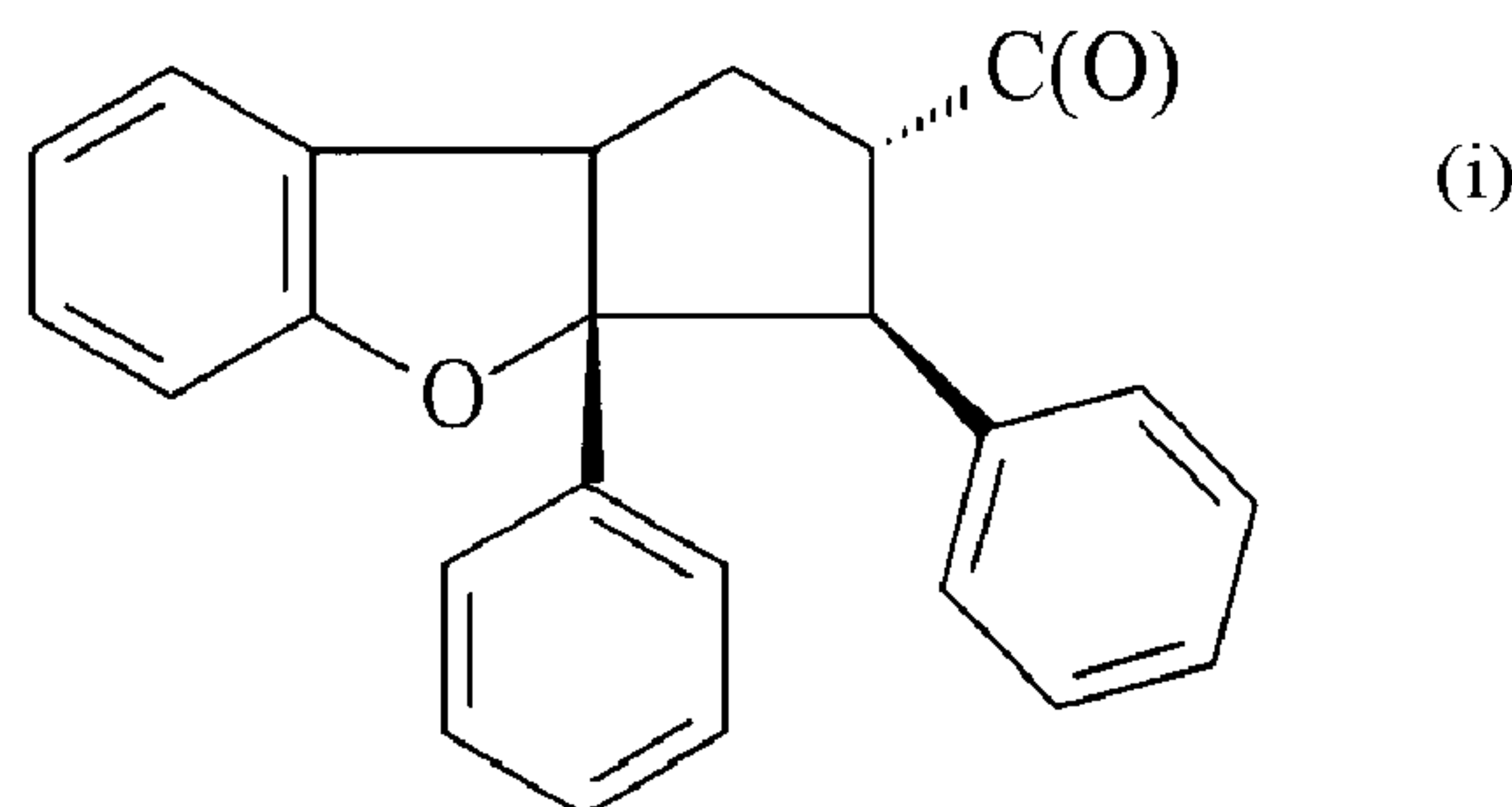
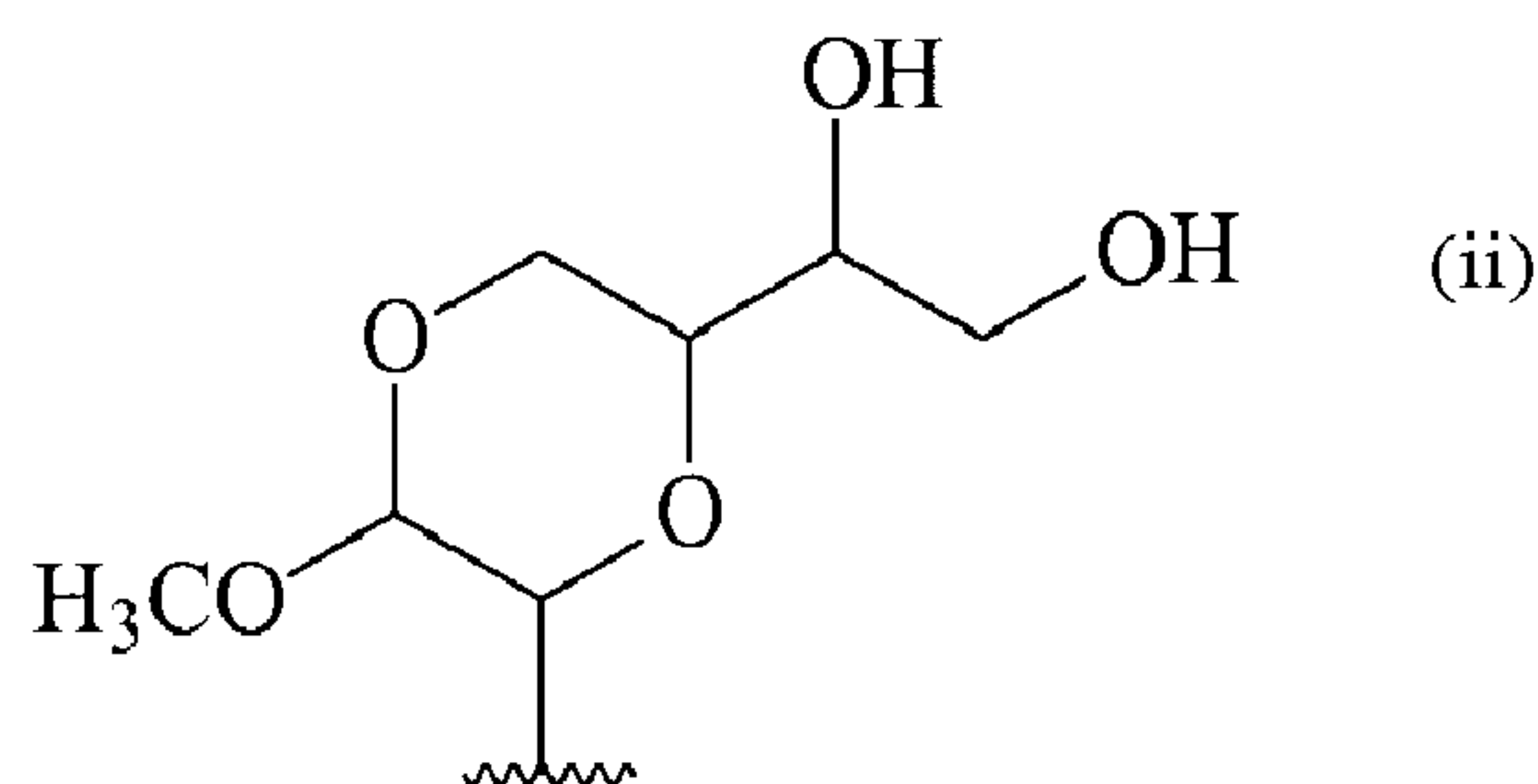




(86) Date de dépôt PCT/PCT Filing Date: 2001/07/05
(87) Date publication PCT/PCT Publication Date: 2002/01/10
(45) Date de délivrance/Issue Date: 2011/02/22
(85) Entrée phase nationale/National Entry: 2002/12/17
(86) N° demande PCT/PCT Application No.: AU 2001/000810
(87) N° publication PCT/PCT Publication No.: 2002/002566
(30) Priorité/Priority: 2000/07/05 (AU PQ 8665)

(51) Cl.Int./Int.Cl. *C07D 493/00* (2006.01),
C07D 307/93 (2006.01), *C07D 319/12* (2006.01),
C07D 407/12 (2006.01)
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(54) Titre : CYCLOPENTABENZOFURANES ET LEURS UTILISATIONS
(54) Title: CYCLOPENTABENZOFURAN COMPOUNDS AND USES THEREOF



