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(57) **Abrégé/Abstract:**

The present invention relates to an antitumor agent containing a histone deacetylase inhibitor and a topoisomerase II inhibitor in combination, and an enhancer of antitumor effect of topoisomerase II inhibitor, which contains a histone deacetylase inhibitor as an active ingredient. The present invention provides an antitumor agent having superior antitumor activity, which is associated with a fewer side effects.



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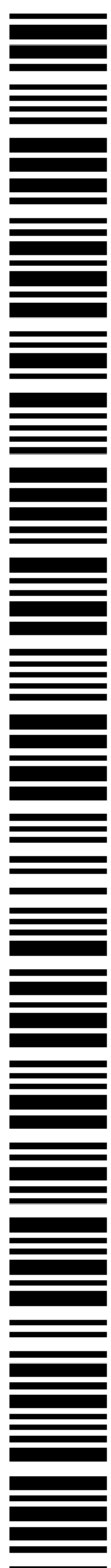
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(54) Title: ANTIMUTOR AGENT COMPRISING A HISTONE DEACETYLASE INHIBITOR AND A TOPOISOMERASE II INHIBITOR

(57) Abstract: The present invention relates to an antitumor agent containing a histone deacetylase inhibitor and a topoisomerase II inhibitor in combination, and an enhancer of antitumor effect of topoisomerase II inhibitor, which contains a histone deacetylase inhibitor as an active ingredient. The present invention provides an antitumor agent having superior antitumor activity, which is associated with a fewer side effects.



WO 2005/030239 A3

DESCRIPTION**ANTITUMOR AGENT****Technical Field**

The present invention relates to a pharmaceutical
5 composition comprising, in combination, a topoisomerase II
inhibitor and a pharmaceutical agent that remarkably enhances
an antitumor effect of the inhibitor, such as a histone
deacetylase inhibitor, and methods of using such topoisomerase
II inhibitors and histone deacetylase inhibitors.

10

Background Art

Generally, in chemotherapy of tumor, particularly
malignant tumor, an exclusive administration of an antitumor
agent rarely results in a desired antitumor effect. To enhance
the effect, a multiple drug therapy using, in combination, 2, 3
15 or more drugs having different action mechanisms, has been
employed in clinical situations. In this combination therapy,
antitumor agents having different action mechanisms are
combined 1) to decrease insensitive cell population, 2) to
prevent or delay appearance of drug-resistance, 3) to disperse
20 toxicity by the combination of pharmaceutical agents having
different toxicities, and the like, thereby decreasing side
effects and enhancing antitumor action. However, aimless
combination of antitumor agents having different action
mechanisms for combination therapy does not necessarily lead to
25 an enhanced antitumor effect, and therefore, a combination
effect afforded by the combination of antitumor agents
exhibiting higher antitumor activities has been studied.

It has been reported that a compound represented by the
formula (I)



(II)

However, there is no report as yet on the combined use of

a histone deacetylase inhibitor such as Compound A and the like, and a topoisomerase II inhibitor, and the effect afforded by the combined use.

Histone deacetylase is a metallo-deacetylating enzyme
5 coordinating Zn at an active center (M.S. Finnin et al., Nature, 401, 188-193 (1999)). This enzyme is considered to change affinity of various acetylated histones for DNA. The direct biological phenomenon brought thereby is a change in the chromatin structure. The minimum unit of the chromatin
10 structure is a nucleosome wherein 146 bp DNA is wound 1.8 times anticlockwise around a histone octamer (H2A, H2B, H3 and H4, each 2 molecules, core histone). The core histone stabilizes the nucleosome structure by interaction of the positive charge of the N-terminus of each histone protein with DNA.
15 Acetylation of histone is controlled by the equilibrium between an acetylation reaction involving histone acetyltransferase and a deacetylation reaction involving histone deacetylase. It is considered that the histone acetylation occurs at a lysine residue where the histone protein N-terminal is evolutionally
20 preserved well, due to which a core histone protein loses charges at the N-terminal, interaction with DNA is attenuated, and the structure of nucleosome becomes unstable. Accordingly, the histone deacetylation is considered to be the reverse thereof, namely, a shift toward stabilization of the nucleosome
25 structure. However, to what degree the acetylation changes the chromatin structure and how it relates to the transcriptional regulation etc. secondarily induced thereby are unclear in many aspects.

An object of the present invention is to provide an
30 antitumor agent causing reduced side-effects and a superior antitumor activity based on the combined use of a topoisomerase II inhibitor as an antitumor agent, and a pharmaceutical agent that strikingly enhances an antitumor effect of the inhibitor.

Disclosure of the Invention

The present inventors have conducted extensive studies, and as a result, have found that histone deacetylase inhibitors, particularly Compound A and FK228, remarkably enhance the antitumor effect of topoisomerase II inhibitors, and completed
5 the present invention. Accordingly, the present invention includes the following:

[1] An antitumor agent comprising a combination of a histone deacetylase inhibitor and a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

10 [2] The antitumor agent of [1] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and
15 pharmaceutically acceptable salts thereof.

[3] The antitumor agent of [2] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and
20 a pharmaceutically acceptable salt thereof.

[4] The antitumor agent of [3] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically
25 acceptable salts thereof.

[5] The antitumor agent of any of [1] to [4] above, which is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

30 [6] An antitumor effect enhancer of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, which comprises a histone deacetylase inhibitor as an active ingredient.

[7] The enhancer of [6] above, wherein the topoisomerase II

inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and
5 pharmaceutically acceptable salts thereof.

[8] The enhancer of [7] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a
10 pharmaceutically acceptable salt thereof.

[9] The enhancer of [8] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically
15 acceptable salts thereof.

[10] The enhancer of any of [6] to [9] above, which is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

20 [11] A method for treating a cancer, which comprises administering, to a mammal, a combination of an effective amount of a histone deacetylase inhibitor and an effective amount of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

25 [12] The method of [11] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and
30 pharmaceutically acceptable salts thereof.

[13] The method of [12] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a

pharmaceutically acceptable salt thereof.

[14] The method of [13] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin,
5 daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[15] The method of any of [11] to [14] above, wherein the cancer is lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder
10 cancer, leukemia, kidney cancer or prostate cancer.

[16] A method of enhancing an antitumor effect of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, which comprises administering an effective amount of a histone deacetylase
15 inhibitor to a mammal.

[17] The method of [16] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide,
20 mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

[18] The method of [17] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically
25 acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[19] The method of [18] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin,
30 daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[20] The method of any of [16] to [19] above, which enhances an antitumor effect against lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer,

chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

[21] Use of a histone deacetylase inhibitor and a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, for producing an antitumor agent.

[22] The use of [21] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

[23] The use of [22] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[24] The use of [23] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[25] The use of any of [21] to [24] above, wherein the antitumor agent is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

[26] Use of a histone deacetylase inhibitor for producing an antitumor effect enhancer of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

[27] The use of [26] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and

pharmaceutically acceptable salts thereof.

[28] The use of [27] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[29] The use of [28] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[30] The use of any of [26] to [29] above, wherein the antitumor effect enhancer is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

[31] A pharmaceutical composition comprising, as active ingredients, a histone deacetylase inhibitor and a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

[32] The pharmaceutical composition of [31] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

[33] The pharmaceutical composition of [32] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[34] The pharmaceutical composition of [33] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin,

epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[35] A commercial package comprising a combination drug containing a histone deacetylase inhibitor and a topoisomerase II inhibitor in combination, except amrubicin and a pharmaceutically acceptable salt thereof, and a written matter associated with the combination drug, the written matter stating that the combination drug can or should be used for an antitumor agent.

[36] The commercial package of [35] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

[37] The commercial package described in [36] above, wherein the histone deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[38] The commercial package of [37] above, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

[39] A commercial package comprising a preparation containing a histone deacetylase inhibitor, and a written matter associated with therewith, the written matter stating that the preparation can or should be used for enhancing the antitumor effect of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

[40] The commercial package of [39] above, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof,

except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

[41] The commercial package of [40] above, wherein the histone
5 deacetylase inhibitor is Compound A, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

[42] The commercial package of [41] above, wherein the
10 anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

Detailed Description of the Invention

15 The "histone deacetylase inhibitor" or (HDAC inhibitors) in the present invention is a compound that binds to an active site of histone deacetylase competitively with substrates, or a compound that binds to a different site from active site of histone deacetylase to change the enzyme activity of histone
20 deacetylase and/or decreases the enzymatic activity of histone deacetylase or otherwise inhibits enzyme activity. To be specific, the aforementioned Compound A, FK228, salts thereof and derivatives thereof (e.g., acetylated Compound A, thiol form with reduced S-S bond described in WO 02/06307, and
25 prodrugs thereof) can be mentioned. Analogs of FK228 are described in US Patent No. 6,403,555. In addition, Trichostatin A, sodium butyrate, suberoylanilide hydroxamic acid (SAHA), MS-275, cyclic hydroxamic-acid-containing peptide, Apicidin, Trapoxin and the like are the compounds reported to have a
30 histone deacetylase inhibitory activity. The inhibitors can be administered or used alone or in combination with one or more additional histone deacetylase inhibitors.

