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(54) Title: NON-PEPTIDIC PROMOTERS OF APOPTOSIS

(57) Abstract: The present invention relates to the use of non-peptidic compounds as medicaments. In particular, these compounds can be used for the treatment of hyperproliferative diseases.

Non-peptidic promoters of apoptosis

The present invention relates to the use of compounds of formula (I) as defined herein as medicaments. In particular, these compounds can be used for the treatment of hyperproliferative diseases. The compounds of the present invention are in particular useful in co-therapy approaches with other drugs, in particular anticancer drugs and anti-proliferative drugs.

Apoptosis is a genetically programmed process of cell death that allows for the removal of excess or damaged cells. In fact, the network of apoptotic signalling mechanisms in most cell types provides a major barrier to the development and progression of human cancer. Most commonly used radiation and chemotherapies rely on activation of apoptotic pathways to kill tumor cells and, importantly, tumor cells which are capable of evading programmed cell death often become resistant to treatment. Apoptotic signalling involves a specific cascade of cysteine proteases, termed caspases, whose activity is initiated by the conversion of a zymogen form to an active form. The subsequent cleavage of caspase substrates results in the disassembly of the cell. Caspases function within the cells in a hierarchical order involving initiator caspases (e.g. caspasae-8 for the extrinsic pathway and caspase-9 for the intrinsic pathway) that cleave effector caspases (e.g. caspase-3).

Regulation of caspase activation and activity is provided by members of the inhibitor of apoptosis protein (IAP) family. IAPs sabotage apoptosis by directly interacting with and neutralizing caspases (Wrzesien-Kus et al., Apoptosis (2004), 9(6), 705-715). Their deregulation in a variety of pathologies especially in cancer and furthermore chemoresistance has placed them in the focus as potential targets for drug discovery (Devi, Drug News Perspect. (2004), 17(2), 127-134; Schimmer et al., Hematology Am. Soc. Hematol. Educ. Program (2005) 215-219; Schimmer et al., Cell Death Differ. (2006), 13(2), 179-188). XIAP is the

prototype IAP and has three functional domains referred to as BIR 1, BIR 2 and BIR 3. BIR 3 interacts directly with caspase-9 and inhibits its ability to bind and cleave procaspase-3, its natural substrate. BIR 2 interacts directly with caspase-3. In each case, however, inhibition of the caspase can be relieved by the binding of the proapoptotic mitochondrial protein SMAC, also known as DIABLO (Wrzesien-Kus et al., Apoptosis (2004), 9(6), 705-715; Arkin, Curr. Opin. Chem. Biol. (2005), 9(3), 317-324).

The state of the art comprises several distinct groups of compounds that resemble the compounds disclosed herein to a certain degree and are also used as medicaments.

For example, WO 2006/114401 discloses cyclic amines, primarily derivatives of pyrrolidine, that are inhibitors of factor Xa and their use for the treatment of thrombotic disorders. Further, their use as antitumor agents is described.

Further, WO 97/28141 describes aromatic piperazine derivatives which are selective serotonine receptor 1D ($5\text{-}HT_{1D}$ receptor) antagonists and their use for the treatment of psychiatric disorders, neurodegenerative disorders and cancer.

WO 2007/067781 relates to derivatives of indene, with at least two carbon atoms of indene being replaced by heteroatoms, as inhibitors of protein kinases, more specifically Aurora kinases, and their use for treating cancer.

WO 2007/054453 describes carbocyclic fused cyclic amines which are inhibitors of factor Xa and their use for the treatment of thrombotic disorders and also tumors.

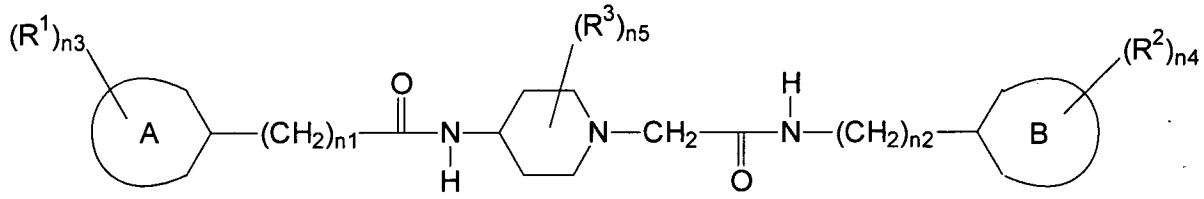
WO 01/44191 discloses derivatives of 4-aminopiperidine exhibiting affinity with somatostatin receptor subtypes and their use for treating acromegaly and certain types of tumor.

It is an object of the present invention to provide compounds that can be used for the treatment of hyperproliferative diseases, such as tumorous diseases, non-solid cancers and metastases. A particular object of the present invention is to overcome chemoresistance upon treatment with conventional chemotherapeutics (e.g. etoposide or taxol) or experimental therapeutics, such as

TRAIL, which are for example described by Li et al., Science (2004), 305(5689), 1471-1474; and Wrzesien-Kus et al., Apoptosis (2004), 9(6), 705-715. A further object of the present invention is the provision of compounds having the above activities which can readily be synthesized. Yet another object of the present invention is the provision of compounds having the above activities which are of low toxicity.

The present inventors surprisingly found that these objects can be achieved by compounds of formula (I) as defined hereinafter.

Thus, the present invention relates to a compound of formula (I)



(I)

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable solvate thereof or a pharmaceutically acceptable prodrug thereof for use as a medicament.

A is a saturated, unsaturated or aromatic 5 to 10-membered mono- or bicyclic ring system, which optionally contains one or more heteroatoms, selected from N, O and S. Preferably, A is a saturated, unsaturated or aromatic 5 or 6-membered monocyclic ring, which optionally contains one or more heteroatoms, selected from N, O and S. More preferably, A is a saturated, unsaturated or aromatic 6-membered monocyclic ring, which optionally contains one or more heteroatoms, selected from N, O and S. Even more preferably, A is a saturated or aromatic 6-membered carbocyclic ring, most preferably a phenyl ring.

B is a saturated, unsaturated or aromatic 5 to 10-membered mono- or bicyclic ring system, which optionally contains one or more heteroatoms, selected from N, O and S. Preferably, B is a saturated, unsaturated or aromatic 5 or 6-membered monocyclic ring, which optionally contains one or more heteroatoms, selected from N, O and S. More preferably, B is a saturated,

unsaturated or aromatic 6-membered monocyclic ring, which optionally contains one or more heteroatoms, selected from N, O and S. Even more preferably, B is an aromatic 6-membered carbocyclic ring, most preferably a phenyl ring.

Each R^1 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen. Preferably, each R^1 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy and halogen (preferably F, Cl or Br, more preferably F or Br, most preferably F).

Each R^2 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen. Preferably, each R^2 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy and halogen (preferably Cl or Br, more preferably Cl). More preferably, each R^2 is independently selected from C_{1-4} alkyl. Most preferably, each R^2 is independently selected from methyl and ethyl.

Each R^3 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen. Preferably, each R^3 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy and halogen (preferably Cl or Br).

In the above definitions of R^1, R^2 and R^3 one or more carbon atoms in the aryl groups may optionally be replaced by a heteroatom selected from N, O and S. The resulting heteroaryl groups include pyrrolidinyl, tetrahydrofuranyl, tetrahydrothiophenyl, piperidinyl, pyrrolyl, furanyl, thiophenyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, indenyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl and thiadiazolyl. In the SO_2NH-heteroaryl groups, the SO_2NH moiety is bonded to a carbon atom of the heteroaryl group.

n1 is an integer from 0 to 3. Preferably, n1 is 0 or 1. In one preferred aspect, n1 is 0. In another preferred aspect, n1 is 1.

n2 is an integer from 0 to 3. Preferably, n2 is 0 or1, more preferably n2 is 0.

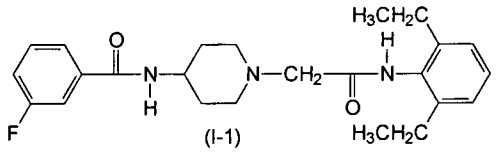
n3 is an integer from 0 to 3. In one preferred aspect, n3 is 0. In another preferred aspect, n3 is 1.

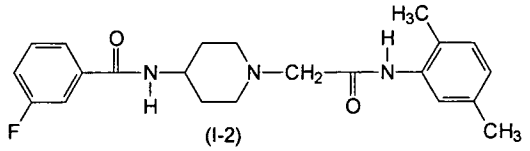
n4 is an integer from 0 to 5. In one preferred aspect, n4 is 1. In another preferred aspect, n4 is 2. In yet another preferred aspect, n4 is 3.

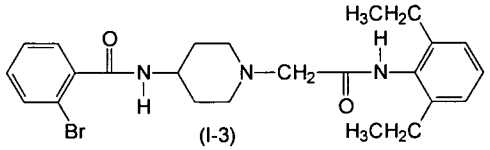
n5 is an integer from 0 to 3. Preferably, n5 is 0.

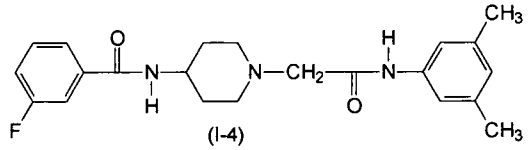
It is to be understood that, if n3, n4 or n5 is 0, the respective ring is substituted with hydrogen instead of R^1, R^2 or R^3.

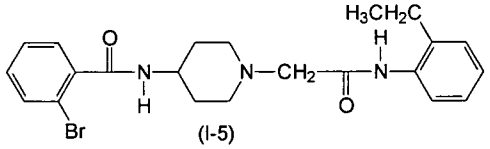
The following compounds of formulae (I-1) to (I-12) are employed in particularly preferred embodiments.

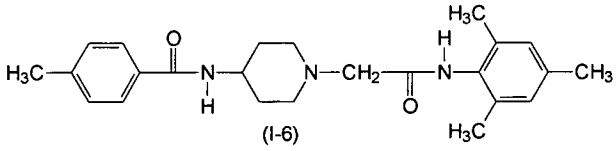

(I-1)

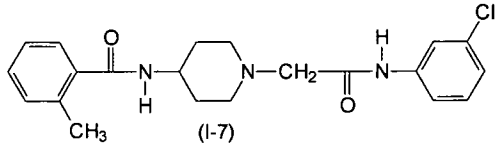

(I-2)

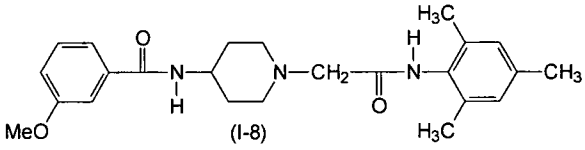

(I-3)

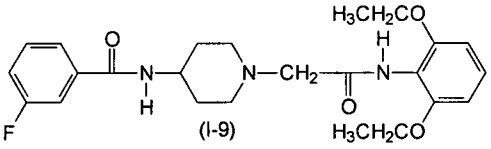

(I-4)

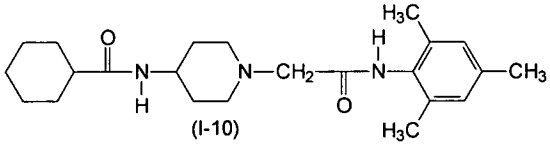

(I-5)

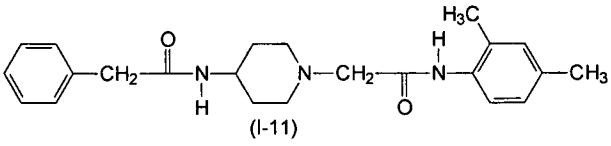

(I-6)

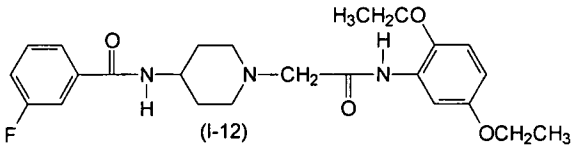

(I-7)


(I-8)


(I-9)


(I-10)


(I-11)


(I-12)

