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Substituted 2-alkyl quinazolinone derivatives as PARP inhibitors

57	ABSTRACT (NOT MORE THAN 150 WORDS)
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The sheet(s) containing the abstract is/are attached.

If no classification is furnished, Form P.9 should accompany this form.
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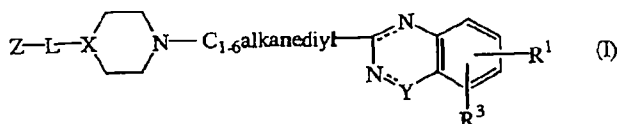
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(54) Title: SUBSTITUTED 2-ALKYL QUINAZOLINONE DERIVATIVES AS PARP INHIBITORS



(57) Abstract: The present invention provides
compounds of formula (I), their use as PARP inhibitors
as well as pharmaceutical compositions comprising said
compounds of formula (I) wherein R¹, R², L, X, Y and
Z have defined meanings.

SUBSTITUTED 2-ALKYL QUINAZOLINONE DERIVATIVES AS PARP
INHIBITORS

5 Field of the invention

The present invention relates to inhibitors of PARP and provides compounds and compositions containing the disclosed compounds. Moreover, the present invention provides methods of using the disclosed PARP inhibitors for instance as a medicine.

10 Background of the invention

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) is a member of the PARP enzyme family. This growing family of enzymes consist of PARPs such as, for example: PARP-1, PARP-2, PARP-3 and Vault-PARP; and Tankyrases (TANKs), such as, for example: TANK-1, TANK-2 and TANK-3. PARP is also referred to as
15 poly(adenosine 5'-diphospho-ribose) polymerase or PARS (poly(ADP-ribose) synthetase).

PARP-1 is a major nuclear protein of 116 kDa consisting of three domains : the N-terminal DNA binding domain containing two zinc fingers, the automodification
20 domain and the C-terminal catalytic domain. It is present in almost all eukaryotes. The enzyme synthesizes poly(ADP-ribose), a branched polymer that can consist of over 200 ADP-ribose units. The protein acceptors of poly(ADP-ribose) are directly or indirectly involved in maintaining DNA integrity. They include histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and Ca²⁺- and Mg²⁺-dependent endonucleases.
25 PARP protein is expressed at a high level in many tissues, most notably in the immune system, heart, brain and germ-line cells. Under normal physiological conditions, there is minimal PARP activity. However, DNA damage causes an immediate activation of PARP by up to 500-fold.

30 Tankyrases (TANKs) were identified as components of the human telomeric complex. They have also been proposed to have a role in vesicle trafficking and may serve as scaffolds for proteins involved in various other cellular processes. Telomeres, which are essential for chromosome maintenance and stability, are maintained by telomerase, a specialized reverse transcriptase. TANKs are (ADP-ribose)transferases with some
35 features of both signalling and cytoskeletal proteins. They contain the PARP domain, which catalyses poly-ADP-ribosylation of substrate proteins, the sterile alpha motif, which is shared with certain signalling molecules and the ANK domain, which contains 24 ankyrin repeats homologues to the cytoskeletal protein ankyrin. The ANK domain

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interacts with a telomeric protein, Telomere Repeat binding Factor-1 (TRF-1). These proteins were therefore named TRF1-interacting, ankyrin-related ADP-ribose polymerase (TANKs).

5 One of the more specific functions of TANK is the ADP-ribosylation of TRF-1. Human telomere function requires two telomere-specific DNA binding proteins, TRF-1 and TRF-2. TRF-2 protects chromosome ends, and TRF-1 regulates telomere length. ADP-ribosylation inhibits the ability of TRF-1 to bind to telomeric DNA. This poly-ADP-ribosylation of TRF-1 releases TRF-1 from the telomeres, opening up the telomeric
10 complex and allow access to telomerase. Therefore, TANK functions as a positive regulator of telomere length., allowing elongation of the telomeres by telomerase.

Among the many functions attributed to PARP, and especially PARP-1, is its major role in facilitating DNA repair by ADP-ribosylation and therefore co-ordinating a
15 number of DNA repair proteins. As a result of PARP activation, NAD^+ levels significantly decline. Extensive PARP activation leads to severe depletion of NAD^+ in cells suffering from massive DNA damage. The short half-life of poly(ADP-ribose) results in a rapid turnover rate. Once poly(ADP-ribose) is formed, it is quickly degraded by the constitutively active poly(ADP-ribose) glycohydrolase (PARG),
20 together with phosphodiesterase and (ADP-ribose) protein lyase. PARP and PARG form a cycle that converts a large amount of NAD^+ to ADP-ribose. In less than an hour, over-stimulation of PARP can cause a drop of NAD^+ and ATP to less than 20% of the normal level. Such a scenario is especially detrimental during ischaemia when deprivation of oxygen has already drastically compromised cellular energy output.
25 Subsequent free radical production during reperfusion is assumed to be a major cause of tissue damage. Part of the ATP drop, which is typical in many organs during ischaemia and reperfusion, could be linked to NAD^+ depletion due to poly(ADP-ribose) turnover. Thus, PARP or PARG inhibition is expected to preserve the cellular energy level thereby potentiating the survival of ischaemic tissues after insult.

30 Poly(ADP-ribose) synthesis is also involved in the induced expression of a number of genes essential for inflammatory response. PARP inhibitors suppress production of inducible nitric oxide synthase (iNOS) in macrophages, P-type selectin and intercellular adhesion molecule-1 (ICAM- 1) in endothelial cells. Such activity underlies the strong
35 anti-inflammation effects exhibited by PARP inhibitors. PARP inhibition is able to reduce necrosis by preventing translocation and infiltration of neutrophils to the injured tissues.

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PARP is activated by damaged DNA fragments and, once activated, catalyzes the attachment of up to 100 ADP-ribose units to a variety of nuclear proteins, including histones and PARP itself. During major cellular stresses the extensive activation of PARP can rapidly lead to cell damage or death through depletion of energy stores. As four molecules of ATP are consumed for every molecule of NAD⁺ regenerated, NAD⁺ is depleted by massive PARP activation, in the efforts to re-synthesize NAD⁺, ATP may also become depleted.

10 It has been reported that PARP activation plays a key role in both NMDA- and NO-induced neurotoxicity. This has been demonstrated in cortical cultures and in hippocampal slices wherein prevention of toxicity is directly correlated to PARP inhibition potency. The potential role of PARP inhibitors in treating neurodegenerative diseases and head trauma has thus been recognized even if the exact mechanism of action has not yet been elucidated.

Similarly, it has been demonstrated that single injections of PARP inhibitors have reduced the infarct size caused by ischemia and reperfusion of the heart or skeletal muscle in rabbits. In these studies, a single injection of 3-amino-benzamide (10 mg/kg), either one minute before occlusion or one minute before reperfusion, caused similar reductions in infarct size in the heart (32-42%) while 1,5-dihydroxyisoquinoline (1 mg/kg), another PARP inhibitor, reduced infarct size by a comparable degree (38-48%). These results make it reasonable to assume that PARP inhibitors could salvage previously ischaemic heart or reperfusion injury of skeletal muscle tissue.

25 PARP activation can also be used as a measure of damage following neurotoxic insults resulting from exposure to any of the following inducers like glutamate (via NMDA receptor stimulation), reactive oxygen intermediates, amyloid β -protein, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its active metabolite N-methyl-4-phenylpyridine (MPP⁺), which participate in pathological conditions such as stroke, Alzheimer's disease and Parkinson's disease. Other studies have continued to explore the role of PARP activation in cerebellar granule cells in vitro and in MPTP neurotoxicity. Excessive neural exposure to glutamate, which serves as the predominate central nervous system neurotransmitter and acts upon the N-methyl D-aspartate (NMDA) receptors and other subtype receptors, most often occurs as a result of stroke or other neurodegenerative processes. Oxygen deprived neurons release glutamate in great quantities during ischaemic brain insult such as during a stroke or heart attack.

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This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors, which open ion channels and permit uncontrolled ion flow (e.g., Ca^{2+} and Na^+ into the cells and K^+ out of the cells) leading to overstimulation of the neurons. The over-stimulated neurons

5 secrete more glutamate, creating a feedback loop or domino effect which ultimately results in cell damage or death via the production of proteases, lipases and free radicals. Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions including epilepsy, stroke, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, schizophrenia,

10 chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Glutamate exposure and stimulation has also been implicated as a basis for compulsive disorders, particularly drug dependence. Evidence includes findings in many animal species, as well as in cerebral cortical cultures treated with glutamate or NMDA, that glutamate receptor antagonists (i.e., compounds which

15 block glutamate from binding to or activating its receptor) block neural damage following vascular stroke. Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind and hence finding an effective mix of antagonists or universal antagonist to prevent binding of glutamate to all of the receptor

20 and allow testing of this theory, has been difficult. Moreover, many of the compositions that are effective in blocking the receptors are also toxic to animals. As such, there is presently no known effective treatment for glutamate abnormalities.

The stimulation of NMDA receptors by glutamate, for example, activates the enzyme neuronal nitric oxide synthase (nNOS), leading to the formation of nitric oxide (NO),

25 which also mediates neurotoxicity. NMDA neurotoxicity may be prevented by treatment with nitric oxide synthase (NOS) inhibitors or through targeted genetic disruption of nNOS in vitro.

Another use for PARP inhibitors is the treatment of peripheral nerve injuries, and the

30 resultant pathological pain syndrome known as neuropathic pain, such as that induced by chronic constriction injury (CCI) of the common sciatic nerve and in which transsynaptic alteration of spinal cord dorsal horn characterized by hyperchromatosis of cytoplasm and nucleoplasm (so-called "dark" neurons) occurs.