"Compound A" or "compound A", used interchangeably herein, includes its stereoisomers (e.g., FK 228) based on an

asymmetric carbon atom or a double bond, such as an optically active form, a geometric isomer, racemic mixtures and the like.

Moreover, polymorphic forms, solvates (e.g., inclusion compounds (e.g., hydrate etc.)) and anhydrous forms of the
5 compounds described herein, such as compound A, FK 228 and pharmaceutically acceptable salts thereof, are also encompassed in the scope of the present invention.

In the present specification, unless particularly specified, a simple reference to compound A includes any member
10 of the group of compounds regardless of stereoisomerism, and includes FK 228 or pharmaceutically acceptable salt thereof.

The compound A or a salt thereof are known and available substances. For example, FK 228, which is one of the stereoisomers of compound A, can be obtained by culturing a
15 strain belonging to the genus *Chromobacterium*, which is capable of producing FK 228, under aerobic conditions, and harvesting the substance from its culture broth. As the strain belonging to the genus *Chromobacterium*, which is capable of producing FK 228, for example, *Chromobacterium violaceum* WB968 (FERM BP-
20 1968) can be mentioned. More specifically, FK 228 can be obtained from a FK 228 producing strain as described in JP-B-7-64872 (corresponding to US Patent No. 4977138, which is incorporated herein by reference). FK 228 is preferably harvested from a strain belonging to the genus *Chromobacterium*,
25 which is capable of producing FK 228, because it can be obtained more easily. Synthetic or semi-synthetic FK 228 is also advantageous in that further purification step is not necessary or the number of steps can be reduced. Similarly, compounds A other than FK 228 can be also obtained by semi-
30 synthesis or total synthesis by conventionally known methods. To be more specific, it can be produced according to the method reported by Khan W. Li, et al. (*J. Am. Chem. Soc.*, Vol. 118, 7237-7238 (1996)).

The form of a pharmaceutically acceptable salt of

compound A, or other HDAC inhibitors, includes salts with a base or an acid addition salt such as salts with inorganic base (e.g., alkali metal salts such as sodium salt, potassium salt etc., alkaline earth metal salts such as calcium salt, 5 magnesium salt etc., ammonium salt), salts with an organic base (e.g., organic amine salts such as triethylamine salt, diisopropylethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt etc.), inorganic acid 10 addition salts (e.g., hydrochloride, hydrobromide, sulfate, phosphate etc.), organic carboxylic acid or sulfonic acid addition salts (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate etc.), salts with a basic or acidic amino acid 15 (e.g., arginine, aspartic acid, glutamic acid etc.) and the like.

The "topoisomerase II inhibitor" in the present invention is a compound that binds to an active site of topoisomerase II competitively with substrates, or a compound that binds to a 20 different site from active site of topoisomerase II to change the enzyme activity of topoisomerase II, and/or a compound that decreases the enzyme activity of topoisomerase II or otherwise inhibits enzyme activity. To be specific, anthracycline anticancer drugs, etoposide, mitoxantrone, sobuzoxane, 25 amsacrine, teniposide and the like, and pharmaceutically acceptable salts thereof and combinations thereof can be mentioned.

The anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, which is used as a topoisomerase II 30 inhibitor in the present invention, is not particularly limited. Generally, amrubicin or a pharmaceutically acceptable salt thereof is not included in the scope of the present invention, which embodiment is described and claimed in copending application No. 334344/2003 filed in Japan on the same date as

the patent application No. 334340/2003 filed in Japan forming the basis of this application. This copending application is incorporated herein by reference in its entirety. For example, aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin
5 (adriamycin), pirarubicin and the like, and pharmaceutically acceptable salts thereof can be mentioned, with preference given to doxorubicin, daunorubicin and pharmaceutically acceptable salts thereof. The anthracycline anticancer drug or a pharmaceutically acceptable salt thereof may be used alone or
10 in a mixture of two or more kinds thereof.

In the present specification, the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is intended to exclude amrubicin and a pharmaceutically acceptable salt thereof, unless otherwise indicated.

15 As pharmaceutically acceptable salts of anthracycline anticancer drug, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide and the like, salts with a base or an acid addition salt such as salts with inorganic base (e.g., alkali metal salts such as sodium salt, potassium salt etc., alkaline earth
20 metal salts such as calcium salt, magnesium salt etc., ammonium salt), salts with an organic base (e.g., organic amine salts such as triethylamine salt, diisopropylethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-
25 dibenzylethylenediamine salt etc.), inorganic acid addition salts (e.g., hydrochloride, hydrobromide, sulfate, phosphate etc.), organic carboxylic acid or sulfonic acid addition salts (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate
30 etc.), salts with a basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid etc.) and the like can be mentioned.

In the present invention, the histone deacetylase inhibitor such as Compound A and the like, markedly enhances

the antitumor activity of topoisomerase II inhibitor such as an anthracycline anticancer drug, a pharmaceutically acceptable salt thereof and the like. Thus, the antitumor agent and the antitumor effect enhancer of the present invention are useful
5 as therapeutic drugs for cancer diseases including blood cancer and solid cancer, which are more specifically lung cancer, malignant lymphoma (e.g., reticulosarcoma, lymphosarcoma, Hodgkin's disease and the like), cancer of digestive organ (e.g., gastric cancer, gallbladder·bile duct cancer, pancreatic
10 cancer, liver cancer, colon cancer, rectal cancer and the like), breast cancer, ovarian cancer, chondrosarcoma (e.g., osteosarcoma and the like), bladder cancer, leukemia (e.g., acute leukemia such as acute inversion of chronic myeloid leukemia and the like), kidney cancer, prostate cancer and the
15 like.

The antitumor agent of the present invention is a combination of a histone deacetylase inhibitor and a topoisomerase II inhibitor (i.e., combination drug), and may be any as long as a histone deacetylase inhibitor and a
20 topoisomerase II inhibitor can be combined when in use for the administration. Therefore, the antitumor agent of the present invention may be a single preparation obtained by simultaneously preparing a histone deacetylase inhibitor and a topoisomerase II inhibitor, or a combined preparation
25 comprising two or more preparations obtained by separately processing a histone deacetylase inhibitor and a topoisomerase II inhibitor, as long as a histone deacetylase inhibitor and a topoisomerase II inhibitor can be combined when in use for the administration.

30 The form of administration is not particularly limited, and, for example, (1) administration of a composition comprising a histone deacetylase inhibitor and a topoisomerase II inhibitor, i.e., a single preparation, (2) simultaneous administration of two kinds of preparations obtained by

separately processing the histone deacetylase inhibitor and the topoisomerase II inhibitor by the same administration route, (3) time staggered administration of two kinds of preparations obtained by separately processing a histone deacetylase inhibitor and a topoisomerase II inhibitor by the same administration route (e.g., administration in the order of a histone deacetylase inhibitor and a topoisomerase II inhibitor, or administration in reverse order), (4) simultaneous administration of two kinds of preparations obtained by separately processing a histone deacetylase inhibitor and a topoisomerase II inhibitor by different administration routes, and (5) time staggered administration of two kinds of preparations obtained by separately preparing a histone deacetylase inhibitor and a topoisomerase II inhibitor by different administration routes (e.g., administration in the order of a histone deacetylase inhibitor and a topoisomerase II inhibitor, or administration in reverse order) and the like can be mentioned.

In the case of the time staggered administration, moreover, it is preferred that a histone deacetylase inhibitor and a topoisomerase II inhibitor be co-present in the body for a time period necessary for the histone deacetylase inhibitor to reinforce the antitumor effect of the topoisomerase II inhibitor. As such, it is preferred that the second inhibitor is administered before the first inhibitor is cleared from the patient.