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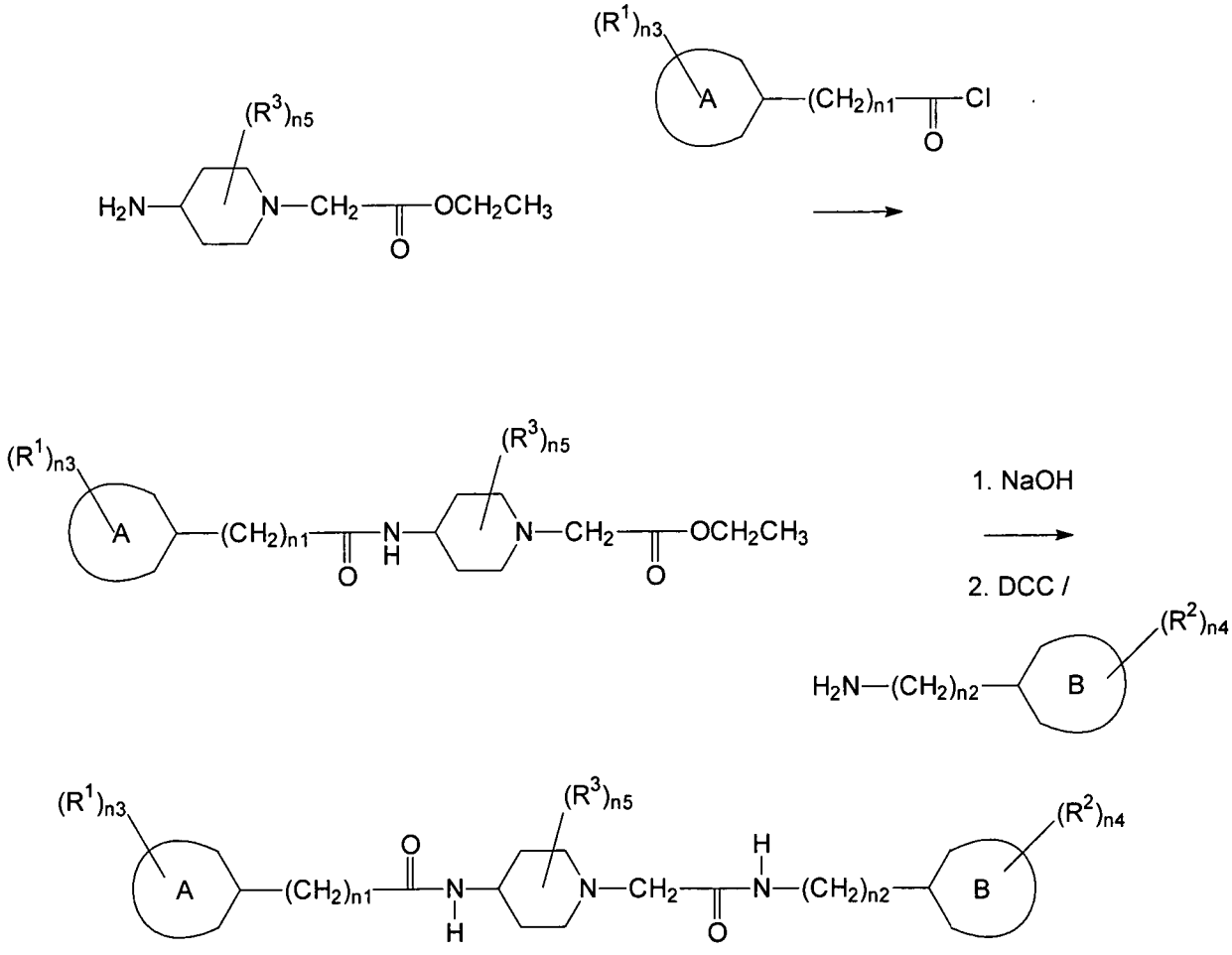
Among compounds of formulae (I-1) to (I-12), the compounds of formulae (I-1), (I-3), (I-9), (I-11) and (I-12) are particularly preferred, the compounds of formulae (I-1) and (I-11) are even more preferred and the compound of formula (I-1) is most preferred. The compound of formula (I-1) is also referred to as "T8".

Compounds falling under formula (I) are commercially available, for example from Asinex Europe B.V. (Rijswijk, The Netherlands). Further, a skilled chemist will be readily in a position to devise synthetic routes to compounds falling under formula (I).

Compounds of formula (I) can, for example, be obtained by the following synthetic route, involving two successive peptide couplings:



DCC stands for N,N'-dicyclohexyl-carbodiimide. The synthesis of the aminopiperdine used as a starting material is, for example, described in Walker et al., J. Org. Chem. (1961) 26, 2740.

Depending on the type of substitution in the target compounds falling under formula (I), the skilled person will employ suitable protection group chemistry, for example based on the principles laid out in P. G. M. Wuts, T. W. Greene "Greene's Protective Groups in Organic Synthesis" Wiley & Sons, 4th edition (2006).

Pharmaceutically acceptable salts of compounds that can be used in the present invention can be formed with various organic and inorganic acids and bases. Exemplary base addition salts comprise, for example, alkali metal salts such as sodium or potassium salts; alkaline-earth metal salts such as calcium or magnesium salts; ammonium salts; aliphatic amine salts such as trimethylamine, triethylamine, dicyclohexylamine, ethanolamine, diethanolamine, triethanolamine, procaine salts, meglumine salts, diethanol amine salts or ethylenediamine salts; aralkyl amine salts such as N,N-dibenzylethylenediamine salts, benetamine salts; heterocyclic aromatic amine salts such as pyridine salts, picoline salts, quinoline salts or isoquinoline salts; quaternary ammonium salts such as tetramethylammonium salts, tetraethylammonium salts, benzyltrimethylammonium salts, benzyltriethylammonium salts, benzyltributylammonium salts, methyltrioctylammonium salts or tetrabutylammonium salts; and basic amino acid salts such as arginine salts or lysine salts. Exemplary acid addition salts comprise, for example, mineral acid salts such as hydrochloride, sulfate salts, nitrate salts, phosphate salts, carbonate salts, hydrogencarbonate salts or perchlorate salts; organic acid salts such as acetate, propionate, lactate, maleate, fumarate, tararic acid salt, malate, citrate or ascorbate salts; sulfonate salts such as methanesulfonate, benzenesulfonate, or p-toluenesulfonate salts; and acidic amino acid salts such as aspartate or glutamate salts.

Pharmaceutically acceptable solvates of compounds that can be used in the present invention may exist in the form of solvates with water, for example hydrates, or with organic solvents such as methanol, ethanol or acetonitrile, i.e. as a methanolate, ethanolate or acetonitrilate, respectively.

Pharmaceutically acceptable prodrugs of compounds that can be used in the present invention are derivatives which have chemically or metabolically cleavable groups and become, by solvolysis or under physiological conditions, the compounds used in the present invention which are pharmaceutically active in vivo. Prodrugs of compounds that can be used in the present invention may be formed in a conventional manner with a functional group of the compounds such as with an amino, hydroxy or carboxy group. The prodrug derivative form often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, Bundgaard, H., Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well known to the person skilled in the art, such as, for example, esters prepared by reaction of the parent acidic compound with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a suitable amine. When a compound employed in the present invention has a carboxyl group, an ester derivative prepared by reacting the carboxyl group with a suitable alcohol or an amide derivative prepared by reacting the carboxyl group with a suitable amine is exemplified as a prodrug. An especially preferred ester derivative as a prodrug is methylester, ethylester, n-propylester, isopropylester, n-butylester, isobutylester, tert-butylester, morpholinoethylester or N,N-diethylglycolamidoester. When a compound employed in the present invention has a hydroxy group, an acyloxy derivative prepared by reacting the hydroxyl group with a suitable acylhalide or a suitable acid anhydride is exemplified as a prodrug. An especially preferred acyloxy derivative as a prodrug is $-O(=O)-CH_3$, $-OC(=O)-C_2H_5$, -OC(=O)-(tert-Bu), $-OC(=O)-C_{15}H_{31}$, -OC(=O)-(m-COONa-Ph), $-OC(=O)-CH_2CH_2COONa$, $-O(C=O)-CH(NH_2)CH_3$ or $-OC(=O)-CH_2-N(CH_3)_2$. When a compound employed in the present invention has an amino group, an amide derivative prepared by reacting the amino group with a suitable acid halide or a suitable mixed anhydride is exemplified as a prodrug. An especially preferred amide derivative as a prodrug is $-NHC(=O)-(CH_2)_2OCH_3$ or $-NHC(=O)-CH(NH_2)CH_3$.

While it is possible for the compounds of the present invention to be administered alone, it is preferable to formulate them into a pharmaceutical composition in accordance with standard pharmaceutical practice. Thus the invention also provides a pharmaceutical composition which comprises a therapeutically effective amount of a compound of formula (I) in admixture with a pharmaceutically acceptable excipient.

Pharmaceutically acceptable excipients are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1991). The pharmaceutical excipient can be selected with regard to the intended route of administration and standard pharmaceutical practice. The excipient must be acceptable in the sense of being not deleterious to the recipient thereof.

Pharmaceutically useful excipients that may be used in the formulation of the pharmaceutical composition of the present invention may comprise, for example, carriers, vehicles, diluents, solvents such as monohydric alcohols such as ethanol, isopropanol and polyhydric alcohols such as glycols and edible oils such as soybean oil, coconut oil, olive oil, safflower oil, cottonseed oil, oily esters such as ethyl oleate, isopropyl myristate, binders, adjuvants, solubilizers, thickening agents, stabilizers, disintegrants, glidants, lubricating agents, buffering agents, emulsifiers, wetting agents, suspending agents, sweetening agents, colorants, flavors, coating agents, preservatives, antioxidants, processing agents, drug delivery modifiers and enhancers such as calcium phosphate, magnesium state, talc, monosaccharides, disaccharides, starch, gelatine, cellulose, methylcellulose, sodium carboxymethyl cellulose, dextrose, hydroxypropyl-ß-cyclodextrin, polyvinylpyrrolidone, low melting waxes, and ion exchange resins.

The compounds of this invention and the pharmaceutical compositions as described herein are in particular useful in the medical intervention of proliferative or even hyperproliferative disorders/diseases, like cancers, tumor diseases and other diseases described herein below, like fibrotic diseases, autoimmune diseases, B-cell mediated diseases and B-cell mediated autoimmune diseases.

The routes for administration (delivery) of the compounds employed in the invention include, but are not limited to, one or more of: oral (e.g. as a tablet, capsule, or as an ingestible solution), topical, mucosal (e.g. as a nasal spray or aerosol for inhalation), nasal, parenteral (e. g. by an injectable form), gastrointestinal, intraperitoneal, intramuscular, intravenous, intrauterine, intraocular, subcutaneous, ophthalmic (including intravitreal or intracameral), rectal, buccal, and sublingual. Preferably, the route for administration is parenteral whereby the compounds may be administered using injection techniques or infusion techniques.

If the compounds of the present invention are administered parenterally, then examples of such administration include one or more of: intravenously, intraarterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intramuscularly or subcutaneously administering the compounds; and/or by using infusion techniques. For parenteral administration, the compounds are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The compounds of the present invention can also be administered orally in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavoring or coloring agents, for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release applications.

The tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycolate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included. Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the agent may be combined with various sweetening or flavoring agents, coloring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

Alternatively, the compounds of the present invention can be administered in the form of a suppository or pessary, or it may be applied topically in the form of a gel, hydrogel, lotion,

solution, cream, ointment or dusting powder. The compounds of the present invention may also be dermally or transdermally administered, for example, by the use of a skin patch.

They may also be administered by the pulmonary or rectal routes. They may also be administered by the ocular route. For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the present invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, 2-octyldodecanol, benzyl alcohol and water.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject. The specific dose level and frequency of dosage for any particular individual may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy.

A proposed, yet non-limiting dose of the compounds according to the present invention for administration to a human (of approximately 70 kg body weight) may be 0.1 mg to 1 g, preferably 1 mg to 500 mg of the active ingredient per unit dose. The unit dose may be administered, for example, 1 to 4 times per day. The dose will depend on the route of administration. It will be appreciated that it may be necessary to make routine variations to the dosage depending on the age and weight of the patient as well as the severity of the condition to

be treated. The precise dose and route of administration will ultimately be at the discretion of the attendant physician or veterinarian.

Accordingly, as also defined herein below, the compounds of the present invention are most preferably used in medical interventions, in particular in the treatment and/or prevention of proliferative or hyperproliferative diseases like cancers and/or tumors in a subject in need of such an intervention. Said subject may be a human as well as an animal, preferably a mammal. A particular gist of the present invention is the use of the compounds of this invention, i.e. 1,4-substituted piperidine derivatives and in particular the compounds of formula (I), preferably compounds I-1 to I-12 and most preferably compound I 1, in cotherapy, i.e. the use of these compounds in combination with other drugs/therapies used in the treatment and/or prevention of hyperproliferative disorders/diseases like cancers and/or tumors.

Cancerous or tumorous diseases that can be treated or prevented with the compounds employed in the present invention include breast (mamma) cancer, genitourinary cancer (for example, a prostate tumor, especially a hormone-refractory prostate tumor), lung cancer (for example, a small cell or non-small cell lung tumor), gastrointestinal cancer (for example, a colorectal tumor), epidermoid cancer (for example, an epidermoid head and/or neck tumor or a mouth tumor), melanoma, ovarian cancer, pancreas cancer, neuroblastoma, head and/or neck cancer, bladder cancer, renal cancer, brain cancer and gastric cancer. However, also lymphoid/lymphatic disorders may be treated with the compounds of this invention.

In a particularly preferred embodiment, the compounds employed in the present invention are used in combination with other therapeutic agents. When the compound is used in combination with a second therapeutic agent active against the same disease, the dose of each compound may differ from that when the compound is used alone. The combination of a compound of this invention with (an) other drug(s) may comprise the administration of said drug(s) with the compound of the invention. Such an administration may comprise simultaneous/concomitant administration. However, also sequential/separate administration is envisaged, as discussed also below.

Preferably, the second therapeutic agent to be administered in combination with the compound(s) of this invention is an anticancer drug, such as etoposide or doxorubicin which have been tested as shown in the examples. The anticancer drug to be administered in combination with the compound(s) of this invention may be a tumor angiogenesis inhibitor (for example, a protease inhibitor, an epidermal growth factor receptor kinase inhibitor, or a vascular endothelial growth factor receptor kinase inhibitor); a cytotoxic drug (for example, an antimetabolite, such as purine and pyrimidine analogue antimetabolites); an antimitotic agent (for example, a microtubule stabilizing drug or an antimitotic alkaloid); a platinum coordination complex; an anti-tumor antibiotic; an alkylating agent (for example, a nitrogen mustard or a nitrosourea); an endocrine agent (for example, an adrenocorticosteroid, an androgen, an anti-androgen, an estrogen, an anti-estrogen, an aromatase inhibitor, a gonadotropin-releasing hormone agonist, or a somatostatin analogue); or a compound that targets an enzyme or receptor that is overexpressed and/or otherwise involved in a specific metabolic pathway that is misregulated in the tumor cell (for example, ATP and GTP phosphodiesterase inhibitors, histone deacetylase inhibitors, protein kinase inhibitors (such as serine, threonine and tyrosine kinase inhibitors (for example, Abelson protein tyrosine kinase)) and the various growth factors, their receptors and kinase inhibitors therefor (such as epidermal growth factor receptor kinase inhibitors, vascular endothelial growth factor receptor kinase inhibitors, fibroblast growth factor inhibitors, insulin-like growth factor receptor inhibitors and platelet-derived growth factor receptor kinase inhibitors)); methionine, aminopeptidase inhibitors, proteasome inhibitors, cyclooxygenase inhibitors (for example, cyclooxygenase-1 or cyclooxygenase-2 inhibitors) and topoisomerase inhibitors (for example, topoisomerase I inhibitors or topoisomerase II inhibitors).