(57) **Abrégé/Abstract:**

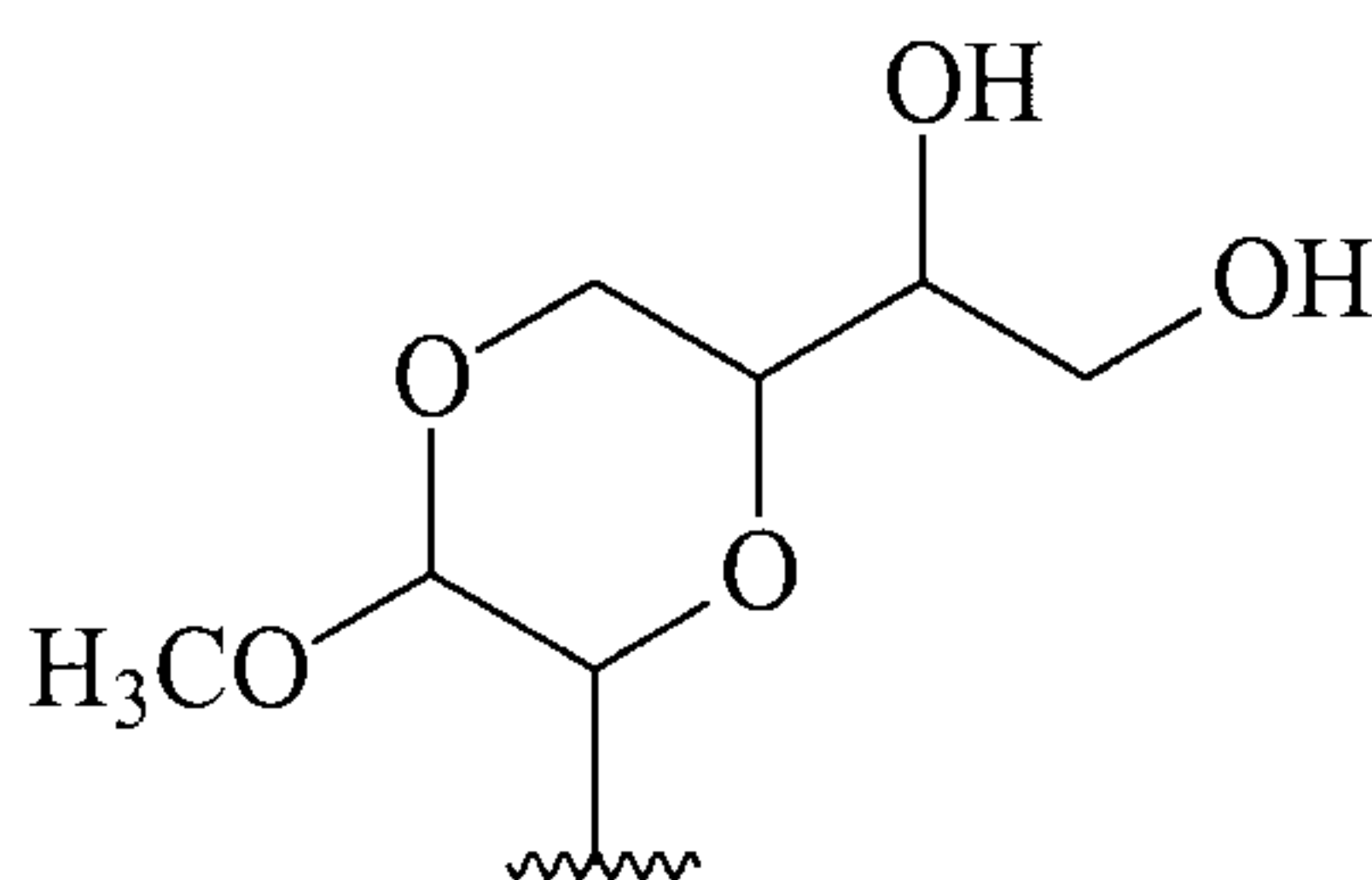
The invention provides compounds having a cyclopentabenzofuran core comprising a group of formula (ii): (see formula ii) attached via an oxygen atom to a cyclopentabenzofuran core of formula (i): (see formula i); compositions comprising said compounds and therapeutic uses of the compounds and compositions for treating a cancer or a cancerous condition or a disease state or condition associated with cellular hyperproliferation.

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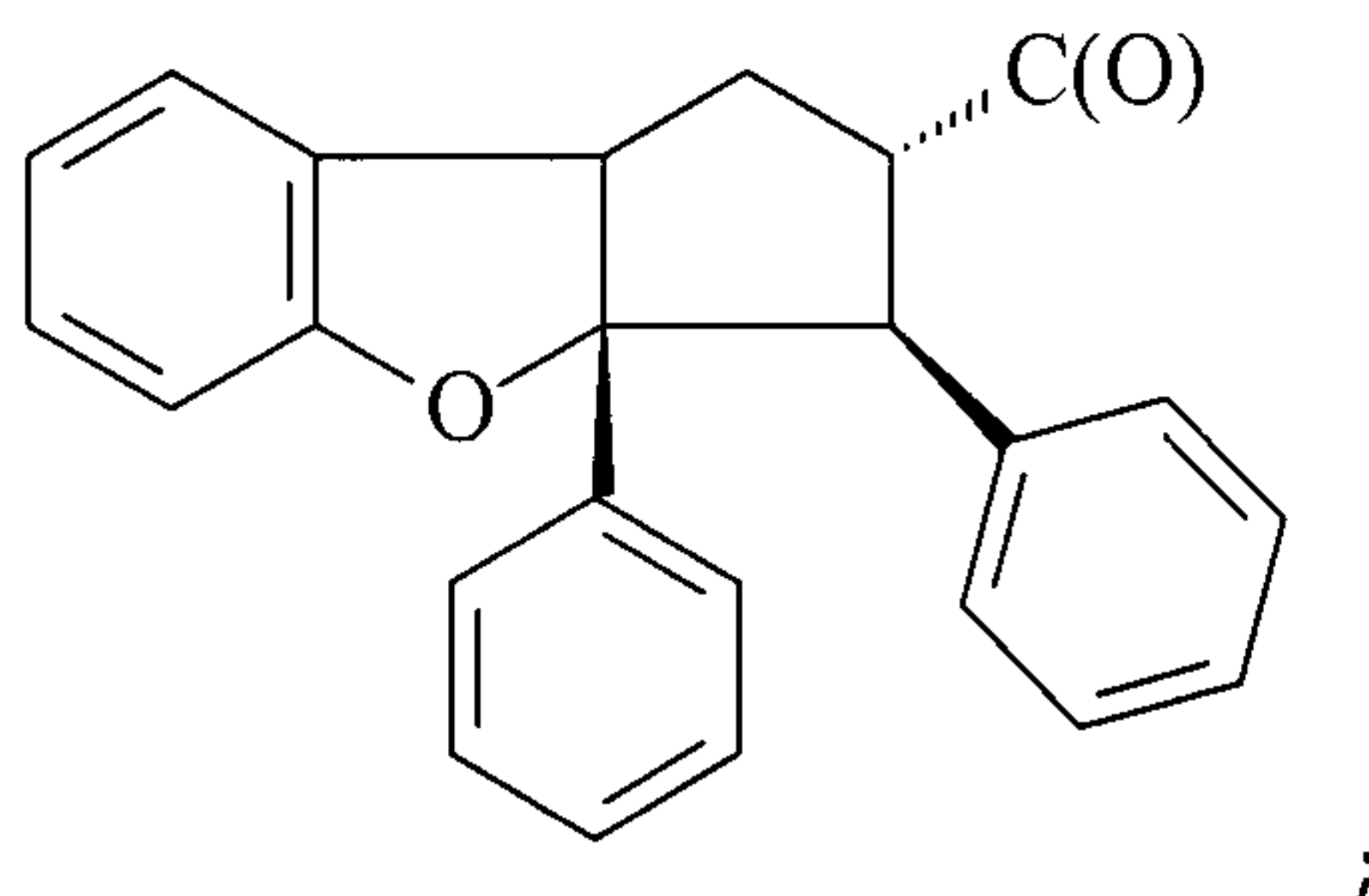
CYCLOPENTABENZOFURAN COMPOUNDS AND USES THEREOF

Abstract

The invention provides compounds having a
cyclopentabenzofuran core comprising a group of
5 formula (ii):



attached via an oxygen atom to a cyclopentabenzofuran core
of formula (i):



10 compositions comprising said compounds and therapeutic uses
of the compounds and compositions for treating a cancer or a
cancerous condition or a disease state or condition
associated with cellular hyperproliferation.

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CYCLOPENTABENZOFURAN COMPOUNDS AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates generally to compounds having a cyclopentabenzofuran core. More particularly, the present invention relates to cyclopentabenzofuran compounds where the cyclopentabenzofuran core is substituted by a dioxanyloxy moiety. The invention also relates to the use of these compounds in therapy and compositions comprising said compounds.

BACKGROUND

Aglaia is a large genus of the family Meliaceae comprising over 100 (mostly woody) species in Indo-Malaysia and the Western Pacific region. Uses include treatment of fever, fractures, parturition and inflammation. Extracts are also used as bactericides, insecticides, in perfumery, as an astringent, tonic, a refrigerant (*Dr Duke's Phytochemical and Ethnobotanical Databases*) and for the treatment of abdominal tumours (Pannel, et al, 1992, *Kew Bull.*, (16) 273-283).

