

COMPOUNDS FOR THE INHIBITION OF APOPTOSIS

The present invention relates to compounds acting as apoptosis inhibitors, as well as to processes for their preparation, to pharmaceutical compositions
5 containing them and their use in medicine.

BACKGROUND ART

Apoptosis is an interesting biological process because of its
10 importance in a wide variety of biological systems, including normal cell turnover, the immune system and embryonic development and its association with different diseases. Inappropriate apoptosis is involved in many human pathologies, including neurodegenerative diseases such as Alzheimer's and
15 Huntington's, ischaemia, autoimmune disorders and several forms of cancer (Reed J.C., Trends Mol. Med. 2001, 7: 314–319).

Diverse apoptotic stimuli, including activation of cell surface death receptors, anticancer agents, irradiation, lack of survival factors,
20 and ischaemia (reviewed in Strasser A. et al., Annu. Rev. Biochem. 2000, 69: 217–245) induce signalling cascades that all activate a family of cysteine aspartyl proteases called caspases. These proteases execute the apoptotic process.

25 Some apoptotic signals activate the mitochondria-mediated or intrinsic pathway that utilises caspase-9 as its initiator. The formation of the macromolecular complex named apoptosome is a key event in this pathway.

The apoptosome is a holoenzyme multiprotein complex formed by cytochrome c-activated Apaf-1 (apoptotic protease-activating factor), dATP and procaspase- 9 (Li P. et al., Cell 1997, 91: 479–489; Acehan D et al., Mol. Cell 2002, 9: 423–432; Rodriguez J. et al., Genes
5 Dev. 1999, 13: 3179–3184; Srinivasula S.M. et al., Mol. Cell 1998, 1: 949–957). In this macromolecular complex, apoptosome associated caspase-9 is activated and then, in turn, activate effector caspases. To identify molecules that could ameliorate disease-associated apoptosis, drug discovery efforts have initially targeted caspase activity, and
10 thus sought specific enzymatic activators or inhibitors (Scott C.W. et al., J. Pharmacol. Exp. Ther. 2003, 304: 433–440; Garcia-Calvo M, J. Biol. Chem. 1998, 273: 32608–32613), rather than modulators in the upstream activation cascade/pathway.

15 Nevertheless, protein–protein interactions upstream of caspase activation can be also relevant points of intervention for the development of modulators of apoptosis pathways. In particular, recent data propose the formation of the apoptosome as an interesting target for the development of apoptotic modulators.

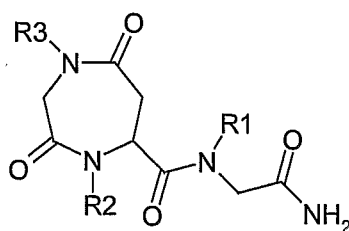
20 Therefore there is an interest in finding molecules that inhibit the apoptosome-mediated activation apoptosis.

DESCRIPTION OF THE INVENTION

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One aspect of the present invention relates to the provision of new compounds of general formula I,

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I

its stereoisomers and mixtures thereof, its polymorphs and mixtures thereof and the pharmaceutically acceptable solvates and addition salts thereof, wherein:

5 R1 represents (C₁-C₅)alkyl, (C₂-C₅)alkenyl, -(CH₂)₁₋₃-NHCO-(C₁-C₃)alkyl, -(CH₂)₁₋₃-CONRaRb, -(CH₂)₀₋₃-(3-6)Cy, -(CH₂)₀₋₃-(5-6)Hetcy, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₂-CH(phenyl)₂, -(CH₂)₂-CH(phenyl)[(5-6)Hetar] or -(CH₂)₂-CH[(5-6)Hetar]₂;

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R2 represents -(CH₂)₀₋₃-(3-6)Cy, -(CH₂)₀₋₃-(5-6)Hetcy, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₂-CH(phenyl)₂, -(CH₂)₂-CH(phenyl)[(5-6)Hetar] or -(CH₂)₂-CH[(5-6)Hetar]₂;

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R3 represents -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), or -(CH₂)₁₋₃-(5-6)Hetar;

(C₁-C₅)alkyl and -(C₂-C₅)alkenyl may be optionally substituted with one or
20 more substituents selected from -O-(C₁-C₂)alkyl, -S-(C₁-C₂)alkyl, -NH₂, -NH-(C₁-C₂)alkyl and -N[(C₁-C₂)alkyl]₂;

(C₁-C₃)alkyl can be optionally substituted with one or more halogen atoms;

25 Ra and Rb independently represent -H or -(C₁-C₃)alkyl

(3-6)Cy represents a radical of a 3- to 6-membered partially unsaturated or saturated carbocyclic ring;

(5-6)Hetcy represents a C- or N- radical of a 5- or 6-membered, partially unsaturated or saturated carbocyclic ring containing from one or two heteroatoms independently selected from O, S and N;

Cy and Hetcy can be optionally substituted with one or more substituents selected from halogen, $-\text{CF}_3$ and $-\text{OH}$;

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(5-6)Hetar and (5-10)Hetar represent a C- or N- radical of an aromatic 5- or 6-membered and 5- to 10-membered ring respectively; containing from one to four heteroatoms independently selected from O, S and N;

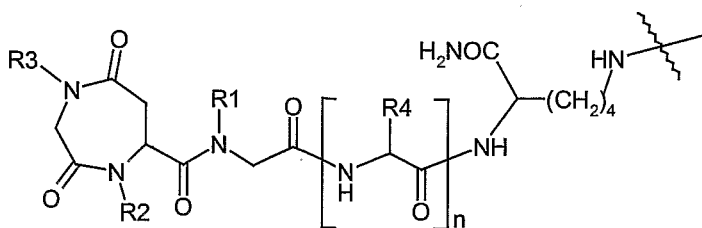
phenyl, 1-naphthyl, 2-naphthyl, (5-6)Hetar and (5-10)Hetar may be optionally substituted with one or more substituents selected from halogen, $-\text{CF}_3$, $-\text{OH}$, $-\text{O}-(\text{C}_1-\text{C}_3)\text{alkyl}$, $-\text{CO}-(\text{C}_1-\text{C}_3)\text{alkyl}$, $-(\text{C}_1-\text{C}_5)\text{alkyl-NRaRb}$, $-\text{NRaRb}$ and $-\text{SO}_2\text{NH}_2$;

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with the proviso that R2 does not represent 2-(4-fluorophenyl)ethyl.

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Another aspect of the present invention relates to a compound which is a drug conjugate comprising a radical of formula II



II

its stereoisomers and mixtures thereof and the pharmaceutically acceptable solvates and addition salts thereof, conjugated to a polyglutamic acid polymer, wherein R1, R2 and R3 have the meanings as defined for the compound of

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formula I without the R2 proviso; each R4 independently represents a side chain from any of the 20 naturally occurring amino acids, and n is 0, 1, 2 or 3.

Thus, compounds of formula II comprise different moieties: a radical of formula I, forming an amide bond to a peptide chain of n L-amino acids and an amide L-lysine derivative, wherein the N of its side chain is linked to the polyglutamic acid polymer.

In the previous definitions, the terms (C₁-C₃)alkyl and (C₁-C₅)alkyl represent straight or branched saturated hydrocarbon chains containing from one to three and from one to five carbon atoms respectively. Examples of (C₁-C₃)alkyl include, but are not limited to, methyl, ethyl, propyl, isopropyl. Examples of (C₁-C₅)alkyl include additionally and without limitation butyl, isobutyl, sec-butyl, *tert*-butyl, 1-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl and 2,2-dimethylpropyl.

The term (C₂-C₅)alkenyl represents an unsaturated straight or branched hydrocarbon chain, containing from two to five carbon atoms and one or more double bonds, for example ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl and 1,3-butadienyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-methyl-2-butenyl, 2-methyl-3-butenyl, 3-methyl-1-butenyl, 1,1-dimethyl-2-propenyl and 1,2-dimethyl-1-propenyl.

The groups (C₁-C₃)alkyl, (C₁-C₅)alkyl and (C₂-C₅)alkenyl may be optionally substituted according to the description whenever appropriate from a chemical view point.

The term (3-6)Cy represents a radical of a 3- to 6-membered partially unsaturated or saturated carbocyclic ring. As mentioned previously, it can be optionally substituted with one or more substituents, which can be placed at any available position of the ring. Examples of (3-6)Cy include, without limitation, radicals of cyclopropane, cyclobutane, cyclopentane, cyclohexane,

1-cyclobutene, 2-cyclobutene, 1-cyclopentene, 2-cyclopentene, 1-cyclohexene, 2-cyclohexene and 3-cyclohexene.

The term (5-6)Hetcy represents a C- or N- radical of a partially unsaturated or saturated 5- to 6-membered carbocyclic ring containing one or two heteroatoms independently selected from O, S and N, that may be substituted according to the description at any available ring position. Examples of (5-6)Hetcy include, without limitation, radicals of dihydrofuran, pyrroline, pyrazoline, imidazolidine, isothiazolidine, isoxazolidine, oxazolidine, pyrazolidine, pyrrolidine, thiazolidine, dioxane, morpholine, piperazine, piperidine, pyran, tetrahydropiran, oxazine, oxazoline, pyrroline, thiazoline, pyrazoline, imidazoline, isoxazoline, and isothiazoline.

The terms (5-6)Hetar and (5-10)Hetar represent a C- or N- radical of an aromatic 5- or 6-membered and 5- to 10-membered ring respectively; containing from one to four heteroatoms independently selected from O, S and N, that may be substituted according to the description at any available ring position. Examples of (5-6)Hetar include, without limitation, radicals of pyrrol, furan, thiophene, imidazole, isoxazole, isothiazole, oxazole, 1,2,4-oxadiazole, 1,2,4-thiadiazole, 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,3-triazole, 1,2,4-triazole, pyridine, pyrimidine, pyridazine and pyrazine. Examples of (5-10)Hetar further include, without limitation, radicals of benzimidazole, benzofuran, benzothiazole, benzothiophene, imidazopyrazine, imidazopyridazine, imidazopyridine, imidazopyrimidine, indazole, indole, isoindole, isoquinoline, pyrazolopyrazine, pyrazolopyridine, pyrazolopyrimidine, purine, quinazoline, quinoline and quinoxaline.

