Title: LEVOGLUCOSEMONONE DERIVATIVES FOR THE TREATMENT OF DISORDERS SUCH AS CANCER, AUTOIMMUNE DISEASES AND HEART DISEASES.

Abstract: A compound of formula (I) or (II) for use as a medicament. A pharmaceutical composition the compound. Use of the compound for preparing a medicament for the treatment of a disorder selected from hyperproliferative diseases, autoimmune diseases, and heart diseases.
LEVOGLUCOSENONE DERIVATIVES FOR THE TREATMENT OF DISORDERS SUCH AS CANCER, AUTOIMMUNE DISEASES AND HEART DISEASES.

Field of the invention
The present invention relates to levoglucosenone derivatives for use in therapy. More particularly, the present invention relates to levoglucosenone derivatives for the treatment and prevention of disorders and diseases such as, for example cancer, autoimmune diseases and heart diseases.

Background
The most common target for mutations in tumors is the p53 gene. The fact that around half of all human tumors carry mutations in this gene is solid testimony as to its critical role as tumor suppressor. p53 halts the cell cycle and/or triggers apoptosis in response to various stress stimuli, including DNA damage, hypoxia, and oncogene activation (Ko and Prives, 1996; Sherr, 1998). Upon activation, p53 initiates the p53-dependent biological responses through transcriptional transactivation of specific target genes carrying p53 DNA binding motifs. In addition, the multifaceted p53 protein may promote apoptosis through repression of certain genes lacking p53 binding sites and transcription-independent mechanisms as well (Bennett et al., 1998; Gottlieb and Oren, 1998; Ko and Prives, 1996). Analyses of a large number of mutant p53 genes in human tumors have revealed a strong selection for mutations that inactivate the specific DNA binding function of p53; most mutations in tumors are point mutations clustered in the core domain of p53 (residues 94-292) that harbours the specific DNA binding activity (Béroud and Soussi, 1998).

Both p53-induced cell cycle arrest and apoptosis could be involved in p53-mediated tumor suppression. While p53-induced cell cycle arrest could conceivably be reversed in different ways, p53-induced cell death would have the advantage of being irreversible. There is indeed evidence from animal in vivo models (Symonds et al., 1994) and human tumors (Bardeesy et al., 1995) indicating that p53-dependent apoptosis plays a major role in the elimination of emerging tumors, particularly in response to oncogenic signaling. Moreover, the ability of p53 to induce apoptosis often determines the efficacy of cancer therapy (Lowe et al., 1994). Taking into account the fact that more than 50% of human tumors carry p53 mutations, it appears highly desirable to restore the function of wild type p53-mediated growth suppression to tumors. The advantage of this approach is that it will allow selective elimination of tumor cells, carrying mutant p53. Tumor cells are particularly sensitive to p53 reactivation, suppos-
edly for two main reasons. First, tumor cells are sensitized to apoptosis due to oncogene activation (reviewed in (Evan and Littlewood, 1998)). Second, mutant p53 proteins tend to accumulate at high levels in tumor cells. Therefore, restoration of the wild type function to the abundant and presumably “activated” mutant p53 should trigger a massive apoptotic response in already sensitized tumor cells, whereas normal cells that express low or undetectable levels of p53 should not be affected. The feasibility of p53 reactivation as an anticancer strategy is supported by the fact that a wide range of mutant p53 proteins are susceptible to reactivation. A therapeutic strategy based on rescuing p53-induced apoptosis should therefore be both powerful and widely applicable.

It may be shown that malfunctioning of the p53 pathway is generally involved in a number of diseases, such as those enumerated herein above.

Taken together, these findings suggest that pharmacological restoration of p53 function would result in elimination of tumor cells. Consequently, there is a need within this field to achieve substances and methods for use therein, which enables such a restoration.

The present inventors have earlier found that the compound PRIMA-1 (i.e. 2,2-bis(hydroxymethyl)-1-azabicyclo[2.2.2]octan-3-one) (disclosed in WO0224692) is able to induce apoptosis of cells carrying mutant p53 and also in melanoma cells carrying wt p53 which are inactivated (WO 04084893). Later they also found some other analogues to Prima-1 that showed similar results (disclosed in WO 05090341).

There however remains a need for still further compounds capable of providing a therapeutic benefit in the treatment of disorders of the kind that are susceptible of being related – directly or indirectly - to p53 abnormality, and one object of the present invention is to provide such compounds.

Summary of the invention

The present inventors now surprisingly have found a group of levoglucosenone derivatives capable of exerting an advantageous biological activity that is susceptible of providing them with a usefulness in the treatment of disorders wherein malfunctioning of the p53 pathway may be involved, and this discovery forms the basis of the present invention.
Consequently, according to one aspect, the present invention provides a compound of formula (I)

![Chemical Structure](image)

wherein

A represents C=O or C(OH)-D wherein D represents a 1-3 membered, substituted or unsubstituted chain of C, N and/or O atoms, attached to R so as to form a 6-7 membered heterocycle;

R represents a saturated or unsaturated, 5- or 6-membered nitrogen-containing heterocycle comprising 1-4 N atoms, 0 or 1 O atom and 0 or 1 S atom; wherein said heterocycle:
- is linked to the dioxa bicycle