More recently, a number of 1H-cyclopenta[b]benzofuran lignans have been isolated from *Aglaia* species (see for example, WO97/08161; JP 97171356; Ohse, et al, *J Nat Prod*, 1996, 59(7):650-52; Lee et al, *Chem. Biol. Interact.*, 1998, 115(3):215-28; Wu et al, *J. Nat. Prod.*, 1997, 60(6):606-08; Bohnenstengel et al, *Z. Naturforsch.*, 1999, 54c (12):55-60 and Bohnenstengel et al, *Z. Naturforsch.*, 1999, 54c (12):1075-83, Xu, Y. J., et al, 2000, *J. Nat. Prod.*, 63, 4732-76). A

number of these compounds have also been noted for their insecticidal activity (Janprasert, et al, 1993, *Phytochemistry*, 32 (1), 67-69; Ishibashi et al, 1993, *Phytochemistry*, 32 (2), 307-310; Hiort, et al, 1999, *J. Nat. Prod.*, 62 (12), 1632-1635). Insecticidal compounds with a closely related core structure were isolated from *Aglaia roxburghiana* and are described in WO 9604284 for use as active ingredients in agrochemical formulations.

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New compounds (Compounds A and B, as described herein) have now been isolated from *Aglaia leptantha*, Miq. (Meliaceae) which uniquely possesses a dioxanyloxy group attached to the cyclopenta[*b*]benzofuran core. Compounds A and B have been shown to exhibit potent cytotoxic and cytostatic effects on cancer cell growth and viability and thus the compounds of the invention may be useful as therapeutic agents in the treatment of cancer and cancerous conditions or other diseases associated with cellular hyperproliferation.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

In a first aspect, the invention relates to compounds A and B and salts, derivatives and prodrugs thereof.

In another aspect, the invention provides a composition comprising a compound A or B, or a salt, derivative or prodrug thereof, together with a pharmaceutically acceptable carrier, excipient or diluent.

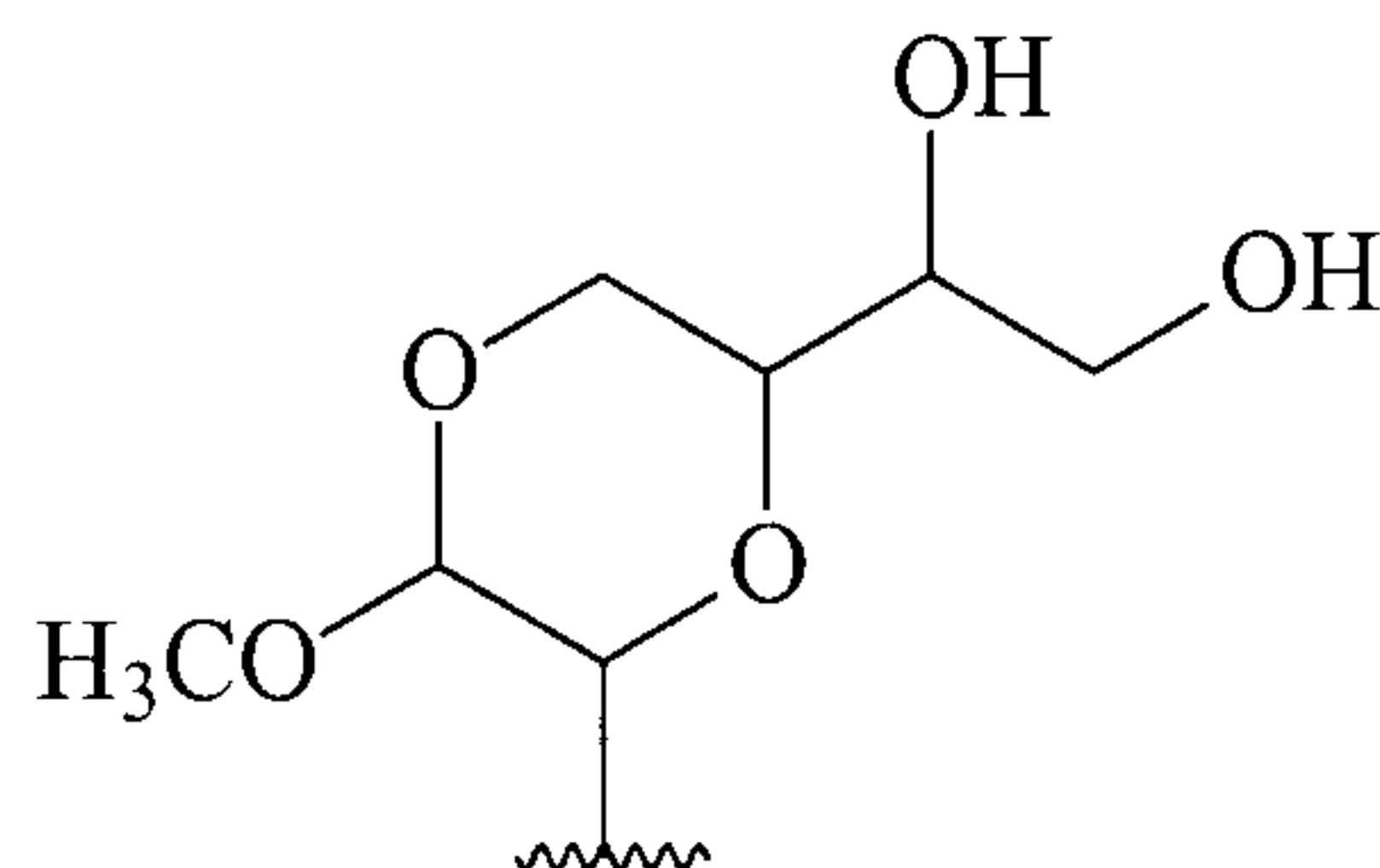
In still a further aspect, the present invention provides a method for the treatment of cancer or a cancerous condition comprising the administration of a treatment effective amount of a compound A or B, or a salt, derivative or prodrug thereof, to a subject in need thereof.

The invention also relates to the use of a compound A or B, or a salt, derivative or prodrug thereof, in the manufacture of a medicament for the treatment of cancer or cancerous conditions.

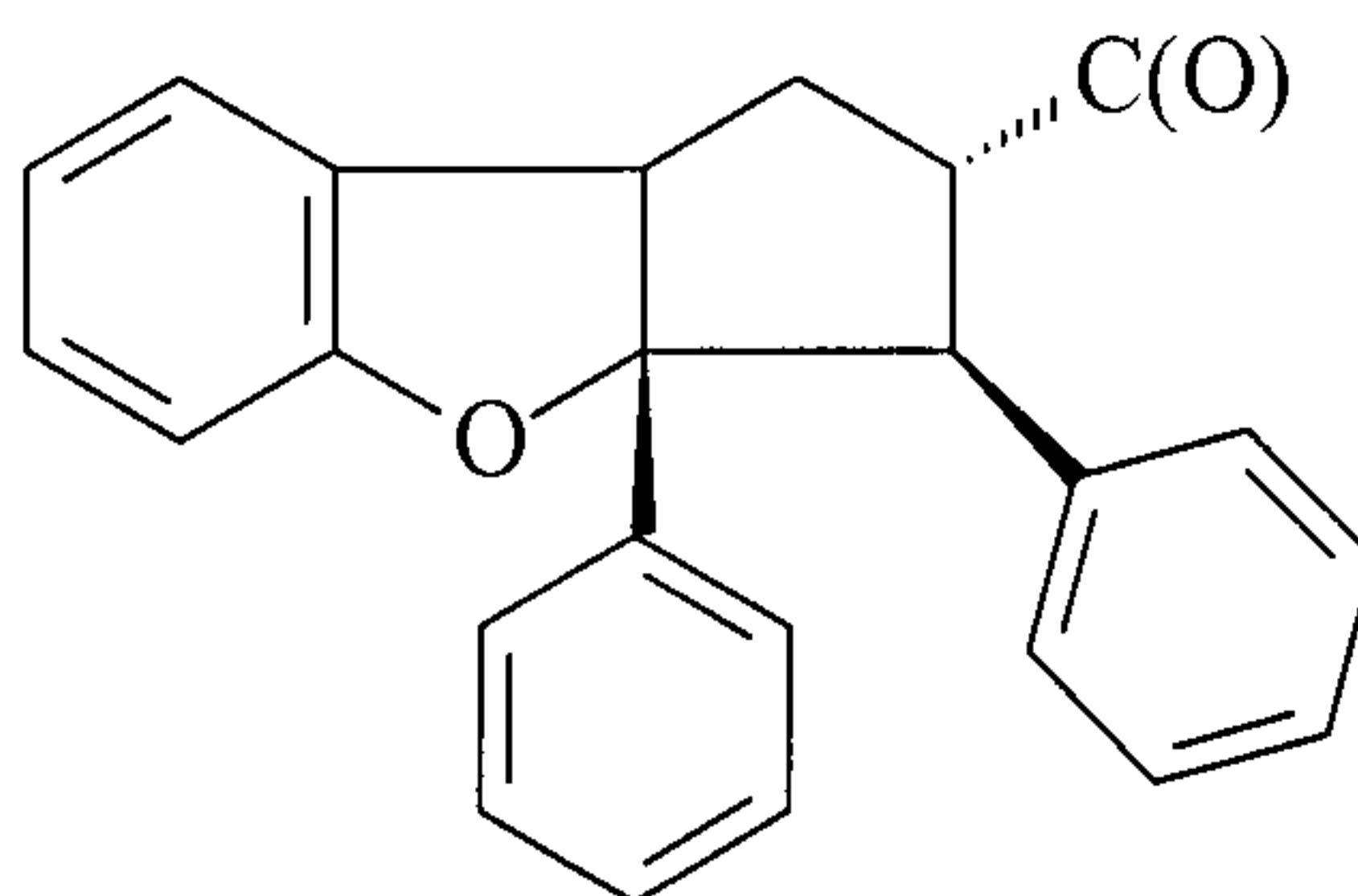
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According to one aspect of the present invention, there is provided a cyclopentabenzofuran compound comprising a group of formula (ii)



