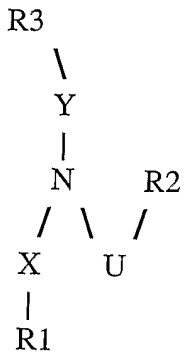
R1 is an optionally substituted aryl, aralkyl, heteroaryl or heteroarylalkyl;

R2 is an optionally substituted heteroalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkyl, heterocycloalkyl or heteroalkylcycloalkyl and

R3 is an optionally substituted alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, heteroarylalkyl or aralkyl; or a pharmacologically acceptable salt, solvate, hydrate or formulation thereof and an agent or inhibitor selected from the group provided in Table I, each of said compound and said agent or inhibitor being present in said composition in an amount effective to attenuate infectivity of said virus.

7. The composition of claim 6, wherein said virus is selected from the group consisting of HHV-8, HHV-4 and hepatitis B virus.

8. A method for prophylaxis of viral infection in a host susceptible to said infection, said method comprising administration of a therapeutically effective amount of a compound having the formula



(I)

wherein U is $(CH_2)_n$, CO, SO_2 or CONH;

n is 0, 1, 2, 3, 4 or 5;

X is CH_2, CO, SO_2 or CONH;

Y is CH_2, CO, SO_2 or CONH;

R1 is an optionally substituted aryl, aralkyl, heteroaryl or heteroarylalkyl;

R2 is an optionally substituted heteroalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkyl, heterocycloalkyl or heteroalkylcycloalkyl and

R3 is an optionally substituted alkyl, alkenyl, alkinyl, heteroalkyl, cycloalkyl, alkylcycloalkyl, heterocycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, heteroarylalkyl or aralkyl;

or a pharmacologically acceptable salt, solvate, hydrate or formulation thereof.

9. The method of claim 8, further comprising the administration of a MAPK pathway inhibitor and optionally an antiproliferative agent.

10. The method of claim 8, wherein said virus is selected from the group consisting of HHV-8, HHV-4 and hepatitis B virus.

11. The method of claim 9, wherein said MAPK pathway inhibitor is selected from the group of inhibitors provided in Table I.

12. The method of claim 9, wherein said anti-proliferative agent is selected from the group of agents provided in Table I.

Figure 1

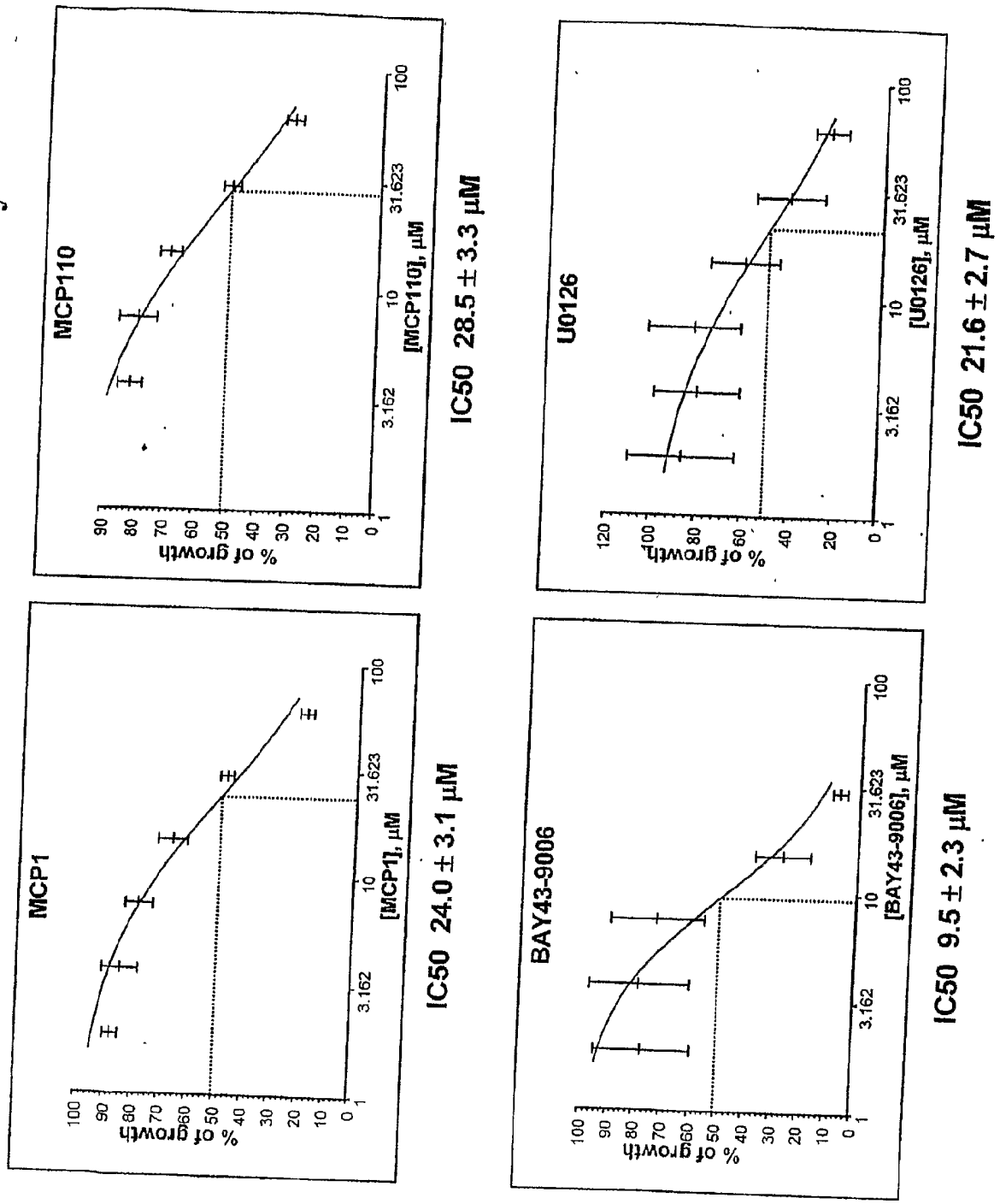

NIH3T3-KSHV-GPCR cells: WST-1 proliferation assay
MCP110
% of growth
[MCP110], μM
IC50 28.5 ± 3.3 μM
MCP1
% of growth
[MCP1], μM
IC50 24.0 ± 3.1 μM
U0126
% of growth
[U0126], μM
IC50 21.6 ± 2.7 μM
BAY43-9006
% of growth
[BAY43-9006], μM
IC50 9.5 ± 2.3 μM