An alkylating agent which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, a nitrogen mustard (such as cyclophosphamide, mechlorethamine (chlormethine), uramustine, melphalan, chlorambucil, ifosfamide, bendamustine, or trofosfamide), a nitrosourea (such as carmustine, streptozocin, fotemustine, lomustine, nimustine, prednimustine, ranimustine, or semustine), an alkyl sulfonate (such as busulfan, mannosulfan, or treosulfan), an aziridine (such as hexamethylmelamine (altretamine), triethylenemelamine, ThioTEPA (N,N'N'-triethylenethiophosphoramide), carboquone, or

triaziquone), a hydrazine (such as procarbazine), a triazene (such as dacarbazine), or an imidazotetrazines (such as temozolomide).

A platinum coordination complex which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin, or triplatin tetranitrate.

A cytotoxic drug which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, an antimetabolite, including folic acid analogue antimetabolites (such as aminopterin, methotrexate, pemetrexed, or raltitrexed), purine analogue antimetabolites (such as cladribine, clofarabine, fludarabine, 6-mercaptopurine (including its prodrug form azathioprine), pentostatin, or 6-thioguanine), and pyrimidine analogue antimetabolites (such as cytarabine, decitabine, 5-fluorouracil (including its prodrug forms capecitabine and tegafur), floxuridine, gemcitabine, enocitabine, or sapacitabine).

An antimitotic agent which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, a taxane (such as docetaxel, larotaxel, ortataxel, paclitaxel/taxol, or tesetaxel), a Vinca alkaloid (such as vinblastine, vincristine, vinflunine, vindesine, or vinorelbine), an epothilone (such as epothilone A, epothilone B, epothilone C, epothilone D, epothilone E, or epothilone F) or an epothilone B analogue (such as ixabepilone/azaepothilone B).

An anti-tumor antibiotic which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, an anthracycline (such as aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, amrubicin, pirarubicin, valrubicin, or zorubicin), an anthracenedione (such as mitoxantrone, or pixantrone) or an anti-tumor antibiotic isolated from Streptomyces (such as actinomycin (including actinomycin D), bleomycin, mitomycin (including mitomycin C), or plicamycin).

A tyrosine kinase inhibitor which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, axitinib, bosutinib, cediranib,

dasatinib, erlotinib, gefitinib, imatinib, lapatinib, lestaurtinib, nilotinib, semaxanib, sorafenib, sunitinib, or vandetanib.

A topoisomerase-inhibitor which can be used as an anticancer drug in combination with a compound of the present invention may be, for example, a topoisomerase I inhibitor (such as irinotecan, topotecan, camptothecin, belotecan, rubitecan, or lamellarin D) or a topoisomerase II inhibitor (such as amsacrine, etoposide, etoposide phosphate, teniposide, or doxorubicin).

Further anticancer drugs may be used in combination with a compound of the present invention, said anticancer drugs may comprise biological or chemical molecules, like TNF-related apoptosis-inducing ligand (TRAIL), tamoxifen, amsacrine, bexarotene, estramustine, irofulven, trabectedin, cetuximab, panitumumab, tositumomab, alemtuzumab, bevacizumab, edrecolomab, gemtuzumab, alvocidib, seliciclib, aminolevulinic acid, methyl aminolevulinate, efaproxiral, porfimer sodium, talaporfin, temoporfin, verteporfin, alitretinoin, tretinoin, anagrelide, arsenic trioxide, atrasentan, bortezomib, carmofur, celecoxib, demecolcine, elesclomol, elsamitrucin, etoglucid, lonidamine, lucanthone, masoprocol, mitobronitol, mitoguazone, mitotane, oblimersen, omacetaxine, sitimagene, ceradenovec, tegafur, testolactone, tiazofurine, tipifarnib, and vorinostat.

Also biological drugs, like antibodies, antibody fragments, antibody constructs (for example, single-chain constructs), and/or modified antibodies (like CDR-grafted antibodies, humanized antibodies, "full humanized" antibodies, etc.) directed against cancer or tumor markers/factors/cytokines involved in proliferative diseases can be employed in co-therapy approaches with the compounds of the invention. Examples of such biological molecules are anti-HER2 antibodies (e.g. trastuzumab, Herceptin®), anti-CD20 antibodies (e.g. Rituximab, Rituxan®, MabThera®, Reditux®), anti-CD19/CD3 constructs (see, e.g., EP-B1 1071752) and anti-TNF antibodies (see, e.g., Taylor PC. Antibody therapy for rheumatoid arthritis. Curr Opin Pharmacol. 2003. 3(3):323-328). Further antibodies, antibody fragments, antibody constructs and/or modified antibodies to be used in co-therapy approaches with the compounds of the invention can be found in Taylor PC. Curr Opin Pharmacol. 2003. 3(3):323-328; Roxana A. Maedica. 2006. 1(1):63-65.

When the compounds of the present invention are used in combination with anticancer drugs, antiproliferative drugs, cytostatic drugs or cytotoxic drugs, the percentage of apoptotic cells unexpectedly increases when compared to the respective drug(s) alone. The percentage of apoptotic cells can be determined as illustrated in the examples, using the tests and testing systems described therein. The compounds of the invention used in combination with the above mentioned further (anti-proliferative, anticancer, cytostatic and/or cytotoxic) drugs thus provide a considerable improvement. In other words, the compounds of the invention used in combination with the above mentioned drugs possess highly advantageous features as compared to the respective drugs alone. Yet, it is also envisaged that the compounds of this invention may be used as singular and individual treatment of proliferative/hyperproliferative disorders/diseases, like cancers and/or tumors or hyperproliferative B-cell diseases and/or autoimmune diseases.

In another particularly preferred embodiment, the compounds employed in the present invention are administered in combination with physical therapy, such as radiotherapy. Radiotherapy may commence before, after, or simultaneously with administration of the compounds. For example, radiotherapy may commence 1-10 minutes, 1-10 hours or 24-72 hours after administration of the compounds. Yet, these time frames are not to be construed as limiting. The subject is exposed to radiation, preferably gamma radiation, whereby the radiation may be provided in a single dose or in multiple doses that are administered over several hours, days and/or weeks. Gamma radiation may be delivered according to standard radiotherapeutic protocols using standard dosages and regimens. Again, and without being bound by theory, the compounds of the present invention may be used to render cells, in particular undesired proliferative/hyperproliferative cells like cancer or tumor cells, more susceptible to such a physical therapy, e.g. radiotherapy.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation. The individual components of such combinations may be administered either sequentially or simultaneously/concomitantly in separate or combined pharmaceutical formulations by any convenient route. When administration is sequential, either the present compound or the second therapeutic agent may be administered first. When

administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition. When combined in the same formulation it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the formulation. When formulated separately they may be provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

The pharmaceutical compositions of the invention can be produced in a manner known per se to the skilled person as described, for example, in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1991).

The compounds employed in the present invention can be used for treating or preventing a proliferative/hyperproliferative disease, such as a tumorous disease, a non-solid cancer and/or metastases. Examples of the non-solid cancer include leukaemia or a lymphoid cancer, which can be a B-cell malignancy, such as B-cell lymphoma, non-Hodgkin lymphoma or B-cell derived chronic lymphatic leukemia (B-CLL). Examples of the proliferative/hyperproliferative disease include hyperplasis, fibrosis, angiogenesis, psoriasis, atherosclerosis or smooth muscle proliferation in the blood vessels. Further proliferative/hyperproliferative diseases that can be treated or prevented using the compounds employed in the present invention include hyperplasis, fibrosis (for example, pulmonary or renal fibrosis), angiogenesis, psoriasis, atherosclerosis or smooth muscle proliferation in the blood vessels (for example, stenosis or restenosis following angioplasty).

The compounds employed in the present invention can further be used for treating or preventing autoimmune diseases, in particular B-cell mediated autoimmune diseases, such as Myasthenia gravis, Morbus Basedow, Hashimoto thyreoiditis or Goodpasture syndrome.

In a further aspect, the present invention relates to the use of a compound of formula (I) as defined above for the preparation of a medicament for the treatment or prevention of a hyperproliferative disease. In yet a further aspect, the present invention relates to a method for treating or preventing a hyperproliferative disease, which comprises administering to a patient

or a subject in need thereof an effective amount of a compound of formula (I). Said patient/subject may be a human.

Again, also in these (non-cancerous, non-tumorous) disorders co-therapy approaches are envisaged, wherein the compounds of this invention can be used in combination with other drugs employed in the medical intervention of these proliferative or hyperproliferative disorders. The corresponding co-therapeutic embodiments as described herein above for the medical intervention in cancerous and/or tumorous diseases applies here mutatis mutandis.

The term "treatment of a disorder or disease" as used herein is well known in the art. "Treatment of a disorder or disease" implies that a disorder or disease has been diagnosed in a patient/subject. A patient/subject suspected of suffering from a disorder or disease typically shows specific clinical and/or pathological symptoms which a skilled person can easily attribute to a specific pathological condition (i.e. diagnose a disorder or disease).

"Treatment of a disorder or disease" may, for example, lead to a halt in the progression of the disorder or disease (e.g. no deterioration of symptoms) or a delay in the progression of the disorder or disease (in case the halt in progression is of a transient nature only). "Treatment of a disorder or disease" may also lead to a partial response (e.g. amelioration of symptoms) or complete response (e.g. disappearance of symptoms) of the subject/patient suffering from the disorder or disease. Such a partial or complete response may be followed by a relapse. It is to be understood that a subject/patient may experience a broad range of responses to a treatment (e.g. the exemplary responses as described herein above).

Treatment of a disorder or disease may, inter alia, comprise curative treatment (preferably leading to a complete response and eventually to healing of the disorder or disease) and palliative treatment (including symptomatic relief).

Also the term "prevention" as used herein is well known in the art. For example, a patient/subject suspected of being prone to suffer from a disorder or disease as defined herein may, in particular, benefit from a prevention of the disorder or disease. Said subject/patient may have a susceptibility or predisposition for a disorder or disease, including but not limited

to hereditary predisposition. Such a predisposition can be determined by standard assays, using, for example, genetic markers or phenotypic indicators. It is to be understood that a disorder or disease to be prevented in accordance with the present invention has not been diagnosed or cannot be diagnosed in said patient/subject (for example, said patient/subject does not show any clinical or pathological symptoms). Thus, the term "prevention" comprises the use of compounds of the present invention before any clinical and/or pathological symptoms are diagnosed or determined or can be diagnosed or determined by the attending physician.

The term "chemosensitizing effect of a compound" as used herein is well-known in the art and means that said compound renders cells, like cancer or tumor cells or other undesired proliferative cells, more sensitive, i.e. more vulnerable, to the effects of anti-proliferative drugs, anticancer drugs, cytostatic and/or cytotoxic drugs, as it is illustrated for the compounds of the invention in the appended examples. However, this also relates to the fact that compounds of this invention may render cancer, tumor or other (undesired) proliferative cells more sensitive to physical therapy, like radiotherapy.

Without being bound by theory, it is believed that the compounds of formula (I) target the BIR 3 domain of XIAP, thereby promoting apoptosis. The chemical structure of the compounds of formula (I) differs from SMAC mimetics which represent tripeptide entities as well as polyphenylureas (Schimmer, Cancer Cell (2004), 5(1), 25-35; Huang et al., Cancer Cell (2994), 5(1), 1-2; Carter et al., Blood (2005), 105(10), 4043-4050), embelin (Nikolovska-Coleska et al., J. Med. Chem. (2004), 47(10), 2430-2440; Chen et al., Bioorg. Med. Chem. Lett. (2006), 16(22), 5805-5808) or a tetrazoyl thioether C2 diyne (Li et al., Science (2004), 305(5689), 1471-1474). The compounds of formula (I) are simple and easy to synthesize. Furthermore and as shown, e.g., for compound I-1 in Figure 9, they possess no significant toxicity even at high concentrations which is a major advantage regarding the therapeutical use and possible side effects. As compared to other XIAP antisense drugs which are presently in phase I/II clinical trials (Cummings et al., Br. J. Cancer (2005), 92(3), 532-538; La Casse et al., Clin. Cancer Res. (2006), 12(17), 5231-5241) the compounds of formula (I), being small molecule inhibitors, possess advantages such as better pharmacokinetic profiles in general.

Without being bound by theory, it is believed that the compounds of formula (I) have a cytotoxic, cytostatic and/or a chemosensitizing effect in certain proliferative diseases, in particular in cancer and/or tumorous diseases. In particular, the chemosensitizing effect of the compounds of this invention, i.e. 1,4-substituted piperidine derivatives and in particular the compounds of formula (I), preferably compounds I-1 to I-12 and most preferably compound I-1, is illustrated herein and documented in the appended examples. A person skilled in the art is readily in a position to measure the chemosensitizing effect of the compounds of this invention, in particular when the compounds of this invention are used in combination with other drugs, in particular anti-proliferative drugs, anticancer drugs, cytostatic drugs and/or cytotoxic drugs. Such tests may comprise in vitro as well as in vivo tests on test animals. The appended examples illustrate corresponding tests and test systems. These tests and test systems can easily be adopted for further evaluation and are within the normal skill of the artisan. For example, cultured cells, like cancer cells or also cultured primary tumor cells may be used to validate and assess the combinatory effect of the compounds of this invention with any given other drug used in the treatment of hyperproliferative disorders. The person skilled in the art can easily obtain corresponding (cultured) cells, for example from corresponding depositories, like the ATCC, DSMZ Braunschweig, Centraalbureau voor Schimmelcultures (CBS) and the like. As shown in the appended examples, such cells comprise Jurkat cells, LnCAP (prostate cancer) cells, breast cancer cells, like MDAMB231 cells, and pancreatic cancer cells, like L3.6pl cells. Other cell lines comprise, but are not limited to, Sk-Br3 cells, CaMa-1 cells, MCF-7 cells (as breast cancer cell examples), DU 145 cells (for example urogenital), HeLa cells, HEK293 cells, DAUDI cells, BJAB (Burkitt's lymphoma) Raji cells, and the like. Also cultured and transformed cells may be employed in corresponding validation assays. Furthermore, animal models may be used to validate the chemosensitizing effect of the compounds of this invention for other anticancer/anti-proliferative drugs. Such animal models may comprise, but are not limited to, the nude mouse model (comprising xenografts of tumors), the SCID mouse models (also comprising, as desired, xenografts of tumors/tumor cells/cancer cells), diverse transgenic mouse models for tumorous diseases and B-cell disorders or lymphomas. Yet, the most commonly employed models are xenografts of human tumors grown subcutaneously in immunodeficient mice such as athymic (nude) or severe combined immune deficient (SCID) mice.