![Dioxa Bicycle](image)

through one of the heterocyclic N atoms;
- optionally comprises one or several ring carbonyl and/or ring thiocarbonyl and/or ring sulfonyl groups;
- optionally is condensed with a C6-C10 aryl; and
- optionally is substituted with one or several moieties selected from branched or unbranched, unsubstituted or substituted, saturated or unsaturated C1-C6 alkyl; unsubstituted or substituted, saturated or unsaturated C3-C12 cycloalkyl; unsubstituted or substituted C6-C10 aryl; unsubstituted or substituted C6-C10 aryl-NH--; unsubstituted or substituted C6-C10 aryloxy; unsubstituted or substituted C4-C5 heteroaryl; unsubstituted or substituted benzyl; and
- C(O)O-C1-C6 alkyl;
or R represents

\[ \begin{array}{c}
\text{S} \\
\text{G} \\
\text{G} \\
\text{R}^{10} \\
\text{R}^{11} \\
\text{R}^{12}
\end{array} \]

wherein

G is selected from N and CR\textsuperscript{13}; and

R\textsuperscript{10}, R\textsuperscript{11} and R\textsuperscript{12} are independently selected from H; unsubstituted or substituted, branched or unbranched C1-C6 alkyl; unsubstituted or substituted C6-C10 aryl and C4-C5 heteroaryl or any two of R\textsuperscript{10}, R\textsuperscript{11} and R\textsuperscript{12}, together with the ring atoms to which they are attached, form a unsubstituted or substituted C5-C7 carbocycle;

R\textsuperscript{13} is, independently for the two locations, selected from H and C1-C6 alkyl;

or a compound of formula (II)

\[ \begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{R} \\
\text{R} \\
\text{n} \\
\text{O} \\
\text{O} \\
\text{O}
\end{array} \]

(II)

wherein

n is 0-3; and

R represents a saturated or unsaturated, 5- or 6-membered nitrogen-containing heterocycle comprising 1-4 N atoms, 0 or 1 O atom and 0 or 1 S atom; wherein said heterocycle: is linked to the bicycle through one of the heterocyclic N atoms;

- optionally comprises one or several ring carbonyl and/or ring thiacarbonyl and/or ring sulfonyl groups; and

- optionally is substituted with one or several moieties selected from branched or unbranched, unsubstituted or substituted, saturated or unsaturated C1-C6 alkyl; unsubstituted or substituted, saturated or unsaturated C3-C12 cycloalkyl; unsubstituted or substituted C6-C10 aryl; unsubstituted or substituted C6-C10 aryl-NH-; unsubstituted or substituted C6-C10 aryloxy; unsubstituted or substituted C4-C5 heteroaryl; unsubstituted or substituted benzyl; and
-C(O)O-C1-C6 alkyl;

as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a medicament.

According to a further aspect, the present invention provides the use of the compounds of formula (I) or (II) or pharmaceutically acceptable salts or prodrugs thereof for the treatment of diseases associated with a malfunctioning p53 signalling pathway.

According to a still further aspect, the invention provides pharmaceutical compositions comprising compounds of formula (I) or (II), or salts or prodrugs thereof.

According to a still further aspect the invention provides a method of medical treatment by administration of a therapeutically effective amount of a compound of formula (I) or (II) or a pharmaceutically acceptable salt or prodrug thereof to a mammal in the need of such treatment.

According to one aspect, the invention provides the use of the inventive compounds, or salts or prodrugs thereof in the manufacture of a medicament for the treatment or prevention of a disorder selected from hypeproliferative diseases, autoimmune diseases and heart diseases.

Any further aspects are as defined in the claims.

**Brief description of the drawings**

**Fig. 1** Fluorometric microculture cytotoxicity assay (FMCA): Percent fluorescence signal intensity vs. concentration of inventive compound 2-(4-methyl-3-phenyl-5-thioxo-4,5-dihydro[1,2,4]triazol-1-yl)-6,8-dioxo-bicyclo[3.2.1]octan-4-one in microtiter plates containing various human tumor cell lines.

**Fig. 2** Fluorometric microculture cytotoxicity assay (FMCA): Percent fluorescence signal intensity vs. concentration of inventive compound 2-[4-(4-chloro-phenyl)-6-trifluoromethyl-pyrimidin-2-ylsulfanyl]-6,8-dioxo-bicyclo[3.2.1]octan-4-one in microtiter plates containing various human tumor cell lines.
Detailed description of the invention

Unless otherwise specified, the alkyl groups that are considered useful in the compounds according to the invention generally may be selected from unbranched or branched, cyclic, saturated or unsaturated (alkenyl or alkynyl) hydrocarbyl radicals. Where cyclic, the alkyl group is preferably C3 to C12, more preferably C5 to C10, most preferably C5-C7. Where acyclic, the alkyl group is preferably C1 to C10, more preferably C1 to C6, more preferably methyl, ethyl, propyl (n-propyl, isopropyl), butyl (branched or unbranched) or pentyl, most preferably methyl.

As used herein, and unless specified otherwise, the term “C6-C10 aryl” means phenyl and naphthyl.

As used herein, and unless specified otherwise, the term “heteroaryl” means an aromatic group containing one or more heteroatom(s) preferably selected from N, O and S, such as pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, imidazolyl, pyrimidinyl, indolyl, pyrazinyl or indazolyl.

As used herein, and unless specified otherwise, the term “heterocycle” means a non-aromatic cyclic group containing one or more heteroatom(s) preferably selected from N, O and S, such as a cyclic amino group such as pyrrolidinyl, piperidyl, piperazinyl, morpholinyl or a cyclic ether such as tetrahydrofuranyl, monosaccharide.

As used herein, and unless specified otherwise, the term “halogen” means a fluorine, chlorine, bromine or iodine.

As used herein, and unless specified otherwise, the term “substituted” means that the entity is substituted with at least one moiety, e.g. 1, 2 or 3 moieties, selected from saturated or unsaturated, branched, unbranched or cyclic alkyl, or at least one functional group such as hydroxyl, amine, sulfide, silyl, carboxylic acid, halogen, aryl, etc. The substitution also may be by a moiety forming, together with the substituted entity, a ring system, e.g. a dioxyethylene forming a cyclic diether, a carboxylic ester group forming a lactone or a chain of C, N, O and/or S atoms forming a heterocyclic or carbocyclic ring.
According to one embodiment of the invention, R is a nitrogen-containing heterocycle as defined herein above.