5 attached, via an oxygen atom to a cyclopentabenzofuran core of formula (i)



and having the following NMR spectral characteristics:

10 ^1H NMR (CDCl_3) (ppm)

3.49, s, 3H; 3.56, dd, 11.7, 2Hz, 1H; 3.61, m, 1H, 3.61, 2H; 3.65, s, 3H; 3.71, s, 3H; 3.87, s, 3H; 3.89, dd, 14.2, 6.7 Hz, 1H; 4.13, t, 11.2 Hz, 1H; 4.23, brt, 11.3 Hz, 1H; 4.28, d, 14.2 Hz, 1H; 4.59, s, 1H; 5.03, d, 6.7 Hz, 1H; 15 5.28, s, 1H; 6.28, d, 2Hz, 1H; 6.43, d, 2Hz, 1H; 6.68, brd, 9Hz, 2H; 6.84, m, 2H; 7.06, m, 2H; 7.06, m, 1H; 7.10, brd, 9Hz, 2H;

^{13}C NMR (CDCl_3) (ppm)

50.03, 52.06, 55.03, 55.05, 55.1, 55.9, 59, 63.3, 20 68.3, 70.6, 79.6, 92.8, 93.4, 93.9, 94, 95.2, 101.9, 109.6,

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112.7, 126.2, 126.6, 127.8. 127.8, 128.9, 136.7, 157.1,
158.8, 160, 160.6, 170.6;

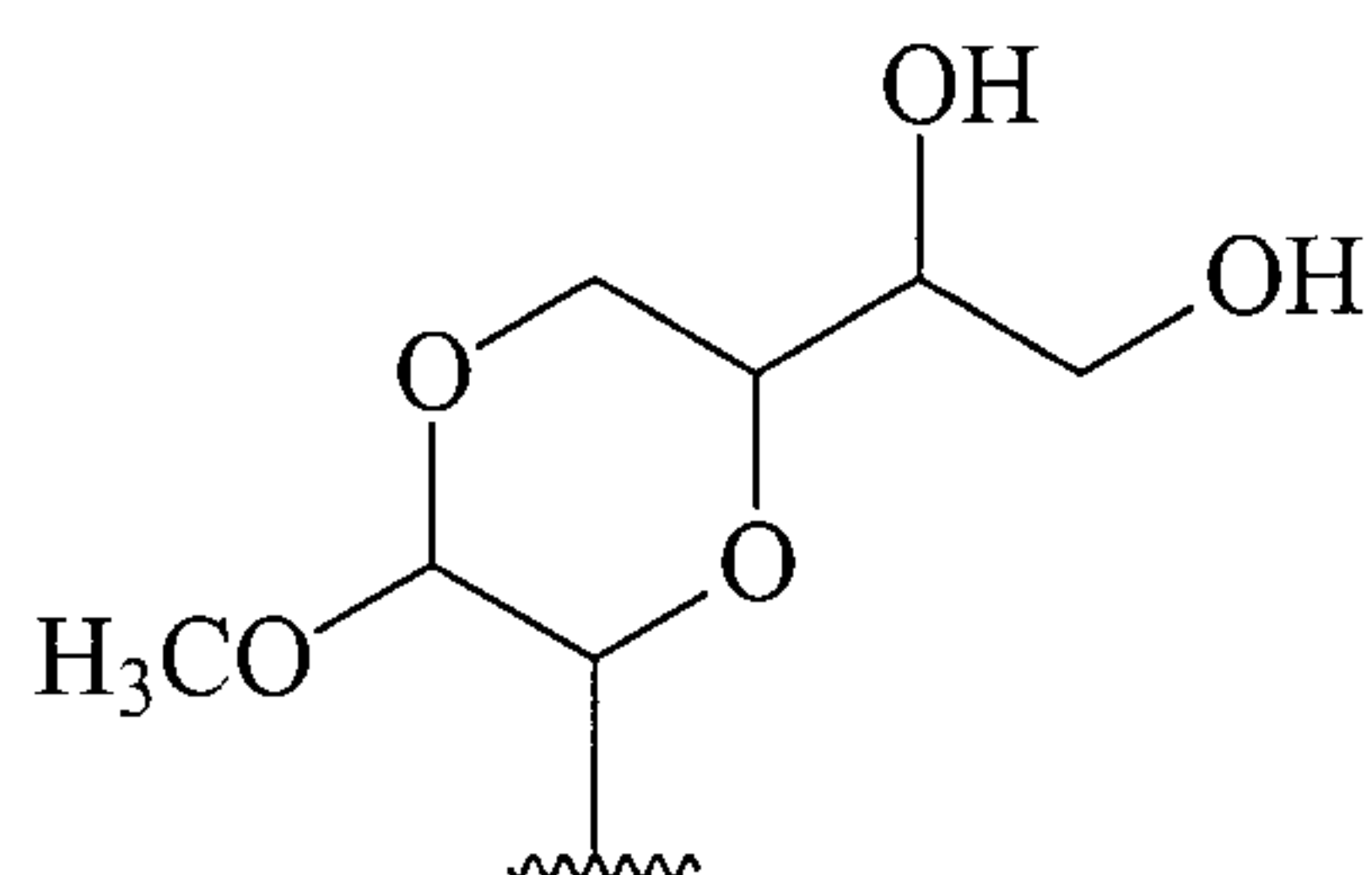
or a salt, derivative or prodrug thereof, wherein
the prodrug or derivative is an ester, glycoside, optionally
5 substituted alkyloxy, optionally substituted cyclic alkoxy,
optionally substituted alkenyloxy, optionally substituted
cyclic alkenyloxy, optionally substituted alkynyloxy,
optionally substituted cyclic alkynyloxy, optionally
substituted acyloxy of formula -O-C(O)-R, where R is an
10 alkyl, cyclic alkyl, alkenyl, cyclic alkenyl, alkynyl,
cyclic alkynyl, heteroaryl or aryl group, or optionally
substituted aryloxy or optionally substituted heteroaryloxy;

and the optional substituents for said optionally
substituted alkyloxy, optionally substituted cyclic alkoxy,
15 optionally substituted alkenyloxy, optionally substituted
cyclic alkenyloxy, optionally substituted alkynyloxy,
optionally substituted cyclic alkynyloxy and optionally
substituted acyloxy are selected from one or more of halo,
hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino, C₁₋₆alkylamino,
20 C₁₋₆dialkylamino, halomethyl, halomethoxy, acetyl, aryl,
heteroaryl and a cyclic alkyl group and the optional
substituents for said optionally substituted aryloxy and
optionally substituted heteroaryloxy are selected from one
or more of halo, hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino,
25 C₁₋₆alkylamino, C₁₋₆dialkylamino, halomethyl, halomethoxy, and
acetyl.