The enhancer of the present invention comprises a histone deacetylase inhibitor, and may be any as long as a histone deacetylase inhibitor and a topoisomerase II inhibitor can be combined when in use for the administration. Therefore, as long as a histone deacetylase inhibitor is contained, the enhancer of the present invention may contain a topoisomerase II inhibitor in a single preparation. When it does not contain a topoisomerase II inhibitor, a topoisomerase II inhibitor can

be administered separately as a combination drug.

The form of administration is not particularly limited, and, for example, (1) administration of an enhancer of the present invention, comprising a histone deacetylase inhibitor
5 and a topoisomerase II inhibitor in a single preparation, (2) simultaneous administration of the enhancer of the present invention and a topoisomerase II inhibitor by the same administration route, (3) time staggered administration of the enhancer of the present invention and a topoisomerase II
10 inhibitor by the same administration route (e.g., administration in the order of a topoisomerase II inhibitor and the enhancer of the present invention, or administration in reverse order), (4) simultaneous administration of the enhancer of the present invention and a topoisomerase II inhibitor by
15 different administration routes, and (5) time staggered administration of the enhancer of the present invention and a topoisomerase II inhibitor by different administration routes (e.g., administration in the order of a topoisomerase II inhibitor and the enhancer of the present invention, or
20 administration in reverse order) and the like can be mentioned.

In addition, in the case of time staggered administration, both ingredients need to be co-present in the body as in the case of the above-mentioned antitumor agent.

In the present invention, the ratio of combination of the
25 histone deacetylase inhibitor and the topoisomerase II inhibitor is generally in the range of 1 : 100 to 100 : 1, preferably in the range of 1 : 10 to 10 : 1, in a weight ratio, whether they are prepared into a single preparation or separate preparations.

30 To enhance the antitumor effect of the present invention, administration along with ATRA (all-trans-retinoic acid) and other antitumor agents is also possible (e.g., administration as a single preparation, or simultaneous or sequential administration as separate preparations). In addition, the

invention includes administering additional active agents that can treat or prevent side effects of one or more of the antitumor agents being administered.

The antitumor agent of the present invention can be used
5 in the form of a pharmaceutical preparation such as a solid, semisolid or liquid preparation (tablet, pellet, troche, capsule, suppository, cream, ointment, aerosol, powder, liquid, emulsion, suspension, syrup, injection etc.) containing a histone deacetylase inhibitor and/or topoisomerase II inhibitor
10 as an active ingredient, which is suitable for transrectal, intranasal, pulmonary, vaginal, external (topical), oral or parenteral (including subcutaneous, implantation, intravenous and intramuscular) administration.

The antitumor effect enhancer of the present invention
15 can be used in the form of a pharmaceutical preparation such as a solid, semisolid or liquid preparation (tablet, pellet, troche, capsule, suppository, cream, ointment, aerosol, powder, liquid, emulsion, suspension, syrup, injection etc.) containing a histone deacetylase inhibitor as an active ingredient, which
20 is suitable for transrectal, intranasal, pulmonary, vaginal, external (topical), oral or parenteral (including subcutaneous, implantation, intravenous and intramuscular) administration.

The antitumor agent and antitumor effect enhancer of the present invention can be also produced by conventional methods
25 using various organic or inorganic carriers conventionally used for forming pharmaceutical preparations, such as excipients (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, talc, calcium phosphate, calcium carbonate etc.), condensation agents (e.g., cellulose, methyl cellulose,
30 hydroxypropyl cellulose, polypropylpyrrolidone, gelatin, gum arabic, polyethylene glycol, sucrose, starch etc.), disintegrants (e.g., starch, carboxymethyl cellulose, carboxymethyl cellulose calcium, hydroxypropyl starch, sodium starch glycolate, sodium hydrogen carbonate, calcium phosphate,

calcium citrate etc.), lubricants (e.g., magnesium stearate, aerosil, talc, sodium lauryl sulfate etc.), corrigents (e.g., citric acid, menthol, glycine, orange powder etc.), preservatives (e.g., sodium benzoate, sodium hydrogen sulfite, methylparaben, propylparaben etc.), stabilizers (citric acid, sodium citrate, acetic acid etc.), suspensions (e.g., methyl cellulose, polyvinyl pyrrolidone, aluminum stearate etc.), dispersants (e.g., hydroxypropylmethyl cellulose etc.), diluents (e.g., water etc.), wax base materials (e.g., cacao butter, polyethylene glycol, white petrolatum etc.) and the like.

The antitumor agent and the antitumor effect enhancer of the present invention can be administered, without any particular limitation, in the form of the above-mentioned conventional pharmaceutical preparations, to mammals inclusive of human. Particularly, they are preferably administered intravenously, intramuscularly or orally.

The dose in the present invention can be set to a lower level, as compared to an exclusive administration of a histone deacetylase inhibitor or a topoisomerase II inhibitor.

For example, the dose is selected appropriately depending on various factors such as the body weight and/or age of patients, and/or the degree of the symptom and an administration route. For example, when Compound A was added as a histone deacetylase inhibitor, and an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof was added as a topoisomerase II inhibitor, the dose of a combination of Compound A and an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof for intravenous administration is generally in the range of 1 to 1000 mg/day/m² human body surface area, preferably in the range of 5 to 100 mg/day/m² human body surface area, and more preferably 10 to 60 mg/day/m² human body surface area by continuous drip infusion administration. In this case, the dose of Compound A is 0.1 to

WO 2005/030239

PCT/JP2004/014451

100 mg/day/m² human body surface area, preferably 1 to 50
mg/day/m² human body surface area, and more preferably 5 to 30
mg/day/m² human body surface area, wherein the dose of the
anthracycline anticancer drug or a pharmaceutically acceptable
5 salt thereof to be administered is an amount obtained by
subtracting the dose of Compound A from the combined dose of
the above-mentioned Compound A and the anthracycline anticancer
drug or the pharmaceutically acceptable salt thereof.

The present invention also provides a commercial package
10 comprising a combination drug containing a histone deacetylase
inhibitor and a topoisomerase II inhibitor in combination,
except amrubicin and a pharmaceutically acceptable salt thereof,
and a written matter associated with the combination drug, the
written matter stating that the combination drug can or should
15 be used for an antitumor agent; and a commercial package
comprising a preparation containing a histone deacetylase
inhibitor, and a written matter associated with the preparation,
the written matter stating that the preparation can or should
be used for enhancing the antitumor effect of a topoisomerase
20 II inhibitor, except amrubicin and a pharmaceutically
acceptable salt thereof.

Examples

To demonstrate the usefulness of the present invention,
the results of a pharmacological test are shown in the
25 following.

Experimental Method 1

For an animal, 6 to 12-week-old female CDF₁ mice (body
weight: 17.2 to 24.7 g) were used at 5 to 12 mice per group.
Furthermore, female DBA/2 mice were used for the subculture of
30 tumor cells. For tumor, L-1210 mouse leukemia cells
(hereinafter to be referred to as L-1210) were used. L-1210
cells subcultured intraperitoneally in DBA/2 mice were
harvested and washed twice with a Hanks' solution. Dead cells
were stained with trypan blue, viable cells were counted, and

then the cells were suspended in a Hanks' solution, whereby the cells were adjusted to a predetermined number of cells. For the experiment, L-1210 cells (1×10^5 cells) were implanted intraperitoneally into the CDF₁ mice.

5 The test compound was administered intraperitoneally into the mice once a day for 4 days, starting from the day after implantation of the tumor cells (dose: diluted test compound is administered at 10 ml/kg). In combined use experiment of the test compound, FK228 and the test compound were administered
10 sequentially.

All the antitumor effects were evaluated using prolonged life of mice as an index.

For the survival period of the mice, the survival was observed over 30 days or 60 days after tumor implantation, a
15 Median Survival Time (hereinafter to be referred to as MST) was determined and the life prolonging rate was calculated from the following formula:

$$20 \quad \text{Life prolonging rate} = \frac{\text{MST of test compound administration group}}{\text{MST of control group}} \times 100$$

[T/C (%)]

The combination effect was determined by calculating a
25 Combination Index (C.I.) from the following formula, wherein C.I. > 1 was evaluated to be synergistic effect; C.I. = 1 was evaluated to be addition effect; and C.I. < 1 was evaluated to be no combination effect.

$$30 \quad \text{C.I.} = \frac{\text{T/C [\%] of FK228 and the test compound combined use group} - 100}{\left[\frac{\text{T/C [\%] of FK228 alone group} - 100}{\text{group} - 100} \right] + \left[\frac{\text{T/C [\%] of test compound alone group} - 100}{\text{group} - 100} \right]}$$

35 Test Compound 1

Adriamycin was dissolved in distilled water, and diluted

with physiological saline. Other test compounds were dissolved in physiological saline, and then diluted with physiological saline.