35 Evidence also exists that PARP inhibitors are useful for treating inflammatory bowel disorders, such as colitis. Specifically, colitis was induced in rats by intraluminal administration of the hapten trinitrobenzene sulfonic acid in 50% ethanol. Treated rats

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received 3-aminobenzamide, a specific inhibitor of PARP activity. Inhibition of PARP activity reduced the inflammatory response and restored the morphology and the energetic status of the distal colon.

- 5 Further evidence suggests that PARP inhibitors are useful for treating arthritis. Further, PARP inhibitors appear to be useful for treating diabetes. PARP inhibitors have been shown to be useful for treating endotoxic shock or septic shock.

10 PARP inhibitors have also been used to extend the lifespan and proliferative capacity of cells including treatment of diseases such as skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, degenerative diseases of skeletal muscle involving replicative senescence, age-related muscular degeneration, immune senescence, AIDS, and other immune senescence disease; and to alter gene expression of senescent cells.

15

It is also known that PARP inhibitors, such as 3-amino benzamide, affect overall DNA repair in response, for example, to hydrogen peroxide or ionizing radiation.

20 The pivotal role of PARP in the repair of DNA strand breaks is well established, especially when caused directly by ionizing radiation or, indirectly after enzymatic repair of DNA lesions induced by methylating agents, topoisomerases I inhibitors and other chemotherapeutic agents as cisplatin and bleomycin. A variety of studies using "knockout" mice, trans-dominant inhibition models (over-expression of the DNA-binding domain), antisense and small molecular weight inhibitors have demonstrated
25 the role of PARP in repair and cell survival after induction of DNA damage. The inhibition of PARP enzymatic activity should lead to an enhanced sensitivity of the tumor cells towards DNA damaging treatments.

30 PARP inhibitors have been reported to be effective in radiosensitizing (hypoxic) tumor cells and effective in preventing tumor cells from recovering from potentially lethal and sublethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA strand break rejoining and by affecting several DNA damage signaling pathways.

35 PARP inhibitors have been used to treat cancer. In addition, U.S. Patent No.5,177,075 discusses several isoquinolines used for enhancing the lethal effects of ionizing radiation or chemotherapeutic agents on tumor cells. Weltin et al., "Effect of 6(5-Phenanthridinone), an Inhibitor of Poly(ADP-ribose) Polymerase, on Cultured Tumor

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Cells", *Oncol. Res.*, 6:9, 399-403 (1994), discusses the inhibition of PARP activity, reduced proliferation of tumor cells, and a marked synergistic effect when tumor cells are co-treated with an alkylating drug.

- 5 Reviews of the state of the art has been published by Li and Zhang in *IDrugs* 2001, 4(7): 804-812, by Ame et al in *Bioassays* 2004, 26: 882-883 and by Nguewa et al., in *Progress in Biophysic & Molecular Biology* 2005, 88: 143-172.

- 10 There continues to be a need for effective and potent PARP inhibitors, and more particularly PARP-1 inhibitors which produce minimal side effects. The present invention provides compounds, compositions for, and methods of, inhibiting PARP activity for treating cancer and/or preventing cellular, tissue and/or organ damage resulting from cell damage or death due to, for example, necrosis or apoptosis. The compounds and compositions of the present invention are especially useful in
- 15 enhancing the effectiveness of chemotherapy and radiotherapy where a primary effect of the treatment is that of causing DNA damage in the targeted cells.

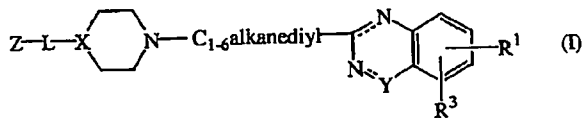
Background prior art

- 20 GB 1062357 published on March 22, 1967 discloses quinazolone derivatives having antihypertensive effects.
DE 2258561 published on June 20, 1973 discloses substituted pyridinone derivatives with antihypertensive action. 4*
EP 13612, published on November 11, 1983, discloses substituted
- 25 piperidinylalkylquinazolinc dcrivatives. The described compounds are serotonin-antagonists. More in particular compound No. 63 of the present invention is disclosed.
EP 669919, published on June 9, 1994, discloses dimethylbenzofurans and dimethylbenzopyrans as 5-HT₃ antagonists.
- 30 US 5374637, published on December 20, 1994, discloses benzamide derivatives. The disclosed compounds have gastrointestinal motility stimulating properties.
EP 885190, published on December 23, 1998 discloses 1,4-disubstituted piperidine derivatives having gastrokinetic properties.
- 35 EP 1036073, published on June 17, 1999, discloses substituted quinazolinodione derivatives. The described compounds have fundic relaxation properties.
EP 1355888 published on 20 June 2002 discloses quinazolinone derivatives as PARP inhibitors.

Description of the invention

This invention concerns compounds of formula (I)

5



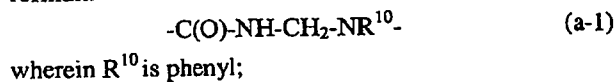
the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

10

the dotted lines represent optional bonds;

X is >N-, >CH- or >CR²- wherein R² is aminocarbonyl; or when X is >CR²- then R² taken together with -L-Z can form a bivalent radical of

15



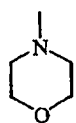
—N^{.....}Y— is -N-C(O)- or -N=CR⁴-, wherein R⁴ is hydroxy;

20

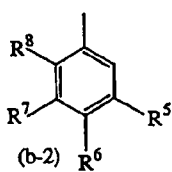
R¹ is hydrogen, halo, C₁₋₆alkyloxy or C₁₋₆alkyl;

R³ is hydrogen, or C₁₋₆alkyloxy;

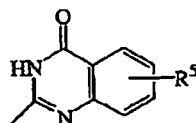
25 Z is a radical selected from



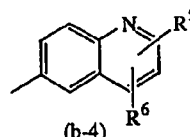
(b-1)



(b-2)

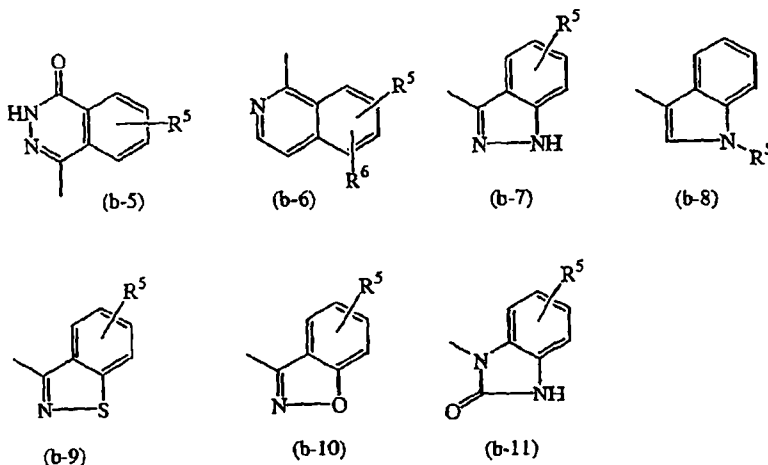


(b-3)



(b-4)

-8-



- 5 wherein each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen, halo, amino, C_{1-6} alkyl or C_{1-6} alkyloxy; or R^7 and R^8 taken together may form a bivalent radical of formula

- $CH_2-CR^9_2-O-$ (c-1),
 -(CH_2)₃-O- (c-2),
 10 -O-(CH_2)₂-O- (c-3) or
 -CH=CH-CH=CH- (c-4)

wherein each R^9 is independently selected from hydrogen or C_{1-6} alkyl; and

- L is a bivalent radical selected from -C(O)-, -C(O)-NH-,
 15 -C(O)- C_{1-6} alkanediyl- or -C(O)-O- C_{1-6} alkanediyl-; or
 L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11);

- with the proviso that when X is $>CH-$, $\overset{\cdot}{N} \cdots Y$ is -N-C(O)-, the second dotted line is not a bond, Z is a radical of formula (b-2) and L is -C(O)-, then - C_{1-6} alkanediyl- is
 20 other than - CH_2-CH_2- .

The compounds of formula (I) may also exist in their tautomeric forms. Such forms although not explicitly indicated in the above formula are intended to be included within the scope of the present invention.

- 25 A number of terms used in the foregoing definitions and hereinafter are explained hereunder. These terms are sometimes used as such or in composite terms.

As used in the foregoing definitions and hereinafter, halo is generic to fluoro, chloro, bromo and iodo; C_{1-6} alkyl defines straight and branched chain saturated hydrocarbon

radicals having from 1 to 6 carbon atoms such as, e.g. methyl, ethyl, propyl, butyl, pentyl, hexyl, 1-methylethyl, 2-methylpropyl, 2-methyl-butyl, 2-methylpentyl and the like; C₁₋₆alkanediyl defines bivalent straight and branched chained saturated

5 hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, methylene, 1,2-ethanediyl, 1,3-propanediyl, 1,4-butanediyl, 1,5-pentanediyl, 1,6-hexanediyl and the branched isomers thereof such as, 2-methylpentanediyl, 3-methylpentanediyl, 2,2-dimethylbutanediyl, 2,3-dimethylbutanediyl and the like.

10 The term "pharmaceutically acceptable salts" means pharmaceutically acceptable acid or base addition salts. The pharmaceutically acceptable acid or base addition salts as mentioned hereinabove are meant to comprise the therapeutically active non-toxic acid and non-toxic base addition salt forms which the compounds of formula (I) are able to form. The compounds of formula (I) which have basic properties can be converted in their pharmaceutically acceptable acid addition salts by treating said base form with an
15 appropriate acid. Appropriate acids comprise, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid; sulfuric; nitric; phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic (*i.e.* butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic,
20 cyclamic, salicylic, *p*-aminosalicylic, pamoic and the like acids.