The expression "optionally substituted with one or more" means that a group can be either unsubstituted or substituted with one or more, preferably with 1, 2, 3 or 4 substituents, provided that this group has 1, 2, 3 or 4 positions susceptible of being substituted.

Throughout the description and claims the word "comprise" and variations of the word, such as "comprising", are not intended to exclude other additives, components, elements or steps.

- 5 As used therein the term "treatment" includes treatment, prevention and management of such condition. The term "pharmaceutically acceptable" as used herein refers to those compounds, compositions, and/or dosage forms which are, within the scope of medical judgement, suitable for use in contact with the tissues of humans and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

In a particular embodiment of the invention R1 represents (C₁-C₅)alkyl, -(CH₂)₁₋₃-CONRaRb, -(CH₂)₀₋₃-(3-6)Cy; -(CH₂)₀₋₃-(5-6)Hetcy; -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl) or -(CH₂)₁₋₃-(2-naphthyl), all of them optionally substituted.

In a particular embodiment of the invention R1 represents -(CH₂)₀₋₃-(5-6)Hetcy, -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl) or -(CH₂)₁₋₃-(2-naphthyl), all of them optionally substituted.

In another embodiment R1 represents -(CH₂)₂-phenyl optionally substituted with one or more substituents selected from halogen. In another embodiment R1 represents 2,4-dichlorophenyl.

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In another embodiment R2 represents -(CH₂)₁₋₃-aryl optionally substituted, or -(CH₂)₂-CH(Ph)₂ optionally substituted, with the proviso that R2 does not represent 2-(4-fluorophenyl)ethyl-. In another embodiment R2 represents 3,3-diphenylpropyl, with the proviso that R2 does not represent 2-(4-fluorophenyl)ethyl-.

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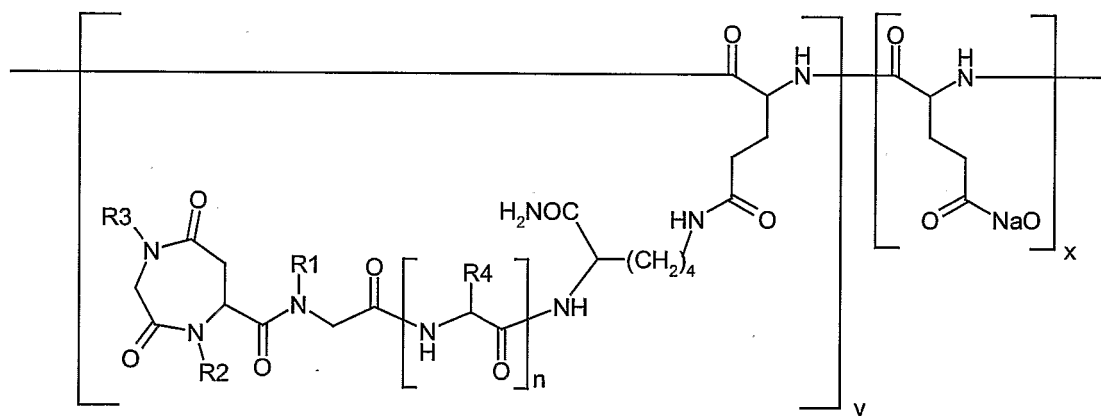
In another embodiment R3 represents $-(CH_2)_{1-3}-(5-6)\text{Heter}$ or $-(CH_2)_2\text{-phenyl}$ both optionally substituted. In another embodiment R3 represents $-(CH_2)_2\text{-phenyl}$ optionally substituted with one or more substituents selected from halogen. In another embodiment R3 represents 2,4-dichlorophenyl.

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In another embodiment R2 represents 3,3-diphenylpropyl and R1 and R3 both represent 2,4-dichlorophenyl.

Another embodiment of the present invention relates to a compound which is a drug conjugate comprising a compound of formula II conjugated to a polyglutamic acid polymer as previously defined, wherein said polymer has a molecular weight of from 20 to 60 kDa.

Another embodiment of the invention relates to a compound of formula III which is a polymer-drug conjugate



III

wherein R1, R2, R3 and R4 have the meanings described in the general formula II and the proportion of y to x is 25-30% to 75-70% by weight.

In another embodiment in a compound of formula III n represents 0. In another embodiment n represents 2 and R4 represents a glycine side chain. In another embodiment n represents 3 and R4 represents a glycine side chain, an arginine side chain and a phenylalanine side chain. In another embodiment n represents 3 and R4 represents a valine side chain, an arginine side chain and a phenylalanine side chain.

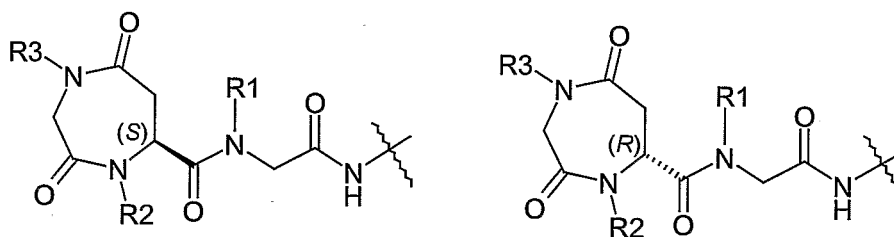
In the polymer drug conjugate, the polyglutamic acid may be poly(L-glutamic acid), poly(D-glutamic acid) or poly(DL-glutamic acid). In one embodiment of the invention polyglutamic acid is poly(L-glutamic acid).

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Furthermore, all possible combinations of the above-mentioned embodiments form also part of this invention.

Compounds of formula I comprise at least one chiral center. The present invention includes both the racemic compounds and the enantiomeric compounds of formula I, i.e. compounds wherein the configuration of the chiral carbon attached to R1 is (S) and compounds wherein the configuration of the chiral carbon attached to R1 is (R) as shown below.

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Compounds of formula II comprise at least two chiral centers. The present invention also includes both mixtures of stereoisomers and isolated stereoisomers of formula II.

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The compounds of the present invention may contain one or more basic nitrogen atoms and, therefore, they may form salts with acids, that also form part of this invention. Examples of pharmaceutically acceptable salts include, among others, addition salts with inorganic acids such as hydrochloric, hydrobromic, hydroiodic, nitric, perchloric, sulphuric and phosphoric acid, as well as addition salts of organic acids such as acetic, methanesulfonic, trifluoromethanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, benzoic, camphorsulfonic, mandelic, oxalic, succinic, fumaric, tartaric, and maleic acid. Likewise, compounds of the present invention may contain one or more acid protons and, therefore, they may form salts with bases, that also form part of this invention. Examples of these salts include salts with metal

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- cations, such as for example an alkaline metal ion, an alkaline-earth metal ion or an aluminium ion; or it may be coordinated with an organic with an organic or inorganic base. An acceptable organic base includes among others diethylamine and triethylamine. An acceptable inorganic base includes
- 5 aluminium hydroxide, calcium hydroxide, potassium hydroxide, sodium carbonate, and sodium hydroxide. There may be more than one cation or anion depending on the number of functions with charge and on the valency of cations and anions.
- 10 Salts derived from pharmaceutically acceptable organic nontoxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, *N,N*-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-
- 15 dimethylaminoethanol, ethanolamine, ethylenediamine, *N*-ethylmorpholine, ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.
- 20
- There is no limitation on the type of salt that can be used, provided that these are pharmaceutically acceptable when they are used for therapeutic purposes. Salts can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be
- 25 prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile or in a mixture of both. The compounds of formula I or formula III and their salts differ in some physical properties but they are equivalent for the purposes of
- 30 the present invention.

Some of the compounds of formula I or formula III of the present invention may exist in unsolvated as well as solvated forms such as, for example, hydrates. The present invention encompasses all such above-mentioned forms which are pharmaceutically active.

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Some of the compounds of general formula I may exhibit polymorphism, encompassing the present invention all the possible polymorphic forms, and mixtures thereof. Various polymorphs may be prepared by crystallization under different conditions or by heating or melting the compound followed by gradual or fast cooling. The presence of polymorphs may be determined by solid NMR spectroscopy, IR spectroscopy, differential scanning calorimetry, powder X-ray diffraction or such other techniques.

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Compounds of formula I of the present invention comprise at least one chiral center. Additionally, they may have further chiral centres. The present invention includes each one of the possible stereoisomers and mixtures thereof, particularly racemic mixtures thereof. A single enantiomer may be prepared by any of the commonly used processes, for example, by chromatographic separation of the racemic mixture on a stationary chiral phase, by resolution of the racemic mixture by fractional crystallisation techniques of the diastereomeric salts thereof, by chiral synthesis, by enzymatic resolution or by biotransformation. This resolution can be carried out on any chiral synthetic intermediate or on products of general formula I. Alternatively, any enantiomer of a compound of the general formula I may be obtained by enantiospecific synthesis using optically pure starting materials or reagents of known configuration.

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Compounds of formula I or formula III may exist as several diastereoisomers, and may be separated by conventional techniques such as chromatography or fractional crystallization. Some compounds of the present invention may exhibit cis/trans isomers. The present invention includes each of the geometric isomers and its mixtures. The present invention covers all isomers and

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mixtures thereof (for example racemic mixtures) whether obtained by synthesis and also by physically mixing them.