Figure 2

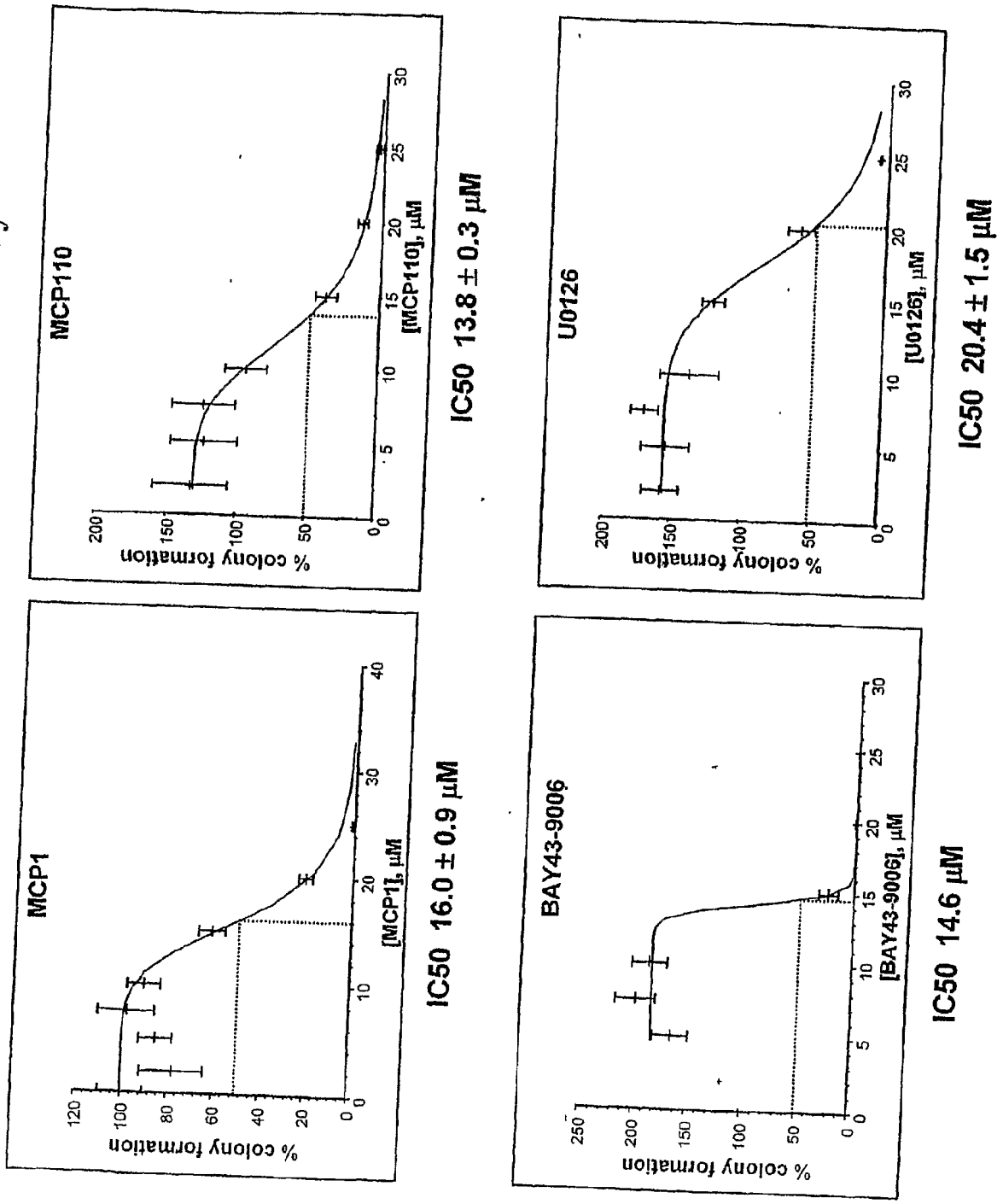

NIH3T3-KSHV-GPCR cells: Colony formation assay
MCP110
% colony formation
[MCP110], µM
IC50 13.8 ± 0.3 µM
MCP1
% colony formation
[MCP1], µM
IC50 16.0 ± 0.9 µM
U0126
% colony formation
[U0126], µM
IC50 20.4 ± 1.5 µM
BAY43-9006
% colony formation
[BAY43-9006], µM
IC50 14.6 µM