The compounds of formula (I) which have acidic properties may be converted in their pharmaceutically acceptable base addition salts by treating said acid form with a suitable organic or inorganic base. Appropriate base salt forms comprise, for example,
25 the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, *N*-methyl-*D*-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

The terms acid or base addition salt also comprise the hydrates and the solvent addition forms which the compounds of formula (I) are able to form. Examples of such forms
30 are e.g. hydrates, alcoholates and the like.

The term stereochemically isomeric forms of compounds of formula (I), as used hereinbefore, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not
35 interchangeable, which the compounds of formula (I) may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms which said compound may

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possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of formula (I) both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

5

The *N*-oxide forms of the compounds of formula (I) are meant to comprise those compounds of formula (I) wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide, particularly those *N*-oxides wherein one or more of the piperidine- or piperazine nitrogens are *N*-oxidized.

10

Whenever used hereinafter, the term "compounds of formula (I)" is meant to include also the *N*-oxide forms, the pharmaceutically acceptable acid or base addition salts and all stereoisomeric forms.

- 15 GB 1062357 discloses quinazolone derivatives having antihypertensive effects. DE 2258561 discloses substituted pyridinone derivatives with antihypertensive action. EP 13612 discloses substituted piperidinylalkylquinazoline derivatives that are serotonin-antagonists. EP 669919 discloses dimethylbenzofurans and dimethylbenzopyrans as 5-HT₃ antagonists. US 5374637 discloses benzamide
20 derivatives that have gastrointestinal motility stimulating properties. EP 885190 discloses 1,4-disubstituted piperidine derivatives having gastrokinetic properties. EP 1036073 discloses substituted quinazolinone derivatives that have fundic relaxation properties. EP 1355888 discloses quinazolinone derivatives as PARP inhibitors
- 25 Unexpectedly, it has been found that the compounds of the present invention show PARP inhibitory activity.

A first group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

- 30 a) X is >N- or >CR²- wherein R² is aminocarbonyl;
b) when X is >CR²- then R² taken together with -L-Z can form a bivalent radical of formula (a-1);
c) $\text{---N}^{\text{---}}\text{---Y}$ is -N-C(O)- or -N=CR⁴-, wherein R⁴ is hydroxy and the second dotted line is a bond;
35 d) R¹ is halo, C₁₋₆alkyloxy or C₁₋₆alkyl;
e) R³ is C₁₋₆alkyloxy;

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- f) Z is a radical selected from (b-1), (b-3), (b-4), (b-5), (b-6), (b-7), (b-8), (b-9), (b-11), (b-11);
- g) each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen, amino, C_{1-6} alkyl or C_{1-6} alkoxy;
- 5 h) L is a bivalent radical selected from $-C(O)-NH-$, $-C(O)-C_{1-6}alkanediy-$ or $-C(O)-O-C_{1-6}alkanediy-$.

A second group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

- 10 a) X is $>CH-$ or $>CR^2-$ wherein R^2 is aminocarbonyl;
- b) when X is $>CR^2-$ then R^2 taken together with $-L-Z$ can form a bivalent radical of formula (a-1);
- c) R^1 is hydrogen;
- d) R^3 is hydrogen;
- 15 e) Z is a radical of formula (b-1), (b-2) or (b-11);
- f) each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen or halo;
- g) L is a bivalent radical selected from $-C(O)-$, $-C(O)-NH-$, or $-C(O)-C_{1-6}alkanediy-$; and
- h) L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11).

20

A third group of interesting compounds consists of those compounds of formula (I) wherein one or more of the following restrictions apply:

- a) X is $>CH-$ or $>CR^2-$ wherein R^2 is aminocarbonyl;
- b) R^1 is hydrogen;
- 25 c) R^3 is hydrogen;
- d) Z is a radical of formula (b-1) or (b-2);
- e) each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen or halo;
- f) L is a bivalent radical selected from $-C(O)-$ or $-C(O)-NH-$; and
- g) L can be a direct bond when X is $>CR^2-$.

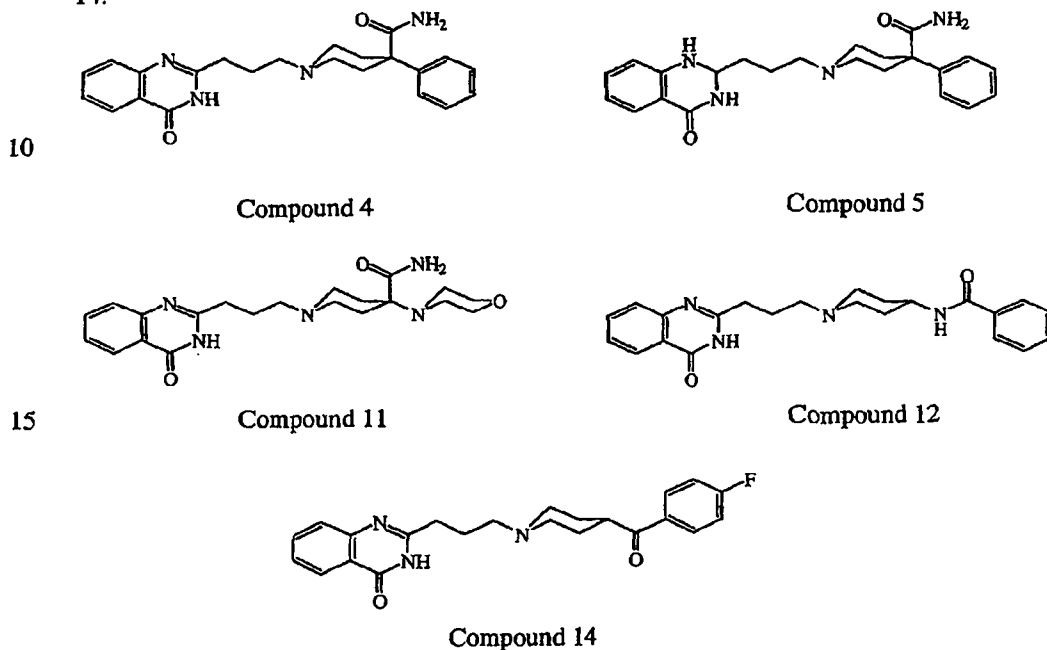
30

- A group of preferred compounds consists of those compounds of formula (I) wherein X is $>CH-$ or $>CR^2-$ wherein R^2 is aminocarbonyl; when X is $>CR^2-$ then R^2 taken together with $-L-Z$ can form a bivalent radical of formula (a-1); R^1 is hydrogen; R^3 is hydrogen; Z is a radical of formula (b-1), (b-2) or (b-11); each R^5 , R^6 , R^7 and R^8 is
- 35 independently selected from hydrogen or halo; L is a bivalent radical selected from $-C(O)-$, $-C(O)-NH-$, or $-C(O)-C_{1-6}alkanediy-$; and L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11).

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A group of preferred compounds consists of those compounds of formula (I) wherein X is >CH- or >CR²- wherein R² is aminocarbonyl; R¹ is hydrogen; R³ is hydrogen; Z is a radical of formula (b-1) or (b-2); each R⁵, R⁶, R⁷ and R⁸ is independently selected from hydrogen or halo; L is a bivalent radical selected from -C(O)- or -C(O)-NH- and L can be a direct bond when X is >CR²-.

The most preferred compounds are compounds No. 4, No. 5, No. 11, No. 12 and No. 14.



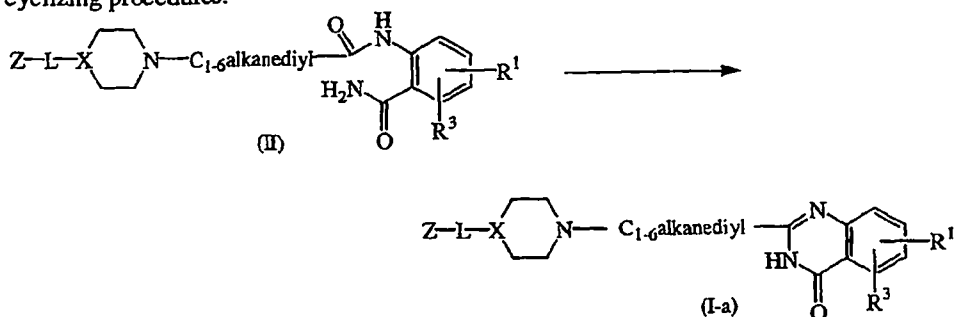
20 The compounds of formula (I) can be prepared according to the general methods described in EP13612. The starting materials and some of the intermediates are known compounds and are commercially available or may be prepared according to conventional reaction procedures generally known in the art.

Some preparation methods will be described hereinafter in more detail. Other methods for obtaining final compounds of formula (I) are described in the examples.

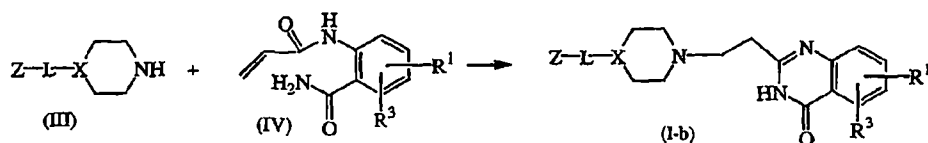
The compounds of formula (I), wherein $\text{---N}^{\text{---}}\text{Y}^{\text{---}}$ is ---N-C(O)- and the second dotted line is a bond, herein referred to as compounds of formula (I-a), can be derived from an appropriate substituted

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2-aminocarbonylbenzamide of formula (II) by cyclizing the later following art-known cyclizing procedures.