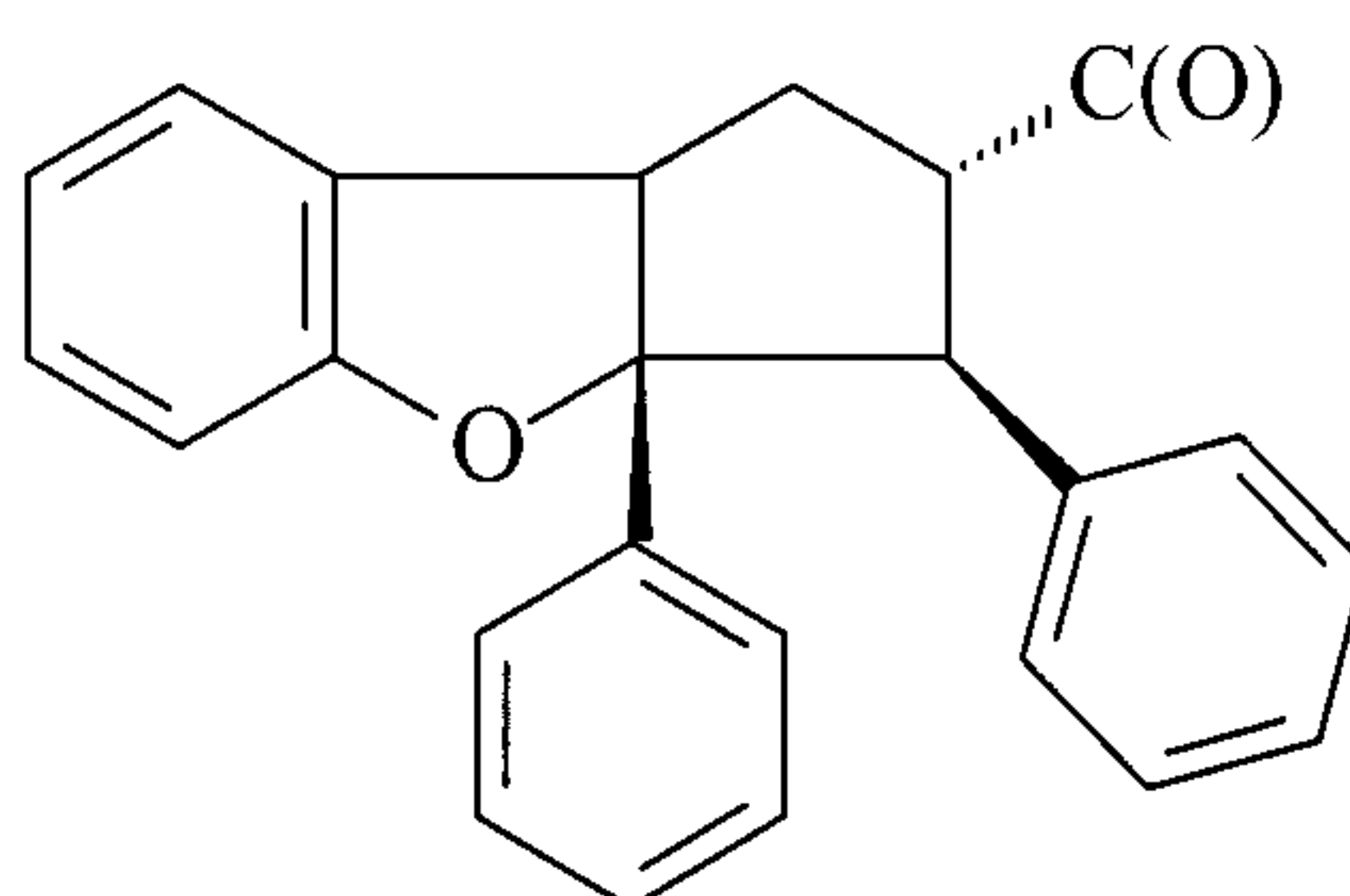
According to another aspect of the present
invention, there is provided a cyclopentabenzofuran compound
comprising a group of formula (ii)

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- 2c -



attached, via an oxygen atom to a
cyclopentabenzofuran core of formula (i)



5 and having the following NMR spectral
characteristics:

^1H NMR (CDCl_3) (ppm)

3.5, s, 3H; 3.61, dd, 10.4, 4.4Hz, 1H; 3.66, m,
1H; 3.66, s, 3H; 3.72, m; 3.72, s, 3H; 3.78, dd, 11.7,
10 2.4 Hz, 1H; 3.86, s, 3H; 3.9, dd, 14, 6.8 Hz, 1H; 4.02, t,
11.2 Hz, 1H; 4.12, ddd, 11, 6.8, 2-8Hz, 1H; 4.28, d, 14Hz,
1H; 4.60, s, 1H; 5.04, d, 6.8Hz, 1H; 5.26, s, 1H; 6.29, d,
2Hz, 1H; 6.45, d, 2Hz, 1H; 6.69, brd, 9Hz, 2H; 6.86, m, 2H;
7.06, m, 2H; 7.06, m, 1H; 7.10, brd, 9Hz, 2H;

15 ^{13}C NMR (CDCl_3) (ppm)

50, 52, 55, 55, 55, 55.8, 59.6, 62.5, 67.6, 71.4,
79.6, 92.8, 93.4, 94.3, 95.2, 101.8, 109.4, 112.8, 126.2,
126.6, 127.5, 127.5, 128.9, 136.6, 157.1, 158.8, 159.8,
160.2, 170.7;

20 or a pharmaceutically acceptable salt, derivative
or prodrug thereof, wherein the prodrug or derivative is an

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ester, glycoside, optionally substituted alkyloxy,
optionally substituted cyclic alkoxy, optionally substituted
alkenyloxy, optionally substituted cyclicalkenyloxy,
optionally substituted alkynyloxy, optionally substituted
5 cyclicalkenynyloxy, optionally substituted acyloxy of formula
-O-C(O)-R, where R is an alkyl, cyclic alkyl, alkenyl,
cyclic alkenyl, alkynyl, cyclic alkynyl, heteroaryl or aryl
group, or optionally substituted aryloxy or optionally
substituted heteroaryloxy;

10 and the optional substituents for said optionally
substituted alkyloxy, optionally substituted cyclic alkoxy,
optionally substituted alkenyloxy, optionally substituted
cyclic alkenyloxy, optionally substituted alkynyloxy,
optionally substituted cyclic alkynyloxy and optionally
15 substituted acyloxy are selected from one or more of halo,
hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino, C₁₋₆alkylamino,
C₁₋₆dialkylamino, halomethyl, halomethoxy, acetyl, aryl,
heteroaryl and a cyclic alkyl group and the optional
substituents for said optionally substituted aryloxy and
20 optionally substituted heteroaryloxy are selected from one
or more of halo, hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino,
C₁₋₆alkylamino, C₁₋₆dialkylamino, halomethyl, halomethoxy, and
acetyl.

According to still another aspect of the present
25 invention, there is provided a use of a compound, salt,
derivative or prodrug as described herein, for treatment of
cancer or a cancerous condition.

According to yet another aspect of the present
invention, there is provided a use of a compound, salt,
30 derivative or prodrug as described herein, for treatment of
a disease state or condition associated with cellular
hyperproliferation.

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According to a further aspect of the present invention, there is provided a compound obtained by:

(a) treating a sample of ground bark from tree species *Aglaia leptantha* with methanol;

5 (b) filtering the resulting extract to form a solution and concentrating the resulting solution under vacuum;

(c) fractionating the resulting extract via solid-phase extraction on a C-18 Varian extraction column
10 (10g) using 0.1% formic acid in acetonitrile/water with increasing acetonitrile concentrations;

(d) collecting the eluate obtained in step (c) with an acetonitrile/water ratio of 7:20;

(e) concentrating the fraction from (d);

15 (f) chromatographing the concentrate obtained under step (e) on a C-18 preparative column at a flow rate of 20ml/min using a linear gradient of from 25% to 45% acetonitrile in water in 30 minutes with 0.1% formic acid;

(g) collecting and concentrating the eluates with
20 UV absorption maxima at 200 and 273 nm and HPLC retention times of approximately 30.67 and 31.05 minutes;

(h) chromatographing each eluate obtained in (g) on a Sephadex LH20 column using methanol as a solvent; collecting and concentrating the fractions obtained with
25 spectral characteristics outlined in step (g).

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BRIEF DESCRIPTION OF THE DRAWINGS**Figure 1: Compound A promotes differentiation of THP-1 leukemic cells.**

THP-1 cells were cultured for 4 days in the presence or absence of 10 nM Compound A as indicated. Where shown cells were also treated with IFN γ (100ng/ml) (3 days) or with PMA (0.1 μ M) (4 days) in the presence or absence of Compound A. Images are of cells visualised by phase contrast microscopy (magnification x200).

Figure 2: Effects of Compound A on cell cycle progression and viability of THP-1 cells.

THP-1 cells were cultured for 2 days with the indicated concentration of Compound A or 1000 nM paclitaxel then collected and fixed in 70% ethanol prior to staining with propidium iodide and DNA content determined by flow cytometry. The numbers indicate the % of cells in the various cell cycle phases relative to all cells with $\geq 2N$ DNA content and also the % dead cells (ie. subdiploid $\leq 2N$ cells) to the left of the marker (the vertical line) that arose during the culture period.

Figure 3: Effects of Compound A on the proliferation of A549 cells.

A549 cells were seeded at $\sim 10,000$ cells/well and cultured in the presence of the indicated concentrations of Compound A or paclitaxel. Cells were collected and the viable cell number determined by haemocytometer counting of trypan blue stained cells at the various times. The results are the averages $\pm$ SEM of triplicate cultures.

Figure 4: Effects of Compound A on cell cycle progression and viability of A549 cells.

A549 cells were cultured for 6 days with the indicated concentration of Compound A or 1 μ M paclitaxel then collected and fixed in 70% ethanol prior to staining with propidium iodide and DNA content determined by flow cytometry. The numbers indicate the % of cells in the

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various cell cycle phases relative to all cells with $\geq 2N$ DNA content and also the % dead cells (ie. subdiploid $\leq 2N$ cells) to the left of the marker that arose during the culture period.