5 Test Results 1

The results are shown in Table 1.

Table 1

Combination effect by combined use 1 [6 mice per group (12 mice per group for control group)]

Test compound	Dose (mg/mg)	T/C (%)	C.I.
FK228 + adriamycin	0.14 + 0	100	-
	0 + 1	133	-
	0.14 + 1	178	2.36

The combined use of FK228 and adriamycin revealed a C.I. of 2.36, which was evaluated to suggest a synergistic effect. In addition, 2 mice out of 6 mice survived for 30 days.

As is clear from the results, the combined use of 0.14 mg/kg of FK228 ineffective by exclusive use and 1 mg/kg of adriamycin synergistically enhanced the antitumor effect thereof. Thus, FK228 is extremely useful as an antitumor effect enhancer.

Formulation Example 1

FK228	20 mg
<u>physiological saline</u>	<u>4 ml</u>

FK228 (20 mg) is dissolved in and diluted with 4 ml of physiological saline to obtain a preparation for injection.

25 Formulation Example 2

FK228	20 mg
doxorubicin hydrochloride	10 mg
<u>physiological saline</u>	<u>4 ml</u>

FK228 (20 mg) and doxorubicin hydrochloride (10 mg) are dissolved in and diluted with 4 ml of physiological saline to obtain a preparation for injection.

5

Industrial Applicability

A histone deacetylase inhibitor (particularly Compound A) remarkably enhances the antitumor effect of a topoisomerase II inhibitor.

Therefore, the antitumor agent of the present invention
10 comprising a histone deacetylase inhibitor and a topoisomerase II inhibitor in combination provides higher cancer treatment effects with a lower dose as compared to an exclusive administration of a histone deacetylase inhibitor or a topoisomerase II inhibitor, and moreover, can decrease the side
15 effects to a lower level.

This application is based on patent application Nos. 334340/2003 and 344315/2003 filed in Japan, the contents of which are hereby incorporated by reference.

20

While this invention has been shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompasses by the appended
25 claims.

All patents, patent publications and other publications identified or referenced herein are incorporated by reference in their entirety.

SEQUENCE LISTING

<110> Fujisawa Pharmaceutical Co., Ltd.

<120> Antitumor agent

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<150> JP 2003-334340

<151> 2003-09-25

<150> JP 2003-344315

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<223> Xaa is an amino acid represented by the formula
 $\text{NH}_2\text{C}(\text{CHCH}_3)\text{COOH}$.

<220>

<221> SITE

<222> (1), (2), (4)

<223> In the formula $\text{COOHCH}_2\text{CH}(\text{CHCHC}_2\text{H}_4\text{SH})\text{OH}$, the carboxylic group is bonded with the amino group of the first amino acid Val, the hydroxyl group is bonded with the carboxylic group of the fourth amino acid Val, and the SH group is bonded with the SH group of the second amino acid Cys via a disulfide bond.

<400> 1

Val Cys Xaa Val

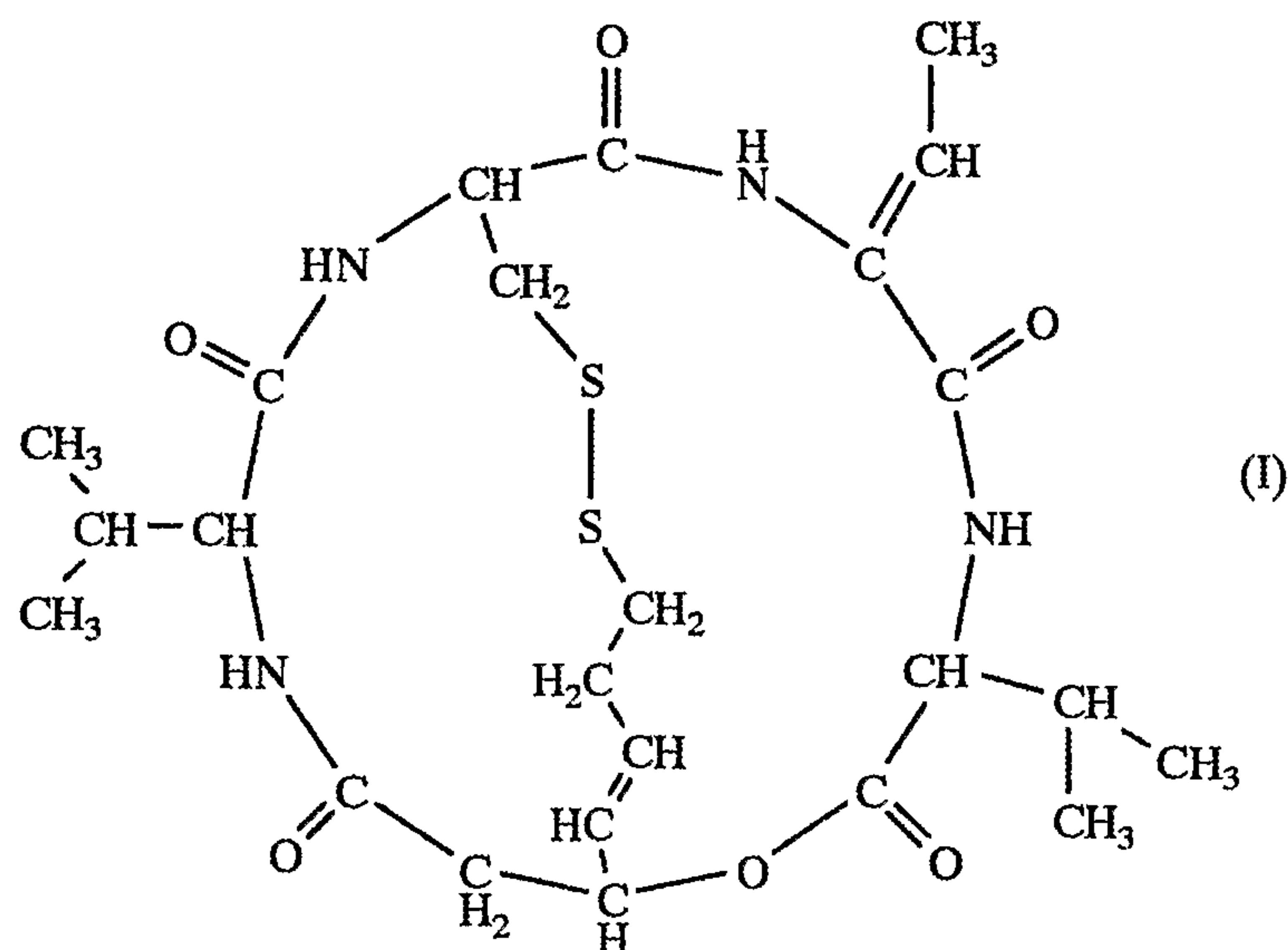
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CLAIMS

1. An antitumor agent comprising a combination of a histone
deacetylase inhibitor and a topoisomerase II inhibitor, except
5 amrubicin and a pharmaceutically acceptable salt thereof.

2. The antitumor agent of claim 1, wherein the topoisomerase II
inhibitor is at least one of an anthracycline anticancer drug
or a pharmaceutically acceptable salt thereof, except amrubicin
10 and a pharmaceutically acceptable salt thereof, etoposide,
mitoxantrone, sobuzoxane, amsacrine, teniposide, and
pharmaceutically acceptable salts thereof.

3. The antitumor agent of claim 2, wherein the histone
15 deacetylase inhibitor is a compound represented by the formula
(I)



or a pharmaceutically acceptable salt thereof, and the
topoisomerase II inhibitor is an anthracycline anticancer drug
or a pharmaceutically acceptable salt thereof, except amrubicin
20 and a pharmaceutically acceptable salt thereof.