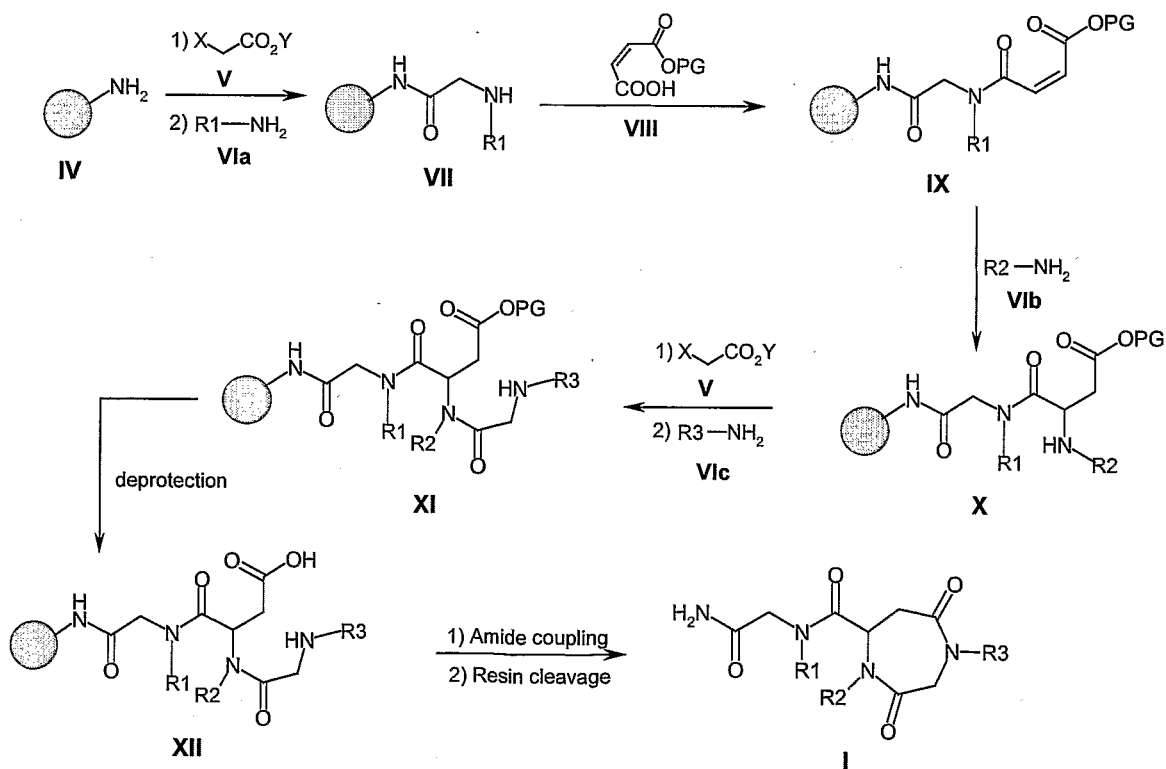
The present invention relates to a process for the preparation of the above said novel compounds, their derivatives, their analogues, their tautomeric forms, their stereoisomers, their polymorphs or their pharmaceutical acceptable salts and solvates.

The compounds of the present invention may be synthesized using the methods described below, as well as by other processes known in the field of organic synthesis. Preferred methods include, but are not limited to, the general processes shown in the attached schemes. Unless otherwise stated the groups R1, R2, R3, R4, n, x and y have the meaning described in general formulas I, II and III.

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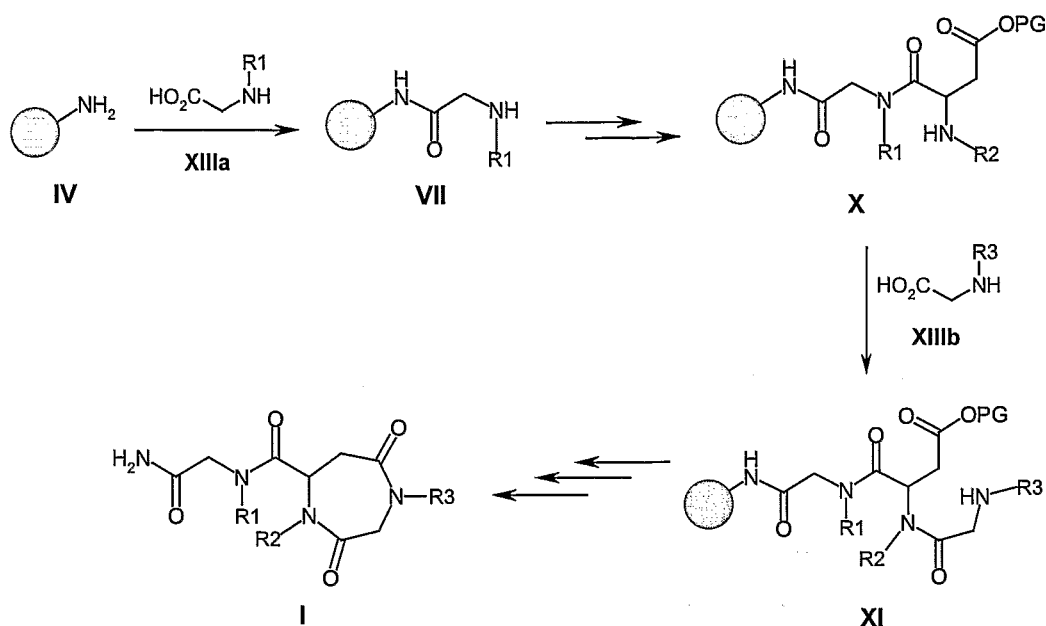
Compounds of formula I can be obtained using the following method:

Method A



- According to Method A, upon deprotection from fluorenylmethoxycarbonyl group, the solid support amine **IV** is acylated with an acylating agent **V**, wherein X represents a leaving group, for example halogen and Y represents -OH or halogen. When Y represents halogen, for example chloroacetylchloride
- 5 the reaction can be carried out in the presence of a tertiary base as triethylamine. When Y represents -OH, for example bromoacetic acid the reaction can be carried out in the presence of a suitable coupling agent, for example *N,N'*-diisopropylcarbodiimide. In both cases, reaction can be performed in an inert solvent capable of swelling correctly the resin, such as
- 10 *N,N'*-dimethylformamide or dichloromethane and at room temperature or using microwave irradiation, decreasing reaction time. Then, an amine **Vla** is coupled using a tertiary amine as a base. The reaction can be carried out by heating, for example at 60°C, or by MW irradiation.
- 15 A carboxylic acid **VIII**, wherein PG represents a protecting group, such as allyl, is reacted with amine **VII** to yield amide **IX** using a coupling agent, for example the combination of *N,N'*-diisopropylcarbodiimide and 1-hydroxybenzotriazole. Next, an amine **Vlb** is introduced by Michael reaction using a base and a solvent such as *N,N'*-dimethylformamide or dimethylsulfoxide to obtain
- 20 intermediate **X**. After repetition of acylation and amination in the same way as described for the intermediate **VII**, **XI** is obtained. Deprotection carried out using PhSiH_3 and $\text{Pd(PPh}_3)_4$ in dichloromethane as a solvent (Tetrahedron Lett 1999, 40:2505-2508; Tetrahedron Lett 1997, 38:7275-7278) or with KOH aqueous in dioxane yields **XII**. Cyclization is promoted by amide formation
- 25 using standard coupling reagents for solid phase peptidic bond procedures as the combination of benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate and 1-hydroxybenzotriazole in presence of a tertiary base as *N,N*-diisopropylethylamine. Final compound **I** can be released from the resin using a mixture of trifluoroacetic acid, dichloromethane and water.
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- An alternative strategy for obtaining a compound of formula **I** involves acylation of the amines **IV** and **X** with amino acids of formulas **XIIla** and **XIIlb**

respectively (Method B).

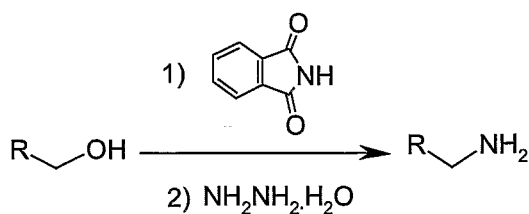


Method B

- As it will be obvious to one skilled in the art, it is possible to combine some steps of method A with some steps of method B to obtain a compound of formula I.

- Starting primary amines **Vla**, **Vlb** and **Vlc** are commercially available, or may be obtained by known methods (March, Advanced Organic Chemistry, 1991, Ed. John Wiley & Sons) or using for example the scheme described below.

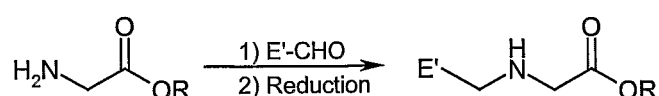
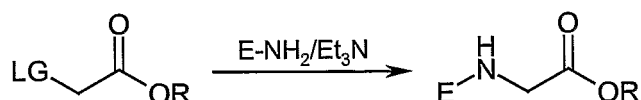
Scheme 1