Figure 3

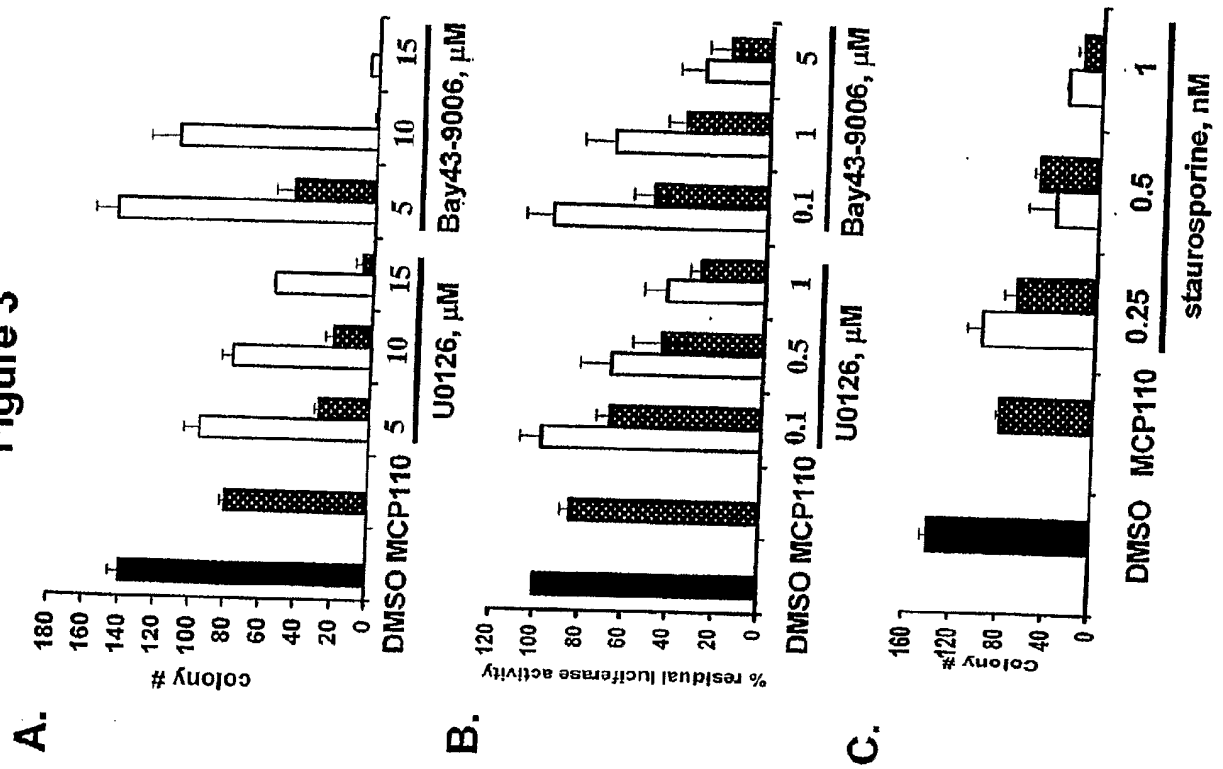
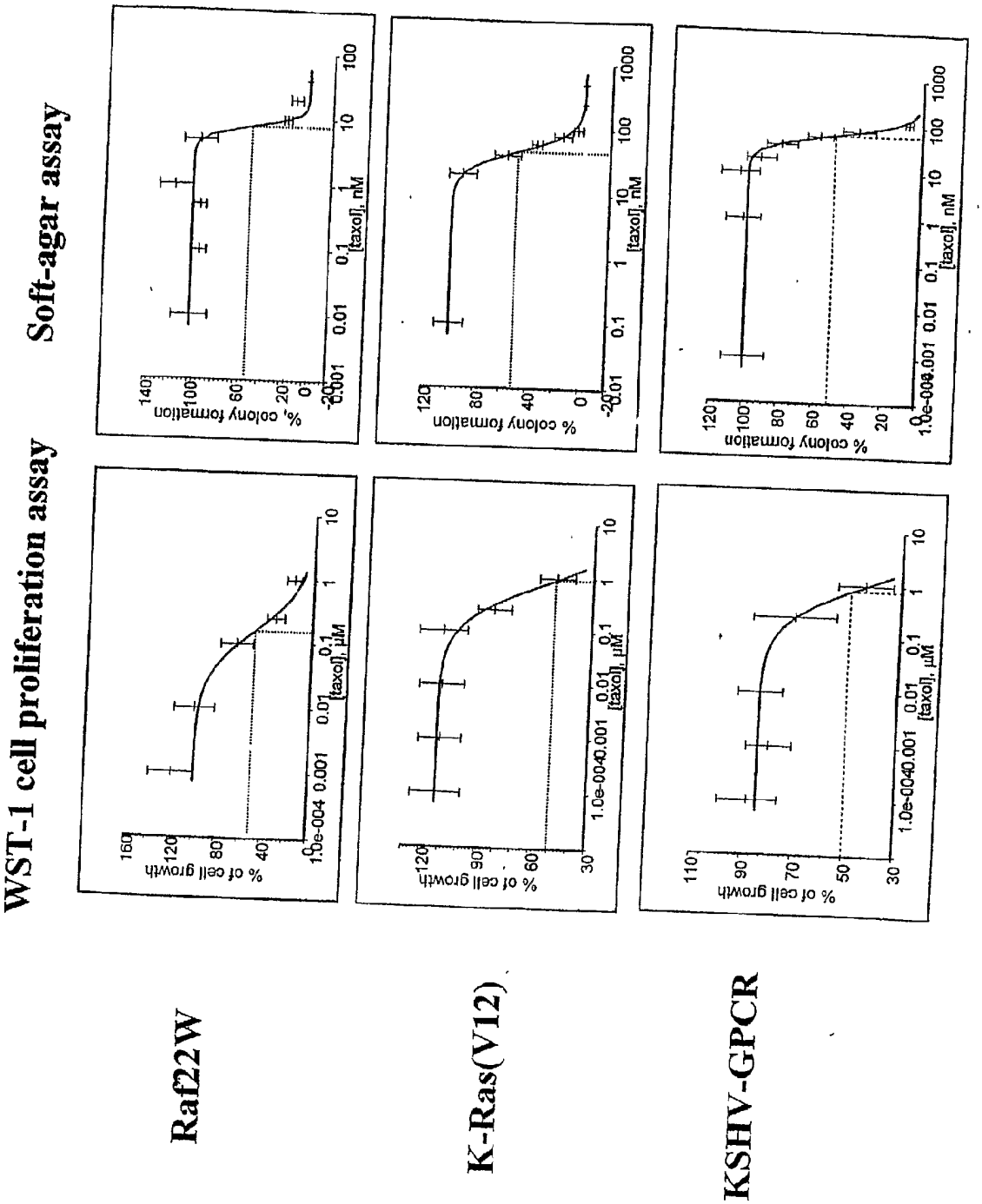

A.
colony #
180
160
140
120
100
80
60
40
20
0
DMSO MCP110 5 10 15 5 10 15
U0126, μM
Bay43-9006, μM
B.
% residual luciferase activity
120
100
80
60
40
20
0
DMSO MCP110 0.1 0.5 1 0.1 1 5
U0126, μM
Bay43-9006, μM
C.
Colony #
160
120
80
40
0
DMSO MCP110 0.25 0.5 1
staurosporine, nM

Figure 4A


WST-1 cell proliferation assay
Soft-agar assay
Raf22W
K-Ras(V12)
KSHV-GPCR
% of cell growth
% colony formation
[taxol], μM
[taxol], nM