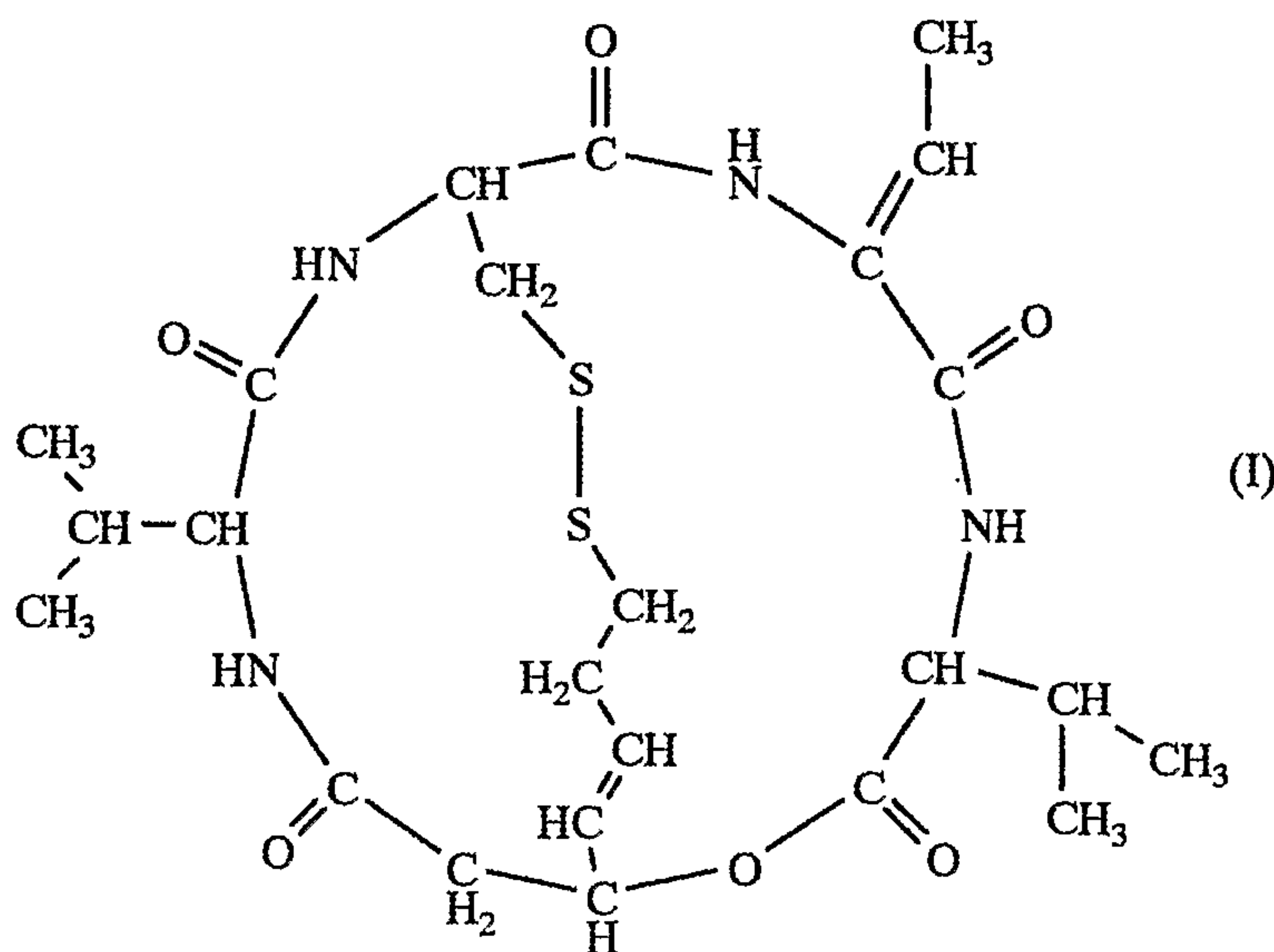
4. The antitumor agent of claim 3, wherein the anthracycline
anticancer drug or a pharmaceutically acceptable salt thereof

WO 2005/030239

PCT/JP2004/014451

is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

- 5 5. The antitumor agent of any of claims 1 to 4, which is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.
- 10 6. An antitumor effect enhancer of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, which comprises a histone deacetylase inhibitor as an active ingredient.
- 15 7. The enhancer of claim 6, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and
- 20 pharmaceutically acceptable salts thereof.
8. The enhancer of claim 7, wherein the histone deacetylase inhibitor is a compound represented by the formula (I)



or a pharmaceutically acceptable salt thereof, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

5

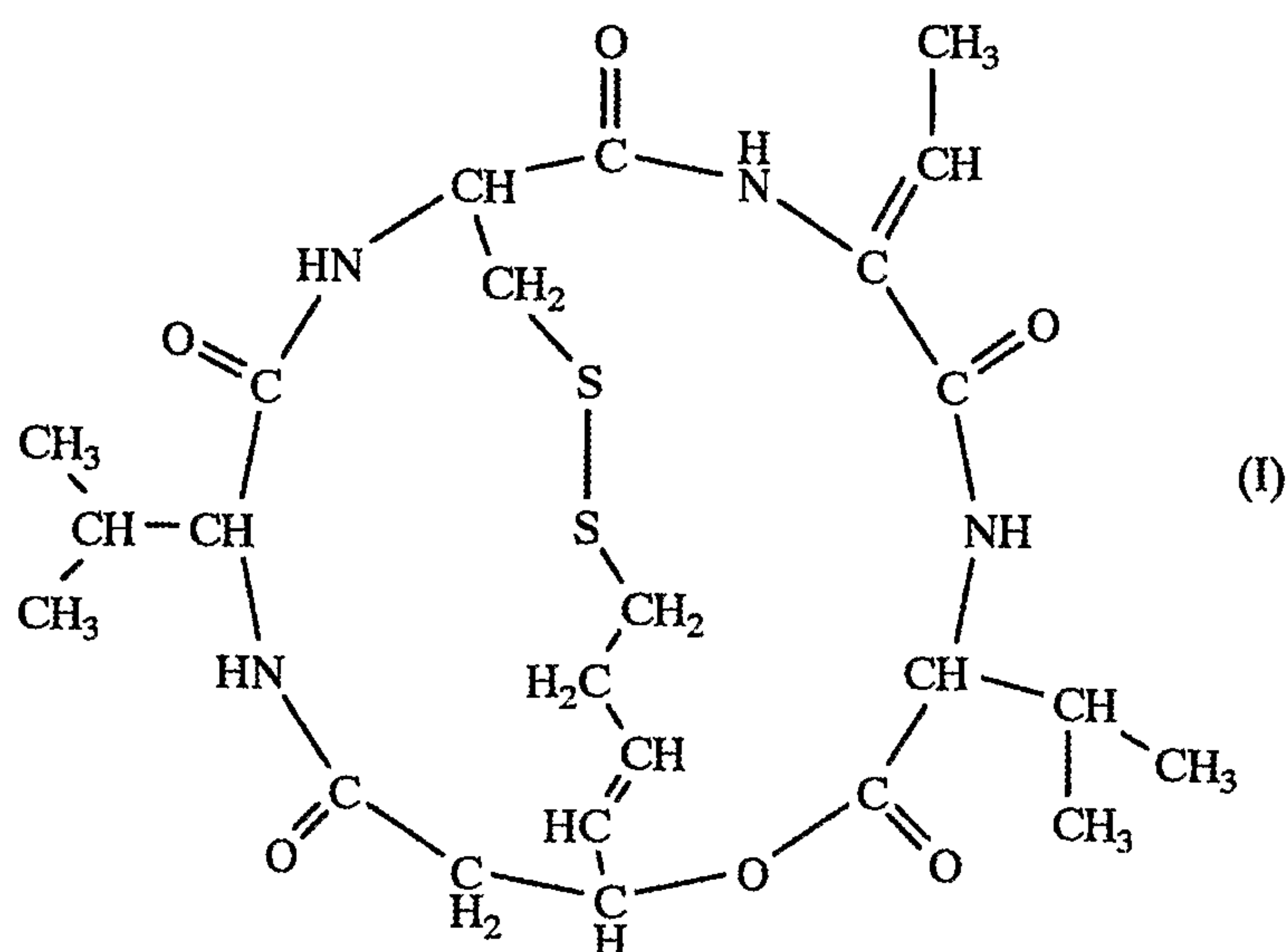
9. The enhancer of claim 8, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically
10 acceptable salts thereof.

10. The enhancer of any of claims 6 to 9, which is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer,
15 leukemia, kidney cancer or prostate cancer.

11. A method for treating a cancer, which comprises administering, to a mammal, a combination of a histone deacetylase inhibitor and a topoisomerase II inhibitor, except
20 amrubicin and a pharmaceutically acceptable salt thereof.