According to one embodiment of the invention, the nitrogen-containing heterocycle comprises 1 or 2 double bonds in the heterocycle ring.

According to one specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle.

According to another embodiment of the invention, the nitrogen-containing heterocycle is a 6-membered heterocycle.

According to one specific embodiment of the invention, the nitrogen-containing heterocycle comprises 4 heterocyclic ring N atoms.

According to another specific embodiment of the invention, the nitrogen-containing heterocycle comprises 3 heterocyclic ring N atoms.

According to still another specific embodiment of the invention, the nitrogen-containing heterocycle comprises 2 heterocyclic ring N atoms.

According to one specific embodiment of the invention, the nitrogen-containing heterocycle comprises 1 double-bond in the heterocycle ring.

According to another embodiment of the invention, the 5-membered nitrogen-containing heterocycle is condensed with a benzene or naphthalene ring.

According to one embodiment of the invention, the nitrogen-containing heterocycle comprises at least one ring carbonyl, thio carbonyl or sulfonyl group. By ring carbonyl or thiocarbonyl group is meant a C atom of the nitrogen-containing heterocycle double-bonded to an O or S atom, respectively. Similarly, by ring sulfonyl group is meant an S atom of the nitrogen-containing heterocycle double-bonded to two O atoms.
According to one embodiment of the invention, the nitrogen-containing heterocycle comprises one ring thiocarbonyl group.

According to another embodiment of the invention, the nitrogen-containing heterocycle comprises one ring thiocarbonyl and one ring carbonyl group.

According to another embodiment of the invention, the nitrogen-containing heterocycle comprises one ring sulfonyl group.

According to another embodiment of the invention, the nitrogen-containing heterocycle comprises one ring sulfonyl and one ring carbonyl group.

According to a specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle having one ring double bond and comprising 3 or 4 heterocyclic N atoms and a ring carbonyl or thiocarbonyl group.

According to another specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle comprising 2 heterocyclic N atoms or 1 heterocyclic N atom and 1 heterocyclic O atom, and a ring carbonyl or thiocarbonyl group, and being condensed to a substituted or unsubstituted benzene ring.

According to another specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered saturated heterocycle comprising 2 heterocyclic N atoms, a ring carbonyl group and a ring thiocarbonyl group.

According to another specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle comprising 2 ring double bonds and 3 or 4 heterocyclic N atoms.

According to still another embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle comprising one heterocyclic N atom and one ring sulfonyl group, said heterocycle being condensed to a substituted or unsubstituted naphthalene ring.
According to still another embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle comprising one heterocyclic N atom, one ring sulfonyl group and one ring carbonyl group, said heterocycle being condensed to a substituted or unsubstituted benzene or naphthalene ring.

According to another specific embodiment of the invention, the nitrogen-containing heterocycle is a 5-membered heterocycle comprising 3 or 4 heterocyclic N atoms and being condensed to a substituted or unsubstituted benzene ring.

According to one embodiment of the invention, the nitrogen-containing heterocycle linked to the bicycle through one of the heterocyclic N atoms is selected from

\[
\begin{align*}
\text{Y is selected from S and O;} \\
\text{X is selected from N and C-R²;} \\
\text{T is selected from S and O;} \\
\text{Z is selected from O and N-R³;} \\
\text{E is selected from C-R⁷ and C attached to D;} \\
\text{W is selected from N and C-R⁸;} \\
\text{Q is selected from N and C-R⁹;} \\
\text{M is selected from N and C attached to D;} \\
\text{R² is selected from H; (iii) unsubstituted or substituted C1-C6 alkyl; and (iv) unsubstituted or substituted C6-C10 aryl, C6-C10 aryl-NH-, C6-C10 arylxy or C4-C5 heteroaryl;}
\end{align*}
\]
R³ is selected from (v) unsubstituted or substituted benzyl;

R⁴ and R⁵ are independently selected from H and C1-C6 alkyl;

R⁶ is selected from C6-C10 aryl;

R⁷ is selected from H; C1-C6 alkyl; and (vi) unsubstituted or substituted C6-C10 aryl;

R⁸ and R⁹ are independently selected from H and -C(O)O-C1-C6 alkyl.

According to one embodiment of the invention, A is C=O. In this embodiment, the compound of formula (I) may be represented by formula (I’)

(II)

wherein R is as defined herein above.

According to one embodiment, R is a nitrogen-containing heterocycle and A represents C(OH)-D wherein D represents a 1-3 membered, substituted or unsubstituted chain of C, N and/or O atoms, attached to a carbon atom of the nitrogen-containing heterocycle so as to form a 6-7 membered heterocycle. In this embodiment, the compound of formula (I) may be represented by formula (I’’)

(I’’)

wherein D represents a 1-3 membered, substituted or unsubstituted chain of C, N and/or O atoms and R is as defined herein above.
According to one embodiment of the invention, in the compound of formula (I'), R is selected from

\[
\begin{align*}
\text{N} & \text{N} \\
E & = W \\
\text{N} & \text{N} \quad \text{and} \quad \text{M} & \text{N} \\
\end{align*}
\]

wherein E and M are both carbon atoms; and

D represents a 1-3 membered, substituted or unsubstituted chain of C, N and/or O atoms, attached to E or M, respectively, so as to form a 6-7 membered heterocycle.

According to one embodiment of the invention, in a compound according to formula (II)

\[
\begin{align*}
\text{O} & \text{O} \\
R & \quad n \quad R \\
\text{O} & \text{O}
\end{align*}
\]

(II)

wherein n is 1 or 2.