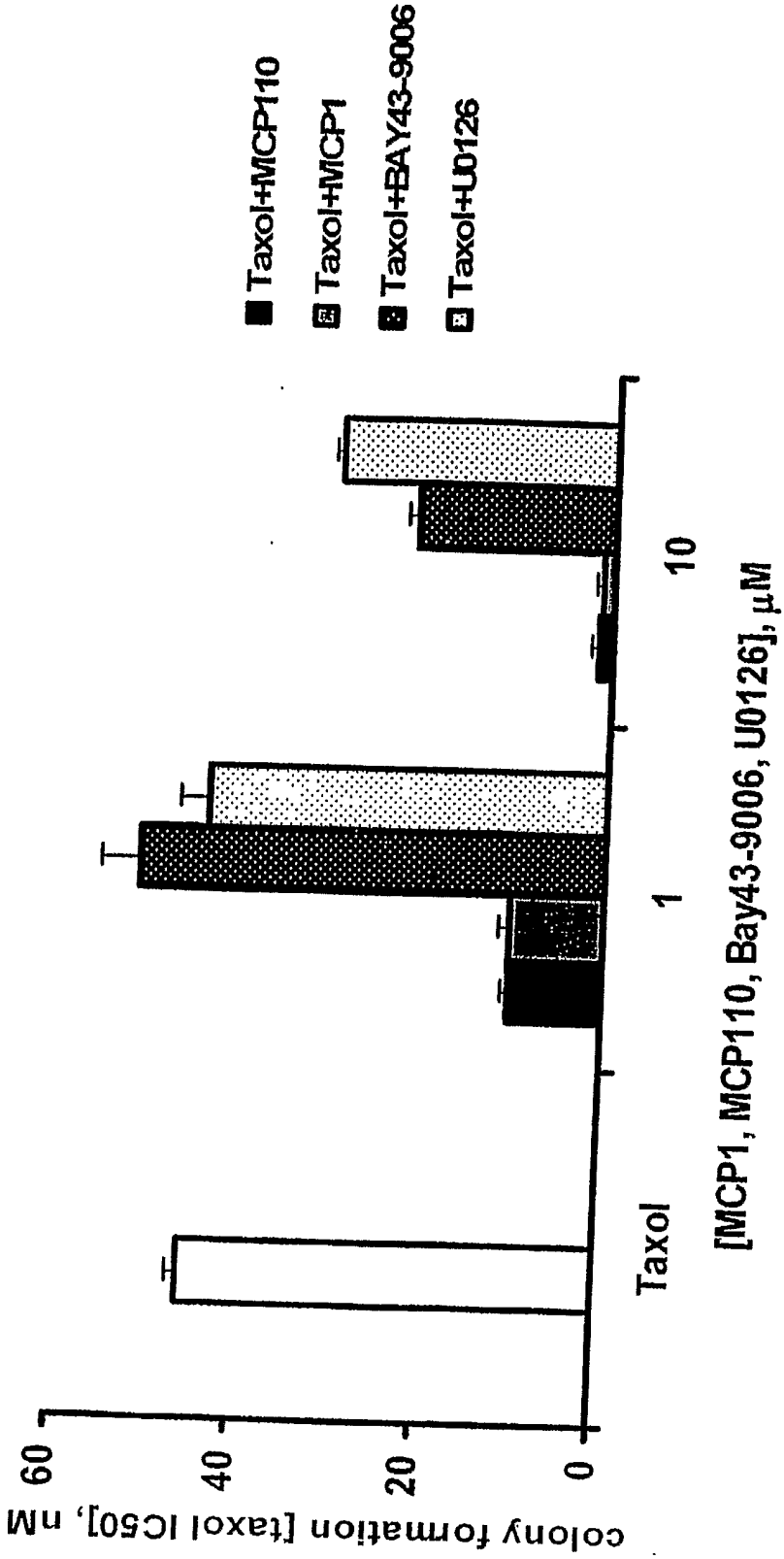

Taxol+MCP110
Taxol+MCP1
Taxol+BAY43-9006
Taxol+U0126
colony formation [taxol IC50], nM
60
40
20
0
Taxol
1
10
[MCP1, MCP110, Bay43-9006, U0126], μM

Figure 4B

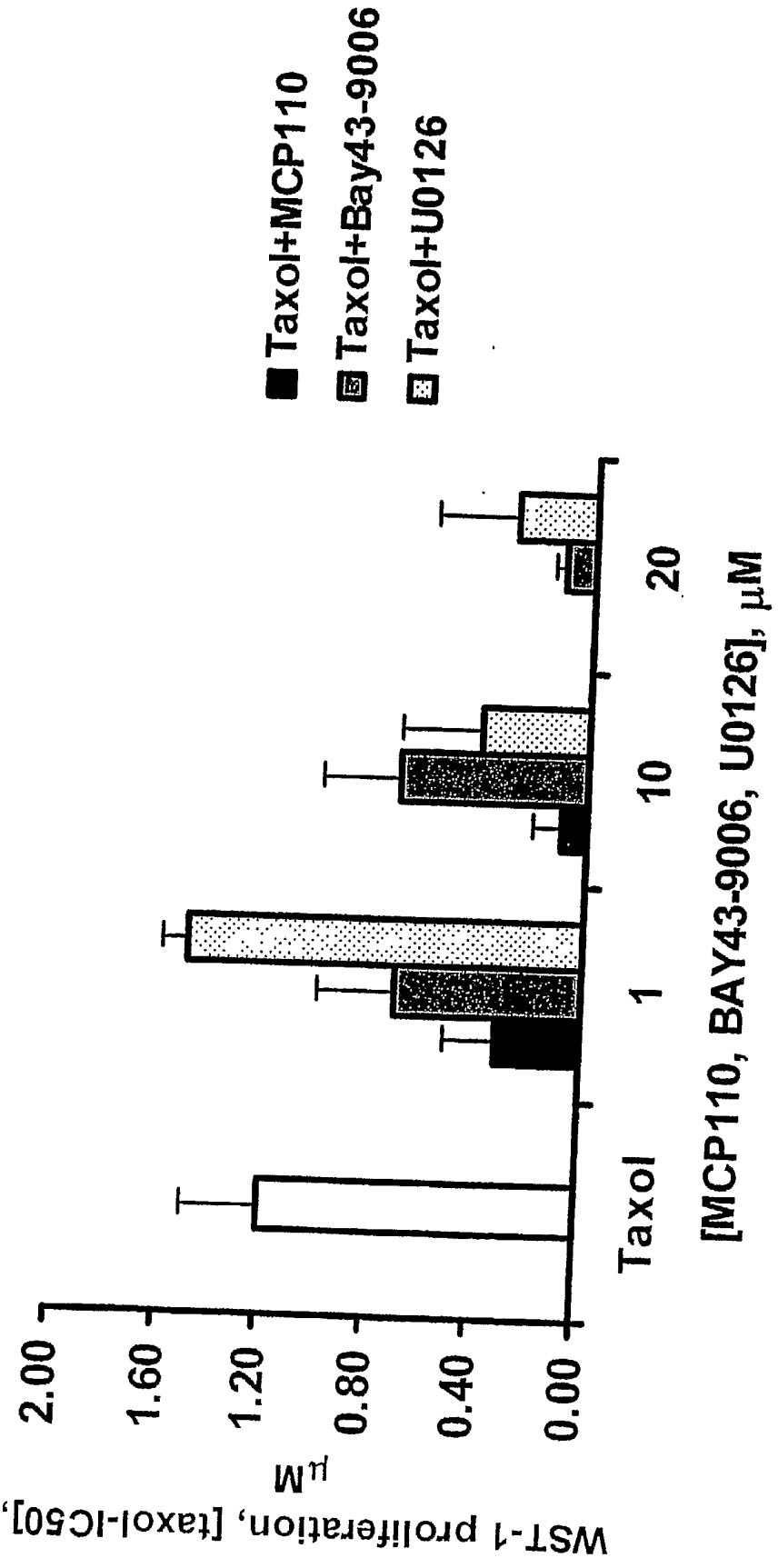

Taxol+MCP110
Taxol+Bay43-9006
Taxol+U0126
2.00
1.60
1.20
0.80
0.40
0.00
μM
WST-1 proliferation, [taxol-IC50],
Taxol
1
10
20
[MCP110, BAY43-9006, U0126], μM