12. The method of claim 11, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin
25 and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

13. The method of claim 12, wherein the histone deacetylase
30 inhibitor is a compound represented by the formula (I)



or a pharmaceutically acceptable salt thereof, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

5

14. The method of claim 13, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

10

15. The method of any of claims 11 to 14, wherein the cancer is lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

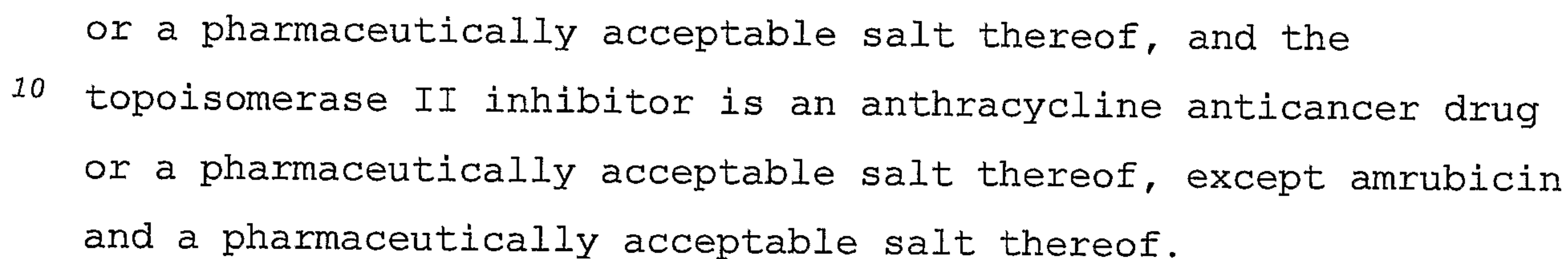
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16. A method of enhancing an antitumor effect of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, which comprises administering an effective amount of a histone deacetylase inhibitor to a mammal.

20

17. The method of claim 16, wherein the topoisomerase II

18. The method of claim 17, wherein the histone deacetylase inhibitor is a compound represented by the formula (I)



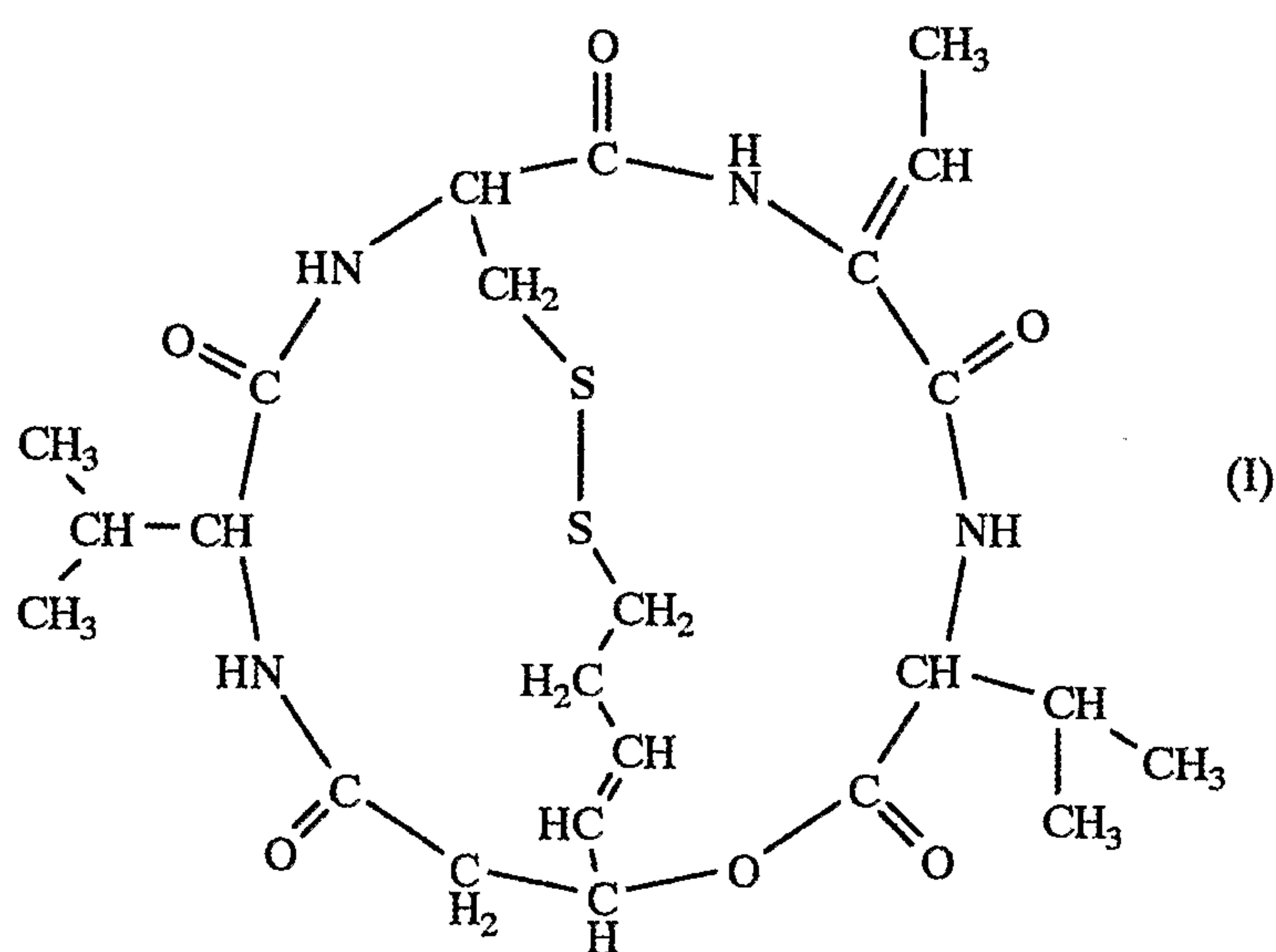
20 20. The method of any of claims 16 to 19, which enhances an antitumor effect against lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or

prostate cancer.

21. Use of a histone deacetylase inhibitor and a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof, for producing an antitumor agent.

22. The use of claim 21, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof, etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