According to one embodiment, in a compound of formula (II) R represents a saturated or unsaturated, 5-membered nitrogen-containing heterocycle comprising 1-4 N atoms, 0 or 1 O atom and 0 or 1 S atom.

According to one embodiment of the invention, the compound of formula (II) is according to the formula (II')
wherein \( n \) is 0-3, preferably 1 or 2, and \( X \) and \( Y \) are as defined herein above.

For the purpose of the invention, and unless otherwise specified, reference to a compound of formula (I) should be understood also as a reference to a compound of formula (I’’) or (I’’’), and reference to a compound of formula (II) should be understood also as a reference to a compound of formula (II’

The compounds according to formula (I) or (II) will be useful for treating or preventing various diseases such as hyperproliferative diseases, e.g. cancer, autoimmune diseases, such as rheumatoid arthritis and Sjogren’s syndrome, and heart diseases such as hereditary idiopathic cardiomyopathy. The treatment may be preventive, palliative or curative.

Examples of pharmaceutically acceptable addition salts for use in the pharmaceutical compositions of the present invention include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids. The pharmaceutically acceptable excipients described herein, for example, vehicles, adjuvants, carriers or diluents, are well-known to those who are skilled in the art and are readily available to the public. The pharmaceutically acceptable carrier may be one which is chemically inert to the active compounds and which have no detrimental side effects or toxicity under the conditions of use. Pharmaceutical formulations are found e.g. in Remington: The Science and Practice of Pharmacy. A. R. Gennaro, Editor. Lippincott, Williams and Wilkins, 20th edition (2000).

Prodrugs of the compounds of formula (I) or (II) may be prepared by modifying functional groups present on the compound in such a way that the modifications are cleaved, in vivo when such prodrug is administered to a mammalian subject. The modifications typically are
achieved by synthesizing the parent compound with a prodrug substituent. Prodrugs include compounds of formula (I) or (II) wherein a hydroxy, amino, sulphydryl, carboxy or carbonyl group in a compound of formula (I) or (II) is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl, amino, or sulphydryl group, respectively.

Examples of prodrugs include, but are not limited to, esters and carbamates of hydroxy functional groups, esters groups of carboxyl functional groups, N-acyl derivatives, N-Mannich bases. General information on prodrugs may be found e.g. in Bundegaard, H. "Design of Prodrugs" pl-92, Elsevier, New York-Oxford (1985).

The composition according to the invention may be prepared for any route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, or intraperitoneal. The precise nature of the carrier or other material will depend on the route of administration. For a parenteral administration, a parenterally acceptable aqueous solution is suitably employed, which is pyrogen free and has requisite pH, isotonicity, and stability. Those skilled in the art are well able to prepare suitable solutions and numerous methods are described in the literature. A brief review of methods of drug delivery is also found in e.g. Langer (1990).

The dose administered to a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the mammal over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors including the potency of the specific compound, the age, condition and body weight of the patient, as well as the stage/severity of the disease. The dose will also be determined by the route (administration form), timing and frequency of administration. In the case of oral administration the dosage can vary from about 0.01 mg to about 1000 mg per day of a compound of formula (I) or (II) or the corresponding amount of a pharmaceutically acceptable salt or prodrug thereof.

The compounds of the present invention may be used or administered in combination with one or more additional drugs useful in the treatment of diseases mediated by mutant p53, or wherein a malfunction of the p53 signalling pathway is involved, such as cytostatic drugs. The components may be in the same formulation or in separate formulations for administration simultaneously or sequentially. The compounds of the present invention may also be used
or administered in combination with other treatment such as irradiation for the treatment of cancer.

Examples of cytostatic compounds for use as indicated herein above are DNA alkylating compounds, topoisomerase I inhibitors, topoisomerase II inhibitors, compounds interfering with RNA and DNA synthesis, compounds polymerising the cytoskeleton, and compounds depolymerising the cytoskeleton.

Compounds according to formula (I) or (II) are commercially available, or may be prepared by methods that are well within the knowledge of the person skilled in the art.

As an example, the compounds of the present invention may be formed in a one step reaction, wherein a suitable nucleophile is reacted with levoglucosenone in a Michael addition reaction under basic conditions.

The nucleophile may be an N-, S-, O- or C- nucleophile. The synthesis with N-nucleophiles has been described earlier and below is just one example based on the method described by Samet et al in 1994 and 1996:

\[ \text{base} \]

Suitable synthetic methods employing S-nucleophiles have been described by Witczak et al (1995), and by Niyazymbetov et al (1994). Shafizadeh et al (1982) have described suitable Michael-addition reactions with C-nucleophiles.

**Biology**

**HTS screening**

*Cell lines*

The human SAOS-2 (human osteosarcoma) cell line lacking p53 expression and its transfected clone SAOS-2-His273 that carries tetracycline-regulated mutant p53 constructs were
used for the HTS screen.

**WST-1 assay**

Cells were plated on 96-well plates at a density of 3000 cells per well per 100ml medium, cultured overnight and treated with compounds at a concentration of 1, 5, 10, 25, or 50μM, respectively. After 96h 10μl of WST-1-cell proliferation reagent were added to each well. Samples were incubated at 37°C for 1-2 h and absorbance of samples was measured at 490nm. Survival of the untreated cells was taken as 100%.

A number of compounds according to the invention, that all showed inhibition of cell proliferation at a concentration ranging from 1 to 50 μM, are illustrated in Table 1 herein below.