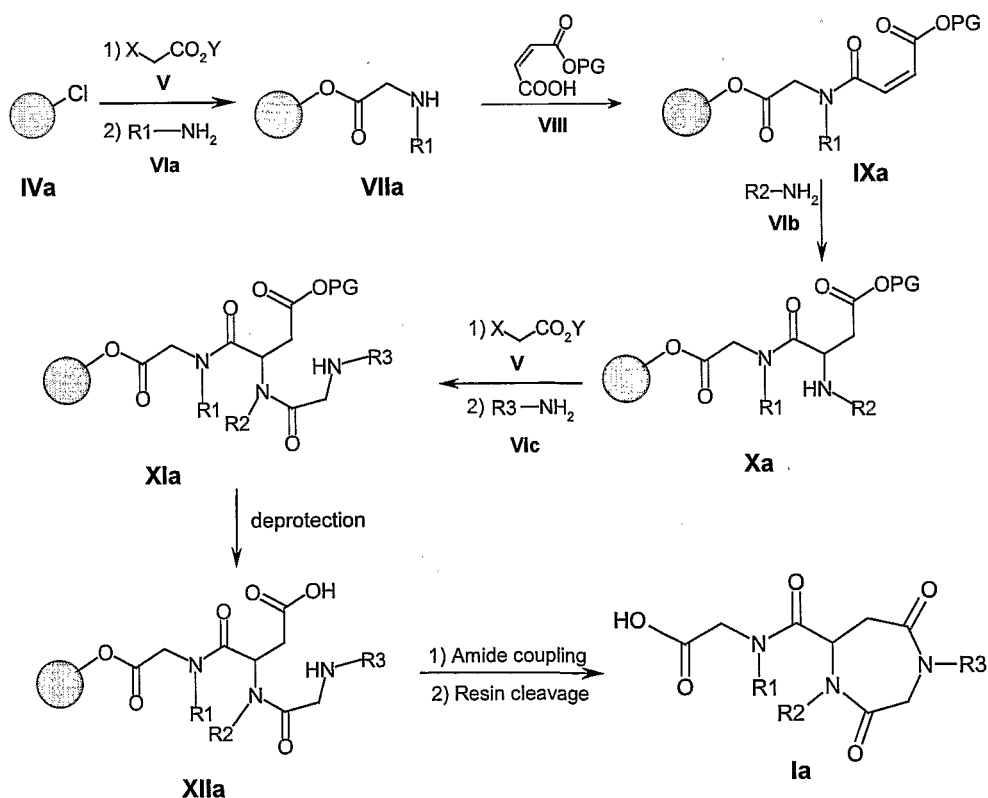
- An amine can be obtained by Mitsunobu reaction starting from an alcohol and potassium phthalimide in the presence of, for example, diethyl azodicarboxylate (DEAD) and triphenylphosphine in tetrahydrofuran as a solvent and further cleavage with hydrazine hydrate (Mitsunobu, *J. Am. Chem.*

Soc. 1972, 94, 679-680)

N-Substituted glycines **XIIla** and **XIIlb** may be synthesized by the methods shown below either by reductive amination of the corresponding glycine with the suitable aldehyde (Scheme 2) using reducing agents such as NaBH₄, NaBH₃CN or NaBH(AcO)₃, or by nucleophilic substitution of an ester with the suitable amine E-NH₂ (Scheme 3).

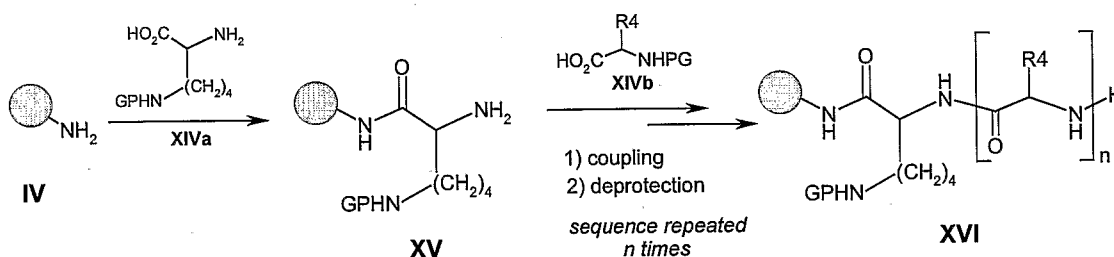
Scheme 210 Scheme 3

Compounds of formula **II** can be obtained using analogous reactions to those described above.



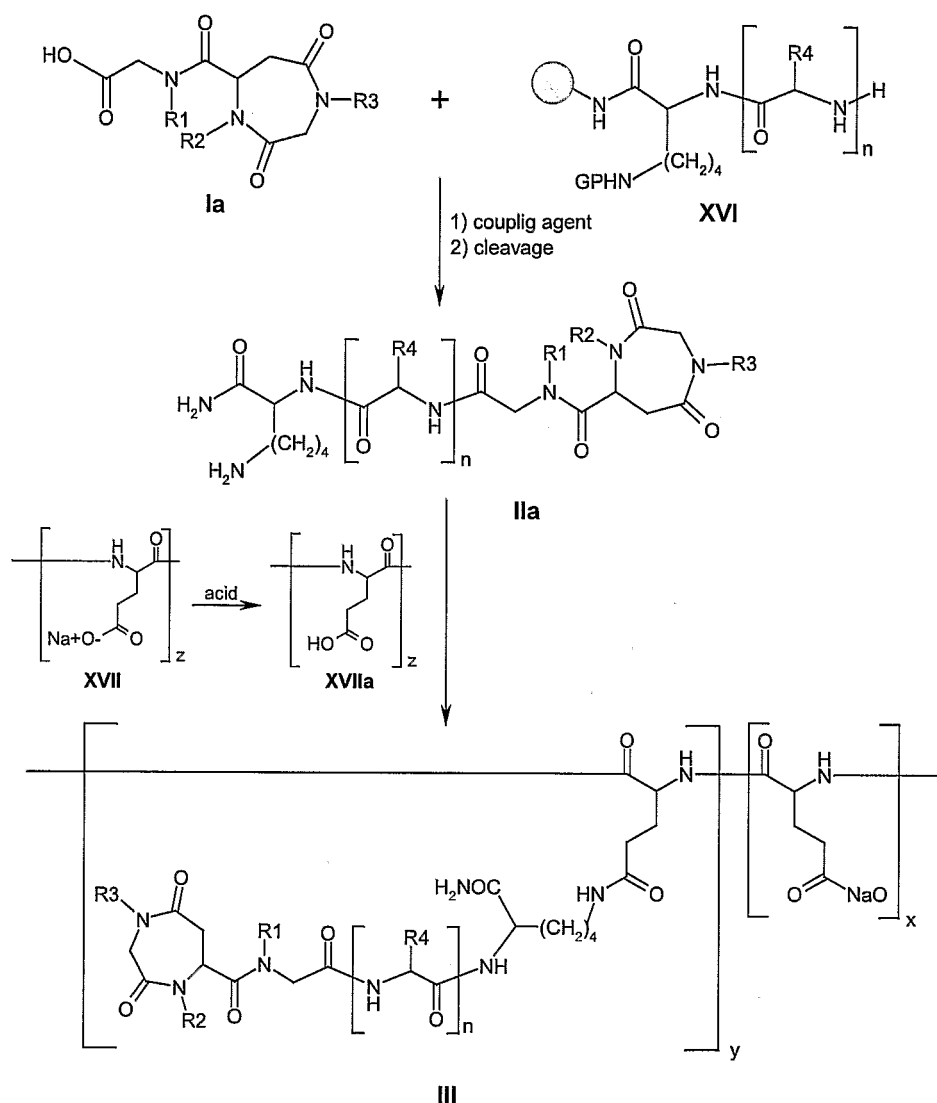
Thus, in order to obtain the corresponding carboxylic acid of formula I, that is, a compound of formula Ia, the same sequence for the synthesis of a compound of formula I is used (using methods A and/or B), except that the starting solid support (**IVa**) has reactive halogen atoms instead of an amino group.

The peptide **XVI** to be bond to compound Ia is obtained also using standard reactions for the synthesis of peptides. Thus, the solid support amine **IV** is firstly reacted with a protected Lysine (**XIVa**) and optionally subsequently reacted with another protected amino acid (**XIVb**). After coupling the protecting group is removed. This sequence can be repeated up to three times.



Carboxylic acid Ia is then reacted with amine **XVI** and after deprotection compound **Ila** is obtained. Amide coupling between **Ila** and polyglutamic acid **XVIIa** (obtained from the corresponding salt **XVII**) yields the drug conjugate **III**.

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The compounds of the present invention are apaf-1 inhibitors. Therefore, they are useful for the treatment or prevention of a condition associated with the excess of apoptosis.

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In adults, post-mitotic cells, such as neurons and heart muscle cells are rarely renewed. Hence their loss can cause neurological and cardiac disorders. These cells undergo apoptotic cell death in a variety of acute and chronic degenerative diseases. Similarly, septic shock, ischaemia, and intoxication can cause massive apoptosis in multiple organs. Infections can cause an elevated apoptotic turnover of specific cell types, for instance, lymphocytes in

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AIDS or gastric mucosa cells in *Helicobacter pylori* infection. Apoptosis events are also of importance in procedures for ischaemic and preservation-reperfusion in organ transplantation. These diseases are therefore candidates for therapeutic cell death suppression.

- 5 Other conditions or diseases related with apoptosis include, without limitation, stroke, traumatism, brain injury, spinal cord injury, meningitis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, multiple sclerosis, prion-related Diseases, as well as other acute or chronic cardiovascular diseases, alcoholic related pathologies, and treatment of
- 10 alopecia.

Thus, the present invention relates to the use of these compounds for the preparation of a medicament for the treatment or prevention of the above mentioned diseases or conditions.

- 15 Another aspect of the present invention relates to the use of a compound of formula I without the R2 proviso or a compound which is a polymer-drug conjugate as previously defined for the manufacture of a medicament for the treatment or prevention of a disease or condition associated with apoptosis.

- 20 Another aspect of the present invention relates to a method for the treatment or prevention of a condition associated with the inhibition of apoptosis comprising administering a compound of formula I without the R2 proviso or a compound which is a polymer-drug conjugate as previously defined and one or more pharmaceutical acceptable excipients.

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- Another aspect of the present invention relates to a method for the treatment or prevention of stroke, traumatism, brain injury, spinal cord injury, meningitis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, multiple sclerosis, prion-related Diseases, myocardial
- 30 ischaemia, as well as other acute or chronic cardiovascular diseases, organ transplantation, alcoholic related pathologies, and treatment of alopecia,

comprising administering a compound of formula I without the R2 proviso or a compound which is a polymer-drug conjugate as previously defined and one or more pharmaceutical acceptable excipients.