23. The use of claim 22, wherein the histone deacetylase inhibitor is a compound represented by the formula (I)

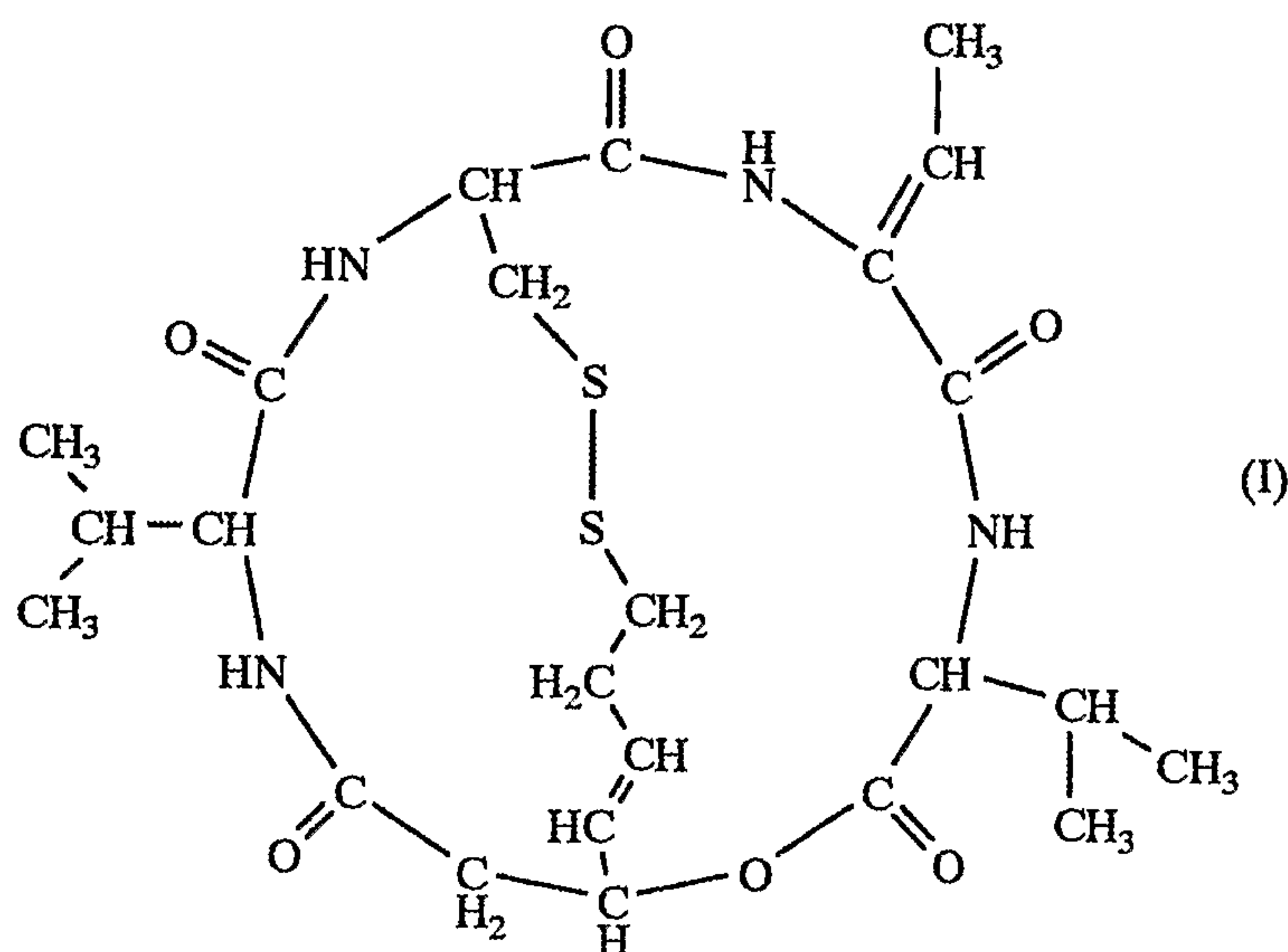


or a pharmaceutically acceptable salt thereof, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

24. The use of claim 23, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin,

26. Use of a histone deacetylase inhibitor for producing an
antitumor effect enhancer of a topoisomerase II inhibitor,
except amrubicin and a pharmaceutically acceptable salt thereof.

20 28. The use of claim 27, wherein the histone deacetylase inhibitor is a compound represented by the formula (I)



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WO 2005/030239

PCT/JP2004/014451

or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

29. The use of claim 28, wherein the anthracycline anticancer
5 drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

10 30. The use of any of claims 26 to 29, wherein the antitumor effect enhancer is used for lung cancer, malignant lymphoma, cancer of digestive organ, breast cancer, ovarian cancer, chondrosarcoma, bladder cancer, leukemia, kidney cancer or prostate cancer.

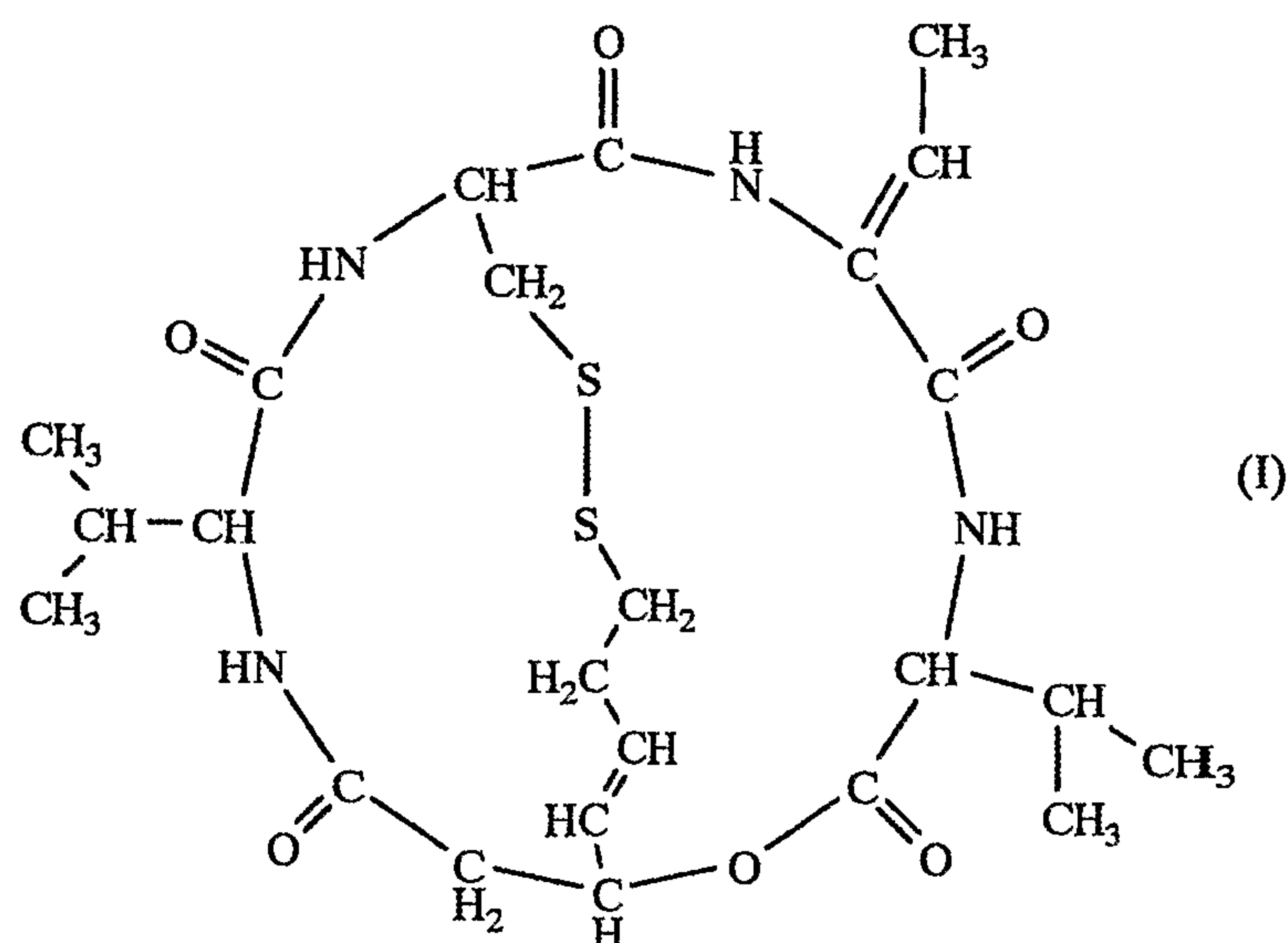
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31. A pharmaceutical composition comprising, as active ingredients, a histone deacetylase inhibitor and a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

20

32. The pharmaceutical composition of claim 31, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof,
25 etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

33. The pharmaceutical composition of claim 32, wherein the histone deacetylase inhibitor is a compound represented by the
30 formula (I)



or a pharmaceutically acceptable salt thereof, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof.

5

34. The pharmaceutical composition of claim 33, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

10

35. A commercial package comprising a combination drug containing a histone deacetylase inhibitor and a topoisomerase II inhibitor in combination, except amrubicin and a pharmaceutically acceptable salt thereof, and a written matter associated with the combination drug, the written matter stating that the combination drug can or should be used for an antitumor agent.

15

36. The commercial package of claim 35, wherein the topoisomerase II inhibitor is at least one of an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin and a pharmaceutically acceptable salt thereof,