**Table 1** Some compounds of the invention showing inhibition of cell proliferation at a concentration ranging from 1 to 50 μM

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>![Diagram 1]</td>
<td>![Diagram 2]</td>
</tr>
<tr>
<td>![Diagram 3]</td>
<td>![Diagram 4]</td>
</tr>
<tr>
<td>![Diagram 5]</td>
<td>![Diagram 6]</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>37</td>
<td>38</td>
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<td>39</td>
<td>40</td>
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<td>41</td>
<td>42</td>
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<tr>
<td>43</td>
<td>44</td>
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<tr>
<td>45</td>
<td>46</td>
</tr>
</tbody>
</table>
Compounds of this invention inherently contain one or more asymmetric centres and may thus give rise to stereoisomers and diastereomers. The present invention includes such stereoisomers and diastereomers; as well as the racemic and resolved, enantiomerically pure stereoisomers and pharmaceutically acceptable salts thereof, which possess the indicated activity. Stereoisomers may be obtained in pure form by standard procedures known to those skilled in the art. It also is contemplated that the invention encompasses all possible regioisomers, and mixtures thereof which possess the indicated activity. Such regioisomers may be obtained in pure form by standard separation procedures known to those skilled in the art.

Some selected results from IC50 determination and the comparison between derivative in human SAOS-2 (human osteosarcoma) cell line lacking p53 expression and in a SAOS-2-His273 cell line that carries tetracycline-regulated mutant p53 constructs are shown in Table 2 herein below, wherein each compound is referred to by the number given to it in Table 1.
Table 2 IC50 values of some inventive compounds

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>IC50 μM in SAOS-2</th>
<th>IC50 μM in SAOS-2-His273</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>13.978</td>
<td>7.974</td>
<td>1.75</td>
</tr>
<tr>
<td>6</td>
<td>13.91</td>
<td>5.825</td>
<td>2.39</td>
</tr>
<tr>
<td>7</td>
<td>8.719</td>
<td>3.288</td>
<td>2.65</td>
</tr>
<tr>
<td>10</td>
<td>not toxic</td>
<td>25</td>
<td>2.00</td>
</tr>
<tr>
<td>13</td>
<td>20.362</td>
<td>5.711</td>
<td>3.57</td>
</tr>
<tr>
<td>14</td>
<td>24.603</td>
<td>9.557</td>
<td>2.57</td>
</tr>
<tr>
<td>30</td>
<td>15.594</td>
<td>1.466</td>
<td>10.64</td>
</tr>
<tr>
<td>56</td>
<td>11.9</td>
<td>5.9</td>
<td>2.02</td>
</tr>
<tr>
<td>85</td>
<td>14.556</td>
<td>5.087</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Human tumor cell-line panel

To evaluate the activity patterns of the drugs a human cell line panel of four sensitive parental cell lines, five drug resistant sublines, representing different mechanisms of resistance, and one cell line with primary resistance were used, cf. Table 3 herein below. The cell lines included were the myeloma cell line RPMI 8226/S and its sublines 8226/Dox40 and 8226/LR-5, the lymphoma cell lines U-937 GTB and U-937-Vcr, the SCLC cell line NCI-H69 and its subline H69AR, the renal adenocarcinoma cell line ACHN (ATCC) and the leukaemic cell line CCRF-CEM and its subline CEM/VM-1. The 8226/Dox40 was selected for doxorubicin resistance and shows the classical MDR phenotype with overexpression of P-glycoprotein 170 (Pgp-170). The 8226/LR-5 was selected for melphalan resistance, proposed to be associated with increased levels of GSH. The U-937-Vcr was selected for vincristine resistance, proposed to be tubulin associated. The H69AR, selected for doxorubicin resistance, expresses a MDR phenotype proposed to be mediated by MRP. The CEM/VM-1, selected for teniposide resistance, expresses an atypical MDR, which is proposed to be topoisomerase II (topoII) associated. The exact mechanism of resistance for the primary resistant ACHN cell line is not known and may be multifactorial.

The cell lines were grown in complete culture medium consisting of carbonate buffered culture medium RPMI-1640 (HyClone, Cramlington, UK) supplemented with 10% inactivated FCS, 2mM glutamine, 50 μg/ml of streptomycin and 60 μg/ml of penicillin, at 37°C in humidified atmosphere containing 5% CO₂. The 8226/Dox40 cell line was treated once a month
with doxorubicin at 0.24 μg/ml and the 8226/LR-5 cell line at each change of medium with melphalan at 1.53 μg/ml. The U-937-Vcr cell line was continuously cultured in presence of 10 ng/ml of vincristine and the H69AR cell line was alternately fed with drug free medium and medium containing 0.46 μg/ml of doxorubicin. The CEM/VM-1 cell line was cultured in drug free medium without any loss of resistance for a period of 6-8 months. The resistance patterns of the cell lines were routinely confirmed in control experiments.

**Table 3** Human tumor cell lines used in the study.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Selecting agent</th>
<th>Resistance associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRF-CEM</td>
<td>Leukemia</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CEM/VM-1</td>
<td>&quot;</td>
<td>teniposide</td>
<td>topoisomerase II</td>
</tr>
<tr>
<td>ACHN</td>
<td>Renal cancer</td>
<td>-</td>
<td>(primary resistance)</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>Small cell lung cancer</td>
<td>-</td>
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<tr>
<td>H69AR</td>
<td>&quot;</td>
<td>doxorubicin</td>
<td>MRP</td>
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<tr>
<td>RPMI 8226/S</td>
<td>Myeloma</td>
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<tr>
<td>8226/dox40</td>
<td>&quot;</td>
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<td>Pgp</td>
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<tr>
<td>8226/LR5</td>
<td>&quot;</td>
<td>melphalan</td>
<td>glutathione</td>
</tr>
<tr>
<td>U-937 GTB</td>
<td>Lymphoma</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>U-937-vcr</td>
<td>&quot;</td>
<td>vincristine</td>
<td>tubulin</td>
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</table>

**The fluorometric microculture cytotoxicity assay (FMCA)**

The fluorometric microculture cytotoxicity assay (FMCA) is based on measurement of fluorescence generated from hydrolysis of FDA (fluorescein diacetate) to fluorescein by cells with intact plasma membranes and has been described in detail previously in the literature.