Figure 5: Compounds A and B induce G2/M phase accumulation of K562 leukemic cells

K562 cells were cultured for 3 days with the indicated concentration of Compounds A or B then collected and fixed in 70% ethanol prior to staining with propidium iodide and DNA content determined by flow cytometry. The numbers indicate the % of cells in G0/G1, S and G2/M phases of the cell cycle respectively relative to all cells with $\geq 2N$ DNA content.

Figure 6: Cytostatic effects of Compound A on A549 cells are reversible

A549 cells were seeded at $\sim 10,000$ cells/well and cultured in the presence of the indicated concentrations of Compound A or paclitaxel and the viable cell numbers determined by haemocytometer counting of trypan blue stained cells at the various times. On day 5 some of the cells were washed, resuspended in fresh medium lacking the various treatments and cultured for another 4 days prior to counting.

Figure 7: Compound A inhibits camptothecin- and paclitaxel-induced cytotoxicity of A549 cells

A549 cells in 96 well plates were cultured for 3 days in the presence or absence of 10 nM Compound A together with the indicated concentrations of (A) camptothecin or (B) paclitaxel. Loss of membrane integrity was then assessed by the addition of the fluorescent DNA-binding dye YOYO-1 and the increased fluorescence accompanying cell death measured using a fluorescent plate reader.

Figure 8: Compound A inhibits cell cycle arrest and cell death induced by anti-cancer agents but not by staurosporine.

A549 cells in 6 well plates were cultured for 3 days in the presence or absence of 10 nM Compound A together with 0.1 μ M camptothecin, 10 μ M vinblastin, 1 μ M paclitaxel or 1

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μ M staurosporine as indicated. The cells were then collected and fixed in 70% ethanol prior to staining with propidium iodide and DNA content determined by flow cytometry. The numbers indicate the % of cells in the various cell cycle phases relative to all cells with $\geq 2N$ DNA content and also the % dead cells (ie. subdiploid $\leq 2N$ cells) to the left of the dotted marker that arose during the culture period.

Figure 9: Compound A does not induce senescence-associated β -galactosidase activity in A549 cells.

A549 cells were seeded at 10,000 cells/well in 6 well plates in the presence or absence of varying concentrations of Compound A (10 –50 nM) or 250 nM doxorubicin for 10 days prior to their processing and staining overnight for senescence-associated β -galactosidase activity as described previously (Dimri et al., 1995, *Proc Natl Acad Sci USA* 1995 92(20):9363-7). For Compound A only the 10 nM treatment is shown but there was no detectable SA- β gal activity at any other concentrations tested. PC, phase contrast microscopy. BF, bright field microscopy. Magnification x200.

Figure 10: Compound A inhibits growth of human tumour cells in a mouse xenograft model.

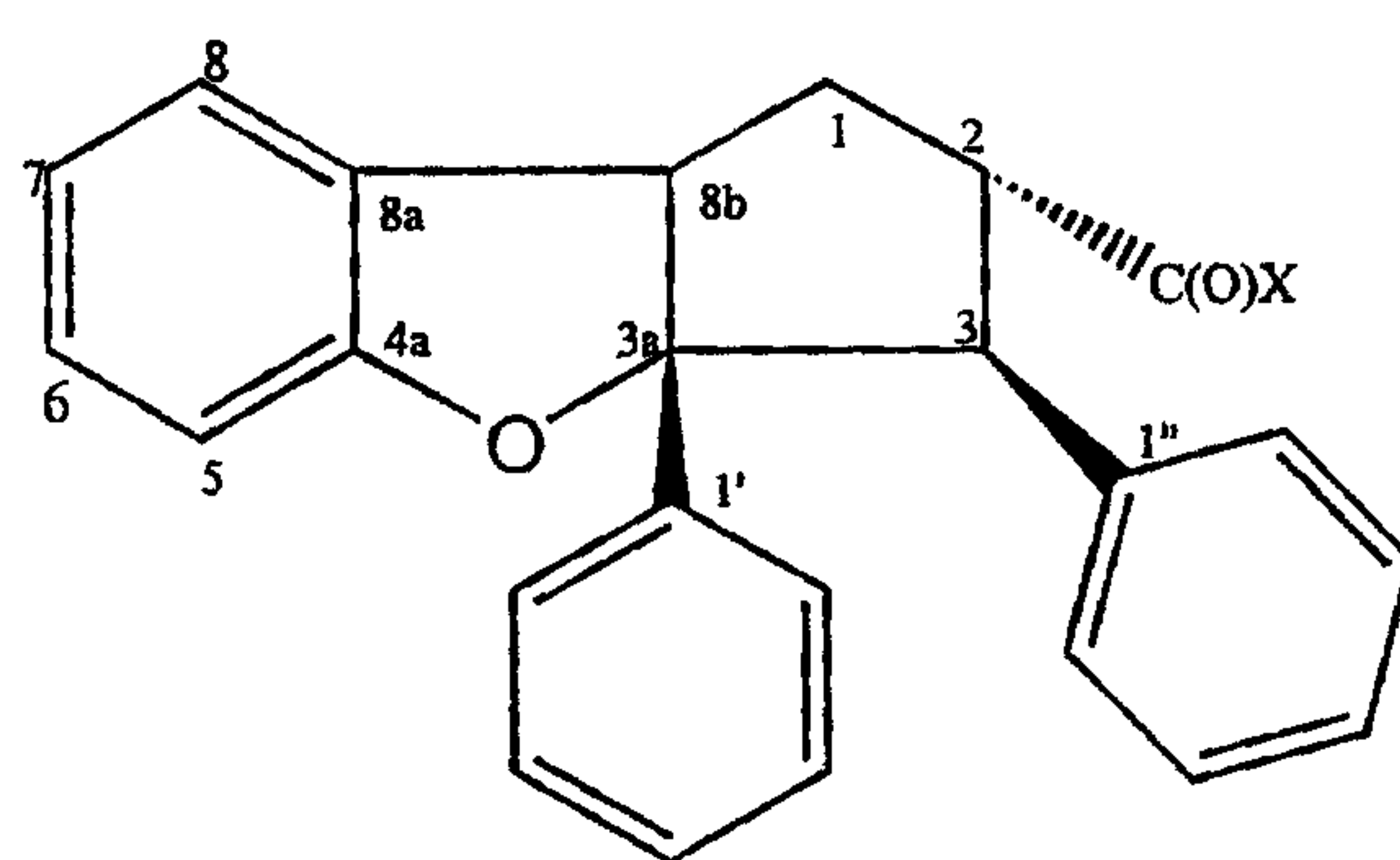
Athymic Balb/c nude mice (Rygaard and Povisen, 1969, *Acta Pathol Microbiol Scand*, 77: 758) were inoculated subcutaneously in the dorsal flank with 2×10^6 PC3 cells. Compound A was administered (3 mg/kg) after eight days when the tumours became palpable by intraperitoneal injection three times a week. Compound A was first solubilized in ethanol then mixed 1:1 with cremaphore and diluted in saline for injection. Control animals were treated in an analogous manner with the same vehicle but lacking Compound A. (A) Effect of Compound A on mean tumour volume. Tumour volumes were measured using a micrometer caliper at the indicated times. The data represents mean tumour volume $\pm$ SEM (B) Effect of Compound A on mean tumour mass. At the end of the experiment (29 days post inoculation of PC3 cells) the mice were sacrificed, the tumours excised and then weighed. The data represents mean tumour weight $\pm$ SEM.