The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

The invention is also illustrated by the following illustrative figures. The appended figures show:

Figure 1: Compound I-1 ("I-1") sensitizes different Jurkat cell lines towards etoposide-induced apoptosis. Compound I-1 was tested in wild type human leukemia Jurkat T cells (**A**), as well as in Jurkat cells stably transfected with empty vector (**B**), full-length XIAP overexpressing Jurkat cells (**C**) or the BIR3/Ring domain overexpressing Jurkat cells (**D**). Cell death was evaluated as described under "Materials and Methods". Further testing yielded very good P-values. *Bars*, the mean ± SE of three independent experiments performed in triplicate. *** $P<0.001$ (Anova/Bonferroni).

Figure 2: Compound I-1 ("I-1") sensitizes to a reduction of etoposide-induced clonogenic survival. Wild type Jurkat cells were left untreated (**A**) or treated for 1h with 25μM of compound I-1 (**B**), 500nM etoposide (**C**) or a combination of both (**D**). After replacement into fresh methylcellulose medium, cells were incubated for 7 days. Afterwards colony number was counted (**E**). *Bars*, the mean ± SE of three independent experiments. *** $P<0.001$ (Anova/Bonferroni).

Figure 3: Compound I-1 ("I-1") increases initiator caspase activities. Wild type Jurkat cells were left untreated or treated with 25μM of compound I-1, 500nM etoposide or a combination of both for the indicated time periods. Fluorescence measurements were performed for caspase-9 (**A**) and caspase-3 (**B**). Protein concentration was used for normalization. *Bars*, the mean ± SE of three independent experiments performed in triplicate. *** $P<0.001$ (Anova/Bonferroni).

Figure 4: Compound I-1 ("I-1") activates initiator caspases and induces PARP cleavage. Wild type Jurkat cells were left untreated or treated with 25μM of compound I-1, 500nM etoposide or a combination of both for the indicated time periods. Expression of caspase-9, caspase-3 and

PARP was assessed by Western Blot analysis. Cleavage products are indicated by arrows. Ponceau S was used as loading control. A representative experiment out of three independent experiments is shown.

Figure 5: XIAP protein level is reduced by compound I-1 ("I-1"). Cells were left untreated (CO) or treated with 25µM of compound I-1, 500nM etoposide or with combination of both for the indicated time periods. XIAP protein level was detected by Western Blot analysis. Ponceau S was used as loading control. A representative experiment out of three independent experiments is shown.

Figure 6: Testing of derivatives of compound I-1 ("T8") for their apoptosis induction and sensitizing potential. Wild type Jurkat cells were left untreated (control) or treated with 25µM test compound (see Table 2 in the Examples section for concordance between compounds of formula (I) and internal numbers), 500nM etoposide or with combination of both. Cell death was evaluated as described under "Materials and Methods". Similar data can be obtained with I-12 (not shown) as with I-9 ("T8-14"). Data are the mean of three independent experiments performed in triplicate. Statistical analysis was performed with ANOVA with Bonferroni multiple comparison post test: 500nM etoposide vs. derivatives of compound I-1 and 500nM etoposide. Further testing yielded very good P-values. * P-value<0,05; ** P<0,01; *** P<0,001.

Figure 7: Compound I-1 ("I-1") sensitizes different tumor cells to low dose of doxorubicin. Compound I-1 was tested in prostate cancer cells LnCAP (**A**) or mamma carcinoma cells MDAMB231 (**B**) for 48h. *% apoptotic cells*, percentage of cells with subdiploid DNA content. *Bars*, the mean ± SE of three independent experiments.

Figure 8: Combination of compound I-1 ("T8") and etoposide ("eto") induces apoptosis and decreases clonogenic survival in pancreas L3.6pl cancer cells. (**A**) Compound I-1 was tested in pancreas cancer cells L3.6pl for 48h. *% apoptotic cells*, percentage of cells with subdiploid DNA content. (**B**) L3.6pl cells were seeded in 6-well plates (0.2x105 cells/well) and stimulated as indicated for 24h. Afterwards cells were washed with PBS, left to grow for 6 days and stained with crystal violet (representative pictures in upper panel). Afterwards intracellular

crystal violet was solved and absorption at 550 nm was measured (lower insert). Untreated control cells were set as 100%. *Bars*, the mean ± SE of three independent experiments.

Figure 9: Compound I-1 ("I-1") in the same combination of the drugs as for tumor cells is not toxic for primary endothelial cells (HUVECs). Compound I-1 and cytostatics (etoposide or doxorubicin) were tested in HUVECs (Human Umbilical Vein Endothelial Cells) for 48h. The cell viability was indicated in MTT assay. Briefly, cells were incubated for 1h with tetrazolium salt MTT. After that, cells were lysed by adding DMSO to each well and shaking the plates for 1h. The absorption of the solubilised formazan crystals was measured at 550 nm in a microplate absorbance reader (Sunrise™, Tecan, Crailsheim, Germany). Untreated control cells were set as 100%. *Bars*, the mean ± SE of three independent experiments.

Examples

Materials and Methods

Test Compounds. The test compounds were purchased from Asinex Europe B.V. (Rijswijk, The Netherlands). Before application, the compounds to be tested were dissolved and further diluted in DMSO. Final DMSO concentration did not exceed 1%, a concentration verified not to interfere with the experiments performed. Etoposide was purchased from Merck Biosciences (Darmstadt, Germany), propidium iodide (PI) from Sigma (Deisenhofen, Germany).

Cell Culture. Human leukemia Jurkat T cells (clone J16), Jurkat T-cells stably transfected with either empty vector (Jurkat vector, pBabe Jurkat) or XIAP (Jurkat-XIAP) as well as the BIR-3 domain only (BIR-3-sp-RING-Jurkat) (kindly provided by Dr. Colin Duckett at the University of Michigan) were cultured (37°C and 5% CO_2) in RPMI 1640 containing 2 mM L-glutamine (PAN Biotech, Aidenbach, Germany) supplemented with 10% FCS gold (PAA Laboratories, Cölbe, Germany) and 1% pyruvate (Sigma Deisenhofen, Germany).

Detection and Quantification of Apoptosis. Quantification of apoptosis was performed according to Nicoletti *et al.* (J Immunol. Methods (1991) 139, 271-279). Briefly, cells were

incubated for 24 h in a hypotonic buffer (0.1% sodium citrate, 0.1% Triton X-100 and 50 µg/ml PI) and analyzed by flow cytometry on a FACSCalibur (Becton Dickinson, Heidelberg, Germany). Nuclei to the left of the G_1-peak containing hypodiploid DNA were considered apoptotic.

Western Blot Analysis. Cells were collected by centrifugation, washed with ice-cold PBS and lysed in 1% Triton X-100, 0.15 M NaCl and 30 mM Tris-HCl pH 7.5 with the protease inhibitor complete™ (Roche, Mannheim, Germany) for 30 min. Lysates were centrifuged at 10,000xg for 10 min at 4°C. Equal amounts of protein were separated by SDS-PAGE (12% for XIAP) and transferred to polyvinylidene difluoride membranes (Immobilon-P™, Millipore, Eschborn, Germany). Equal protein loading was controlled by Coomassie staining of gels. Membranes were blocked with 5% fat-free milk powder in PBS containing 0.05% Tween-20 (1 h) and incubated with specific antibodies against XIAP (mouse antibody IgG1, clone 28, BD Transduction Laboratories, Heidelberg, Germany) overnight at 4°C. Specific proteins were visualized by secondary antibodies conjugated to horseradish peroxidase and the ECL Plus™ Western Blotting detection reagent (Amersham Biosciences, Freiburg, Germany). Membranes were exposed to X-ray film for the appropriate time periods and subsequently developed in a tabletop film processor (Curix 60, Agfa, Cologne, Germany). Equal protein loading was controlled by Ponceau S staining of membranes.

Clonogenic assay. Wildtype Jurkat cells were left untreated or treated for 1h with 25µM test compound, 500nM etoposide or a combination of both. Subsequently, cells were washed with PBS and resuspended in culture medium (5x105 cells/ml). Cell suspensions were diluted 1:10 with methylcellulose (0.52%) medium containing 40% FCS. Cells were seeded in 96-well plates (100 µl) and colonies were scored after 7 days of culture.

Caspase-9/3 activity assay. Caspase-9/3 activity was measured using caspase-9(Ac-LEHD-AFC) or caspase 3(Ac-DEVD-AFC) substrates (both from Bachem AG, Bubendorf, Germany). Jurkat leukemia T cells were left untreated or treated with test compound, etoposide or a combination of both for the indicated time period. Cells were collected by centrifugation, washed with ice-cold PBS and lysed at –85°C in 5mM $MgCl_2$, 1mM EGTA, 0,1% Triton X-100, 25mM HEPES, pH 7.5. Afterwards lysates were centrifuged (14,000 rpm, 40°C) and

supernatants were incubated with caspase substrate in 96-well plates. The reading was performed in a plate-reading multifunction photometer (SPECTRAFLUOR PLUS, Tecan, Crailsheim, Germany). The activity was calculated from the difference between fluorescence in the 0 point and after 1h (for caspase-3) or after 3h (for caspase-9). Protein concentration was used for normalization.

Statistical analysis. All experiments were performed at least three times in triplicate. Results are expressed as mean value ± SE (standard error). One-way ANOVA with Bonferroni's post test was performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego California, USA). One-way ANOVA (analysis of variance) tests compare two or more, usually three or more, independent groups whereby the data are categorized in one way. The Bonferroni correction states that if an experimenter is testing n dependent or independent hypotheses on a set of data, then one way of maintaining the familywise error rate is to test each individual hypothesis at a statistical significance level of 1/n times what it would be if only one hypothesis were tested. Statistically significant simply means that a given result is unlikely to have occurred by chance. Herein, the level of significance was chosen as $P<0.05$, for the probability of error to be within 5%. Accordingly, P-values < 0.05 were considered significant.

Results

Compounds were first tested for their apoptotic potential in Jurkat cells alone as well as in combination with sub-optimal concentrations of etoposide. Table 1 shows the percentage of apoptotic cells including standard deviation. The results of the cell tests revealed a distinct apoptosis enhancing effect for compound I-1. Whereas 25 µM of compound I-1 and 0.75 µM etoposide, respectively, showed some apoptotic effects themselves, their combination strongly induced cell death (65.7% apoptotic cells).

Table 1: Percentage of apoptotic Jurkat cells measured after application of medium control, 0.75 μM etoposide, 25 μM test substance (compound I-1), and a mixture of 0.75 μM etoposide and 25 μM test substance (compound I-1), respectively.[a]

Compound	Control (DMSO)	Etoposide 0.75μM	Test substance 25μM	Etoposide 0.75μM and Test substance 25μM
I-1	9.07±1.47	30.66±9.89	13.16±2.28	65.74±6.0

[a] mean value and standard deviation ($C.I._{95}$); $n = 3$

In a further step Jurkat cells either overexpressing the full length XIAP gene or the smaller BIR3 domain were used to gain insights on the mechanism of action of the compounds of the present invention. Compound I-1 was tested alone or with low dose of etoposide in wild type Jurkat cells and Jurkat cells overexpressing full-length XIAP or BIR-3/Ring. Clearly compound I-1 potentiates apoptosis induced by subtoxic concentrations of etoposide (Figure 1).

Compound I-1 does not only sensitize etoposide-treated cells for apoptotic cell death, it also sensitizes for reduction of clonogenic survival of etoposide treated Jurkat cells (Figure 2).

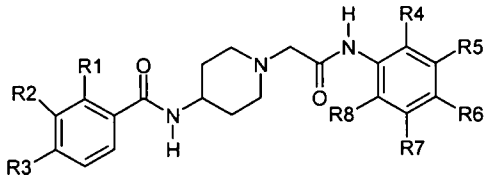
Caspase activities induced by etoposide are significantly increased by compound I-1 (Figure 3). Since caspase-3 is activated by caspase-9 which is targeted by the BIR-3 domain, compound I-1 not only potentiates etoposide induced caspase-9 but also the downstream effector caspase-3 activity.

Figure 4 confirms the caspase-activity data by showing the strong impact of compound I-1 on cleavage of caspase-9 and -3 proforms as well as PARP the substrate of caspase-3 induced by etoposide in a time dependent manner.

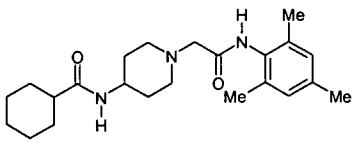
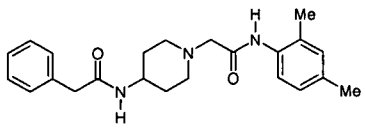
Figure 5 shows another interesting feature of compound I-1. Compound I-1 alone as well as in combination with etoposide is able to reduce XIAP protein after 16 and 24 h.

In addition to compound I-1, derivatives thereof were investigated.