- 5 The present invention further provides for pharmaceutical compositions comprising a compound of formula I or a compound which is a polymer-drug conjugate as previously defined, a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable excipients, in either single or multiple doses. The examples of the excipients mentioned
10 below are given by way of illustration only and are not to be construed as limiting the scope of the invention.

The compounds of the present invention can be administered in the form of any pharmaceutical formulation. The pharmaceutical formulation will depend
15 upon the nature of the active compound and its route of administration. Any route of administration may be used, for example such as oral, buccal, pulmonary, topical, parenteral (including subcutaneous, intramuscular, and intravenous), transdermal, ocular (ophthalmic), inhalation, intranasal, otic, transmucosal, implant or rectal administration. However oral, topical or
20 parenteral administration are preferred.

Solid compositions for oral administration include among others tablets, granulates and hard gelatin capsules, formulated both as immediate release or modified release formulations.

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Alternatively, the compounds of the present invention may be incorporated into oral liquid preparations such as emulsions, solutions, dispersions, suspensions, syrups, elixirs or in the form of soft gelatin capsules. They may contain commonly-used inert diluents, such as purified water, ethanol, sorbitol,
30 glycerol, polyethylene glycols (macrogols) and propylene glycol. Aid compositions can also contain coadjuvants such as wetting, suspending,

sweetening, flavouring agents, preservatives, buffers, chelating agents and antioxidants.

Injectable preparations for parenteral administration comprise sterile solutions,
5 suspensions or emulsions in oily or aqueous vehicles, and may contain
coadjuvants, such as suspending, stabilizing, tonicity agents or dispersing
agents.

The compound can also be formulated for its topical application. Formulations
10 include creams, lotions, gels, powders, solutions, shampoo preparations, oral
paste, mouth wash preparations and patches wherein the compound is
dispersed or dissolved in suitable excipients.

In one embodiment of the invention the pharmaceutical composition is in the
15 form of nanospheres, microparticles and nanoparticles.

The effective dosage of active ingredient may vary depending on the particular
compound administered, the route of administration, the nature and severity of
the disease to be treated, as well as the age, the general condition and body
20 weight of the patient, among other factors. A representative example of a
suitable dosage range is from about 0.001 to about 100 mg/Kg body weight
per day, which can be administered as a single or divided doses. However,
the dosage administered will be generally left to the discretion of the
physician.

25

Toxicity and cell recovery through the MTT assay

The MTT assay measures cell proliferation as well as reduction in cell viability
when metabolic events lead to apoptosis or necrosis. It can also be used to
assess the rate of survival of a given cell line when it is incubated in the
30 presence of xenobiotics. The reduction of tetrazolium salts is accepted as a
reliable way to analyse cell proliferation. The yellow tetrazolium MTT (3-(4,5-
dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by

metabolically active cells and results in the intracellular formation of purple formazan that can be spectrophotometrically measured after solubilisation with DMSO.

The MTT assay was used in this invention to assess the toxicity of compounds at different concentrations. To analyse their potential impairment in cell viability, compounds were added to Saos-2 and U937 cell lines at different concentrations and incubated at different times. Results are expressed by percentage cell viability.

		% Cell viability					
		24 h, μ M.			48 h, μ M.		
		10	5	1	10	5	1
Saos-2 cell line	I.1	96	100	99	100	100	100
	I.10	91	100	76	100	100	100
	I.2	99	99	100	91	100	100
	I.11	82	98	100	74	91	100
	I.4	100	97	100	99	98	100
	I.9	82	96	100	66	76	97
	I.3	100	93	97	100	100	100
	I.7	98	99	95	100	100	100
U937 cell line	I.8	99	100	100	100	100	97
	I.1	95	98	100	99	97	100
	I.10	89	99	96	99	98	100
	I.2	97	100	100	83	99	100
	I.11	79	95	100	75	89	98
	I.4	87	99	100	95	94	99
	I.9	88	98	100	59	69	93
	I.3	95	95	100	98	100	100
	I.7	93	95	99	97	100	100
	I.8	99	98	100	99	98	100

10

	Time (h)	% Cell viability of I.1 (μ M.)					% Cell viability of IIa.2 (μ M.)				
		50	25	10	5	1	50	25	10	5	1
Saos-2	24	19	63	100	100	99	10	25	77	100	98
	48	11	19	97	94	97	ND	11	98	97	100
	72	ND	8	80	88	89	ND	ND	ND	ND	ND
U937	24	27	59	87	90	100	ND	ND	ND	ND	ND
	48	ND	ND	83	94	100	ND	ND	ND	ND	ND
	72	ND	ND	67	89	98	ND	ND	ND	ND	ND

	Time (h)	% Cell viability of III.1 (μ M drug-eq.)				
		500	250	100	50	20
Saos-2	24	35	75	93	99	96
	48	ND	ND	95	87	95
	72	ND	ND	68	63	86
U937	24	27	50	84	96	98
	48	ND	ND	ND	ND	ND
	72	ND	ND	ND	ND	ND

The MTT assay was also used to assess cell recovery by the addition of compounds in apoptosis-induced cell lines. For Saos-2 cell line, apoptosis induction was carried out by the addition of doxycyclin 2 μ M; in the case of U937, HeLa, U2-OS, apoptosis was induced by doxorubicin 0.25 μ M, 1.5 μ M, 2 μ M, respectively. Results are expressed as the % of cell viability considering that the addition of doxycyclin and doxorubicin induces 100 % of cell death and 0 % of cell recovery in Saos-2 and HeLa, U2-OS or U937, respectively.

Cell Lines	% cell recovery I.1			
	Time (h)	10 μ M	5 μ M	1 μ M
HeLa	24	30	31	29
	48	3	78	75
	72	2	33	34
U2-OS	24	72	0	0
	48	18	54	58
	72	3	25	1
U937	24	52	19	0
	48	3	19	14
	72	4	46	12
Saos-2	24	15	6	76
	48	8	25	23
	72	2	1	3

Cell Lines	% Cell recovery																
		I.10		I.2		I.11		I.9		I.4		I.3		I.7		I.8	
	Time (h)	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M	5 μ M	1 μ M
Saos-2	24	57	0	10	41	0	22	16	36	9	34	12	18	41	64	23	57
	48	24	0	21	21	8	20	22	31	10	24	21	14	19	27	51	24

ND: Not determined

		% Cell recovery III.1		
		100 μ M	50 μ M	20 μ M
Saos-2	24	25	28	15
	48	18	49	13
	72	15	31	14
U937	24	9	34	5
	48	20	9	1
	72	10	15	0

5 Caspase inhibition assay

The time-dependent inhibition of caspase induced by the compounds described in this invention was analysed. Cell lines were grown in the presence or absence of compounds (50 μ M) at different times. Caspase activity was assayed using Saos-2 or U937 cell extracts and Ac-DEVD-afc (N-acetyl-Asp-Glu-Val-Asp-afc(7-amino-4-trifluoromethyl coumarin)) as substrate (20 μ M). Caspase activity was continuously monitored by the liberation of fluorescent afc using a Cytofluor 4000 fluorimeter (excitation 400 nm; emission 508 nm) for 1 hour. The effect of the compounds on the caspase activity was expressed as the percentage of inhibition in the fluorescence signal of treated cells vs the non-treated cells (control).

Cell Lines	% Caspase inhibition										
		I.1		I.10	I.2	I.11	I.9	I.4	I.3	I.7	I.8
	Time (h)	5 μ M	1 μ M	5 μ M	5 μ M	5 μ M	5 μ M	1 μ M	5 μ M	5 μ M	5 μ M
HeLa	24	31	29	1	1	14	0	48	9	6	5
	48	78	75	6	46	0	0	14	21	7	3
	72	33	34	ND	59	62	41	35	29	11	6

24

U2-OS	24	0	0	0	0	0	0	0	1	0	0
	48	54	58	22	54	31	34	23	29	31	16
	72	25	1	64	27	50	61	54	22	33	24
U937	24	19	0	0	0	11	3	0	12	10	5
	48	19	14	5	3	0	4	12	16	8	15
	72	46	12	2	0	4	63	0	1	2	23
Saos-2	24	53	21	13	11	45	50	55	29	0	1
	48	19	17	4	0	7	2	9	16	24	0
	72	4	4	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not determined

	Time (h)	% Caspase Inhibition IIa.2 (5 μ M.)
Saos-2	24	41
	48	23
	72	1
U937	24	23
	48	11
	72	5

Cell lines	Time (h)	III.1 % Inh. caspase (50 μ M drug-eq.)
Saos-2	24	67
	48	72
	72	100
U937	24	74
	48	85
	72	100

5

Inhibition of Apaf-1-induced apoptosis

The activity of the compounds regards their specific target within the apoptotic machinery was assessed. Apaf-1 oligomerisation is a molecular event that leads to the activation of the apoptosome formation, which ends up in the induction of apoptosis. These experiments were performed in cell-free and in cellular assays.

10

For the cell-free assays, Hek-293 cytoplasmatic extracts were prepared as described before (Malet *et al*, 2005) and a partitioning of the complete cell

extract was carried out. Concomitantly, recombinant Apaf-1 was purified according to Malet *et al*, 2005. The FT fraction (containing caspase-3 and -9 but lacking Apaf-1), was incubated with recombinant Apaf-1 in the presence of different concentrations of the compounds (see figure 1). The activity of the compounds was analysed by the inhibition of caspase activity (see above).

For the cellular assays, Saos-2 and U937 cells were treated with an apoptosome activator (Nguyen and Wells, Proc. Natl. Acad. Sci. USA 2003, 100:7533-7538), which specifically induces Apaf-1 oligomerisation. Afterwards, the compounds were added to the cells (50 μ M) and incubated for 24 or 48 hours. The capacity of the compounds of inhibiting the Apaf-1 induced apoptosis was analysed by the inhibition of caspase activity (see above).