Figure 4C

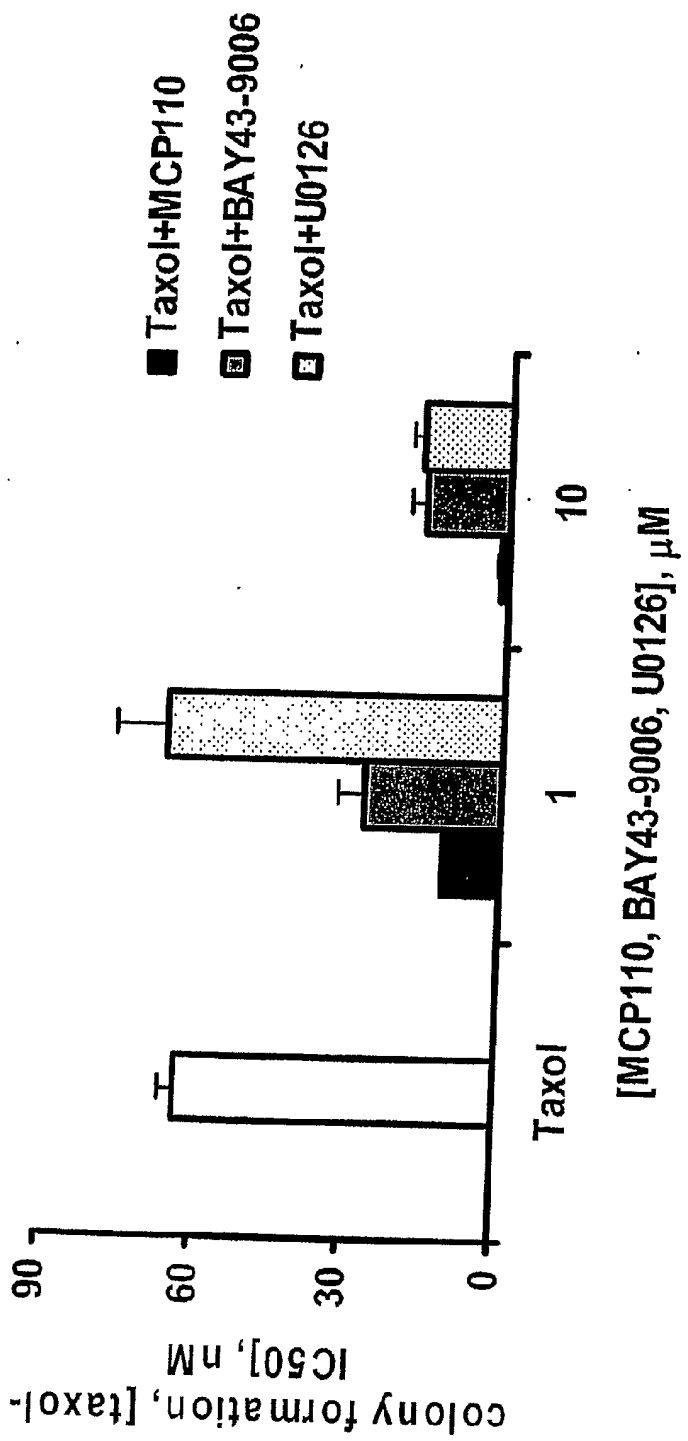

Taxol+MCP110
Taxol+BAY43-9006
Taxol+U0126
colony formation, [taxol-IC50], nM
90
60
30
0
Taxol
1
10
[MCP110, BAY43-9006, U0126], μM

Figure 4D

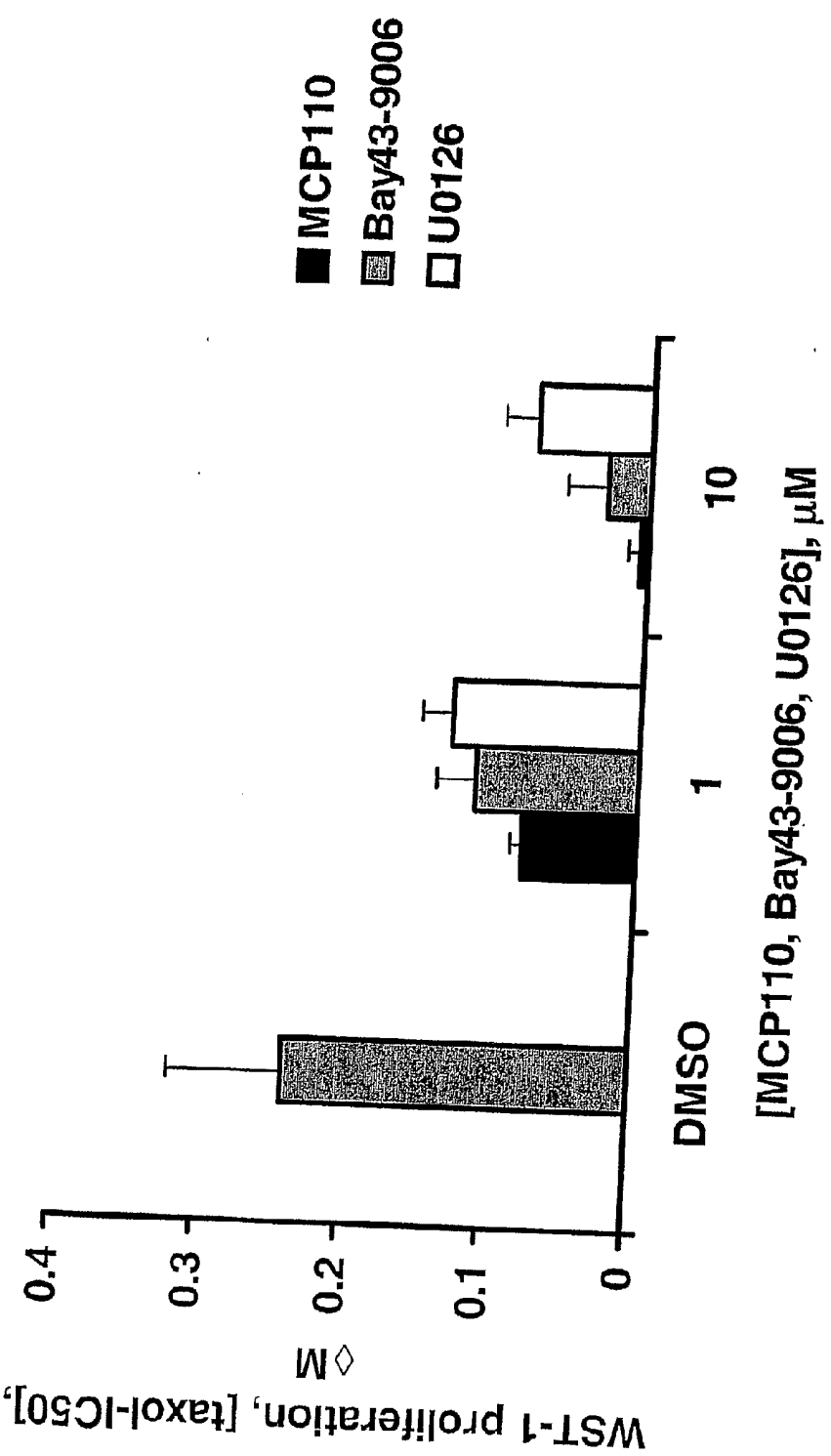

MCP110
Bay43-9006
U0126
WST-1 proliferation, [taxol-IC50], ◊M
0.4
0.3
0.2
0.1
0
DMSO
1
10
[MCP110, Bay43-9006, U0126], μM