Table 2: Derivatives of compound I-1.



Compound	Internal number	R1	R2	R3	R4	R5	R6	R7	R8
I-1	T8	H	F	H	Et	H	H	H	Et
I-2	T8-1	H	F	H	Me	H	H	Me	H
I-3	T8-2	Br	H	H	Et	H	H	H	Et
I-4	T8-3	H	F	H	H	Me	H	Me	H
I-5	T8-4	Br	H	H	Et	H	H	H	H
I-6	T8-9	H	H	Me	Me	H	Me	H	Me
I-7	T8-12	Me	H	H	H	Cl	H	H	H
I-8	T8-13	H	OMe	H	Me	H	Me	H	Me
I-9	T8-14	H	F	H	OEt	H	H	H	OEt

Compound I-10	Compound I-11
(internal number: T8-17)	(internal number: T8-18)
	

Derivatives of compound I-1 were tested for apoptosis-induction. Compounds (see Table 2; internal numbers for derivatives of compound I-1 have also been used in appended Figure 6) were either tested alone or in combination with suboptimal concentration of etoposide. As shown in Figure 6, a combination of the present compounds and etoposide significantly increases the percentage of apoptotic cells as compared to etoposide alone and the effect is

clearly more than additive. In addition, compounds falling within the scope of the present invention can induce apoptosis themselves suggesting additional targets. Without being bound by theory, these results indicate that inhibition of XIAP using the present compounds may overcome the protective effect of XIAP protein to cells and, therefore, sensitizes cells to etoposide induced apoptosis.

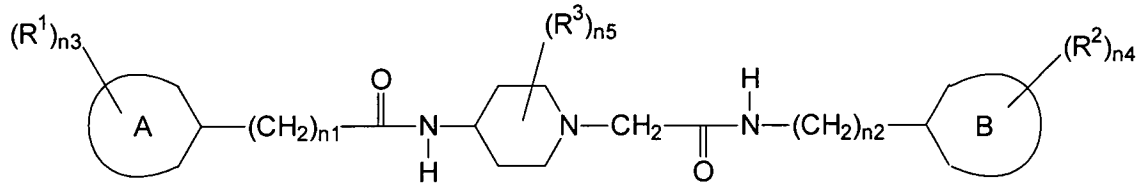
Without being bound by theory, the above pharmacological data indicates XIAP as a potential target upon which the apoptotic effects of the present compounds may be based.

As shown in Figure 7, compound I-1 in combination with doxorubicin increases significantly the percentage of apoptotic cells as compared to doxorubicin alone, whereby the increase is unexpectedly high.

Figure 8 shows that a combination of compound I-1 and etoposide yields an increase in the percentage of apoptotic cells which is more than additive as compared to each of compound I-1 and etoposide alone. Therefore, compound I-1 in combination with etoposide proves highly advantageous over etoposide alone, i.e., compound I-1 in combination with etoposide clearly is an improvement over etoposide alone.

CLAIMS

1. A compound of formula (I)



(I)

wherein

A is a saturated, unsaturated or aromatic 5 to 10-membered mono- or bicyclic ring system, which optionally contains one or more heteroatoms, selected from N, O and S;

B is a saturated, unsaturated or aromatic 5 to 10-membered mono- or bicyclic ring system, which optionally contains one or more heteroatoms, selected from N, O and S;

each R^1 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen;

each R^2 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen;

each R^3 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy, C_{6-10} aryl, SO_2NH-C_{1-4} alkyl, SO_2NH-C_{6-10} aryl and halogen;

n1 is an integer from 0 to 3;

n2 is an integer from 0 to 3;

n3 is an integer from 0 to 3;

n4 is an integer from 0 to 5; and

n5 is an integer from 0 to 3,

wherein in each aryl group one or more carbon atoms may optionally be replaced by a heteroatom selected from N, O and S,

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable solvate thereof or a pharmaceutically acceptable prodrug thereof,

for use as a medicament.

2. The compound of claim 1, wherein A is a saturated or aromatic 6-membered carbocyclic ring.

3. The compound of claim 1 or 2, wherein B is an aromatic 6-membered carbocyclic ring.

4. The compound of any of claims 1 to 3, wherein each R^1 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy and halogen.

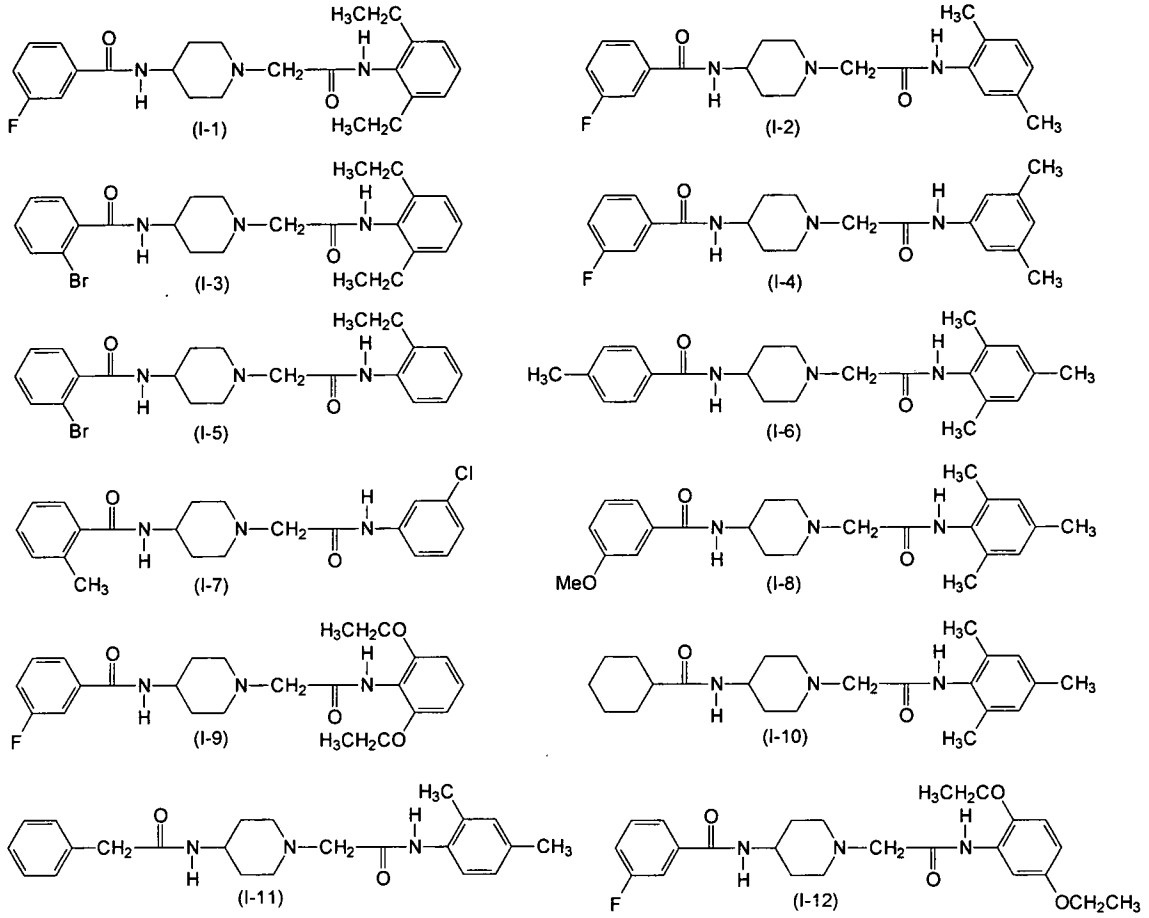
5. The compound of any of claims 1 to 4, wherein each R^2 is independently selected from C_{1-4} alkyl, C_{1-4} alkoxy and halogen.

6. The compound of any of claims 1 to 5, wherein n1 is 0 or 1.

7. The compound of any of claims 1 to 6, wherein n2 is 0.

8. The compound of any of claims 1 to 7, wherein n5 is 0.

9. The compound of claim 1, wherein the compound of formula (I) has one of the following formulae (I-1) to (I-12):



or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable solvate thereof or a pharmaceutically acceptable prodrug thereof.

10. The compound of any of claims 1 to 9 for use in treating or preventing a hyperproliferative disease.

11. Use of a compound as defined in any of claims 1 to 9 for the preparation of a medicament for the treatment or prevention of a hyperproliferative disease.

12. A method for treating or preventing a hyperproliferative disease comprising the administration of a compound as defined in any of claims 1 to 9 to a subject in need of such a treatment or prevention.

13. The compound of claim 10 or the use of claim 11 or the method of claim 12, wherein the hyperproliferative disease is a tumorous disease, a non-solid cancer and/or metastases.

14. The compound of claim 13 or the use of claim 13 or the method of claim 13, wherein the non-solid cancer is leukaemia or a lymphoid cancer.

15. The compound of claim 14 or the use of claim 14 or the method of claim 14, wherein the lymphoid cancer is a B-cell malignancy.

16. The compound of claim 15 or the use of claim 15 or the method of claim 15, wherein the B-cell malignancy is B-cell lymphoma, non-Hodgkin lymphoma or B-cell derived chronic lymphatic leukemia (B-CLL).

17. The compound of claim 10 or the use of claim 11 or the method of claim 12, wherein the hyperproliferative disease is hyperplasis, fibrosis, angiogenesis, psoriasis, atherosclerosis or smooth muscle proliferation in the blood vessels.

18. The compound of any of claims 1 to 9 for use in treating or preventing B-cell mediated autoimmune diseases.

19. Use of a compound as defined in any of claims 1 to 9 for the preparation of a medicament for the treatment or prevention of B-cell mediated autoimmune diseases.

20. A method for treating or preventing B-cell mediated autoimmune diseases comprising the administration of a compound as defined in any of claims 1 to 9 to a subject in need of such a treatment or prevention.

21. The compound of claim 18 or the use of claim 19 or the method of claim 20, wherein the B-cell mediated autoimmune disease is Myasthenia gravis, Morbus Basedow, Hashimoto thyreoiditis or Goodpasture syndrome.

22. The compound of any of claims 1 to 10 and 13 to 17 or the use of any of claims 11 and 13 to 17 or the method of any of claims 12 to 17, whereby said compound is to be administered in combination with an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy.

23. The method of any of claims 12 to 17 and 20 to 22, wherein said subject is a human.

Fig. 1

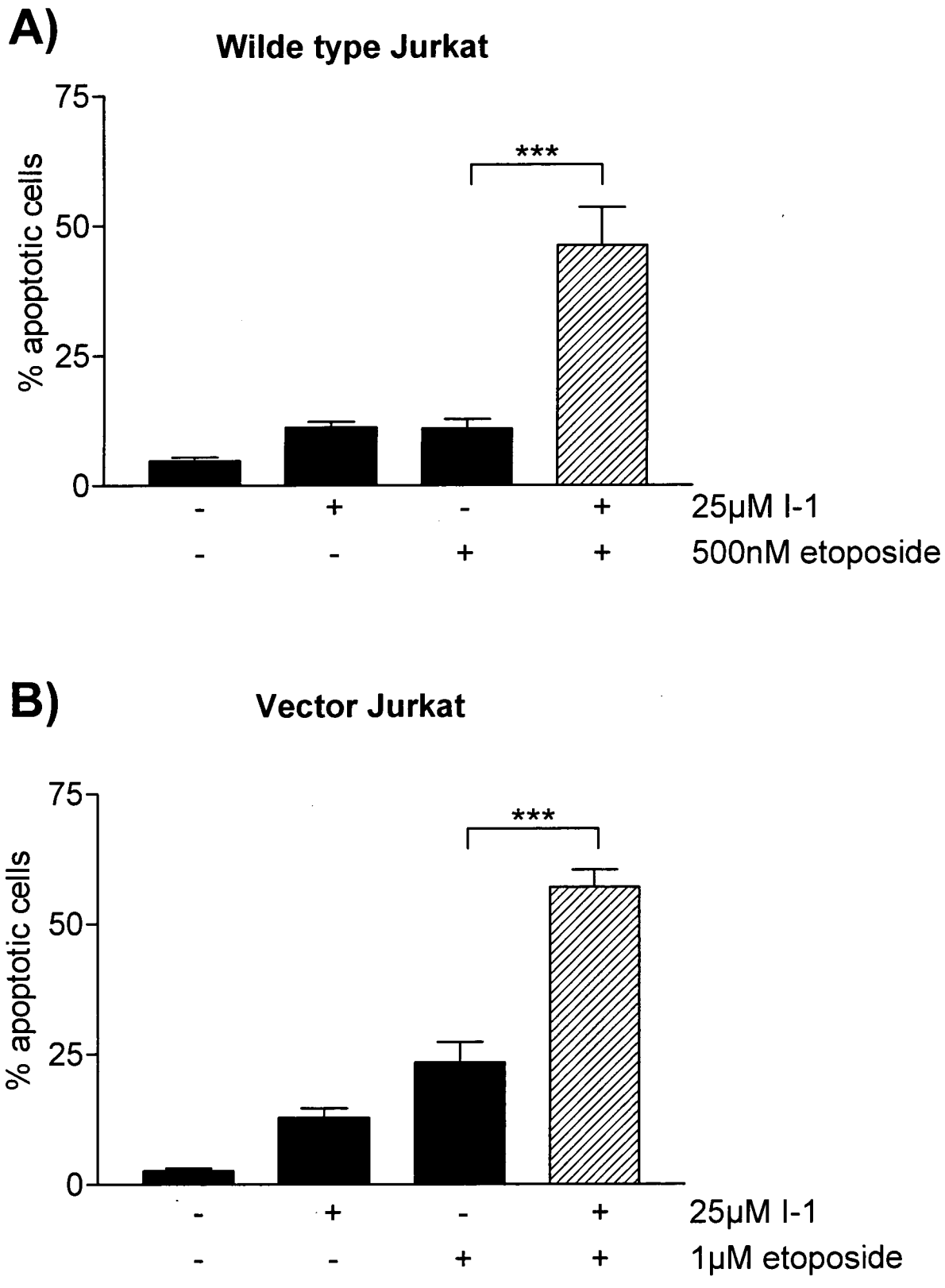
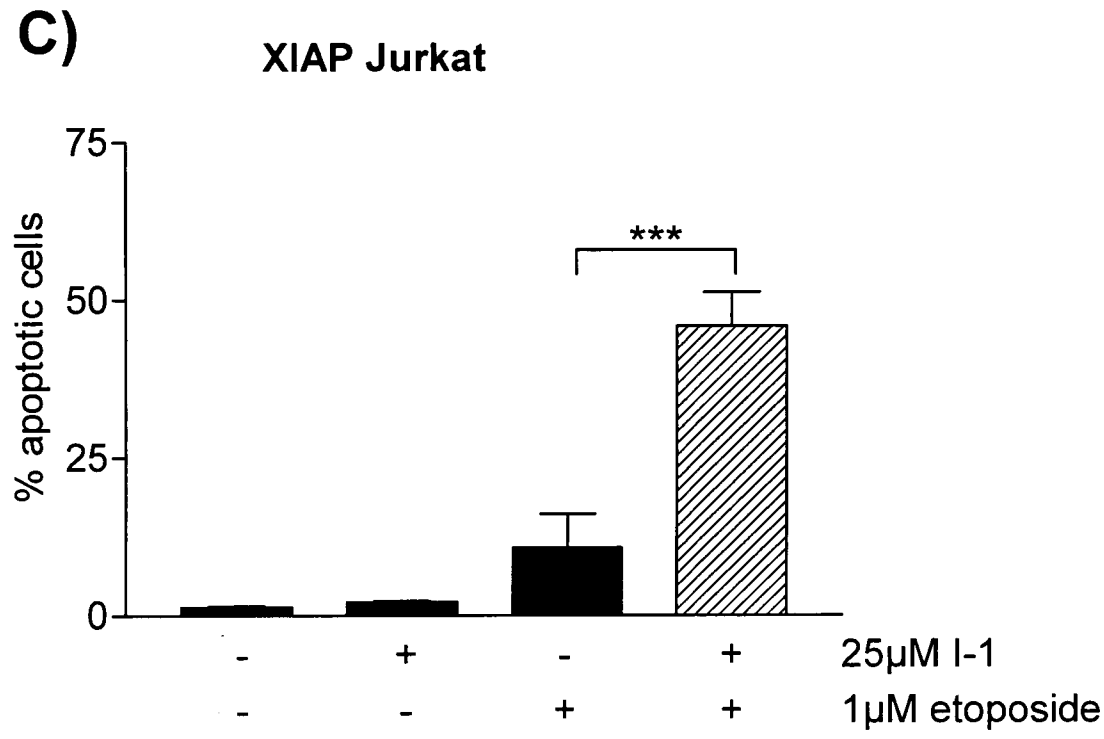