Cell lines	Time (h)	III.1 % Inh. caspase activity (50 μ M drug-eq).
Saos-2	24	22
	48	54
U937	24	27
	48	95

Binding of **Ila.1** to Apaf-1 CARD domain: Fluorescence polarisation assay

Fluorescence polarisation measurements were made in a Victor2V 1420 Multilabel HTS Counter. A 60 nM solution of 5'-6'-carboxyfluoresceinlabelled **Ila.1** (named CF-Ila.1; λ_{exc} =480 nm; λ_{em} =535 nm) in buffer A (total reaction volume was 200 μ l) was titrated with concentrated protein solutions. Data were recorded by using a Wallac 1420 Workstation software. The K_d values were calculated using a two state model (see Figure 2).

To initially characterise the binding site of the peptoids to the apoptosome, we synthesised a fluorescent analogue of **Ila.1** (**CF-Ila.1**). **CF-Ila.1** bound to Apaf-1 CARD domain, but not to cytochrome c and other control proteins (Malet *et al*, 2005), with dissociation constant (K_d) 60 nM as determined in a fluorescence polarisation assay (Figure 2). Nevertheless, the binding constant value contrasted with the concentration of **Ila.1** that was needed to inhibit 50%

of caspase activity in our assays ($IC_{50} \approx 30 \mu M$, Figure 1). A putative explanation to reconcile these data is that the reticulocyte lysate used to in vitro translate procaspase-9 contained components that diminished the effective concentration of **Ila.1**.

5

EXAMPLES

The following abbreviations have been used in the examples:

Boc: *tert*-butoxycarbonyl

10 DCM: dichloromethane

DIC: *N,N'*-diisopropylcarbodiimide

DIPEA: diisopropylethylamine

DMF: *N,N'*-dimethylformamide

eq: molar equivalent

15 EtOAc: ethyl acetate

Fmoc: fluorenylmethoxycarbonyl

Glycine: G

HATU: O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

20 HOBT: 1-hydroxybenzotriazole

Lisine: K

PGA: poly(L-glutamic acid)

Phenylalanine: F

PyBOP: benzotriazole-1-yloxy-tris-pyrrolidinophosphonium

25 hexafluorophosphate

R: Arginine

rt: retention time

TFA: trifluoroacetic acid

Valine: V

30

The present invention will be further illustrated by the following examples. The examples are given by way of illustration only and are not to be construed as

limiting the scope of the invention.

Polystyrene AM RAM resin (0.75 mmol/g) was purchased from Rapp Polymere GmbH (Germany). 2-Chlorotrityl chloride resin (1.4 mmol/g) was purchased from Novabiochem. Semi-preparative RP-HPLC was performed with a Waters (Milford, MA, U.S.A.) system using a X-Terra C₁₈ (19 × 25 cm, 5 μm) column; eluent: A= 0.1% TFA in water, B = 0.1% TFA in acetonitrile, gradient: 0 min 20% B - 20 min 80% B.. High resolution mass spectra (HRMS-FAB) were registered at the Mass Spectrometry Service of the University of Santiago de Compostela (Spain).

The nomenclature used in this document is based on AUTONOM (Automatic Nomenclature), a Beilstein Institute computerized system for the generation of IUPAC systematic nomenclature.

15 Intermediate VIII

VIII: (Z)-but-2-enedioic acid monoallyl ester

To a solution of 2 g of maleic anhydride (20 mmol) in chloroform, 1.8 ml of allyl alcohol (26 mmol, 1.3 eq.) were added. The reaction mixture was stirred at 60°C for 50 min under microwave irradiation. The resulting solution was treated with 1N HCl and extracted with chloroform. The organic extracts were washed with brine, then dried over anhydrous magnesium sulfate and filtered. The solvent was distilled off under reduced pressure, and the obtained residue was purified by column chromatography to obtain intermediate **VIII** as an oil (purity 95%, yield 85%).

25

Compounds of formula I

I.1: 1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]-diazepane-5-carboxylic acid carbamoylmethyl-[2-(2,4-dichlorophenyl)-ethyl]amide

30 The Rink amide resin (500 mg, 0.4 mmol) was treated with 3 mL of 20% piperidine in DMF and the mixture was stirred in a microwave reactor for 2 min at 60 °C. The resin was filtered and washed with DMF (3 x 3 mL), isopropyl

alcohol (3 x 3 mL) and DCM (3 x 3 mL). The resin was treated with a solution of bromoacetic acid (**V**, 275 mg, 5 eq.) and DIC (306 μ L, 5 eq.) in DMF (3 mL). The reaction mixture was stirred for 2 min at 60 °C in a microwave reactor. The resin was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). A solution of 2,4-dichlorophenethylamine (**Vla**, 300 μ L, 5 eq.) and triethylamine (275 μ L, 5 eq.) in 3 mL of DMF was added to the resin and the suspension was stirred for 2 min at 90 °C under microwave activation. The supernatant was removed and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Then, the resin was treated with a solution of (Z)-but-2-enedioic acid monoallyl ester (**VIII**, 310 mg, 5 eq.), HOBT (267 mg, 5 eq.) and DIC (306 μ L, 5 eq.) in DCM: DMF (2:1, 3 mL). The reaction mixture was stirred at room temperature for 30 min, filtered and the reaction was repeated under the same conditions. The resin was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). Then, a solution of 3,3-diphenylpropylamine (**Vlb**, 418 mg, 5 eq.) and triethylamine (275 μ L, 5 eq.) in 3 mL of DMF was added to the resin and the suspension was stirred for 3 h at room temperature. The reaction was repeated for overnight at the same temperature. The supernatant was removed and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Then, the resin was treated with a solution of bromoacetic acid (**V**, 275 mg, 5 eq.) and DIC (306 μ L, 5 eq.) in DMF (3 mL). The reaction mixture was stirred for 2 min at 60 °C in a microwave reactor. The resin was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). A solution of 2,4-dichlorophenethylamine (**Vlc**, 200 μ L, 5 eq.) and triethylamine (210 μ L, 5 eq.) in 3 mL of DMF was added to the resin and the suspension was stirred for 2 min at 90 °C under microwave activation. The supernatant was removed, and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Then, the resin was treated with tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.1 eq.) and phenylsilane (490 μ L, 10 eq.) in anhydrous DCM for 15 min at room temperature under an argon atmosphere. This procedure was

repeated three times. The supernatant was removed and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). The cyclization was promoted by treatment with PyBOP (308 mg, 1.5 eq.), HOBT (80 mg, 1.5 eq.) and DIPEA (203 μ L, 3 eq.) in DMF (3 mL). The reaction mixture was stirred at room temperature for 16 h and filtered. The resin was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). Finally, treatment of the resin with a mixture of 60:40:2 TFA/DCM/water released a crude reaction mixture containing the titled compound **I.1**, which was filtered. Solvents from the filtrate were removed by evaporation under reduced pressure followed by lyophilization. The residue obtained was purified by semipreparative RP-HPLC using aqueous acetonitrile gradient (42% acetonitrile $\rightarrow$ 80% acetonitrile, 35 min) to give 91 mg of the desired product (yield 30%, $\geq$ 98% purity).

HRMS ($M + H$)⁺: Calcd. for $C_{39}H_{38}Cl_4N_4O_4$, 767.1647; found, 767.1720

Following an analogous procedure to that described for example **I.1** but using different amines, the following products were obtained:

Example	Compound name	HRMS ($M + H$) ⁺ :	
		Calculated	found
I.2	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethylcyclopropylamide	$C_{34}H_{36}Cl_2N_4O_4$ 635.2114	635.2186
I.3	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid (2-acetylaminoethyl)carbamoylmethylamide	$C_{35}H_{39}Cl_2N_5O_5$ 680.2328	680.2401
I.4	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl(tetrahydrofuran-2-ylmethyl)amide	$C_{35}H_{40}Cl_2N_4O_5$ 679.2376	679.2449
I.5	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl-[2-(4-methoxyphenyl)ethyl]amide	$C_{40}H_{42}Cl_2N_4O_5$ 729.2532	729.2605
I.6	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethylcyclohexylamide	$C_{37}H_{42}Cl_2N_4O_4$ 677.2583	677.2656
I.7	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl-[2-(2-methoxyphenyl)ethyl]amide	$C_{40}H_{42}Cl_2N_4O_5$ 729.2532	729.2605

Example	Compound name	HRMS (M + H) ⁺ :	
		Calculated	found
I.8	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl[2-(4-chlorophenyl)ethyl]amide	C ₃₉ H ₃₉ Cl ₃ N ₄ O ₄ 733.2037	733.2110
I.9	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl[2-(2-fluorophenyl)-ethyl]amide	C ₃₉ H ₃₉ Cl ₂ FN ₄ O ₄ 717.2332	717.2405
I.10	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid carbamoylmethyl[2-(4-fluorophenyl)ethyl]amide	C ₃₉ H ₃₉ Cl ₂ FN ₄ O ₄ 717.2332	717.2405
I.11	1-[2-(2,4-Dichloro-phenyl)ethyl]-4-(3,3-diphenyl-propyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid butyl-carbamoylmethylamide	C ₃₅ H ₄₀ Cl ₂ N ₄ O ₄ 651.2427	651.2499

Intermediate Ia

Ia: {[2-(2,4-Dichlorophenyl)ethyl]-[1-[2-(2,4-dichlorophenyl)ethyl]-4-(3,3-di-phenylpropyl)-3,7-dioxo[1,4]diazepane-5-carbonyl]amino}acetic acid