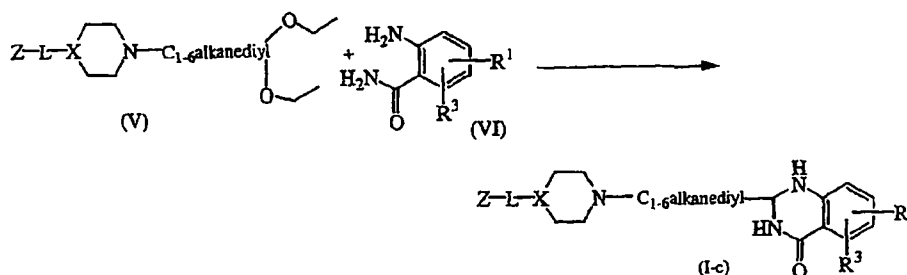


- 5 Alternatively, compounds of formula (I) wherein $-\text{C}_{1-6}\text{alkanediy}-$ is $-\text{CH}_2-\text{CH}_2-$ and $-\text{N} \cdots \text{Y}-$ is $-\text{N}-\text{C}(\text{O})-$ and the second dotted line is a bond, herein referred to as compounds of formula (I-b), can be prepared by cyclization an intermediate of formula (III), with an appropriate substituted 2-aminocarbonylbenzamide of formula (IV) following art-known cyclizing procedures.
- 10



- The compounds of formula (I), wherein $-\text{N} \cdots \text{Y}-$ is $-\text{N}-\text{C}(\text{O})-$ and the second dotted line is not a bond, herein referred to as compounds of formula (I-c) can be derived from an appropriate substituted 2-aminobenzamide of formula (V) by cyclizing the later with an appropriate substituted intermediate of formula (VI). Said cyclization-reaction may conveniently be carried out by stirring the reactants together in the presence of an appropriate solvent, for example an alcohol, e.g., methanol, ethanol, propanol and the like. Somewhat elevated temperature and the addition of a catalytic amount of an appropriate strong acid, e.g. hydrochloric acid and the like, may be used to enhance the rate of the reaction.
- 15
- 20

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The compounds of formula (I) may also be converted into each other via art-known reactions or functional group transformations. Some of such transformations are already described hereinabove. Other examples are hydrolysis of carboxylic esters to the corresponding carboxylic acid or alcohol; hydrolysis of amides to the corresponding carboxylic acids or amines; hydrolysis of nitriles to the corresponding amides; amino groups on imidazole or phenyl may be replaced by a hydrogen by art-known diazotation reactions and subsequent replacement of the diazo-group by hydrogen; alcohols may be converted into esters and ethers; primary amines may be converted into secondary or tertiary amines; double bonds may be hydrogenated to the corresponding single bond; an iodo radical on a phenyl group may be converted in to an ester group by carbon monoxide insertion in the presence of a suitable palladium catalyst.

15

The present invention also relates to a compound of formula (I) as defined above for use as a medicine.

The compounds of the present invention have PARP inhibiting properties as can be seen from the experimental part hereinafter.

20

The term "PARP" is used herein to mean a protein having poly-ADP-ribosylation activity. Within the meaning of this term, PARP encompass all proteins encoded by a parp gene, mutants thereof, and alternative splice proteins thereof. Additionally, as used herein, the term "PARP" includes PARP analogues, homologues and analogues of other animals.

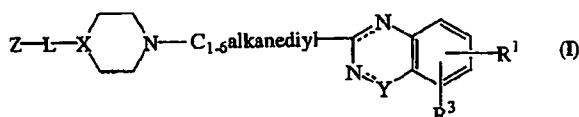
25

The term "PARP", includes but is not limited to PARP-1. Within the meaning of this term PARP-2, PARP-3, Vault-PARP (PARP-4), PARP-7 (TiPARP), PARP-8, PARP-9 (Bal), PARP-10, PARP-11, PARP-12, PARP-13, PARP-14, PARP-15, PARP-16, TANK-1, TANK-2, and TANK-3 may be encompassed.

30

Compounds that inhibit both PARP-1 and tankyrase 2 can have advantageous properties in that they have enhanced growth inhibiting activities in cancer cells.

The present invention also contemplates the use of compounds in the preparation of a medicament for the treatment of any of the diseases and disorders in an animal described herein, wherein said compounds are compounds of formula (I).



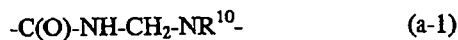
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the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

the dotted lines represent optional bonds;

15

X is >N-, >CH- or >CR²- wherein R² is aminocarbonyl; or when X is >CR²- then R² taken together with -L-Z can form a bivalent radical of formula



20

wherein R¹⁰ is phenyl;

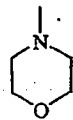
—N^{.....}Y— is -N-C(O)- or -N=CR⁴-, wherein R⁴ is hydroxy;

R¹ is hydrogen, halo, C₁₋₆alkyloxy or C₁₋₆alkyl;

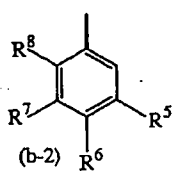
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R³ is hydrogen, or C₁₋₆alkyloxy;

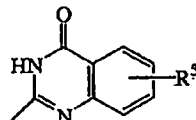
Z is a radical selected from



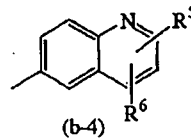
(b-1)



(b-2)



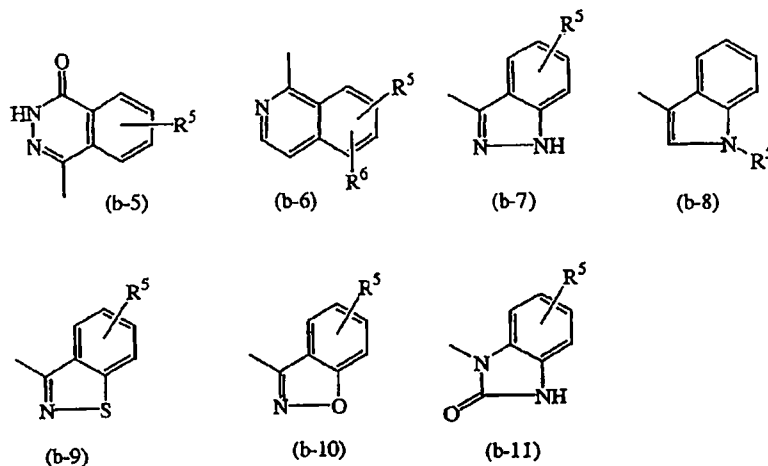
(b-3)



(b-4)

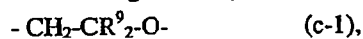
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5 wherein each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen, halo, amino, C_{1-6} alkyl or C_{1-6} alkyloxy; or

R^7 and R^8 taken together may form a bivalent radical of formula



10 -O-(CH_2)₂-O- (c-3) or



wherein each R^9 is independently selected from hydrogen or C_{1-6} alkyl; and

L is a bivalent radical selected from -C(O)-, -C(O)-NH-,

15 -C(O)- C_{1-6} alkanediyl- or -C(O)-O- C_{1-6} alkanediyl-; or

L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11).

In view of their PARP binding properties the compounds of the present invention may be used as reference compounds or tracer compounds in which case one of the

20 atoms of the molecule may be replaced with, for instance, a radioactive isotope.

To prepare the pharmaceutical compositions of this invention, an effective amount of a particular compound, in base or acid addition salt form, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which

25 carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirably in unitary dosage form suitable, preferably, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed; such as, for example, water,

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glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules and tablets. Because of their ease in administration, tablets and capsules

5 represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, to aid solubility for example, may be included. Injectable solutions, for

10 example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which

15 additives do not cause a significant deleterious effect to the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on, as an ointment. It is especially advantageous to

20 formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including

25 scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

The compounds of the present invention can treat or prevent tissue damage resulting

30 from cell damage or death due to necrosis or apoptosis; can ameliorate neural or cardiovascular tissue damage, including that following focal ischemia, myocardial infarction, and reperfusion injury; can treat various diseases and conditions caused or exacerbated by PARP activity; can extend or increase the lifespan or proliferative capacity of cells; can alter the gene expression of senescent cells; can radiosensitize

35 and/or chemosensitize cells. Generally, inhibition of PARP activity spares the cells from energy loss, preventing, in the case of neural cells, irreversible depolarization of the neurons, and thus, provides neuroprotection.

For the foregoing reasons, the present invention further relates to a method of administering a therapeutically effective amount of the above-identified compounds in an amount sufficient to inhibit PARP activity, to treat or prevent tissue damage
5 resulting from cell damage or death due to necrosis or apoptosis, to effect a neuronal activity not mediated by NMDA toxicity, to effect a neuronal activity mediated by NMDA toxicity, to treat neural tissue damage resulting from ischemia and reperfusion injury, neurological disorders and neurodegenerative diseases; to prevent or treat
10 vascular stroke; to treat or prevent cardiovascular disorders; to treat other conditions and/or disorders such as age- related muscular degeneration, AIDS and other immune senescence diseases, inflammation, gout, arthritis, atherosclerosis, cachexia, cancer, degenerative diseases of skeletal muscle involving replicative senescence, diabetes, head trauma, inflammatory bowel disorders (such as colitis and Crohn's disease), muscular dystrophy, osteoarthritis, osteoporosis, chronic and/or acute pain (such as
15 neuropathic pain), renal failure, retinal ischemia, septic shock (such as endotoxic shock), and skin aging, to extend the lifespan and proliferative capacity of cells; to alter gene expression of senescent cells; chemosensitize and/or radiosensitize (hypoxic) tumor cells. The present invention also relates to treating diseases and conditions in an animal which comprises administering to said animal a therapeutically effective
20 amount of the above-identified compounds.