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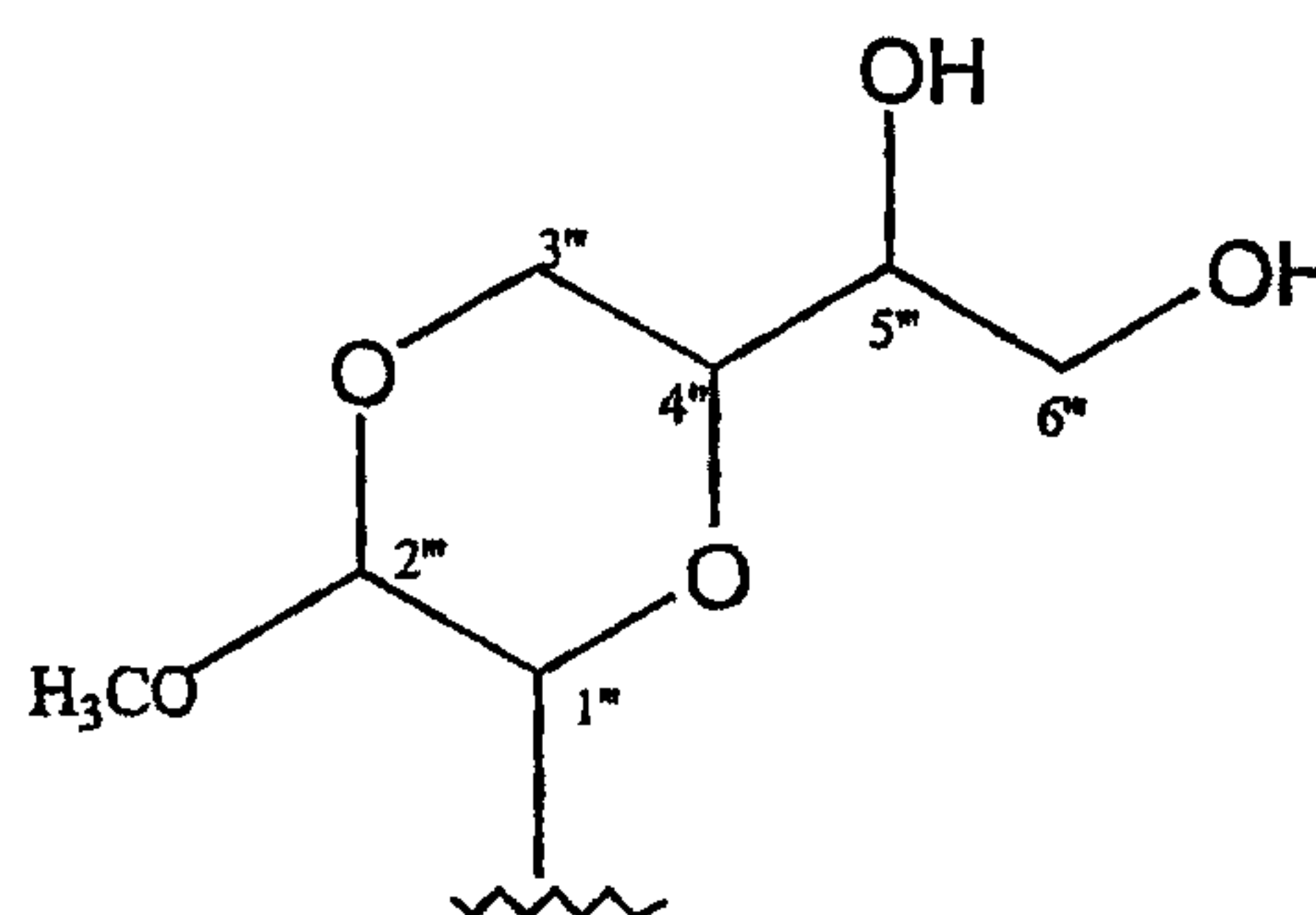
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DETAILED DESCRIPTION OF THE INVENTION

A number of cyclopenta [b] benzofurans have previously been reported, Greger et al, 2001, Phytochemistry, 57, (1); 57-64. The compounds A and B of the present invention carry a dioxanyl group (ii) attached to a cyclopental[b]benzofuran core (i).



(i)



(ii)

The dioxanyl group of Formula (ii) has not previously been reported from a natural source. Without intending to limit the invention by theory, it is believed that the presence of a sterically bulky group, ie spatially larger than a methoxy group, may confer both cytotoxic and cytostatic properties on the compounds having a cyclopenta [b] benzofuran core.

The invention includes within its scope pharmaceutically acceptable salts, derivatives, or prodrugs of Compounds A and B.

The term " salt, or prodrug" includes any pharmaceutically acceptable salt, ester, glycoside, solvate, hydrate or any other compound which, upon administration to the recipient subject is capable of providing (directly or indirectly) a compound of the invention as described herein.

Suitable pharmaceutically acceptable salts include salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids. Base salts include, but is not limited to those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium.

The preparation of salts can be carried out by methods known in the art. It will also be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the invention, since these may be useful as intermediates in the preparation of pharmaceutically acceptable salts.

The compounds of the invention may be in crystalline form or as a solvate (e.g., hydrates). Methods of solution will be known to those skilled in the art.

Pro-drugs of compounds A or B are also within the scope of the invention. The term "pro-drug" includes derivatives that are converted *in vivo* to the compounds of the invention and include for example, ester, acetate and glycoside derivatives of free hydroxy groups.

The derivatisation of hydroxy groups of Compounds A and B can be carried out by methods known in the art for alkylating, arylating or acylating hydroxy groups, for example as described in *Protective Groups in Organic Synthesis* T.W. Greene and P.G.M. Wutz, (1999) Wiley Interscience, New York, and *Advanced Organic Chemistry*, J. March, (4th Edition), Wiley-InterScience.

For example, hydroxy groups can be alkylated using alkyl halides such as methyl iodide or dialkyl sulfates such as dimethyl and diethyl sulfate. Acylation can be effected by

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treatment with appropriate carboxylic acids, acid halides and acid anhydrides in the presence of a base or coupling agent. Benzylation may be effected by treatment with a benzyl halide compound such as benzyl bromide, chloride or iodide. De-esterification of the methyl ester can be effected by treatment of the ester with aqueous base. Esterification of a carboxylic acid can be achieved by conventional means including treatment with an appropriate alcohol in the presence of acid, or treatment with alkyl sulfates or alkyl halides.

As used herein, the term "alkyl", when used alone or in compound words such as "arylalkyl" refers to a straight chain, branched or cyclic hydrocarbon group, preferably C₁₋₂₀, such as C₁₋₁₀. The term "C₁₋₆ alkyl" refers to a straight chain, branched or cyclic alkyl group of 1 to 6 carbon atoms. Examples of "C₁₋₆ alkyl" include methyl, ethyl, *iso*-propyl, *n*-propyl, *n*-butyl, *sec*-butyl, *t*-butyl, *n*-pentyl, isopentyl, 2,2-dimethylpropyl, *n*-hexyl, 2-methylpentyl, 2,2-dimethylbutyl, 3-methylpentyl and 2,3-dimethylbutyl. Examples of cyclic C₁₋₆ alkyl include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Other examples of alkyl include: heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-methyl-octyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2-pentylheptyl and the like. An alkyl group may be optionally substituted by one or more optional substituents as herein defined. Optionally, the straight, branched or cyclic hydrocarbon group (having at least 2 carbon atoms) may contain one, two or more degrees of unsaturation so as to form an alkenyl or alkynyl group, preferably a C₂₋₂₀ alkenyl, more preferably a C₂₋₆ alkenyl, or a C₂₋₂₀ alkynyl, more preferably a C₂₋₆ alkynyl.

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Examples thereof include a hydrocarbon residue containing one or two or more double bonds, or one or two or more triple bonds. Thus, "alkyl" is taken to include alkenyl and alkynyl.

The term "aryl", when used alone or in compound words such as "arylalkyl", denotes single, polynuclear, conjugated or fused residues of aromatic hydrocarbons or aromatic heterocyclic (heteroaryl) ring systems, wherein one or more carbon atoms of a cyclic hydrocarbon residue is substituted with a heteroatom to provide an aromatic residue. Where two or more carbon atoms are replaced, this may be by two or more of the same heteroatom or by different heteroatoms. Suitable heteroatoms include O, N, S and Se.

Examples of "aryl" include phenyl, biphenyl, terphenyl, quaterphenyl, naphthyl, tetrahydronaphthyl, anthracenyl, dihydroanthracenyl, benzanthracenyl, dibenzanthracenyl, phenanthrenyl, fluorenyl, pyrenyl, idenyl, azulenyl, chrysenyl, pyridyl, 4-phenylpyridyl, 3-phenylpyridyl, thienyl, furyl, pyrrolyl, indolyl, pyridazinyl, pyrazolyl, pyrazinyl, thiazolyl, pyrimidinyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothienyl, purinyl, quinazolinyl, phenazinyl, acridinyl, benoxazolyl, benzothiazolyl and the like. Preferred hydrocarbon aryl groups include phenyl and naphthyl. Preferred heterocyclic aryl groups include pyridyl, thienyl, furyl, pyrrolyl. An aryl group may be optionally substituted by one or more optional substituents as herein defined.

The term "acyl" refers to a group $-C(O)-R$ wherein R is an alkyl or aryl group. Examples of acyl include straight chain or branched alkanoyl such as, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl and icosanoyl; cycloalkylcarbonyl, such as cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl and cyclohexylcarbonyl; aroyl such as benzoyl, toluoyl and naphthoyl; aralkanoyl such as phenylalkanoyl (e.g. phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutyl, phenylpentanoyl and phenylhexanoyl) and naphthylalkanoyl (e.g. naphthylacetyl,

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naphthylpropanoyl and naphthylbutanoyl]. Since the R group may be optionally substituted as described above, "acyl" is taken to refer to optionally substituted acyl.

Optional substituents for alkyl, aryl or acyl include halo (bromo, fluoro, chloro, iodo), hydroxy, C₁₋₆alkyl (eg methyl, ethyl, propyl (*n*- and *i*- isomers)), C₁₋₆alkoxy (eg methoxy, ethoxy, propoxy (*n*- and *i*- isomers), butoxy (*n*-, *sec*- and *t*-isomers), nitro, amino, C₁₋₆alkylamino (eg methyl amino, ethyl amino, propyl (*n*- and *i*- isomers)amino), C₁₋₆dialkylamino (eg dimethylamino, diethylamino, diisopropylamino), halomethyl (eg trifluoromethyl, tribromomethyl, trichloromethyl), halomethoxy (eg trifluoromethoxy, tribromomethoxy, trichloromethoxy) and acetyl.