- 5 The 2-chlorotrityl chloride resin (**IVa**, 700 mg, 0.98 mmol) was treated with a solution of chloroacetic acid (**V**, 463 mg, 5 eq.) and DIPEA (840 μ L, 5 eq.) in DCM (4 mL). The mixture was stirred at room temperature for 1 h, the reaction was quenched with methanol (550 μ L) and stirring was prolonged for 10 min. The supernatant was removed and the residue was drained and washed with
- 10 DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). A solution of 2,4-dichlorophenethylamine (**VIa**, 740 μ L, 5 eq.) and DIPEA (840 μ L, 5 eq.) in 4 mL of DMF was added to the resin and the suspension was stirred for 3 h at room temperature. The supernatant was removed and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and
- 15 DCM (3 x 3 mL). Then, the resin was treated with a solution of (Z)-but-2-enedioic acid monoallyl ester (**VIII**) (765 mg, 5 eq.), HOBT (662 mg, 5 eq.) and DIC (760 μ L, 5 eq.) in 2:1 DCM:DMF (4 mL). The reaction mixture was stirred for 30 min at room temperature, filtered and repeated under the same conditions. Then, a solution of 3,3-diphenylpropylamine (**VIb**, 1.0 g, 5 eq.) and
- 20 DIPEA (840 μ L, 5 eq.) in 4 mL of DMF was added to the resin and the suspension was stirred for 3 h at room temperature. The reaction was repeated stirring was prolonged for 16 h at the same temperature. The

supernatant was removed and the residue was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Then, the resin was treated with a solution of chloroacetic acid (**V**, 463 mg, 5 eq.) and DIC (760 μ L, 5 eq.) in 2:1 DCM: DMF (4 mL). The reaction mixture was stirred for 5 30 min at room temperature. The resin was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). A solution of 2,4-dichlorophenethylamine (**VIc**, 740 μ L, 5 eq.) and DIPEA (840 μ L, 5 eq.) in 4 mL of DMF was added to the resin and the suspension was stirred for 3 h at room temperature. The supernatant was removed, and the residue was 10 drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Then, the resin was treated with tetrakis(triphenylphosphine)palladium(0) (114 mg, 0.1 eq.) and phenylsilane (1.2 mL, 10 eq.) in anhydrous DCM for 15 min at room temperature under an argon atmosphere. This synthetic step was carried out by triplicate. The 15 supernatant was removed and the residue was drained and washed with DCM (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DMF (3 x 3 mL). The cyclization was promoted by treatment with PyBOP (765 mg, 1.5 eq.), HOBT (199 mg, 1.5 eq.) and DIPEA (505 μ L, 3 eq.) in DMF (4 mL). The mixture was stirred at room temperature for 48 h and filtered. The resin was drained and washed 20 with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Finally, treatment of the resin with a mixture of 5:95 TFA/DCM released a crude reaction mixture which was filtered. Solvents from the filtrate were removed under reduced pressure followed by lyophilization to give the compound **Ia** (460 mg, yield 61%, $\geq$ 55% purity) as a colourless solid.

25

Intermediates IIa

The Rink amide resin (**IV**, 505 mg, 0.4 mmol) was deprotected with 3 mL of 20% piperidine in DMF. The mixture was stirred in a microwave reactor for 2 min at 60 °C. The resin was filtered and washed with DMF (3 x 3 mL), 30 isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Fmoc-L-Lys(Boc)-OH (**XIVa**, 2 eq) was linked to the resin using HOBT (108 mg, 2 eq.) and DIC (125 μ L, 2 eq.). The mixture was stirred for 1 h at room temperature. The resin was

drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Upon removal of the Fmoc group with 3 mL of 20% piperidine in DMF (30 min), the resin was washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). If necessary, the other amino acids (**XIVb**) were coupled under the same conditions as described above to yield intermediate **XVI**.

To the intermediate **XVI** swelled in DMF, acid **la** (460 mg, 1.5 eq.) was added in the presence of HATU (228 mg, 1.5 eq.) and DIPEA (205 μ L, 3 eq.) in DMF (3 mL). The reaction mixture was stirred at room temperature for 16 h and filtered. The resin was drained and washed with DMF (3 x 3 mL), isopropyl alcohol (3 x 3 mL) and DCM (3 x 3 mL). Treatment of the resin with a mixture of 80:20:2.5:2.5 TFA/DCM/water/ triisopropylsilane afforded released a crude reaction mixture containing compound **Ila**, that was filtered. Solvents from the filtrate were removed under reduced pressure, followed by lyophilization. The residue obtained was purified by semipreparative RP-HPLC using an aqueous-acetonitrile gradient mixture.

Example	Compound name	HPLC-MS	
		rt	m/z
Ila.1 (KGG-Ia.1)	1-[2-(2,4-Dichlorophenyl)ethyl]-3-(3,3-diphenyl-propyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid {[[[(5-amino-1-carbamoylpentylcarbamoyl)methyl]-carbamoyl)methyl]carbamoyl]methyl}-[2-(2,4-dichlorophenyl)ethyl]amide	17.0	1011,506
Ila.2 (K-Ia.1)	1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenyl-propyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid [(5-amino-1-carbamoylpentylcarbamoyl)methyl]-[2-(2,4-dichlorophenyl)ethyl]amide	17.6	895
Ila.3 (KRFG-Ia.1)	1-[2-(2,4-Dichlorophenyl)ethyl]- 4-(3,3-diphenylpro-pyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid {[[[1-[1-(5-amino-1-carbamoylpentylcarbamoyl)-4-guanidinobutylcarbamoyl]-2-phenylethylcarbamoyl]methyl]carbamoyl]methyl}-[2-(2,4-dichlorophenyl)ethyl]amide	13.6	1257,629
Ila.4 (KRFV-Ia.1)	1-[2-(2,4-dichlorophenyl)ethyl]- 4-(3,3-diphenylpropyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid [(1-[1-[1-(5-amino-1-carbamoylpentylcarbamoyl)-4-guanidinobutylcarbamoyl]-2-phenylethylcarbamoyl]-2-methylpropylcarbamoyl)methyl]- 2-(2,4-dichlorophenyl)ethylamide	17.4	1299,650

Compounds of formula III

III.1: PGA-IIa.1

To a solution of PGA sodium salt (**XVII**, 0.1 g, 0.5 mmol) with a molecular weight (M_w) range 17000-43000 Da in water, 0.2 M HCl was added until the pH of the solution was adjusted to 2.0. The precipitate was collected, dialyzed against water, and lyophilized. Then PGA in its protinic form (**XVIIa**) was dissolved in dry DMF (7.5 mL) and DIC (3 eq) and HOBt (3 eq) were added and left stirring for 15 min. Then intermediate **IIa.1** (0.052 mmol (12 mol%) 1 eq) was added and the pH adjusted to 8 with DIPEA (1.5 mL). The reaction was allowed to proceed at room temperature for 36 h. Thin layer chromatography (TLC, silica) showed complete conversion of **IIa.1** ($R_f=0.5$) to polymer conjugate ($R_f=0$, MeOH:H₂O:AcOH=85:10:5). To stop the reaction, the mixture was poured into CHCl₃. The resulting precipitate was collected and dried in vacuum. The sodium salt of PGA-peptoid conjugate **III.1** was obtained by dissolving the product in 1.0 M NaHCO₃. This aqueous solution was dialysed against distilled water (M_w CO 6,000-8000 Da). Lyophilization of the dialysate yielded the product as a white powder. The total peptoid content in the polymer as determined by UV ($\lambda=282$ nm) was in the range of 25-30 % (w/w) (10-12 mol% functionalization), free drug content < 1wt% total drug.

The average molecular weight (M_w), polydispersity (M_w/M_n) and the behavior of **III.1** and control **XVII** in solution were analyzed by size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC). Both techniques showed **III.1** as a more compact conformational structure with smaller hydrodynamic volume and greater sedimentation coefficient (S) than **XVII** ($t_r = 12.5$ min and $S = 3.5 \pm 0.4$ s vs. $t_r = 11.6$ min and $S = 1.9 \pm 0.1$ s for **III.1** and **XVII**, respectively). By sedimentation equilibrium M_w was determined as 26700 Da for **XVII** and 58600 Da for **III.1**, however M_w of 36000 Da ($M_w/M_n = 1.8$) and 38000 Da ($M_w/M_n = 1.5$) were determined for **XVII** and **III.1**, respectively, by GPC using PEG standards.

Determination of total drug content by UV spectroscopy

A stock solution of **IIa** in MeOH was prepared (1 mg/mL). To obtain a calibration curve, samples were diluted using MeOH to give a concentration range of 0–1 mg/mL. The total drug loading of the conjugates was determined by measuring the optical density at 272 nm and 281 nm in H₂O. PGA in the same concentration range as **III** conjugate analyzed (0–5 mg/mL) in H₂O was used as blank. The extinction coefficient found for **IIa** at 281 nm in MeOH at RT was $\epsilon = 713 \text{ L mol}^{-1} \text{ cm}^{-1}$.

10 Determination of free drug content by HPLC

Aqueous solutions of **III.1** (1 mg/mL) were prepared, and an aliquot (100 μL) was added to a polypropylene tube and made up to 1 mL with water. The pH of the samples was adjusted to 8 with ammonium formate buffer (100 μL , 1 M, pH 8.5), and then CHCl_3 (5 mL) was added. Samples were then thoroughly extracted by vortexing (3 x 10 sec). The upper aqueous layer was carefully removed and the solvent was evaporated under N_2 . The dry residue was dissolved in 200 μL of HPLC grade acetonitrile. In parallel the same procedure was carried out for the parent compound **IIa.1** (using 200 μL of a 1 mg/mL stock aqueous solution). Addition of 1 mL of acetonitrile to redissolve the product gave a 200 $\mu\text{g/mL}$ stock from which a range of concentrations was prepared (2 to 100 $\mu\text{g/mL}$). The free amount of drug in the conjugates was determined by HPLC using a Lichrospher[®] C18 (150 x 3.9 mm) column. Flow rate 1 mL/min using an acetonitrile gradient in aqueous 0.1 % TFA (from 20 to 100% of acetonitrile in 20 min). The retention time was $t_r = 14.3 \text{ min}$ for **IIa.1** ($\lambda = 220 \text{ nm}$).