In particular, the present invention relates to a method of treating, preventing or inhibiting a neurological disorder in an animal, which comprises administering to said animal a therapeutically effective amount of the above-identified compounds. The neurological disorder is selected from the group consisting of peripheral neuropathy
25 caused by physical injury or disease state, traumatic brain injury, physical damage to the spinal cord, stroke associated with brain damage, focal ischemia, global ischemia, reperfusion injury, demyelinating disease and neurological disorder relating to neurodegeneration.

30 The present invention also contemplates the use of compounds of formula (I) for inhibiting PARP activity, for treating, preventing or inhibiting tissue damage resulting from cell damage or death due to necrosis or apoptosis, for treating, preventing or inhibiting a neurological disorder in an animal.

35 The term "preventing neurodegeneration" includes the ability to prevent neurodegeneration in patients newly diagnosed as having a neurodegenerative disease, or at risk of developing a new degenerative disease and for preventing further

neurodegeneration in patients who are already suffering from or have symptoms of a neurodegenerative disease.

5 The term "treatment" as used herein covers any treatment of a disease and/or condition in an animal, particularly a human, and includes: (i) preventing a disease and/or condition from occurring in a subject which may be predisposed to the disease and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease and/or condition, i.e., arresting its development; (iii) relieving the disease and/or condition, i.e., causing regression of the disease and/or condition.

10

The term "radiosensitizer", as used herein, is defined as a molecule, preferably a low molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of the cells to ionizing radiation and/or to promote the treatment of diseases which are treatable with ionizing radiation. Diseases which
15 are treatable with ionizing radiation include neoplastic diseases, benign and malignant tumors, and cancerous cells. Ionizing radiation treatment of other diseases not listed herein are also contemplated by the present invention.

20 The term "chemosensitizer", as used herein, is defined as a molecule, preferably a low molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of cells to chemotherapy and/or promote the treatment of diseases which are treatable with chemotherapeutics. Diseases which are treatable with chemotherapy include neoplastic diseases, benign and malignant tumors and cancerous cells. Chemotherapy treatment of other diseases not listed herein are also
25 contemplated by the present invention.

The compounds, compositions and methods of the present invention are particularly useful for treating or preventing tissue damage resulting from cell death or damage due to necrosis or apoptosis.

30 The compounds of the present invention can be "anti-cancer agents", which term also encompasses "anti-tumor cell growth agents" and "anti-neoplastic agents". For example, the methods of the invention are useful for treating cancers and chemosensitizing and/or radiosensitizing tumor cells in cancers such as ACTH-producing tumors, acute lymphocytic leukemia, acute nonlymphocytic leukemia,
35 cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma

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- gallbladder cancer, hairy cell leukemia, head & neck cancer, Hodgkin's Lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and/or non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, non- Hodgkin's Lymphoma,
- 5 osteosarcoma, ovarian cancer, ovary (germ cell) cancer, prostate cancer, pancreatic cancer, penile cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva and Wilm's tumor.
- 10 Hence the compounds of the present invention can be used as "radiosensitizer" and/or "chemosensitizer".

Radiosensitizers are known to increase the sensitivity of cancerous cells to the toxic effects of ionizing radiation. Several mechanisms for the mode of action of

15 radiosensitizers have been suggested in the literature including: hypoxic cell radiosensitizers (e.g., 2- nitroimidazole compounds, and benzotriazine dioxide compounds) mimicking oxygen or alternatively behave like bioreductive agents under hypoxia; non-hypoxic cell radiosensitizers (e.g., halogenated pyrimidines) can be

20 analogs of DNA bases and preferentially incorporate into the DNA of cancer cells and thereby promote the radiation-induced breaking of DNA molecules and/or prevent the normal DNA repair mechanisms; and various other potential mechanisms of action have been hypothesized for radiosensitizers in the treatment of disease.

Many cancer treatment protocols currently employ radiosensitizers in conjunction with radiation of x-rays. Examples of x-ray activated radiosensitizers include, but are not

25 limited to, the following: metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5- iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FudR), hydroxyurea, cisplatin, and therapeutically effective analogs and derivatives of the same.

30 Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the following, but are not limited to: hematoporphyrin derivatives, Photofrin, benzoporphyrin derivatives, tin etioporphyrin, pheorbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective

35 analogs and derivatives of the same.

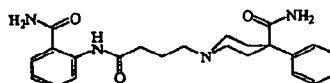
Radiosensitizers may be administered in conjunction with a therapeutically effective amount of one or more other compounds, including but not limited to: compounds

- which promote the incorporation of radiosensitizers to the target cells; compounds which control the flow of therapeutics, nutrients, and/or oxygen to the target cells; chemotherapeutic agents which act on the tumor with or without additional radiation; or other therapeutically effective compounds for treating cancer or other disease.
- 5 Examples of additional therapeutic agents that may be used in conjunction with radiosensitizers include, but are not limited to: 5-fluorouracil, leucovorin, 5'-amino-5-deoxythymidine, oxygen, carbogen, red cell transfusions, perfluorocarbons (e.g., Fluosol 10 DA), 2,3-DPG, BW12C, calcium channel blockers, pentoxifylline, antiangiogenesis compounds, hydralazine, and LBSO. Examples of chemotherapeutic
- 10 agents that may be used in conjunction with radiosensitizers include, but are not limited to: adriamycin, camptothecin, carboplatin, cisplatin, daunorubicin, docetaxel, doxorubicin, interferon (alpha, beta, gamma), interleukin 2, irinotecan, paclitaxel, topotecan, and therapeutically effective analogs and derivatives of the same.
- 15 Chemosensitizers may be administered in conjunction with a therapeutically effective amount of one or more other compounds, including but not limited to : compounds which promote the incorporation of chemosensitizers to the target cells; compounds which control the flow of therapeutics, nutrients, and/or oxygen to the target cells;
- 20 chemotherapeutic agents which act on the tumor or other therapeutically effective compounds for treating cancer or other disease. Examples of additional therapeutical agents that may be used in conjunction with chemosensitizers include, but are not limited to : methylating agents, topoisomerase I inhibitors and other chemotherapeutic agents such as cisplatin and bleomycin.
- 25 The compounds of formula (I) can also be used to detect or identify the PARP, and more in particular the PARP-1 receptor. For that purpose the compounds of formula (I) can be labeled. Said label can be selected from the group consisting of a radioisotope, spin label, antigen label, enzyme label fluorescent group or a chemiluminiscent group.
- 30 Those skilled in the art could easily determine the effective amount from the test results presented hereinafter. In general it is contemplated that an effective amount would be from 0.001 mg/kg to 100 mg/kg body weight, and in particular from 0.005 mg/kg to 10 mg/kg body weight. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses
- 35 may be formulated as unit dosage forms, for example, containing 0.05 to 500 mg, and in particular 0.1 mg to 200 mg of active ingredient per unit dosage form.

Experimental part

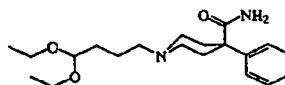
Hereinafter, "DCM" is defined as dichloromethane, "DMF" is defined as *N,N*-dimethylformamide, "MeOH" is defined as methanol, "MIK" is defined as methyl isobutyl keton, "MEK" is defined as methyl ethyl keton, "TEA" is defined as

5 triethylamine and "THF" is defined as tetrahydrofuran.

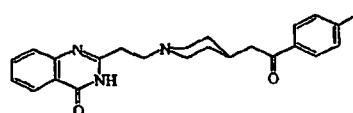
A. Preparation of the intermediate compoundsExample A1Preparation of intermediate 1

A mixture of 2-[(4-chloro-1-oxobutyl)amino]-benzamide (0.03 mol), 4-phenyl-4-
 10 piperidinecarboxamide (0.03 mol) and TEA (0.1 mol) in acetonitrile (150 ml) was
 stirred and refluxed overnight, then the solvent was evaporated and water was added to
 the residue. The oily layer was extracted with trichloromethane, dried, filtered and the
 solvent was evaporated. The residue was purified over silica gel on a glass filter
 (eluent: trichloromethane/MeOH 95/5). The product fractions were collected and the
 15 solvent was evaporated, yielding 5 g (40.8 %) of intermediate 1.

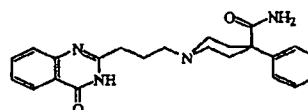
intermediate 2; Ex. [A1]; mp. 144.2°C	intermediate 3; Ex. [A1]
intermediate 4; Ex. [A1]	intermediate 5; Ex. [A1]; mp. 210°C

Example A2Preparation of intermediate 6

A mixture of 4-chloro-1,1-diethoxy-butane (0.05 mol), 4-phenyl-4-
 20 piperidinecarboxamide (0.03 mol), sodium carbonate (0.05 mol) and potassium iodide
 (q.s.) in 4-methyl-2-pentanone (250 ml) was stirred and refluxed overnight, then the
 reaction mixture was cooled, water was added and the layers were separated. The
 organic layer was dried, filtered and the solvent was evaporated, yielding 9 g (86 %) of
 intermediate 6.

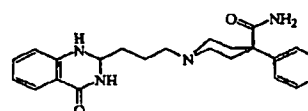
B. Preparation of the final compoundsExample B1Preparation of compound 3

A mixture of 2-[(1-oxo-2-propenyl)amino]-benzamide (0.02 mol), 1-(4-fluorophenyl)-
 5 2-(4-piperidinyl)-ethanone (0.02 mol) and sodium bicarbonate (4 g) in 2-propanol (150 ml) was stirred and refluxed overnight, then the reaction mixture was filtered hot and the solvent was evaporated. The residue was purified twice by column chromatography over silica gel (eluent: trichloromethane/MeOH 95/5). The product fractions were collected and the solvent was evaporated. The residue was crystallised from 2-propanol
 10 and the resulting precipitate was collected, yielding 2 g (25 %) of compound 3, melting point 159.1°C.