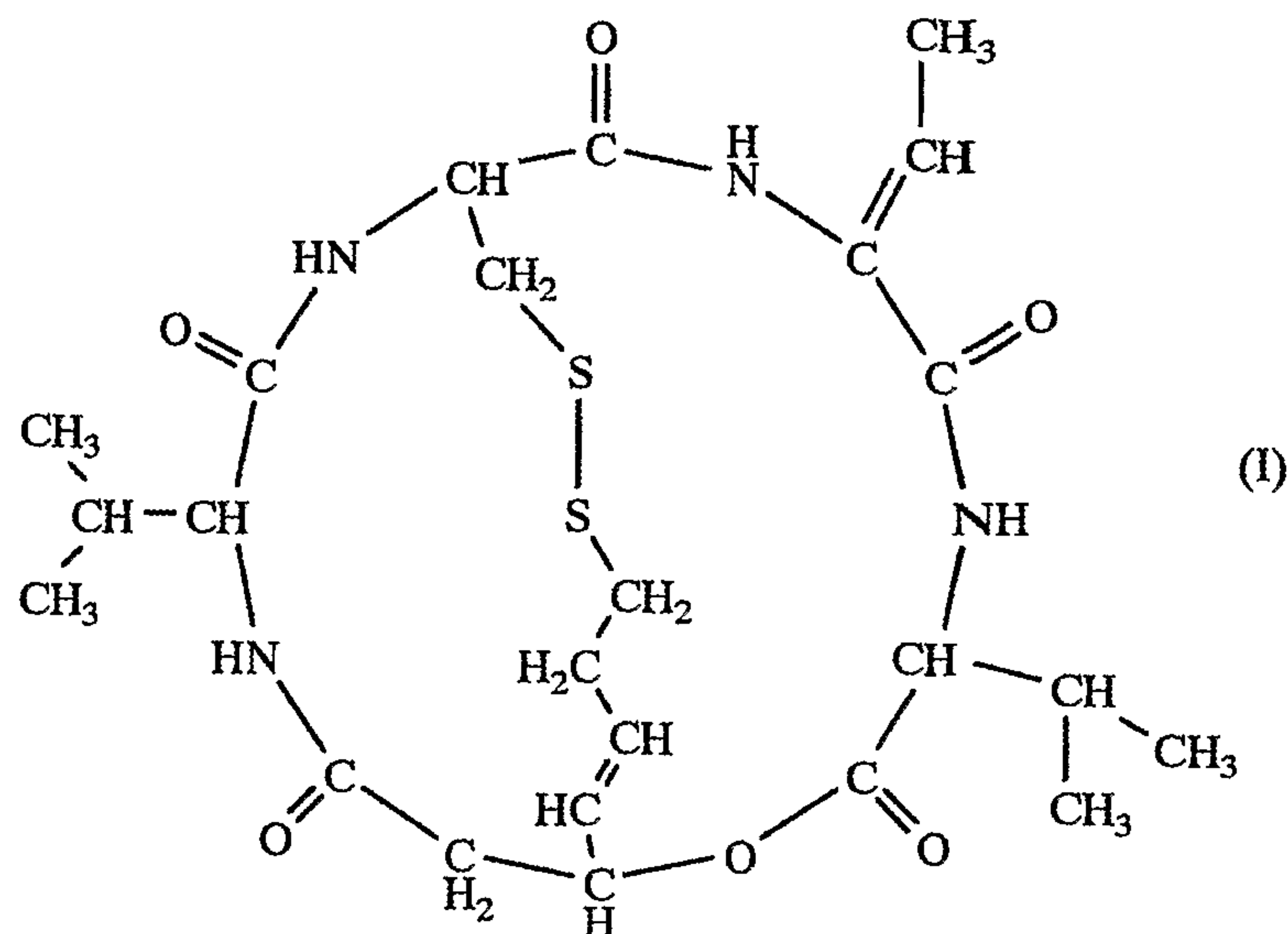
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WO 2005/030239

PCT/JP2004/014451

etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and pharmaceutically acceptable salts thereof.

37. The commercial package described in claim 36, wherein the
5 histone deacetylase inhibitor is a compound represented by the
formula (I)



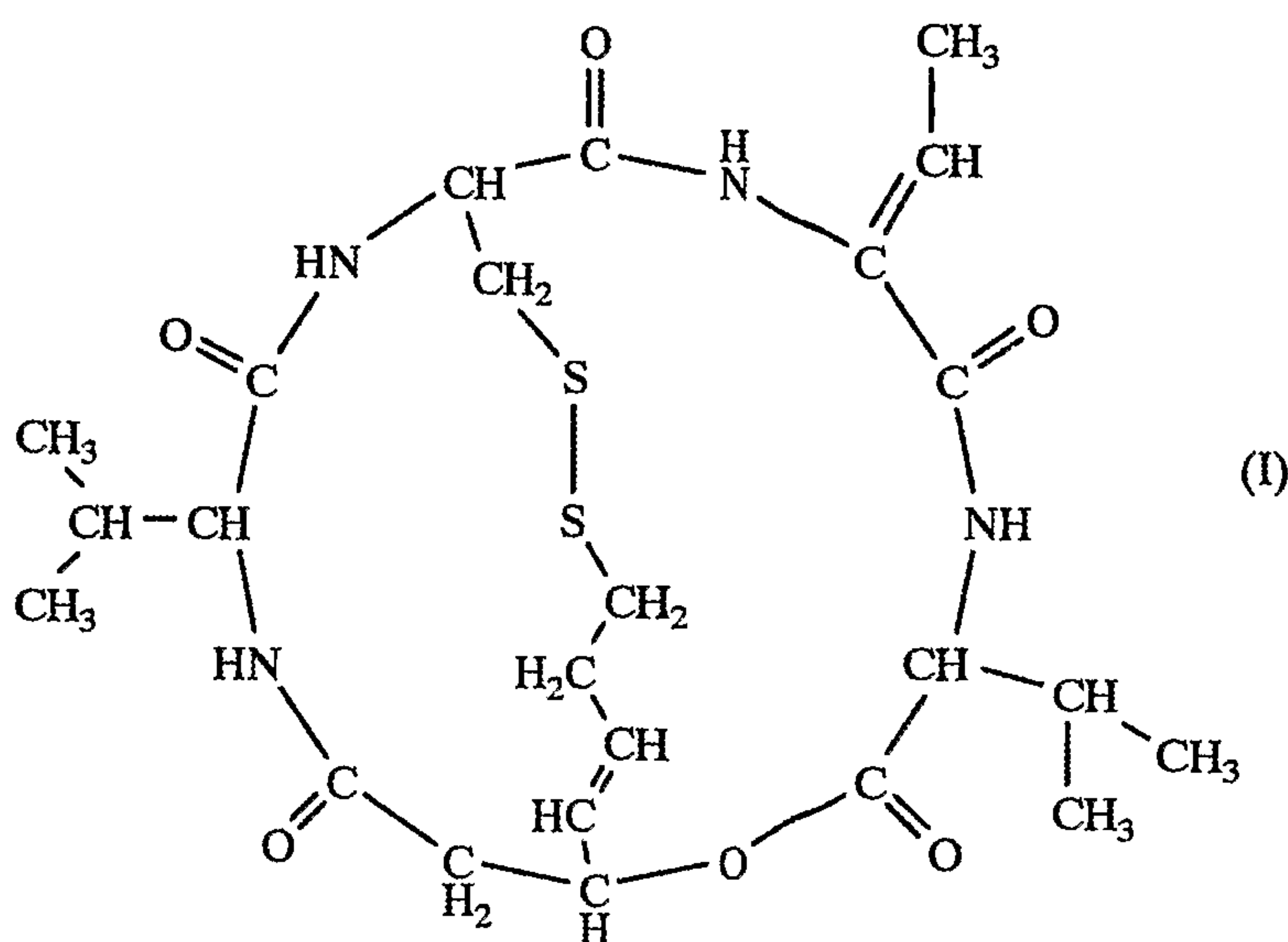
or a pharmaceutically acceptable salt thereof, and the topoisomerase II inhibitor is an anthracycline anticancer drug or a pharmaceutically acceptable salt thereof, except amrubicin
10 and a pharmaceutically acceptable salt thereof.

38. The commercial package of claim 37, wherein the anthracycline anticancer drug or a pharmaceutically acceptable salt thereof is at least one of aclarubicin, idarubicin, epirubicin, daunorubicin, doxorubicin, pirarubicin, and pharmaceutically acceptable salts thereof.

39. A commercial package comprising a preparation containing a histone deacetylase inhibitor, and a written matter associated with therewith, the written matter stating that the preparation can or should be used for enhancing the antitumor effect of a topoisomerase II inhibitor, except amrubicin and a pharmaceutically acceptable salt thereof.

40. The commercial package of claim 39, wherein the
 topoisomerase II inhibitor is at least one of an anthracycline
 anticancer drug or a pharmaceutically acceptable salt thereof,
 5 except amrubicin and a pharmaceutically acceptable salt thereof,
 etoposide, mitoxantrone, sobuzoxane, amsacrine, teniposide, and
 pharmaceutically acceptable salts thereof.

41. The commercial package of claim 40, wherein the histone
 10 deacetylase inhibitor is a compound represented by the formula
 (I)



or a pharmaceutically acceptable salt thereof, and the
 topoisomerase II inhibitor is an anthracycline anticancer drug
 or a pharmaceutically acceptable salt thereof, except amrubicin
 15 and a pharmaceutically acceptable salt thereof.

42. The commercial package of claim 41, wherein the
 anthracycline anticancer drug or a pharmaceutically acceptable
 salt thereof is at least one of aclarubicin, idarubicin,
 20 epirubicin, daunorubicin, doxorubicin, pirarubicin, and
 pharmaceutically acceptable salts thereof.