Figure 4E

Figure 5

A. NIH3T3-K-Ras(V12)
B. NIH3T3-Raf22W

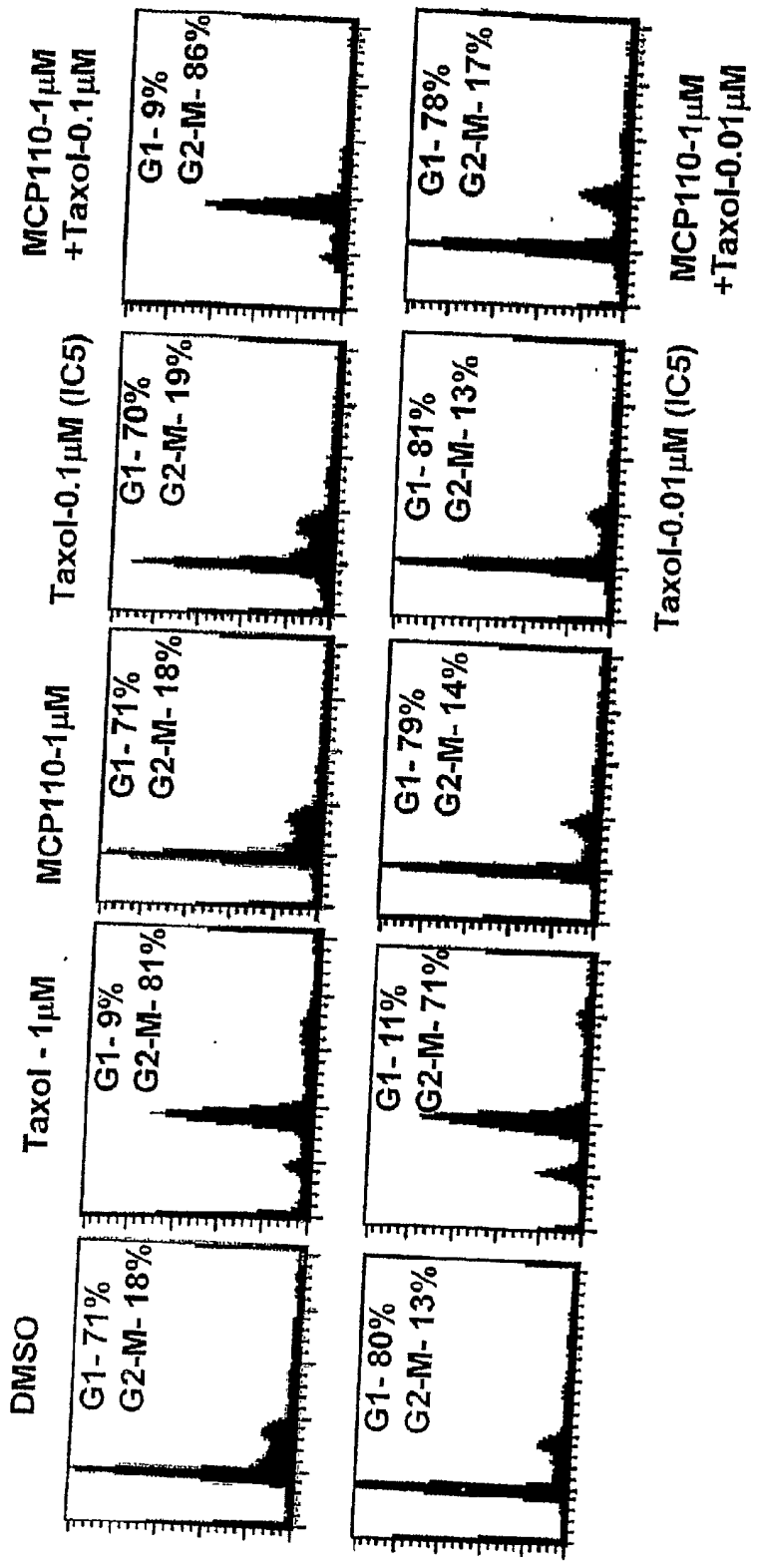
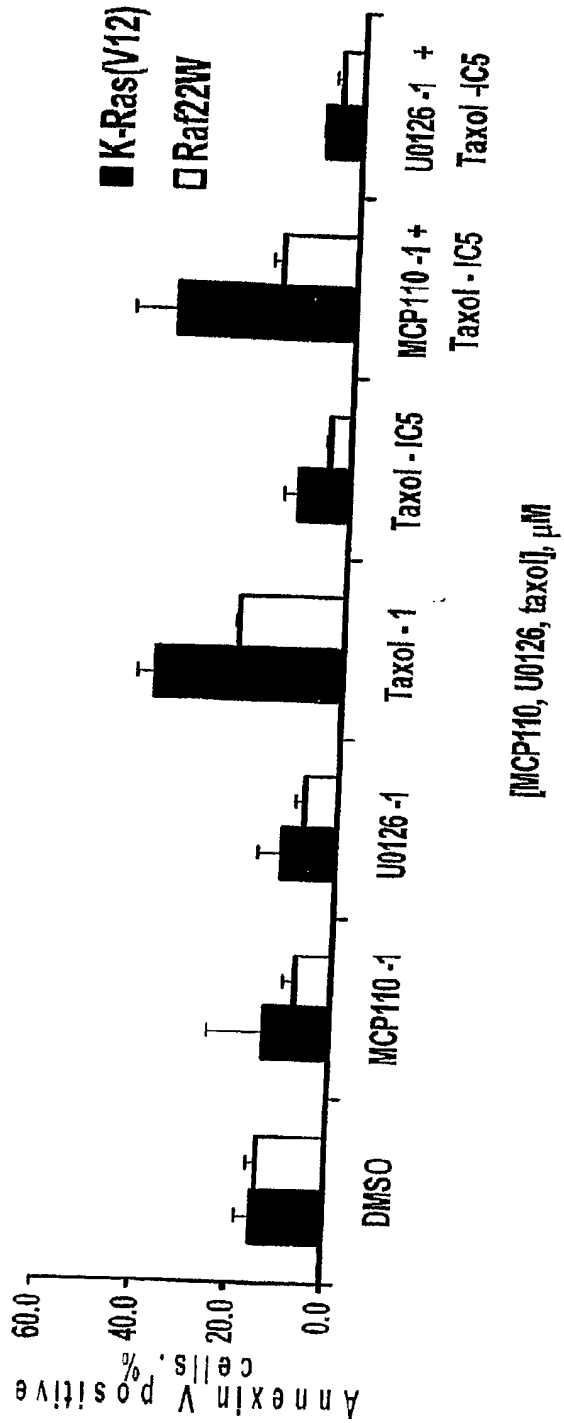