Tumor cells were seeded in the drug prepared 384-well microtiter plates at a cell density of 5,000 cells/well. The plates were incubated at 37°C in humidified atmosphere containing 5% CO₂ for 72 hrs. At the end of the incubation period the medium was removed by aspiration. After one wash in PBS, 50 μl/well of FDA dissolved in a physiological buffer (10 μg/ml) was added. The plates were incubated for 45 minutes and the generated fluorescence from each well was measured in a 384-well scanning fluorometer. The fluorescence is proportional to the number of intact cells in the well.
Quality criteria for a successful analysis included a fluorescence signal in the control wells of more than five times mean blank value, a mean coefficient of variation (CV) in the control wells of less than 30%. Experiments were performed twice (2 for ten-fold dilutions and 2 for 5-fold), mean values are used throughout.

In Figs.1 and 2 test results are shown for two inventive compounds, viz. compound No. 21 (cf. Table 1): 2-(4-methyl-3-phenyl-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-yl)-6,8-dioxabicyclo[3.2.1]octan-4-one (Fig. 1) and compound No. 85 (cf. Table 1): 2-[4-(4-chlorophenyl)-6-trifluoromethyl-pyrimidin-2-ylsulfonyl]-6,8-dioxabicyclo[3.2.1]octan-4-one (Fig. 2). Results are expressed as percent fluorescence signal intensity as a function of the concentration of inventive compound in the medium of microtiter plate well and taking the blank signal as 100%. In Table 4, the corresponding EC50s calculated for the two compounds are shown.

**Table 4** Activity in cell line panel presented as EC50 in μM

<table>
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<tr>
<th>Cell line</th>
<th>Compound No. 21</th>
<th>Compound No. 85</th>
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<tbody>
<tr>
<td>CCRF-CEM</td>
<td>8.2</td>
<td>9</td>
</tr>
<tr>
<td>CEM/VM-1</td>
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<td>9</td>
</tr>
<tr>
<td>ACHN</td>
<td>40</td>
<td>29</td>
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<td>3.6</td>
</tr>
<tr>
<td>H69AR</td>
<td>8.3</td>
<td>8.3</td>
</tr>
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<td>9.1</td>
</tr>
<tr>
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<td>9.3</td>
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</tr>
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<td>6.8</td>
</tr>
<tr>
<td>U-937-vcr</td>
<td>4.3</td>
<td>7.4</td>
</tr>
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</table>
References

Elkin et al (US 3726877).
Lowe et al., 1994 Science 266, 807-10 (1994).
Shafizadeh et al., Carbohydrate Res. 102, 217-230 (1982)
Symonds et al., Cell 78, 703-711 (1994).
Claims

1. A compound of formula (I)

\[
\begin{align*}
\text{(I)}
\end{align*}
\]

wherein

A represents C=O or C(OH)-D wherein D represents a 1-3 membered, substituted or unsubstituted chain of C, N and/or O atoms, attached to R so as to form a 6-7 membered heterocycle;

R represents a saturated or unsaturated, 5- or 6-membered nitrogen-containing heterocycle comprising 1-4 N atoms, 0 or 1 O atom and 0 or 1 S atom; wherein said heterocycle:
- is linked to the dioxa bicycle

\[
\begin{align*}
\text{(II)}
\end{align*}
\]

through one of the heterocyclic N atoms;
- optionally comprises one or several ring carbonyl and/or ring thiocarbonyl and/or ring sulfonyl groups;
- optionally is condensed with a C6-C10 aryl; and
- optionally is substituted with one or several moieties selected from branched or unbranched, unsubstituted or substituted, saturated or unsaturated C1-C6 alkyl; unsubstituted or substituted, saturated or unsaturated C3-C12 cycloalkyl; unsubstituted or substituted C6-C10 aryl; unsubstituted or substituted C6-C10 aryl-NH-; unsubstituted or substituted C6-C10 aryloxy; unsubstituted or substituted C4-C5 heteroaryl; unsubstituted or substituted benzyl; and
- C(O)O-C1-C6 alkyl;
or R represents

\[
\begin{array}{c}
\text{G} \\
\text{S}
\end{array}
\]

wherein

G is selected from N and CR\text{\textsuperscript{13}}; and

R\text{\textsuperscript{10}}, R\text{\textsuperscript{11}} and R\text{\textsuperscript{12}} are independently selected from H; unsubstituted or substituted, branched or unbranched C\text{\textsuperscript{1}}-C\text{\textsuperscript{6}} alkyl; unsubstituted or substituted C\text{\textsuperscript{6}}-C\text{\textsuperscript{10}} aryl and C\text{\textsuperscript{4}}-C\text{\textsuperscript{5}} heteroaryl or any two of R\text{\textsuperscript{10}}, R\text{\textsuperscript{11}} and R\text{\textsuperscript{12}}, together with the ring atoms to which they are attached, form a unsubstituted or substituted C\text{\textsuperscript{5}}-C\text{\textsuperscript{7}} carbocycle;

R\text{\textsuperscript{13}} is, independently for the two locations, selected from H and C\text{\textsuperscript{1}}-C\text{\textsuperscript{6}} alkyl;

or a compound of formula (II)

\[
\text{(II)}
\]

wherein

n is 0-3; and

R represents a saturated or unsaturated, 5- or 6-membered nitrogen-containing heterocycle comprising 1-4 N atoms, 0 or 1 O atom and 0 or 1 S atom; wherein said heterocycle is linked to the bicycle through one of the heterocyclic N atoms;