Example B2Preparation of compound 4

A mixture of intermediate 1 (0.012 mol) in sodium hydroxide (60 ml) and ethanol (100
 15 ml) was stirred and refluxed for 1.5 hour, then the solvent was evaporated and water was added to the residue. The oily layer was extracted with trichloromethane, dried, filtered and the solvent was evaporated. The residue was crystallised from MIK/2-propanol, then the desired product was collected and dried, yielding 2 g (41.7%) of compound 4, melting point 138.9°C.

20

Example B3Preparation of compound 5

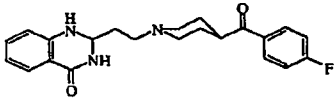
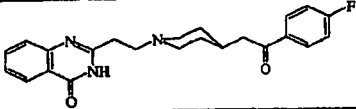
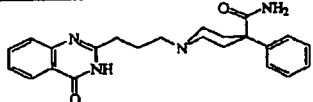
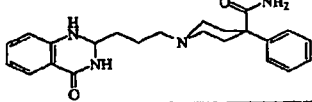
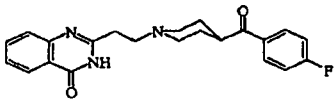
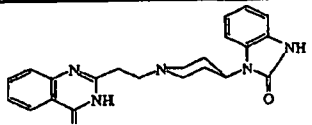
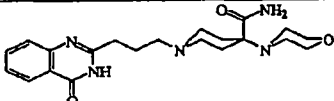
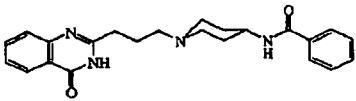
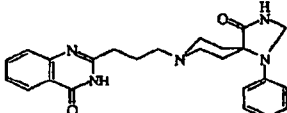
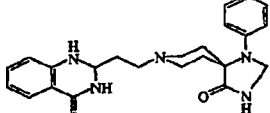
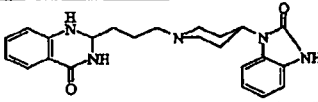
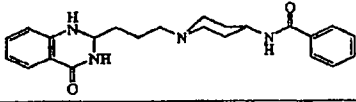
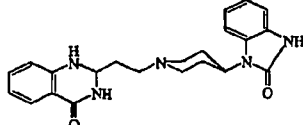
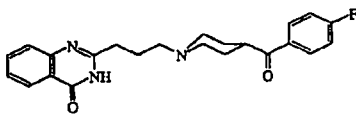
A mixture of 2-amino-benzamide (0.027 mol) and intermediate 6 (0.027 mol) in ethanol (100 ml) was warmed and the mixture was acidified with hydrochloric acid c.p. (3 ml). The reaction mixture was stirred and refluxed overnight, then the solvent was
 25 evaporated and water and concentrated NH₄OH solution were added to the residue. The resulting precipitate was filtered off and dissolved in trichloromethane, then dried and filtered. The solvent was evaporated and the residue was crystallised from MeOH, then

-24-

the resulting precipitate was collected and dried, yielding 3 g (28 %) of compound 5, melting point 216.1°C.

Table F-1 lists the compounds that were prepared according to one of the above

5 Examples.

	
Co.No. 1, EP 13612	Co. No. 3; Ex. [B1]; mp. 159.1°C
	
Co. No. 4; Ex. [B2]; mp. 138.9°C	Co. No. 5; Ex. [B3]; mp. 216.1°C
	
Co. No. 9; Ex. [B1]; mp. 188.2°C	Co. No. 10; Ex. [B2]; mp. 234.3°C
	
Co. No. 11; Ex. [B2]	Co. No. 12; Ex. [B2]; mp. > 300°C
	
Co. No. 2; Ex. [B2]; mp. 243°C	Co. No.6; Ex. [B3]; mp. 251°C
	
Co. No. 7; Ex. [B3]; mp. 239.8°C	Co. No. 8; Ex. [B3]; mp. 194.4°C
	
Co. No. 13; Ex. [B3]; mp. 232.9°C	Co. No. 14; Ex. [B2]

Pharmacological example*In vitro* Scintillation Proximity Assay (SPA) for PARP-1 inhibitory activity

Compounds of the present invention were tested in an *in vitro* assay based on SPA technology (proprietary to Amersham Pharmacia Biotech).

In principle, the assay relies upon the well established SPA technology for the detection of poly(ADP-ribosylation) of biotinylated target proteins, i.e histones. This ribosylation is induced using nicked DNA activated PARP-1 enzyme and [³H]-nicotinamide adenine dinucleotide ([³H]-NAD⁺) as ADP-ribosyl donor.

As inducer of PARP-1 enzyme activity, nicked DNA was prepared. For this, 25 mg of DNA (supplier: Sigma) was dissolved in 25 ml DNase buffer (10 mM Tris-HCl, pH 7.4; 0.5 mg/ml Bovine Serum Albumine (BSA); 5 mM MgCl₂·6H₂O and 1 mM KCl) to which 50 μl DNase solution (1mg/ml in 0.15 M NaCl) was added. After an incubation of 90 min. at 37 °C, the reaction was terminated by adding 1.45 g NaCl, followed by a further incubation at 58 °C for 15 min. The reaction mixture was cooled on ice and dialysed at 4 °C for respectively 1.5 and 2 hours against 1.5 l of 0.2 M KCl, and twice against 1.5 l of 0.01 M KCl for 1.5 and 2 h respectively. The mixture was aliquoted and stored at -20 °C. Histones (1 mg/ml, type II-A, supplier: Sigma) were biotinylated using the biotinylation kit of Amersham and stored aliquoted at -20 °C. A stock solution of 100 mg/ml SPA poly(vinyl toluene) (PVT) beads (supplier: Amersham) was made in PBS. A stock solution of [³H]-NAD⁺ was made by adding 120 μl of [³H]-NAD⁺ (0.1 mCi/ml, supplier: NEN) to 6 ml incubation buffer (50 mM Tris/HCl, pH 8; 0.2 mM DTT; 4 mM MgCl₂). A solution of 4 mM NAD⁺ (supplier: Roche) was made in incubation buffer (from a 100 mM stock solution in water stored at -20 °C). The PARP-1 enzyme was produced using art known techniques, i.e. cloning and expression of the protein starting from human liver cDNA. Information concerning the used protein sequence of the PARP-1 enzyme including literature references can be found in the Swiss-Prot database under primary accession number P09874. Biotinylated histones and PVT-SPA beads were mixed and pre-incubated for 30 min. at room temperature. PARP-1 enzyme (concentration was lot dependent) was mixed with the nicked DNA and the mixture was pre-incubated for 30 min. at 4 °C. Equal parts of this histones/PVT-SPA beads solution and PARP-1 enzyme/DNA solution were mixed and 75 μl of this mixture together with 1 μl of compound in DMSO and 25 μl of [³H]-NAD⁺ was added per well into a 96-well microtiterplate. The final concentrations in the incubation mixture were 2 μg/ml for the biotinylated histones, 2 mg/ml for the PVT-SPA beads, 2 μg/ml for the nicked DNA and between 5 - 10 μg/ml for the PARP-1 enzyme. After incubation of the mixture for 15 min. at room temperature, the reaction

-26-

was terminated by adding 100 μ l of 4 mM NAD^+ in incubation buffer (final concentration 2 mM) and plates were mixed.

The beads were allowed to sediment for at least 15 min. and plates transferred to a TopCountNXTTM (Packard) for scintillation counting, values were expressed as counts per minute (cpm). For each experiment, controls (containing PARP-1 enzyme and DMSO without compound), a blank incubation (containing DMSO but no PARP-1 enzyme or compound) and samples (containing PARP-1 enzyme and compound dissolved in DMSO) were run in parallel. All compounds tested were dissolved and eventually further diluted in DMSO. In first instance, compounds were tested at a concentration of 10^{-5} M. When the compounds showed activity at 10^{-5} M, a dose-response curve was made wherein the compounds were tested at concentrations between 10^{-5} M and 10^{-8} M. In each test, the blank value was subtracted from both the control and the sample values. The control sample represented maximal PARP-1 enzyme activity. For each sample, the amount of cpm was expressed as a percentage of the mean cpm value of the controls. When appropriate, IC_{50} -values (concentration of the drug, needed to reduce the PARP-1 enzyme activity to 50% of the control) were computed using linear interpolation between the experimental points just above and below the 50 % level. Herein the effects of test compounds are expressed as pIC_{50} (the negative log value of the IC_{50} -value). As a reference compound, 4-amino-1,8-naphthalimide was included to validate the SPA assay. The tested compounds showed inhibitory activity at the initial test concentration of 10^{-5} M (see Tabel-2).