A)
Wilde type Jurkat
% apoptotic cells
75
50
25
0
***
- + - +
25µM I-1
- - + +
500nM etoposide
B)
Vector Jurkat
% apoptotic cells
75
50
25
0
***
- + - +
25µM I-1
- - + +
1µM etoposide

Fig. 1 (cont.)


C)
XIAP Jurkat
% apoptotic cells
75
50
25
0
***
- + - +
25µM I-1
- - + +
1µM etoposide

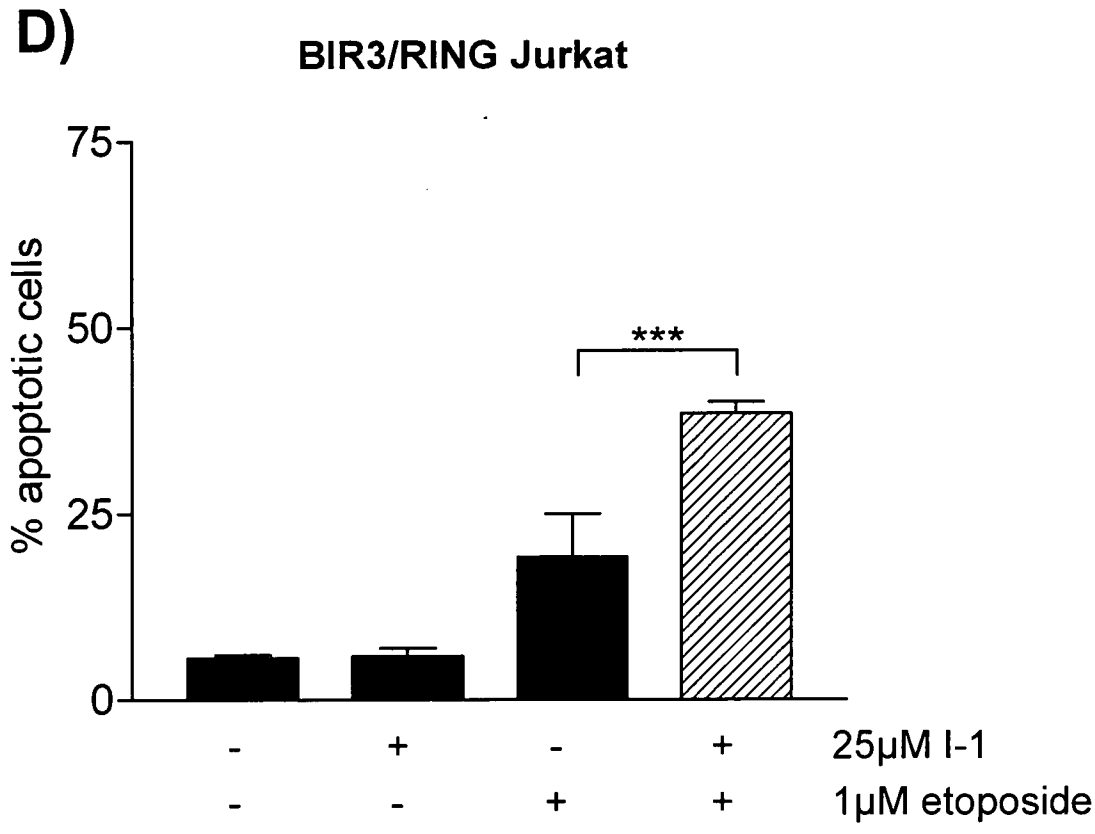

D)
BIR3/RING Jurkat
% apoptotic cells
75
50
25
0
***
- + - +
25µM I-1
- - + +
1µM etoposide

Fig. 2

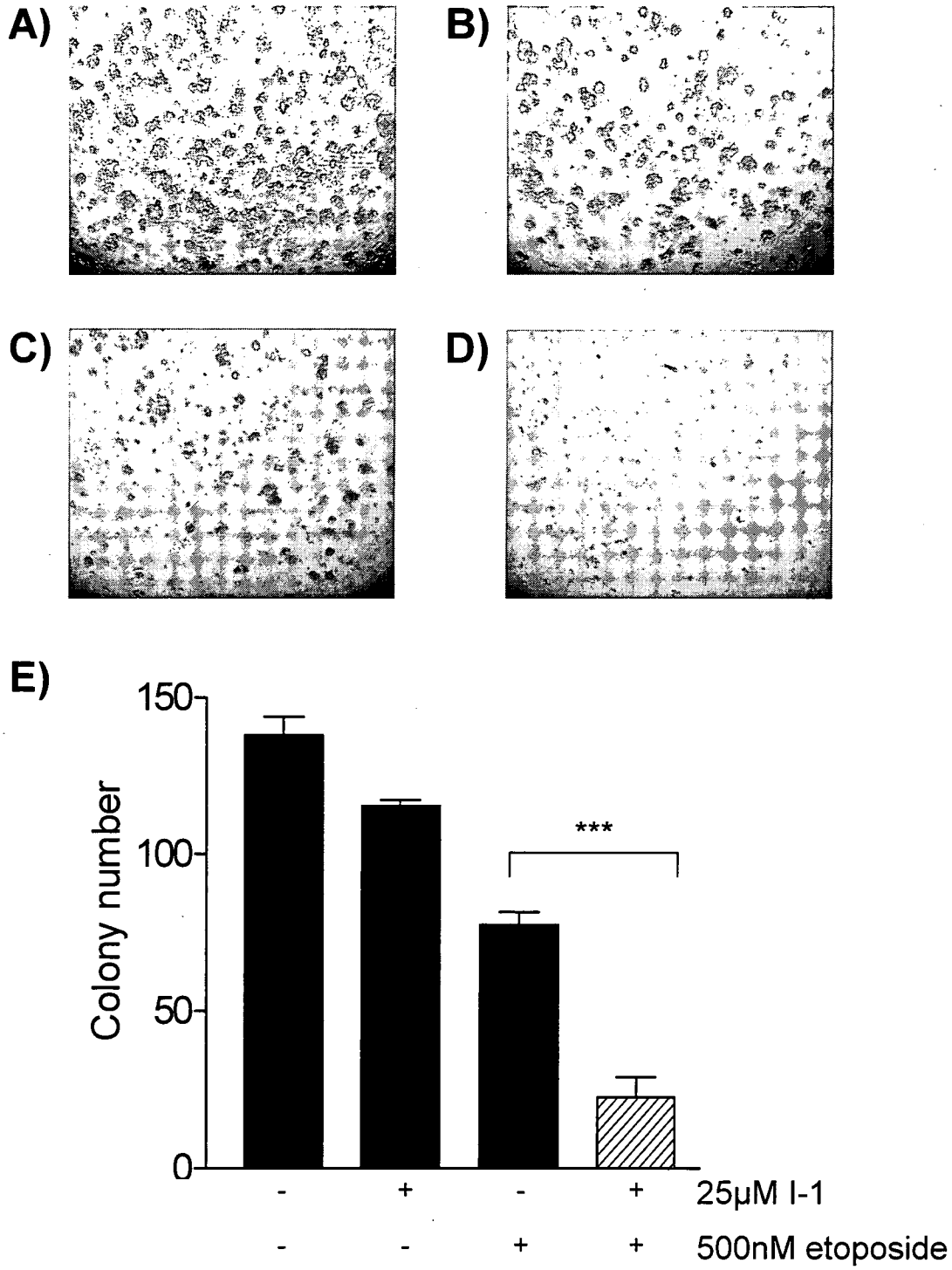


Fig. 3

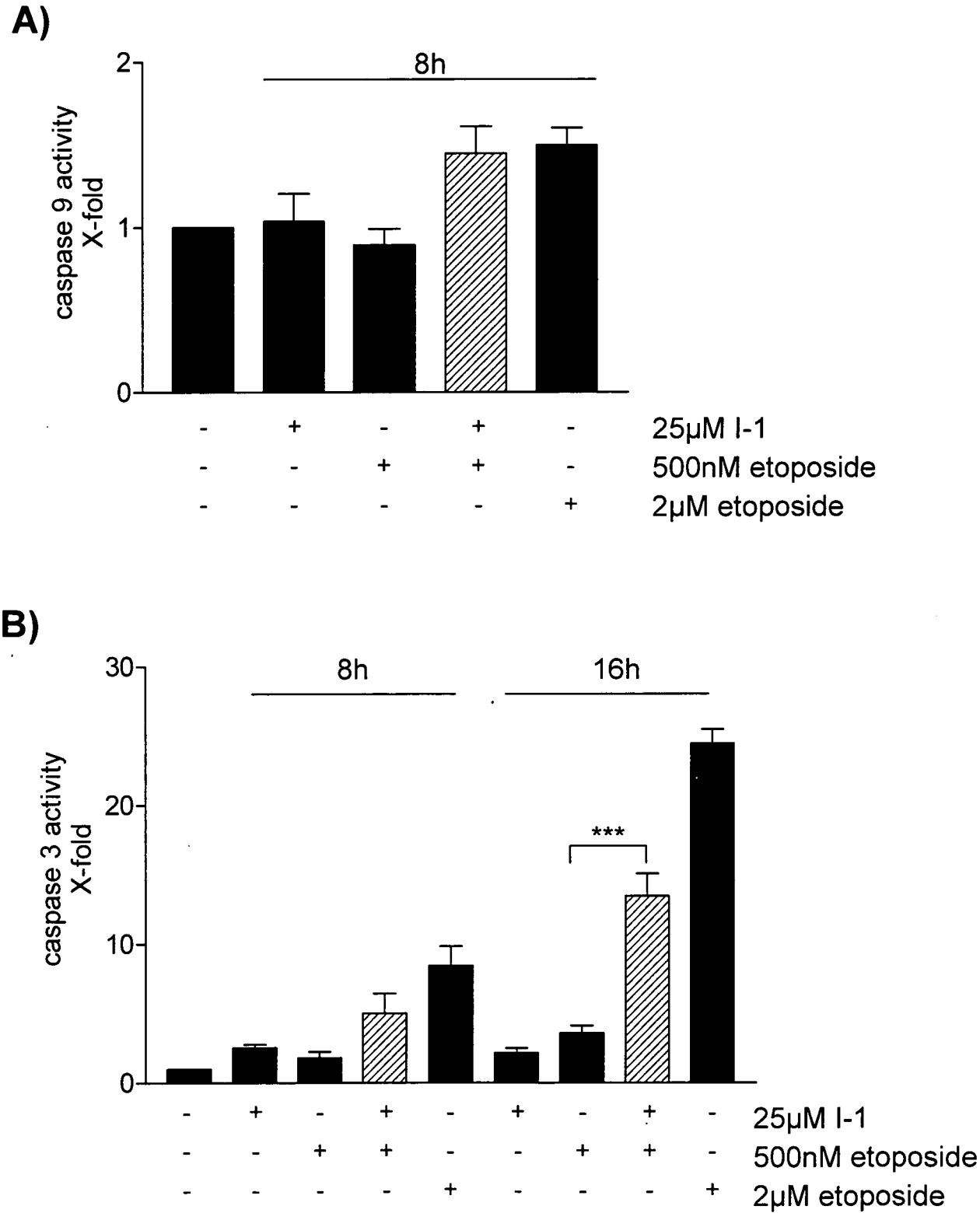

A)
8h
caspase 9 activity
X-fold
2
1
0
- + - + - 25µM I-1
- - + + - 500nM etoposide
- - - - + 2µM etoposide
B)
8h
16h
caspase 3 activity
X-fold
30
20
10
0
***
- + - + - + - + - 25µM I-1
- - + + - - + + - 500nM etoposide
- - - - + - - - + 2µM etoposide