- optionally comprises one or several ring carbonyl and/or ring thiocarbonyl and/or ring sulfonyl groups; and

- optionally is substituted with one or several moities selected from branched or unbranched, unsubstituted or substituted, saturated or unsaturated C\text{\textsuperscript{1}}-C\text{\textsuperscript{6}} alkyl; unsubstituted or substituted, saturated or unsaturated C\text{\textsuperscript{3}}-C\text{\textsuperscript{12}} cycloalkyl; unsubstituted or substituted C\text{\textsuperscript{6}}-C\text{\textsuperscript{10}} aryl; unsubstituted or substituted C\text{\textsuperscript{6}}-C\text{\textsuperscript{10}} aryl-NH\text{-}; unsubstituted or substituted C\text{\textsuperscript{6}}-C\text{\textsuperscript{10}} aryloxy; unsubstituted or substituted C\text{\textsuperscript{4}}-C\text{\textsuperscript{5}} heteroaryl; unsubstituted or substituted benzyl; and
-C(O)O-C1-C6 alkyl;
as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a medicament.

2. A compound according to claim 1, wherein the nitrogen-containing heterocycle is 5-membered.

3. A compound according to claim 1 or 2, wherein the nitrogen-containing heterocycle comprises 2-4 N atoms.

4. A compound according to any one of the claims 1-3, wherein the nitrogen-containing heterocycle comprises 1 or 2 ring double bonds.

5. A compound according to any of the claims 1-4, wherein the nitrogen-containing heterocycle comprises a ring carbonyl and/or thiocarbonyl and/or sulfonyle group.

6. A compound according to any of the claims 1-5, wherein the substituents of any of D, C1-C6 alkyl, C3-C12 cycloalkyl, C6-C10 aryl, C6-C10 aryl-NH-, C6-C10 arylalkoxy, C4-C5 heteroaryl, benzyl; -C(O)O-C1-C6 alkyl, C4-C5 heteroaryl and C5-C7 carbocycle of formula (I), are selected from halogen; C6-C10 aryl; branched or unbranched C1-C6 alkyl; branched or unbranched C1-C6 alkoxy; branched or unbranched C1-C6 thioalkoxy; branched or unbranched C1-C6 alkoxy substituted with at least one halogen; OH; branched or unbranched C1-C6 alkyl-C(O)-; and C4-C5 heteroaryl; or wherein two substituents, together with the carbon atoms to which they are attached, form a C3-C7 heterocycle.

7. A compound according to any of the claims 1-6, wherein in formula (I) the nitrogen-containing heterocycle is condensed with a benzene or naphthalene ring.

8. A compound according to any of the claims 1-6, wherein a compound of formula (II) is according to the formula (II’).
wherein

n is 0-3;

Y is selected from S and O;

X is selected from N and C-R^2;

R^2 is selected from H; (iii) unsubstituted or substituted C1-C6 alkyl; and (iv) unsubstituted or substituted C6-C10 aryl, C6-C10 aryl-NH- or C6-C10 aryloxy.

9. A compound according to any of the claims 1-7, wherein in formula (I)

R is selected from

Y is selected from S and O;

X is selected from N and C-R^2;

T is selected from S and O;

Z is selected from O and N-R^3;

E is selected from C-R^7 and C attached to D;

W is selected from N and C-R^8;

Q is selected from N and C-R^9;
G is selected from N and CR\textsubscript{13};
M is selected from N and C attached to D;

R\textsubscript{1} is selected from (i) branched or unbranched, saturated or unsaturated, unsubstituted or substituted C1-C6 alkyl and C3-C12 cycloalkyl, and (ii) unsubstituted or substituted C6-C10 aryl;

R\textsubscript{2} is selected from H; (iii) unsubstituted or substituted C1-C6 alkyl; and (iv) unsubstituted or substituted C6-C10 aryl, C6-C10 aryl-NH- or C6-C10 aryloxy;

R\textsubscript{3} is selected from (v) unsubstituted or substituted benzyl;

R\textsubscript{3} and R\textsubscript{5} are independently selected from H and C1-C6 alkyl;

R\textsubscript{6} is selected from C6-C10 aryl;

R\textsubscript{7} is selected from H; C1-C6 alkyl; and (vi) unsubstituted or substituted C6-C10 aryl;

R\textsubscript{8} and R\textsubscript{9} are independently selected from H and -C(O)O-C1-C6 alkyl;

R\textsubscript{10}, R\textsubscript{11} and R\textsubscript{12} are independently selected from H; and (vii) branched or unbranched, unsubstituted or substituted C1-C6 alkyl; and (viii) unsubstituted or substituted C6-C10 aryl; or any two of R\textsubscript{10}, R\textsubscript{11} and R\textsubscript{12}, together with the ring atoms to which they are attached, form a (ix) unsubstituted or substituted C5-C7 carbocycle; and

R\textsubscript{13} is, independently for the two locations, selected from H and C1-C6 alkyl;

as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a medicament.

10. A compound according to claim 8 or 9, wherein Y is S.

11. A compound according to claim 9 or 10 wherein in formula (I) the substituent(s) on the C1-C6 alkyl and C3-C12 cycloalkyl in (i) is/are selected from halogen and C6-C10 aryl.
12. A compound according to any of the claims 9-11, wherein in formula (I) the substituent(s) of the C1-C6 aryl in (ii) is/are selected from halogen; branched or unbranched C1-C6 alkyl; branched or unbranched C1-C6 alkoxy; branched or unbranched C1-C6 alkoxy substituted with at least one halogen; OH; branched or unbranched C1-C6 alkyl-S-; and branched or unbranched C1-C6 alkyl-C(O)-; or wherein two substituents, together with the carbon atoms of the C6-C10 aryl to which they are attached, form a C5-C7-membered heterocycle.