In vitro filtration assay for PARP-1 inhibitory activity

Compounds of the present invention were tested in an *in vitro* filtration assay assessing PARP-1 activity (triggered in the presence of nicked DNA) by means of its histone poly (ADP-ribosyl)ation activity using [³²P]-NAD as ADP-ribosyl donor. The radioactive ribosylated histones were precipitated by trichloroacetic acid (TCA) in 96-well filterplates and the incorporated [³²P] measured using a scintillation counter

30

A mixture of histones (stock solution: 5 mg/ml in H_2O), NAD^+ (stock solution: 100 mM in H_2O), and [³²P]- NAD^+ in incubation buffer (50 mM Tris/HCl, pH 8; 0.2 mM DTT; 4 mM MgCl_2) was made. A mixture of the PARP-1 enzyme (5 – 10 μ g/ml) and nicked DNA was also made. The nicked DNA was prepared as described in the *in vitro* SPA for PARP-1 inhibitory activity. Seventy-five μ l of the PARP-1 enzyme/DNA mixture together with 1 μ l of compound in DMSO and 25 μ l of histones- NAD^+ /[³²P]- NAD^+ mixture was added per well of a 96-well filterplate (0.45 μ m, supplier

35

-27-

Millipore). The final concentrations in the incubation mixture were 2 $\mu\text{g/ml}$ for the histones, 0.1 mM for the NAD^+ , 200 μM (0.5 μC) for the [^{32}P]- NAD^+ and 2 $\mu\text{g/ml}$ for the nicked DNA. Plates were incubated for 15 min. at room temperature and the reaction was terminated by the addition of 10 μl ice cold 100% TCA followed by the addition of 10 μl ice-cold BSA solution (1 % in H_2O). The protein fraction was allowed to precipitate for 10 min. at 4 $^\circ\text{C}$ and plates were vacuum filtered. The plates were subsequently washed with, for each well, 1 ml of 10 % ice cold TCA, 1 ml of 5 % ice cold TCA and 1 ml of 5 % TCA at room temperature. Finally 100 μl of scintillation solution (Microscint 40, Packard) was added to each well and the plates were transferred to a TopCountNXTTM (supplier: Packard) for scintillation counting and values were expressed as counts per minute (cpm). For each experiment, controls (containing PARP-1 enzyme and DMSO without compound), a blank incubation (containing DMSO but no PARP-1 enzyme or compound) and samples (containing PARP-1 enzyme and compound dissolved in DMSO) were run in parallel. All compounds tested were dissolved and eventually further diluted in DMSO. In first instance, compounds were tested at a concentration of 10^{-5}M . When the compounds showed activity at 10^{-5}M , a dose-response curve was made wherein the compounds were tested at concentrations between 10^{-5}M and 10^{-8}M . In each test, the blank value was subtracted from both the control and the sample values. The control sample represented maximal PARP-1 enzyme activity. For each sample, the amount of cpm was expressed as a percentage of the mean cpm value of the controls. When appropriate, IC_{50} -values (concentration of the drug, needed to reduce the PARP-1 enzyme activity to 50% of the control) were computed using linear interpolation between the experimental points just above and below the 50 % level. Herein the effects of test compounds are expressed as pIC_{50} (the negative log value of the IC_{50} -value). As a reference compound, 4-amino-1,8-naphthalimide was included to validate the filtration assay. The tested compounds showed inhibitory activity at the initial test concentration of 10^{-5}M (see Tabel-2).

30 *In vitro* Scintillation Proximity Assay (SPA) for TANK-2 inhibitory activity

Compounds of the present invention were tested in an *in vitro* assay based on SPA technology with Ni Flash plates (96 or 384 well).

In principle, the assay relies upon SPA technology for the detection of auto-poly(ADP-ribosyl)ation of TANK-2 protein using [^3H]-nicotinamide adenine dinucleotide ([^3H]- NAD^+) as ADP-ribosyl donor.

-28-

A stock solution of [³H]-NAD⁺/NAD was made by adding 64.6 μl of [³H]-NAD⁺ (0.1 mCi/ml, supplier: Perkin Elmer) and 46.7 μl NAD-stock (10.7 mM, stored at - 20 °C, supplier Roche) to 1888.7 μl assay buffer (60 mM Tris/HCl, pH 7.4; 0.9 mM DTT; 6 mM MgCl₂). The TANK-2 enzyme was produced as described in EP1238063 . 60 μl of assay buffer, together with 1 μl of compound in DMSO, 20 μl of [³H]-NAD⁺/NAD and 20 μl of TANK-2 enzyme (final concentration 6 μg/ml) was added per well into a 96-well Ni-coated flash plate (Perkin Elmer). After incubation of the mixture for 120 min. at room temperature, the reaction was terminated by adding 60 μl of stop solution (42.6 mg NAD in 6 ml H₂O). The plates were covered with a plate sealer and placed in a TopCountNXT™ (Packard) for scintillation counting. Values were expressed as counts per minute (cpm). For each experiment, controls (containing TANK-2 enzyme and DMSO without compound), a blank incubation (containing DMSO but no TANK-2 enzyme or compound) and samples (containing TANK-2 enzyme and compound dissolved in DMSO) were run in parallel. All compounds tested were dissolved and eventually further diluted in DMSO. In first instance, compounds were tested at a concentration of 10⁻⁵ M. When the compounds showed activity at 10⁻⁵ M, a dose-response curve was made wherein the compounds were tested at concentrations between 10⁻⁵ M and 10⁻⁹ M. In each test, the blank value was subtracted from both the control and the sample values. The control sample represented maximal TANK-2 enzyme activity. For each sample, the amount of cpm was expressed as a percentage of the mean cpm value of the controls. When appropriate, IC₅₀-values (concentration of the drug, needed to reduce the TANK-2 enzyme activity to 50% of the control) were computed using linear interpolation between the experimental points just above and below the 50 % level. Herein the effects of test compounds are expressed as pIC₅₀ (the negative log value of the IC₅₀-value). As reference compounds, 3-aminobenzamide and 4-amino-1,8-naphtalimide were included to validate the SPA assay. Herein the assay was described using 96-well plates. In the assay using 384-well plates the same final concentrations were used and volumes were adapted. If 96-well plate results were available these results were incorporated in Table-2, otherwise the results from the 384-well plate assay were shown.

Tabel-2

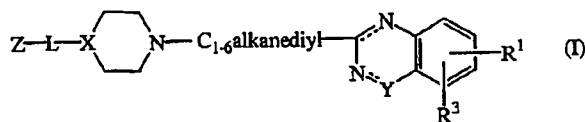
Compound No	in vitro filter assay PARP-1 pIC50	in vitro SPA assay PARP-1 pIC50	in vitro SPA TANK-2 pIC50
1			<5
2	5.303	6.414	<5
3	5.371	6.252	5.605
4	7.115	7.699	<5
5	6.523	7.64	<5
6	5.669	6.75	5.129
7	5.381	6.233	>5
8	5.804	6.627	<5
9	5.243	6.214	<5
10	5	6.376	5.674
11	5.942	6.725	<5
12	6.172	6.931	<5
13	5.332	6.497	6.028
14	6.263	7.232	<5

The compounds can be further evaluated in a cellular chemo- and/or radiosensitization assay, an assay measuring inhibition of endogenous PARP-1 activity in cancer cell lines and eventually in an *in vivo* radiosensitization test.

5

CLAIMS

1. This invention concerns compounds of formula (I)



the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

10 the dotted lines represent optional bonds;

X is >N-, >CH- or >CR²- wherein R² is aminocarbonyl; or when X is >CR²- then R² taken together with -L-Z can form a bivalent radical of formula



wherein R¹⁰ is phenyl;

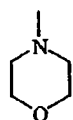
$\text{---}N\text{---}Y$ is -N-C(O)- or -N=CR⁴-, wherein R⁴ is hydroxy;

20 R¹ is hydrogen, halo, C₁₋₆alkyloxy or C₁₋₆alkyl;

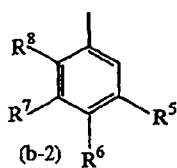
R³ is hydrogen, or C₁₋₆alkyloxy;

Z is a radical selected from

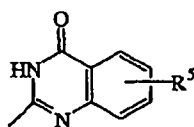
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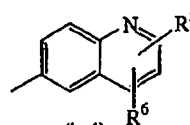
(b-1)



(b-2)

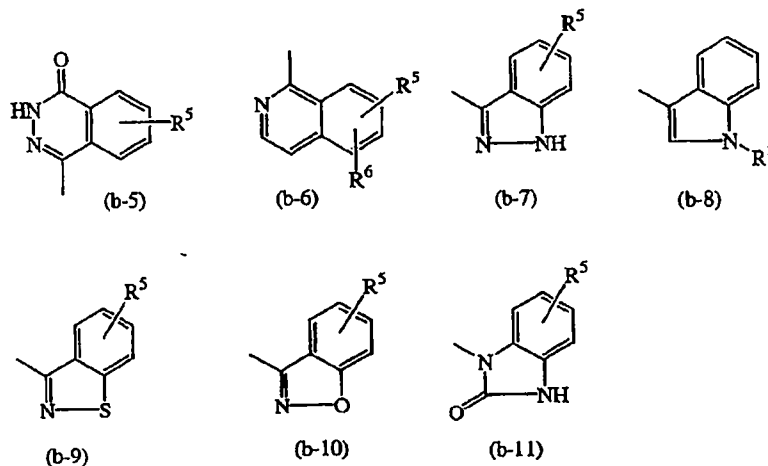


(b-3)



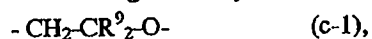
(b-4)

-31-



- 5 wherein each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen, halo, amino, C_{1-6} alkyl or C_{1-6} alkyloxy; or

R^7 and R^8 taken together may form a bivalent radical of formula



- 10 - $O-(CH_2)_2-O-$ (c-3) or



wherein each R^9 is independently selected from hydrogen or C_{1-6} alkyl; and

- 15 L is a bivalent radical selected from $-C(O)-$, $-C(O)-NH-$,

$-C(O)-C_{1-6}$ alkanediy- or $-C(O)-O-C_{1-6}$ alkanediy-; or

L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11);

- 20 with the proviso that when X is $>CH-$, $\overset{\cdot}{N} \equiv \overset{\cdot}{Y}$ is $-N-C(O)-$, the second dotted line is not a bond, Z is a radical of formula (b-2) and L is $-C(O)-$, then $-C_{1-6}$ alkanediy- is other than $-CH_2-CH_2-$.