Figure 6

Taxol IC5 – 0.1μM (3T3-K-RasV12)
Taxol IC5 – 0.01μM (3T3-Raf22W)



SUBSTITUTE SHEET (RULE 26)

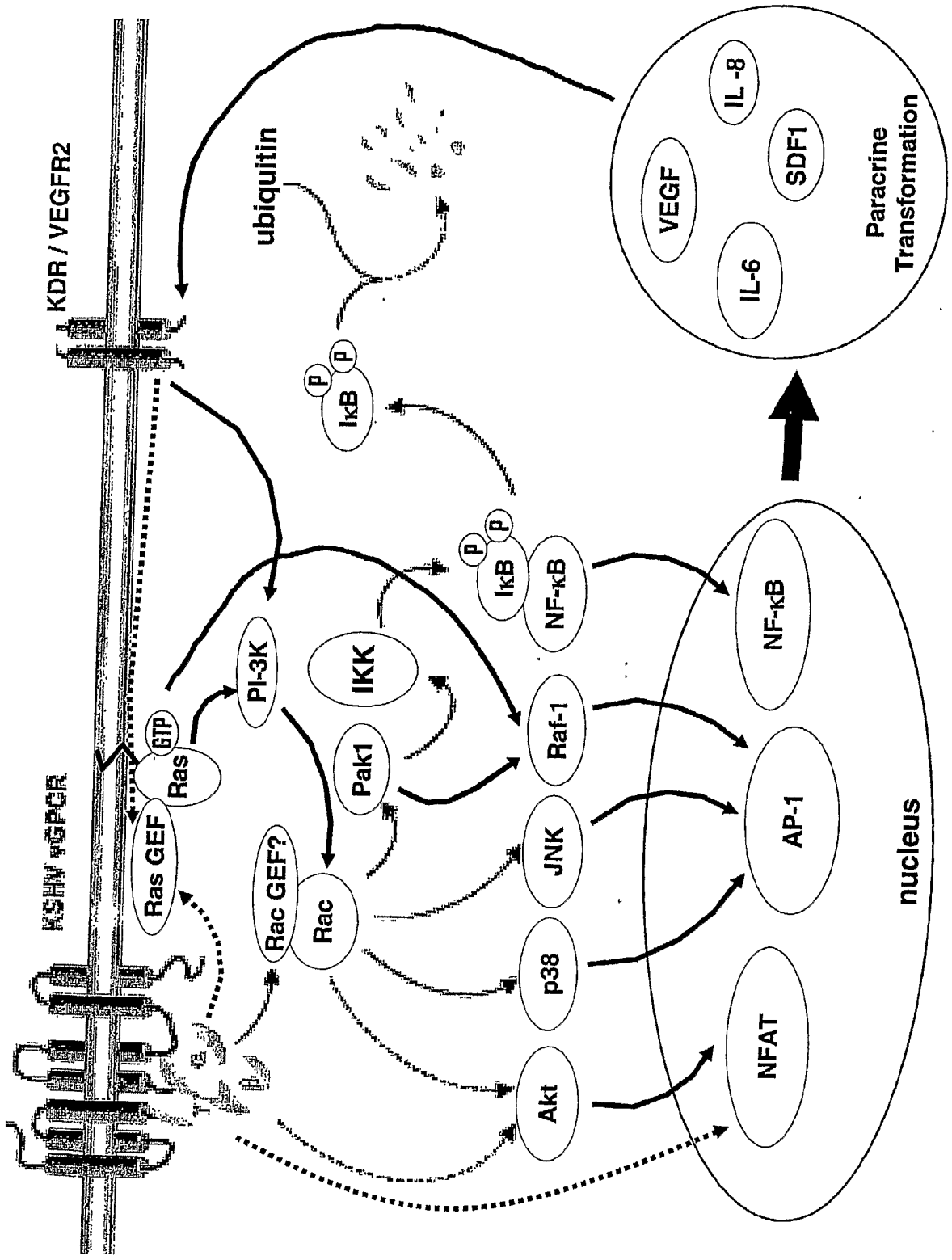

KDR / VEGFR2
KSHV vGPCR
ubiquitin
P
P
IκB
VEGF
IL-8
SDF1
IL-6
Paracrine
Transformation
IκB
NF-κB
PI-3K
IKK
GTP
Ras
Raf-1
Pak1
Ras GEF
Rac GEF?
Rac
JNK
p38
Akt
NF-κB
AP-1
NFAT
nucleus