13. A compound according to any of the claims 8-12, wherein the substituent(s) of the C1-C6 alkyl in (iii) is/are selected from OH, unsubstituted or substituted C6-C10 aryl, C6-C10 aryl-alkoxy and C4-C5 heteroaryl.

14. A compound according to any of the claims 8-13, wherein the substituent(s) on the C6-C10 aryl, C6-C10 aryl-alkoxy, C6-C10 aryl-NH- and C4-C5 heteroaryl in (iv) is/are selected from halogen, OH, C1-C6 alkyl and C1-C6 alkoxy.

15. A compound according to claim 11, wherein in formula (I) the substituent(s) on the benzyl in (v) is/are selected from halogen.

16. A compound according to any of the claims 9-15, wherein in formula (I) the substituent(s) on the C6-C10 aryl in (vi) is/are selected from halogen, C1-C6 alkyl, C1-C6 alkoxy, C1-C6 alkyl-S-.

17. A compound according to any of the claims 9-16, wherein in formula (I) the substituent(s) on the C1-C6 alkyl in (vii) is/are selected from halogen.

18. A compound according to claim 17, wherein in formula (I) the substituent(s) on the C6-C10 aryl in (viii) is/are selected from halogen, C1-C6 alkyl, and C1-C6 alkoxy; or two substituents together form a C3-C6 heterocycle.

19. A compound according to claim 11, wherein in formula (I) the C3-C6 heterocycle is an oxygen-containing C3 heterocycle.

20. A compound according to any of the claims 9-19 wherein in formula (I) the substituent(s) on the C5-C7 carbocycle in (ix) is/are selected from C1-C6 alkyl.
21. A compound according to any of the claims 8-20, wherein C6-C10 aryl is phenyl.

22. A compound according to any of the claims 8-21, wherein C1-C6 alkyl is C1-C4 alkyl.

23. A compound according to any of the claims 9-22, wherein in formula (I) the C3-C12 cycloalkyl is C5-C7 cycloalkyl.

24. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to any of the claims 1-23, or a pharmaceutically acceptable salt or prodrug thereof, and at least one pharmaceutically acceptable excipient.

25. Use of a compound according to any of the claims 1-23, for preparing a medicament for the treatment of a disorder selected from hyperproliferative diseases, autoimmune diseases, and heart diseases.

26. The use according to claim 25, wherein the disorder is a cancer.

27. A method of treatment of a disease selected from hyperproliferative diseases, autoimmune diseases, and heart diseases by administration of a therapeutically effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt or prodrug thereof to a mammal in the need of such treatment.
Figure 1

Figure 2
# INTERNATIONAL SEARCH REPORT

**International application No.**
PCT/SE2007/050363

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC:** see extra sheet
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC:** A61K, C07D

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

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## EPO-INTERNAL, WPI DATA, PAJ, CHEM.ABS DATA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>WO 2004037159 A2 (OBETHERY BIOTECHNOLOGY), 6 May 2004 (06.05.2004), page 241, line 6 - line 24; page 29, line 13 - line 19, page 230, compound 9, RN:686301-66-6, 161720-97-4, 686301-65-5</td>
<td>1-4, 6-7, 9, 24-26</td>
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![X] [Further documents are listed in the continuation of Box C.](X)

See patent family annex.

| * Special categories of cited documents: |
| **A** document defining the general state of the art which is not considered to be of particular relevance |
| **E** earlier application or patent but published on or after the international filing date |
| **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) |
| **O** document referring to an oral disclosure, use, exhibition or other means |
| **P** document published prior to the international filing date but later than the priority date claimed |
| **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| **X** document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| **Y** document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| **&** document member of the same patent family |

Date of the actual completion of the international search

**28 August 2007**

Date of mailing of the international search report

**03-09-2007**

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Helena Melander/ELY
Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (April 2007)
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<td>A</td>
<td>US 5457192 A (MATSUMOTO ET AL), 10 October 1995 (10.10.1995), column 1, line 30 - line 33, claims 1-11</td>
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International patent classification (IPC)

A61K 31/357 (2006.01)
A61K 31/41 (2006.01)
A61K 31/4155 (2006.01)
A61K 31/4178 (2006.01)
A61K 31/4184 (2006.01)
A61K 31/4196 (2006.01)
A61K 31/423 (2006.01)
A61K 31/428 (2006.01)
A61K 31/439 (2006.01)
A61K 31/501 (2006.01)
A61K 31/506 (2006.01)
A61K 31/517 (2006.01)
A61K 31/529 (2006.01)
A61K 31/553 (2006.01)

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Use the application number as username.
The password is BKYQZSGWN.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.
**INTERNATIONAL SEARCH REPORT**

<table>
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<th>Box No. II</th>
<th>Observations where certain claims were found unsearable (Continuation of item 2 of first sheet)</th>
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<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
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<tr>
<td>1. ☒ Claims Nos.: 27 because they relate to subject matter not required to be searched by this Authority, namely:</td>
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<tr>
<td>Claim 27 relates to a method of treatment of the human or animal body by surgery or by therapy, as well as diagnostic</td>
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<td>2. ☒ Claims Nos.: 1, 9, 24, 27 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
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<tr>
<td>The scope of the claims 1, 9, 24 and 27, in as far as the expression “prodrug thereof” is concerned, is so unclear (Article 6 PCT) that a meaningful search is impossible with regard to this expression.</td>
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<tr>
<td>3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<td>2.</td>
<td>☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</td>
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<tr>
<td>3.</td>
<td>☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
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<td>4.</td>
<td>☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:</td>
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**Remark on Protest**

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Box II.1

methods /Rule 39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compounds.
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<td>EP 0530367 A 10/03/1993</td>
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