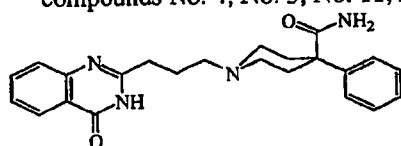
2. A compound as claimed in claim 1 wherein

- 25 X is $>CH-$ or $>CR^2-$ wherein R^2 is aminocarbonyl; when X is $>CR^2-$ then R^2 taken together with $-L-Z$ can form a bivalent radical of formula (a-1); R^1 is hydrogen; R^3 is hydrogen; Z is a radical of formula (b-1), (b-2) or (b-11); each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen or halo; L is a bivalent radical selected from $-C(O)-$, $-C(O)-NH-$, or $-C(O)-C_{1-6}$ alkanediy-; and L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11).

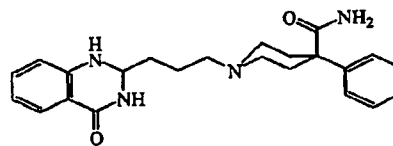
-32-

3. A compound according to claim 1 or 2 wherein
 X is >CH- or >CR²- wherein R² is aminocarbonyl; R¹ is hydrogen; R³ is hydrogen;
 Z is a radical of formula (b-1) or (b-2); each R⁵, R⁶, R⁷ and R⁸ is independently
 selected from hydrogen or halo; L is a bivalent radical selected from
 5 -C(O)- or -C(O)-NH- and L can be a direct bond when X is >CR²-.

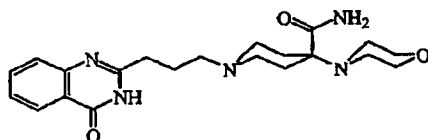
4. A compound according to claim 1, 2 and 3 wherein the compound is selected from
 compounds No. 4, No. 5, No. 11, No. 12 and No. 14.



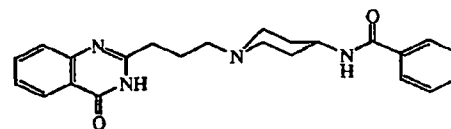
Compound 4



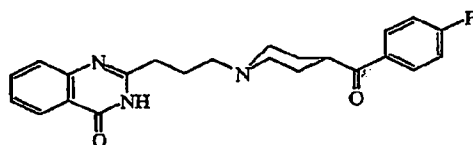
Compound 5



Compound 11

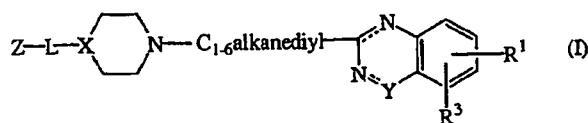


Compound 12



Compound 14

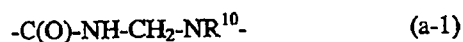
5. A compound as claimed in any of claims 1 to 4 for use as a medicine.
 20
6. A pharmaceutical composition comprising pharmaceutically acceptable carriers and
 as an active ingredient a therapeutically effective amount of a compound as claimed
 in claim 1 to 5.
- 25 7. A process of preparing a pharmaceutical composition as claimed in claim 6 wherein
 the pharmaceutically acceptable carriers and a compound as claimed in claim 1 to 4
 are intimately mixed.
- 30 8. Use of a compound for the manufacture of a medicament for the treatment of a
 PARP mediated disorder, wherein the compound is a compound of formula (I)



- 5 the *N*-oxide forms, the pharmaceutically acceptable addition salts and the stereochemically isomeric forms thereof, wherein

the dotted lines represent optional bonds;

- 10 X is >N-, >CH- or >CR²- wherein R² is aminocarbonyl; or when X is >CR²- then R² taken together with -L-Z can form a bivalent radical of formula



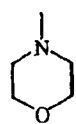
wherein R¹⁰ is phenyl;

- 15 $-N \cdots Y$ is -N-C(O)- or -N=CR⁴-, wherein R⁴ is hydroxy;

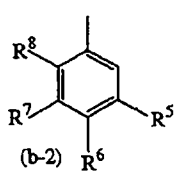
R¹ is hydrogen, halo, C₁₋₆alkyloxy or C₁₋₆alkyl;

- 20 R³ is hydrogen, or C₁₋₆alkyloxy;

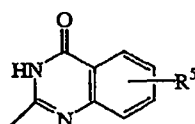
Z is a radical selected from



(b-1)



(b-2)

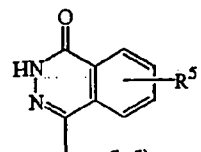


(b-3)

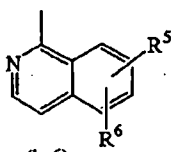


(b-4)

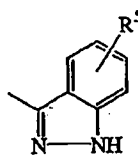
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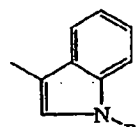
(b-5)



(b-6)

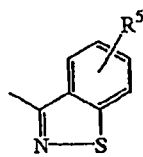


(b-7)

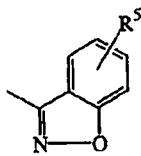


(b-8)

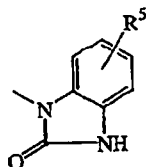
-34-



(b-9)



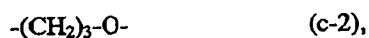
(b-10)



(b-11)

wherein each R^5 , R^6 , R^7 and R^8 is independently selected from hydrogen, halo, amino, C_{1-6} alkyl or C_{1-6} alkyloxy; or

5 R^7 and R^8 taken together may form a bivalent radical of formula



10 wherein each R^9 is independently selected from hydrogen or C_{1-6} alkyl; and

L is a bivalent radical selected from $-C(O)-$, $-C(O)-NH-$,

$-C(O)-C_{1-6}$ alkanediyl- or $-C(O)-O-C_{1-6}$ alkanediyl-; or

L can be a direct bond when X is $>CR^2-$ or when Z is a radical of formula (b-11).

15

9. Use according to claim 8 of a PARP inhibitor of formula (I) for the manufacture of a medicament for the treatment of a PARP-1 mediated disorder.

10. Use according to claim 8 and 9 wherein the treatment involves chemosensitization.

20

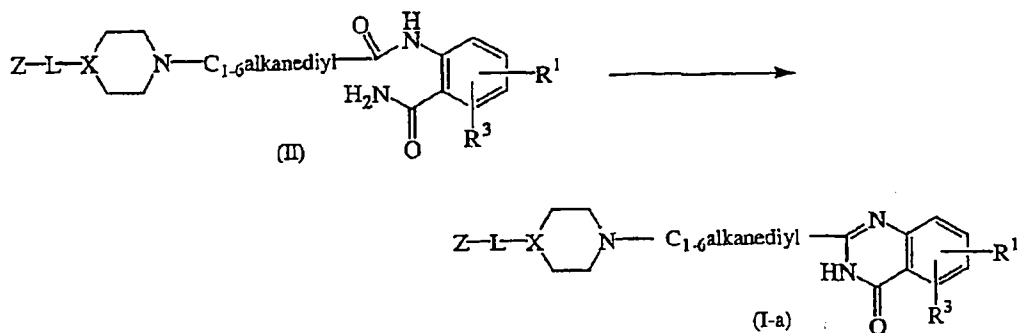
11. Use according to claims 8 and 9 wherein the treatment involves radiosensitization.

12. A combination of a compound of formula (I) with a chemotherapeutic agent.

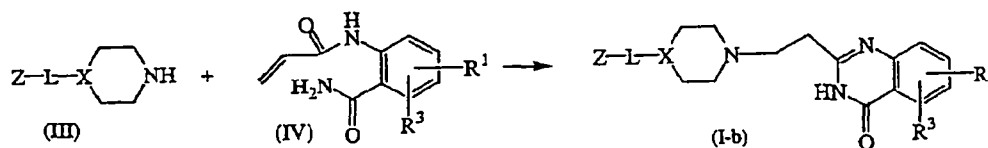
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13. A process for preparing a compound as claimed in claim 1, characterized by a) cyclizing an appropriate substituted 2-aminocarbonylbenzamide of formula (II) into compounds of formula (I-a), wherein both dotted lines can be a bond,

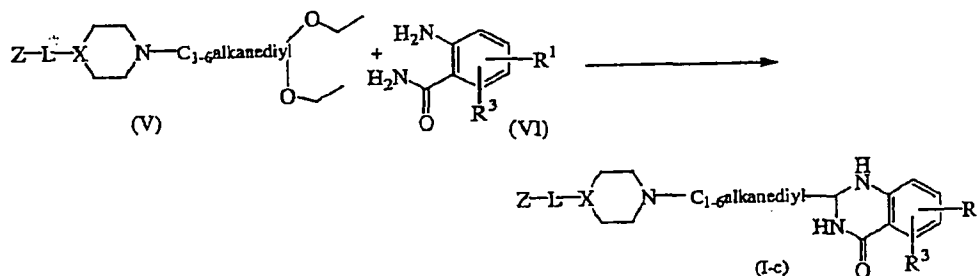
-35-



- b) cyclizing an appropriate substituted 2-aminocarbonylbenzamide of formula (VI) with an intermediate of formula (III) into compounds of formula (I-b), wherein
 5 -C₁₋₆alkanediy- is -CH₂-CH₂- and both dotted lines can be a bond, or



- c) cyclizing an appropriate substituted 2-aminobenzamide of formula (V) with an intermediate of formula (VI) into compounds of formula (I-c), wherein only one of the
 10 dotted lines can be a bond.



- 15 14. A compound according to any one of claims 1 to 5, substantially as herein described with reference to and as illustrated in any of the examples.
15. A composition according to claim 6, substantially as herein described with reference to and as illustrated in any of the examples.
- 20 16. A process according to claim 7 or claim 13, substantially as herein described with reference to and as illustrated in any of the examples.
17. Use according to any one of claims 8 to 11, substantially as herein described with
 25 reference to and as illustrated in any of the examples.
18. A combination according to claim 12, substantially as herein described with reference to and as illustrated in any of the examples.