Figure 7

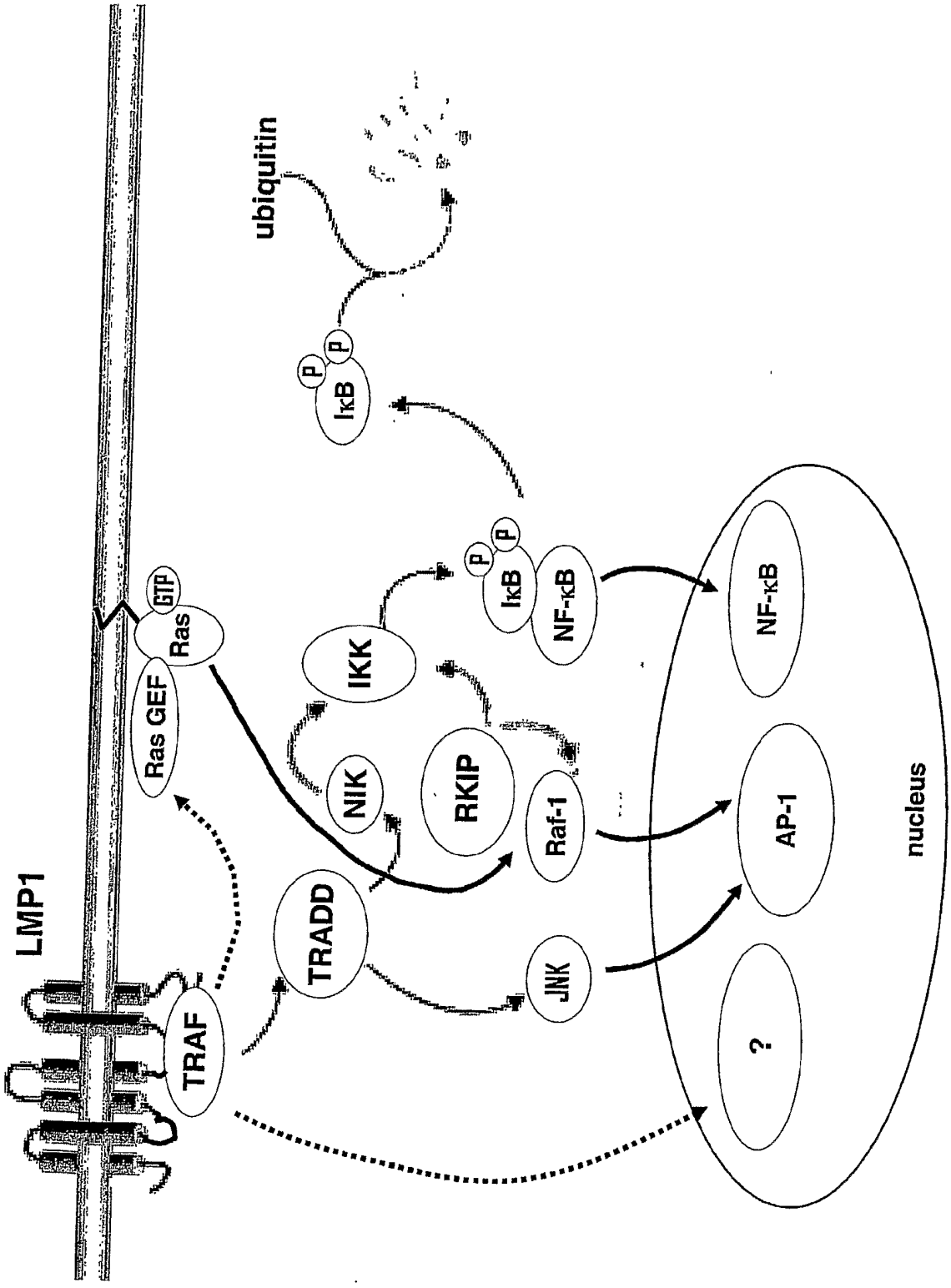
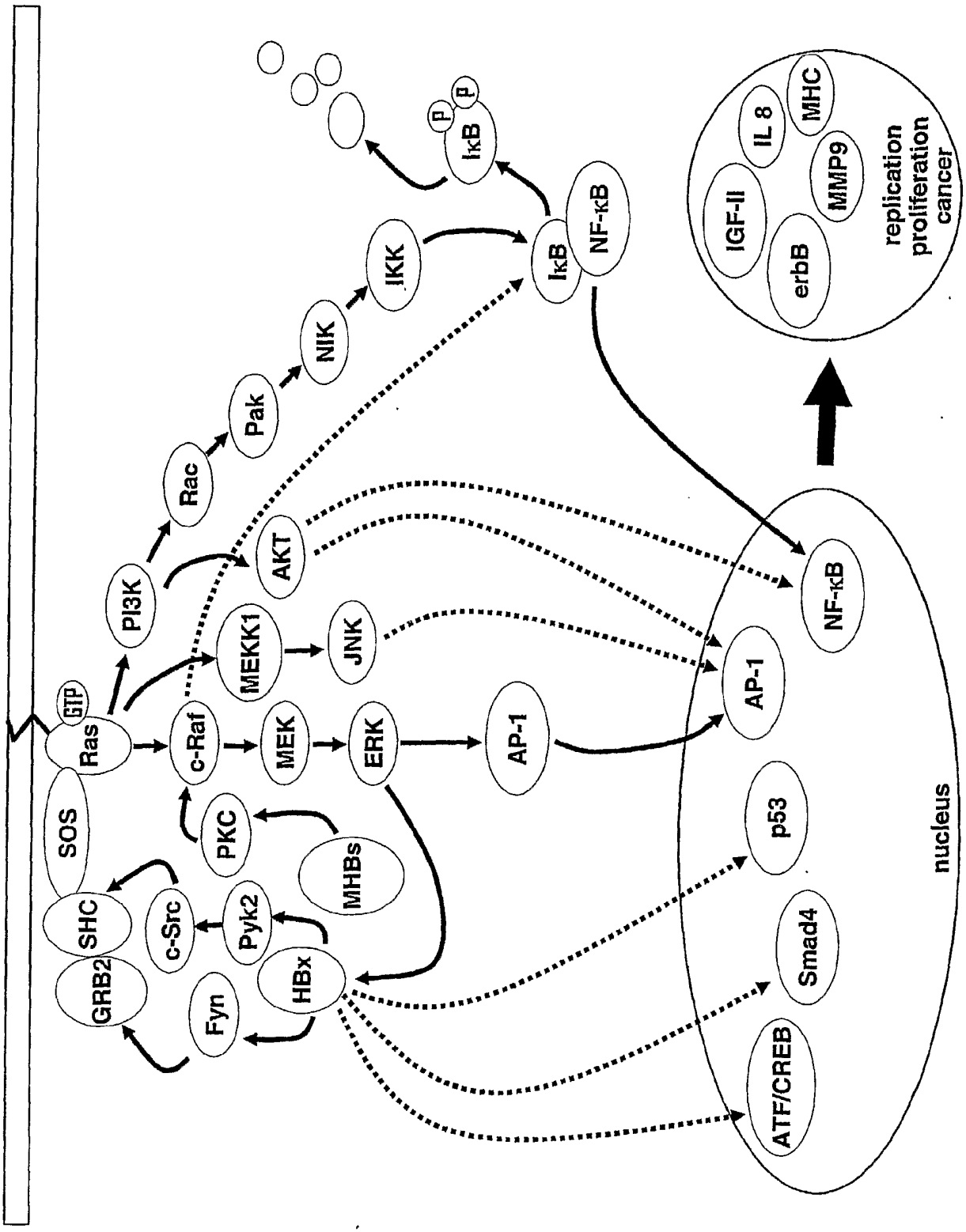

LMP1
TRAF
TRADD
Ras GEF
GTP
Ras
NIK
IKK
RKIP
Raf-1
JNK
ubiquitin
P
P
IκB
IκB
NF-κB
NF-κB
AP-1
?
nucleus

Figure 8

Figure 9


GTP
Ras
SOS
SHC
GRB2
c-Src
Fyn
Pyk2
HBx
PKC
MHBs
c-Raf
MEK
ERK
MEKK1
JNK
PI3K
Rac
Pak
NIK
IKK
AKT
IκB
NF-κB
P
P
IκB
AP-1
AP-1
NF-κB
p53
Smad4
ATF/CREB
nucleus
IGF-II
IL 8
MHC
MMP9
erbB
replication
proliferation
cancer