Fig. 4

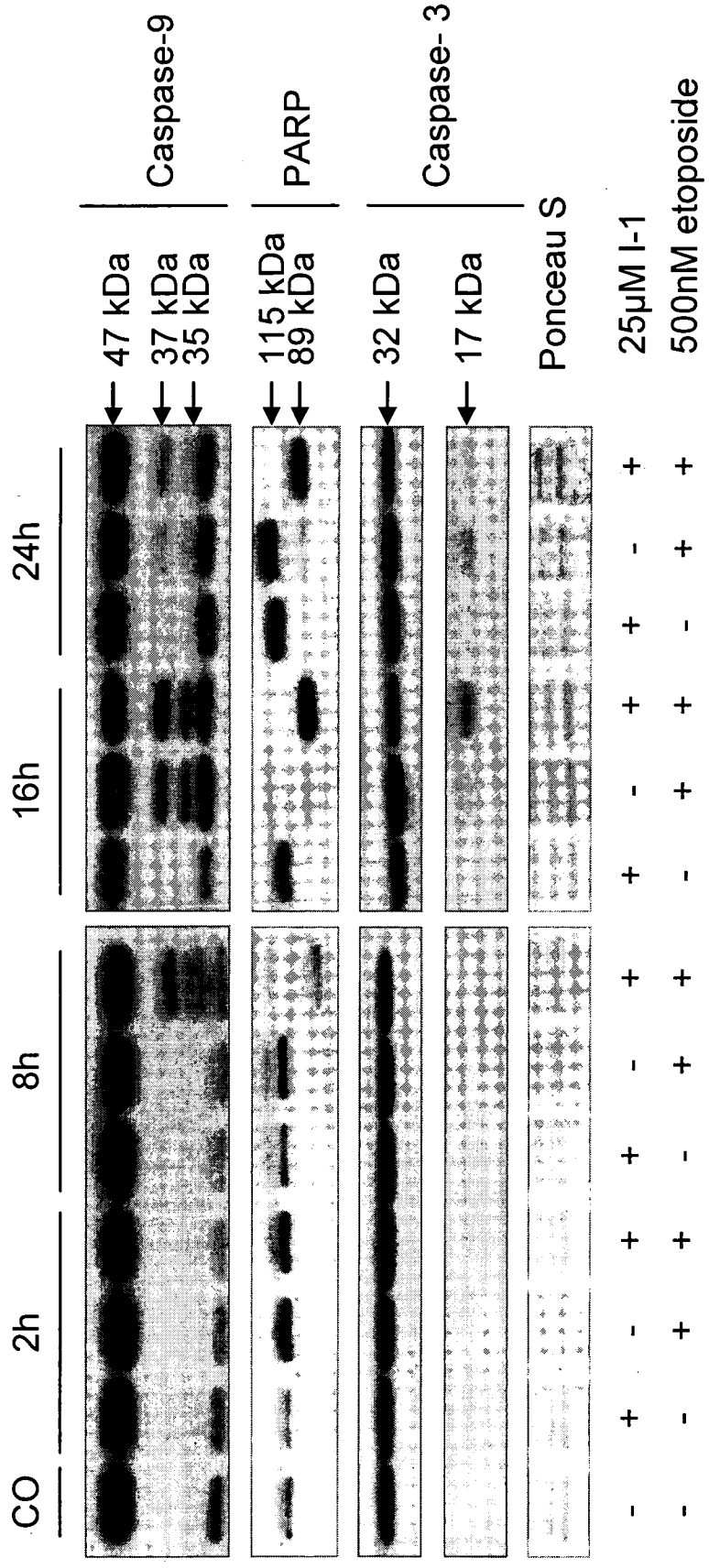

Caspase-9
PARP
Caspase- 3
47 kDa
37 kDa
35 kDa
115 kDa
89 kDa
32 kDa
17 kDa
Ponceau S
25µM I-1
500nM etoposide
CO
2h
8h
16h
24h

Fig. 5

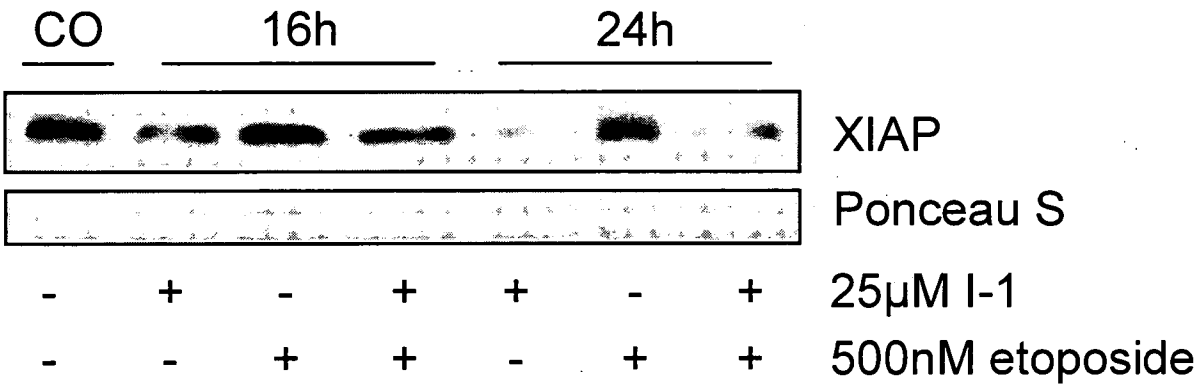


Fig. 6

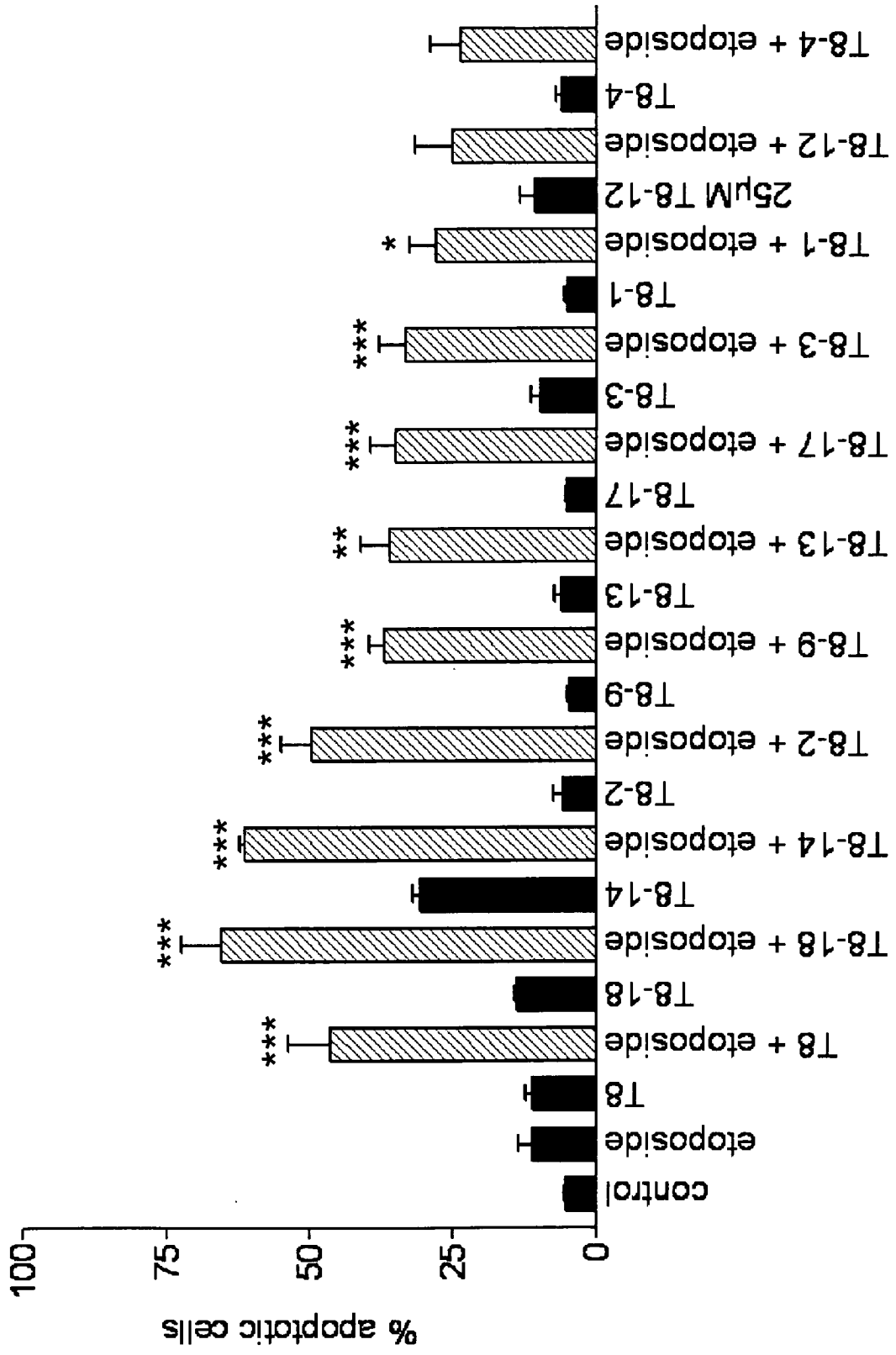

% apoptotic cells
0
25
50
75
100
control
etoposide
T8
T8 + etoposide
T8-18
T8-18 + etoposide
T8-14
T8-14 + etoposide
T8-2
T8-2 + etoposide
T8-9
T8-9 + etoposide
T8-13
T8-13 + etoposide
T8-17
T8-17 + etoposide
T8-3
T8-3 + etoposide
T8-1
T8-1 + etoposide
25µM T8-12
T8-12 + etoposide
T8-4
T8-4 + etoposide

Fig. 7

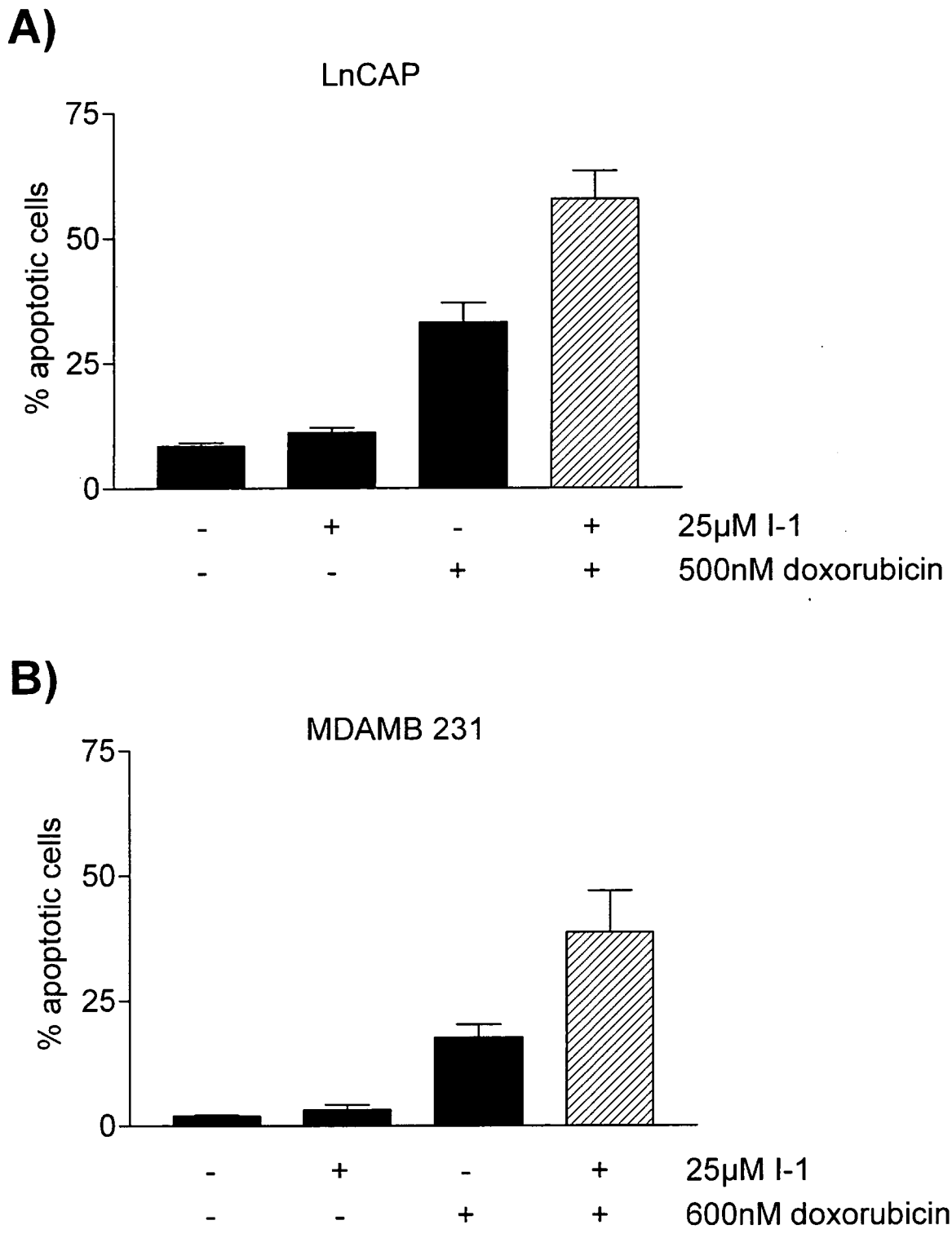

A)
LnCAP
% apoptotic cells
75
50
25
0
- + - +
- - + +
25µM I-1
500nM doxorubicin
B)
MDAMB 231
% apoptotic cells
75
50
25
0
- + - +
- - + +
25µM I-1
600nM doxorubicin