Following these procedure as for **III.1**, the following examples were obtained:

Example	Compound name	SEC ^a	
		rt	Mw
III.1: PGA- IIa.1	Poly-L-glutamic acid-[1-[2-(2,4-Dichlorophenyl)ethyl]-3-(3,3-diphenyl-propyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid {[(5-amino-1-carbamoylpentylcarbamoyl)methyl]-	12.5	36000

Example	Compound name	SEC ^a	
		rt	Mw
conjugate	carbamoyl)methyl)carbamoyl)methyl)-[2-(2,4-dichlorophenyl)ethyl]amide] conjugate		
III.2: PGA- IIa.2 conjugate	Poly-L-glutamic acid-[1-[2-(2,4-Dichlorophenyl)ethyl]-4-(3,3-diphenyl-propyl)-3,7-dioxo[1,4]diazepane-5-carboxylic acid [(5-amino-1-carbamoylpentylcarbonyl)methyl]-[2-(2,4-dichlorophenyl)ethyl]amide] conjugate	13.3	34000

^a Determined by Size Exclusion Chromatography (SEC)

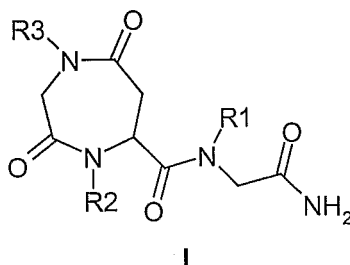
DESCRIPTION OF THE DRAWINGS

FIGURE 1: Cell-free caspase inhibition assay using Hek-293 FT cytoplasmatic fraction incubated with recombinant Apaf-1 in the presence of different concentrations the compound **IIa.1**.

FIGURE 2: Analysis of the CARD/**IIa.1** interaction. A 240 nM solution of CF-**IIa.1** in PBS buffer was titrated with increasing concentrations of CARD-Apaf and fluorescence polarization was measured at 30 °C. The data (mean s.d.; n=3) were recorded in millipolarization units (mP).

CLAIMS

1.- A compound of general formula I,



5 its stereoisomers and mixtures thereof, its polymorphs and mixtures thereof and the pharmaceutically acceptable solvates and addition salts thereof, wherein:

R1 represents (C₁-C₅)alkyl, (C₂-C₅)alkenyl, -(CH₂)₁₋₃-NHCO-(C₁-C₃)alkyl, -(CH₂)₁₋₃-CONRaRb, -(CH₂)₀₋₃-(3-6)Cy, -(CH₂)₀₋₃-(5-6)Hetcy, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₂-CH(phenyl)₂, -(CH₂)₂-CH(phenyl)[(5-6)Hetar] or -(CH₂)₂-CH[(5-6)Hetar]₂;

R2 represents -(CH₂)₀₋₃-(3-6)Cy, -(CH₂)₀₋₃-(5-6)Hetcy, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₂-CH(phenyl)₂, -(CH₂)₂-CH(phenyl)[(5-6)Hetar] or -(CH₂)₂-CH[(5-6)Hetar]₂;

R3 represents -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl), or -(CH₂)₁₋₃-(5-6)Hetar;

(C₁-C₅)alkyl and -(C₂-C₅)alkenyl may be optionally substituted with one or more substituents selected from -O-(C₁-C₂)alkyl, -S-(C₁-C₂)alkyl, -NH₂, -NH-(C₁-C₂)alkyl and -N[(C₁-C₂)alkyl]₂;

(C₁-C₃)alkyl can be optionally substituted with one or more halogen atoms;

Ra and Rb independently represent -H or -(C₁-C₃)alkyl

(3-6)Cy represents a radical of a 3- to 6-membered partially unsaturated or
5 saturated carbocyclic ring;

(5-6)Hetcy represents a C- or N- radical of a 5- or 6-membered, partially
unsaturated or saturated carbocyclic ring containing from one or two
heteroatoms independently selected from O, S and N;
10

Cy and Hetcy can be optionally substituted with one or more substituents
selected from halogen, -CF₃ and -OH;

(5-6)Hetar and (5-10)Hetar represent a C- or N- radical of an aromatic 5- or 6-
15 membered and 5- to 10-membered ring respectively; containing from one to
four heteroatoms independently selected from O, S and N;

phenyl, 1-naphthyl, 2-naphthyl, (5-6)Hetar and (5-10)Hetar may be optionally
substituted with one or more substituents selected from halogen, -CF₃, -OH,
20 -O-(C₁-C₃)alkyl, -CO-(C₁-C₃)alkyl, -(C₁-C₅)alkyl-NRaRb, -NRaRb and
-SO₂NH₂;

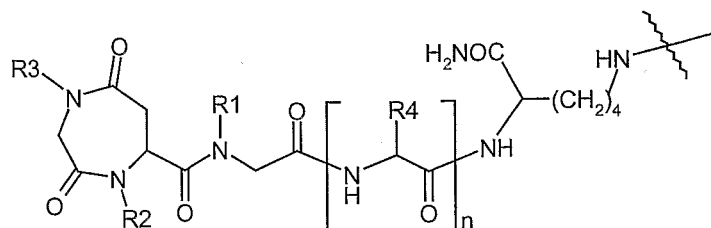
with the proviso that R₂ does not represent 2-(4-fluorophenyl)ethyl.

25 2. A compound according to claim 1 wherein R₁ represents -(CH₂)₀₋₃-
(5-6)Hetcy, -(CH₂)₁₋₃-(5-10)Hetar, -(CH₂)₁₋₃-phenyl, -(CH₂)₁₋₃-(1-naphthyl) or
-(CH₂)₁₋₃-(2-naphthyl), all of them optionally substituted.

30 3. A compound according to claim 1 or 2 wherein R₂ represents -(CH₂)₁₋₃-
phenyl, -(CH₂)₁₋₃-(1-naphthyl), -(CH₂)₁₋₃-(2-naphthyl) or -(CH₂)₂-CH(phenyl)₂,
all of them optionally substituted, with the proviso that R₂ does not represent
2-(4-fluorophenyl)ethyl.

4. A compound according to any of claims 1 to 3 wherein R3 represents -
 (CH₂)₁₋₃-(5-6)Heter or -(CH₂)₂-phenyl, both optionally substituted.

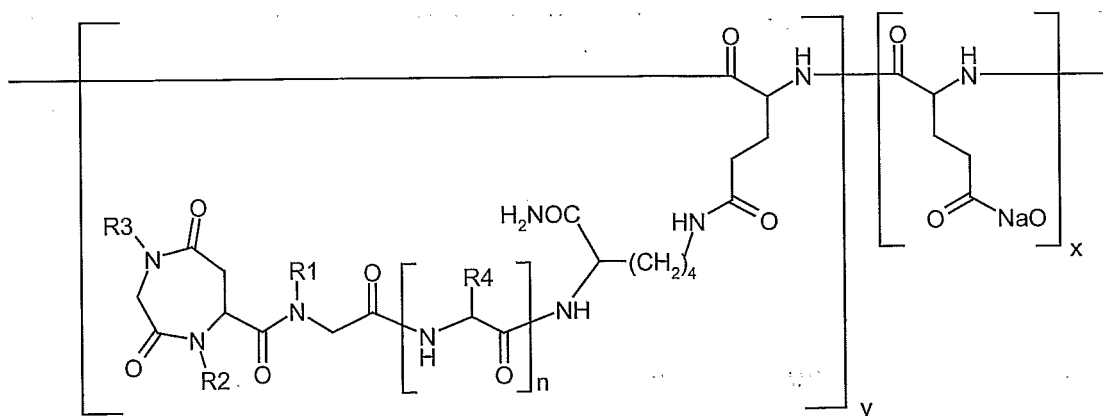
5 5.- A compound which is a drug conjugate comprising a radical of formula II



II

its stereoisomers and mixtures thereof and the pharmaceutically acceptable
 solvates and addition salts thereof, conjugated to a polyglutamic acid polymer,
 wherein R1, R2 and R3 have the meanings as defined in claim 1 without the
 R2 proviso; each R4 independently represents a side chain from any of the 20
 10 naturally occurring amino acids, and n is 0,1, 2 or 3.

6.- A compound of formula III which is a drug conjugate according to claim 2



III

wherein the proportion of y to x is 25-30% to 75-70% by weight.

15 7.- A compound according to claim 5 or 6 wherein the polyglutamic acid is
 poly(L-glutamic acid); n represents 2 and R4 represents a glycine side chain.

8.- Use of a compound of formula I as defined in claim 1 without the R2 proviso or a compound as defined in claim 5 for the manufacture of a medicament for the treatment or prevention of a condition associated with the inhibition of apoptosis.

9.- Use according to claim 8 wherein the condition is selected from stroke, traumatism, brain injury, spinal cord injury, meningitis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, multiple sclerosis, prion-related Diseases, myocardial ischaemia, as well as other acute or chronic cardiovascular diseases, organ transplantation, alcoholic related pathologies or treatment of alopecia.

10.- A method for the treatment or prevention of a condition associated with the inhibition of apoptosis comprising administering a compound of formula I as defined in claim 1 without the R2 proviso or a compound as defined in claim 5 and one or more pharmaceutical acceptable excipients.

11.- A method according to claim 10 wherein the condition is selected from stroke, traumatism, brain injury, spinal cord injury, meningitis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, multiple sclerosis, prion-related Diseases, myocardial ischaemia, as well as other acute or chronic cardiovascular diseases, organ transplanted alcoholic related pathologies or treatment of alopecia.

12.- A pharmaceutical composition which comprises an effective amount of a compound of formula I as defined in claim 1 or a compound as defined in claim 5, a pharmaceutically acceptable salt or solvate thereof, and one or more pharmaceutical acceptable excipients.

13.- A pharmaceutical composition according to claim 12 in the form of nanospheres, microparticles and nanoparticles.