Fig. 8

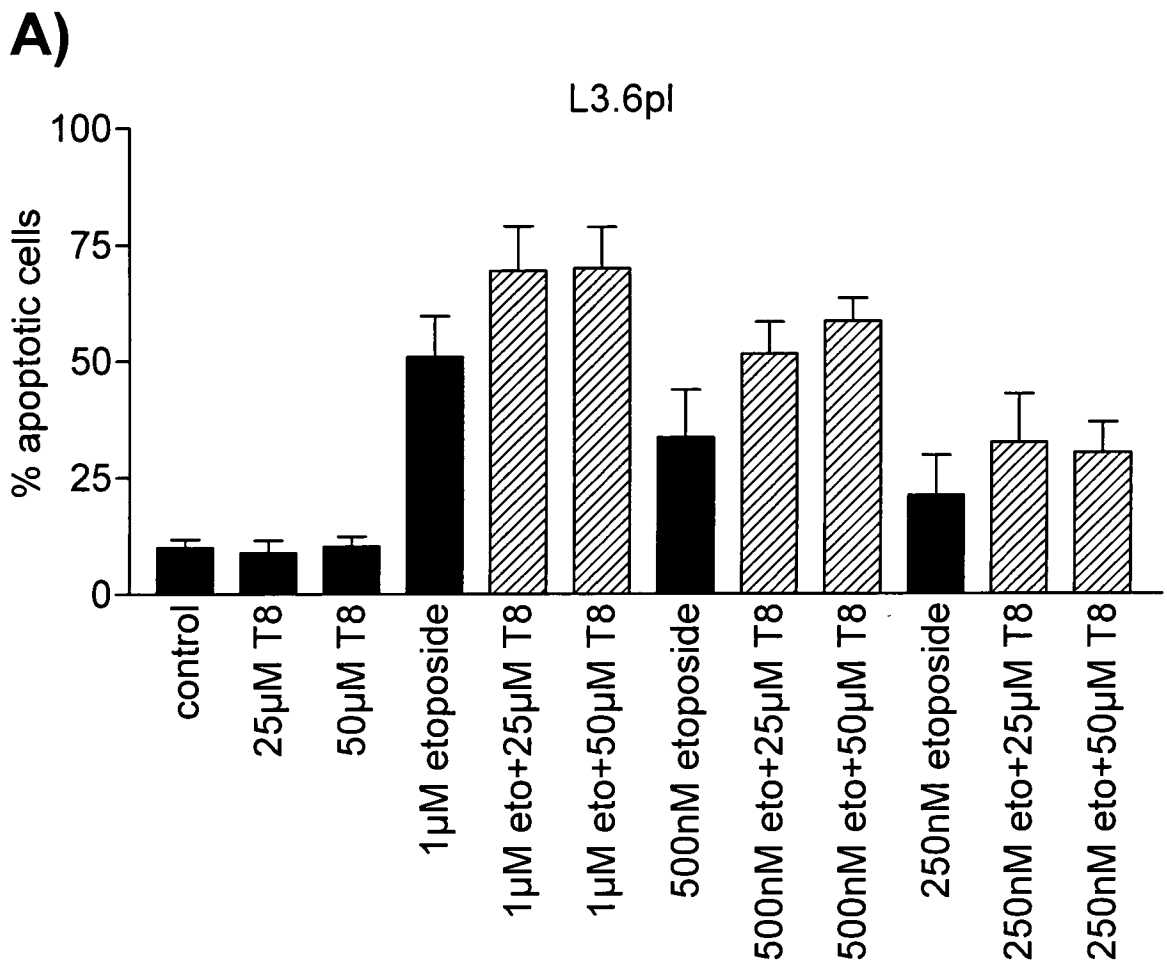
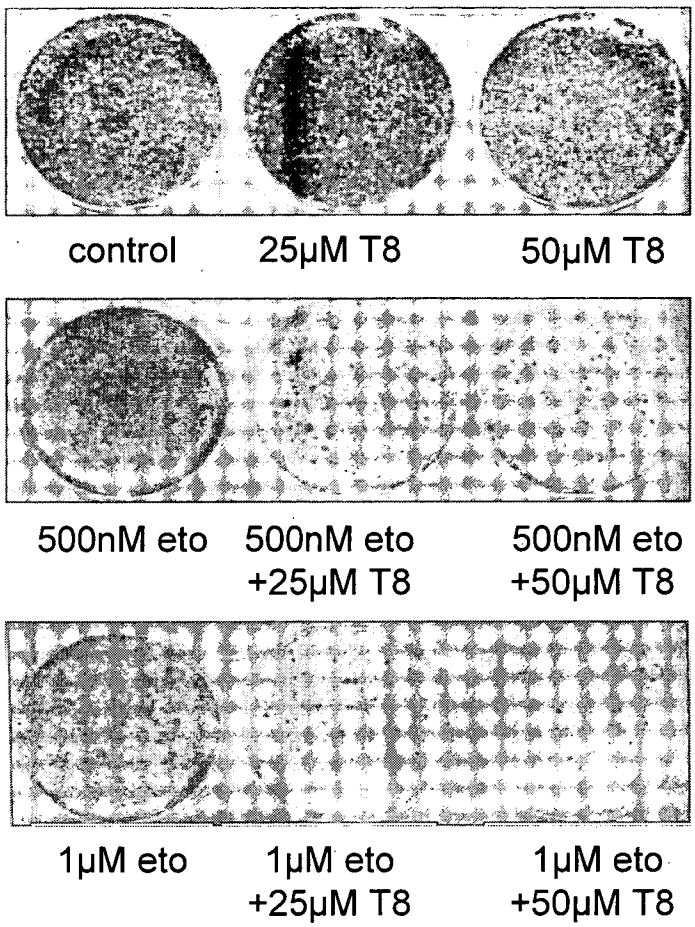

A)
L3.6pl
% apoptotic cells
100
75
50
25
0
control
25µM T8
50µM T8
1µM etoposide
1µM eto+25µM T8
1µM eto+50µM T8
500nM etoposide
500nM eto+25µM T8
500nM eto+50µM T8
250nM etoposide
250nM eto+25µM T8
250nM eto+50µM T8

Fig. 8 (cont.)

B)


control
25µM T8
50µM T8
500nM eto
500nM eto +25µM T8
500nM eto +50µM T8
1µM eto
1µM eto +25µM T8
1µM eto +50µM T8

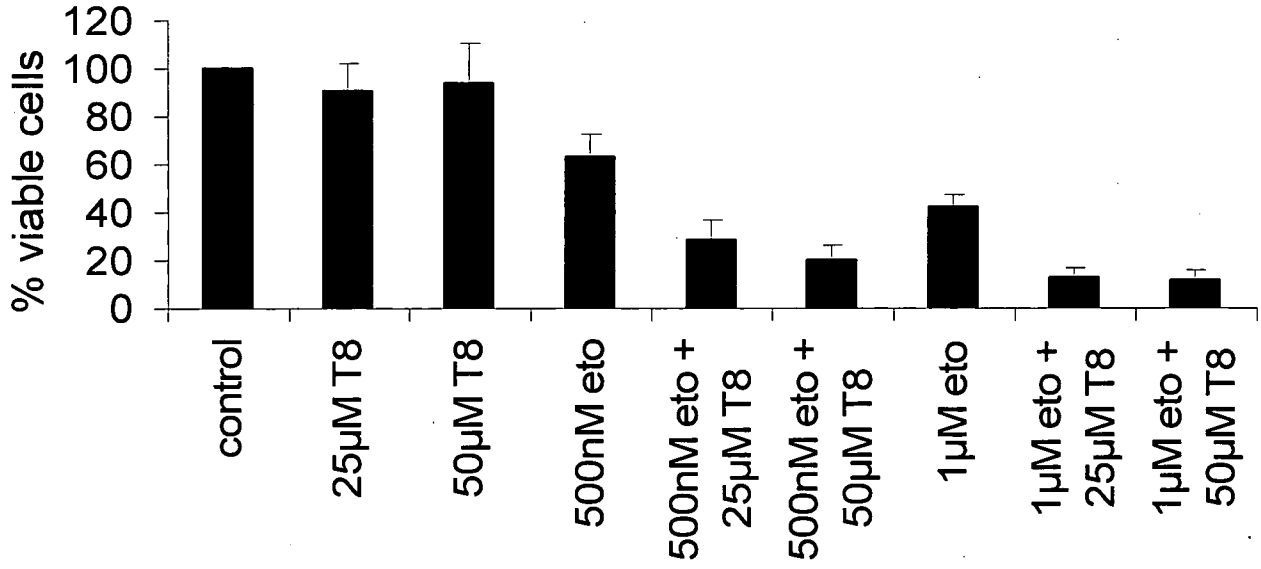
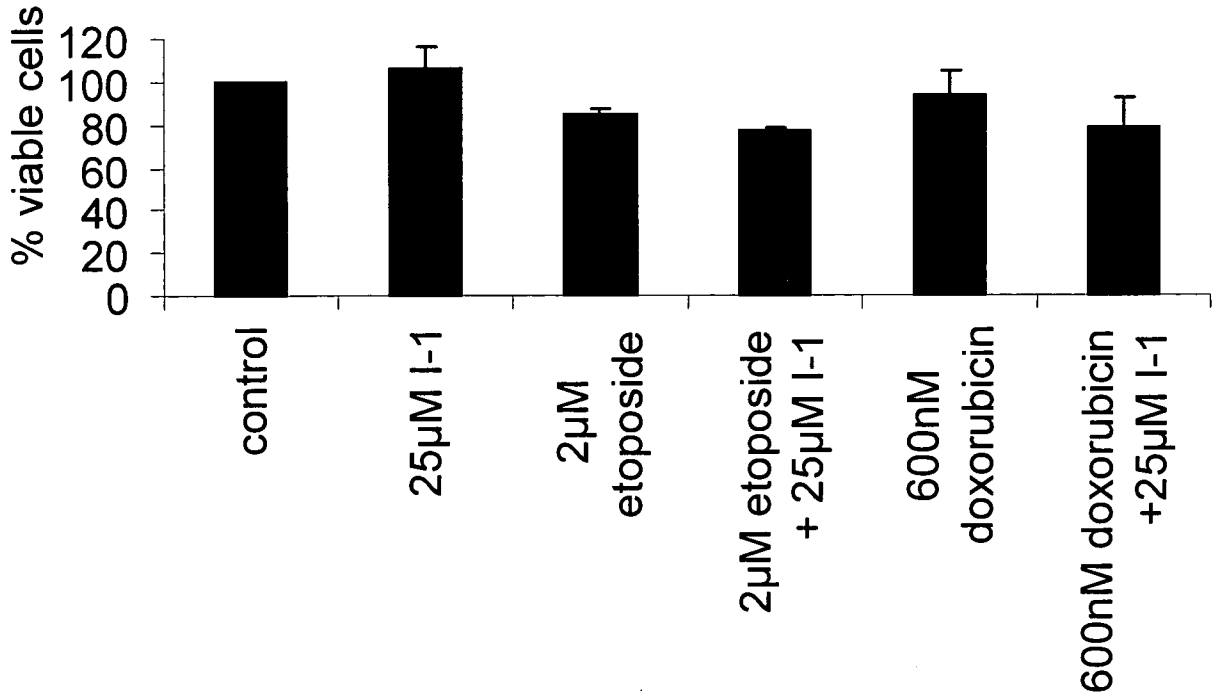

% viable cells
120
100
80
60
40
20
0
control
25µM T8
50µM T8
500nM eto
500nM eto + 25µM T8
500nM eto + 50µM T8
1µM eto
1µM eto + 25µM T8
1µM eto + 50µM T8

Fig. 9


% viable cells
120
100
80
60
40
20
0
control
25µM I-1
2µM etoposide
2µM etoposide + 25µM I-1
600nM doxorubicin
600nM doxorubicin +25µM I-1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/009778

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D211/58 A61K31/4468

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/114401 A (HOFFMANN LA ROCHE [CH]; GROEBKE-ZBINDEN KATRIN [CH]; HAAP WOLFGANG [DE]) 2 November 2006 (2006-11-02) cited in the application examples 93,94	1,4-8, 10,23,24
Y	page 6; claims	1-23
Y	WO 97/28141 A (PF MEDICAMENT [FR]; HALAZY SERGE [FR]; JORAND LEBRUN CATHERINE [FR]; P) 7 August 1997 (1997-08-07) claims 1,18	1-23
Y	WO 2007/067781 A (ABBOTT LAB [US]; MICHAELIDES MICHAEL R [US]; MCCLELLAN WILLIAM J [US];) 14 June 2007 (2007-06-14) cited in the application claims 1,6-9	1-23

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2009	02/03/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Frelon, Didier

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/009778

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/054453 A (HOFFMANN LA ROCHE [CH]; BOEHRINGER MARKUS [CH]; GROEBKE ZBINDEN KATRIN) 18 May 2007 (2007-05-18) cited in the application * ex. 38,39,42-44; page 25; claim 50 *	1-23
Y	WO 01/44191 A (SOD CONSEILS RECH APPLIC [FR]; THURIEAU CHRISTOPHE [FR]; GONZALEZ JERO) 21 June 2001 (2001-06-21) cited in the application page 2; claims	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/009778

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		KR 20070014235 A	31-01-2007
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		PL 356365 A1	28-06-2004
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