An alkyl group may also be substituted (preferably terminally) by an aryl or a cyclic alkyl group. An acyl group may be substituted (for example, terminally substituted) by an aryl or a cyclic alkyl group.

Glycosidic formation may be effected chemically, eg by reacting the starting compound with a protected sugar compound in which C-1 has been activated by halogenation for coupling with the hydroxyl or carboxyl groups and the sugar hydroxyls have been blocked by protecting groups. Alternatively, glycoside formation may be effected enzymatically using an appropriate glycosyltransferase such as UDP-galactose dependent galactocyltransferase and UDP-glucose dependent glycoyltransferase (SIGMA).

Preferred C-1 linked saccharides are a furanose or pyranose saccharide (sugar) substituent which is linked to the backbone structure shown in Formula (I) through the saccharides's 1-carbon (conventional chemical numbering) to form an acetal linkage at any one of positions R₁, R₂, R₃, R₄, R₅, R₆, or R₇ or an ester linkage at the R₈ or an amide at R₉ or R₁₀ position. Exemplary saccharide groups include reducing sugars such as glucose, ribose, arabinose, xylose, mannose and galactoses, each being linked to an oxygen atom of the structure of Formula (I) through the C-1 carbon of the saccharide group.

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The skilled person will recognise that in order to selectively install any one or more of the derivative groups as defined herein, this may require the judicious protection and/or deprotection, of one or more of the oxy and/or carboxy groups of the cyclopentabenzofuran core. Selective derivatisation of one or more hydroxy or carboxy groups may be achieved via conventional techniques by the use of protecting groups with different degrees of stability under appropriate conditions.

Methods for the conversion of a carboxylic acid or ester group to an amide are known to the skilled person and may include treatment of a carboxylic acid with an appropriate amine in the presence of a coupling reagent such as DCC or treatment of an acid halide with the appropriate amine. Other methods which may be suitable are described in Larock, R.E, *Comprehensive Organic Transformations* pp 963-995, VCH Publishers (1989).

As used herein, the term "protecting group", refers to an introduced functionality which may temporarily render a particular functional group, eg hydroxy or carboxylic acid, inactive under certain conditions in which the group might otherwise be reactive. Suitable protecting groups are known to those skilled in the art, for example as described in *Protective Groups in Organic Synthesis (supra)*. Suitable protecting groups for hydroxy include alkyl, (such as C₁-C₆alkyl), acyl (such as C(O)C₁-C₆alkyl, benzoyl and the like), benzyl, and silyl groups (such as trimethylsilyl, *t*-butyldimethyl silyl, *t*-butyldiphenylsilyl and the like). Other suitable groups for hydroxy substituents and a carboxy substituent (acid, amide etc) can be found within Greene *supra*. The stability of various groups under certain conditions is understood by the skilled person and is further exemplified in *Protective Groups in Organic Synthesis (supra)*.

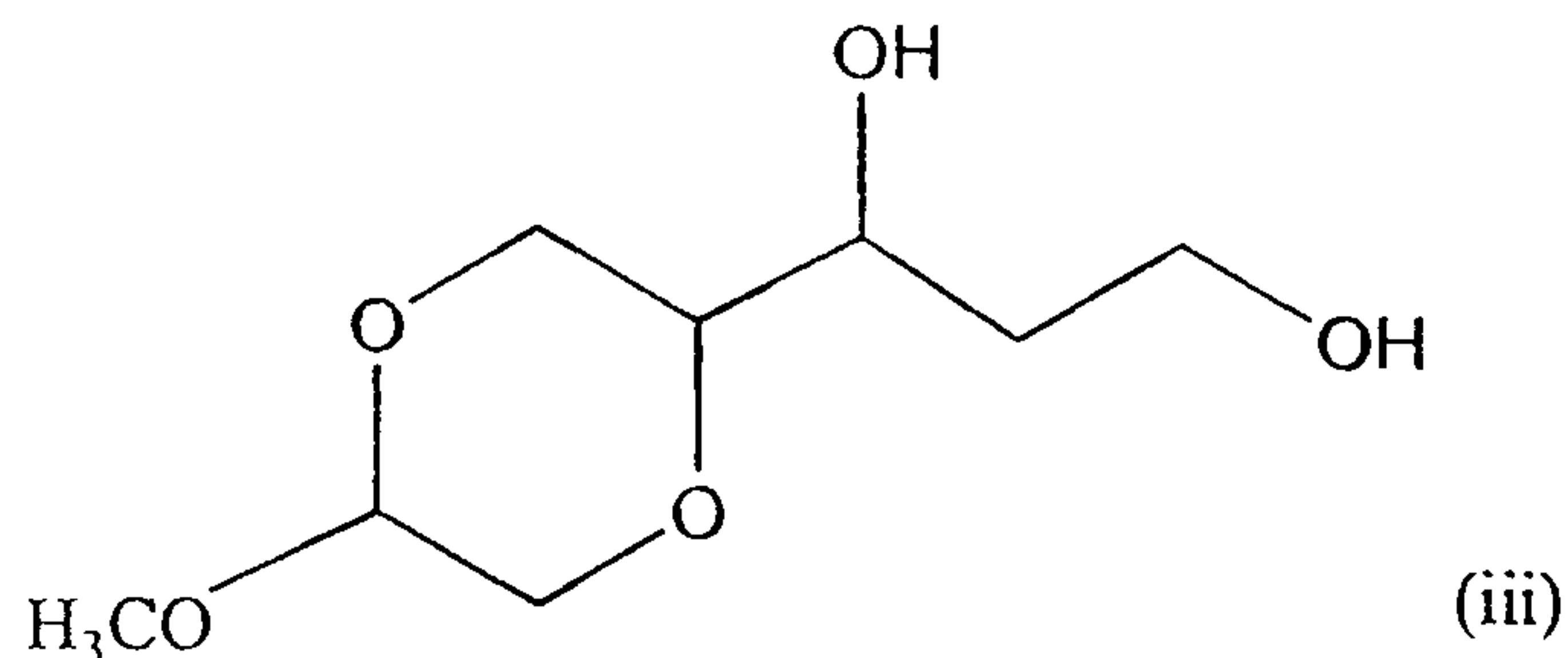
It will also be recognised that the dioxanyl group of Formula (ii), may possess asymmetric centres and is therefore capable of existing in more than one stereoisomeric form. The invention thus also relates to compounds in substantially pure isomeric form at one or

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more asymmetric (chiral) centres of the dioxanyl group eg., greater than about 90% ee, such as about 95% or 97% ee, preferably greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be resolved by conventional methods, eg, chromatography, or use of a resolving agent. The present invention thus provides Compounds A and B.

The dioxanyl group may be cleaved from the cyclopentabenzofuran core using known methods to afford a dioxane compound formula (iii). The resulting dioxane compound could be used to substitute other compounds, such as oxy-substituted compounds, or other oxy positions, on other cyclopentabenzofuran compounds such as those described in the references herein.



It will also be understood that cyclopentabenzofuran compounds, having a methoxy or hydroxy substituent, such as those described in the references cited herein, eg Reference Compounds 1-3 (as described in Example 3), can, where appropriate be demethylated, and the resulting hydroxy group reacted with a suitable Y precursor to form an OY group. Methods therefor are known in the art, for example one method may involve reacting the OH group with a Y-halogen compound where halogen includes Cl, Br and I. Such OY compounds form a further aspect of the invention.

The compounds of the invention may have use in the treatment of cancerous conditions, or other conditions associated with cellular hyperproliferation, in a subject. Subjects which may be treated by the compounds of the invention include mammals, for example, humans, primates, livestock animals (eg. sheep, cows, horses, goats, pigs),

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companion animals, (eg. dogs, cats, rabbits, guinea pigs), laboratory test animals, (eg, rats, mice, guinea pigs, dogs, rabbits, primates) or captured wild animals. Most preferably, humans are the subjects to be treated.

As used herein the term "treatment" is intended to include the prevention, slowing, interruption or halting of the growth of a cancer, tumour or hyperproliferative cell, or a reduction in the number of targeted cells (or size of the growth mass) or the total destruction of said cell, wherein said cells are cancer, tumour or hyperproliferative cells.

Cancerous conditions which may be treated by the compounds of the present invention include conditions wherein the cancers or tumours may be simple (monoclonal, ie composed of a single neoplastic cell type), mixed (polyclonal, ie. composed of more than one neoplastic cell type) or compound (ie. composed of more than one neoplastic cell type and derived from more than one germ layer) and may include benign and malignant neoplasia/hyperplasia. Some examples of cancerous conditions which may be treated by the present invention include leukemia and breast, colon, uterus, prostate, lung, ovarian, brain, skin, liver, bowel and stomach cancers, tumours and melanomas. Examples of benign hyperplasias include those of vascular (eg hemangioma), prostate, renal, adrenal, hepatic, colon (eg colonic crypt), parathyroid gland and other tissues.

As the compounds of the invention may have cytostatic as well as cytotoxic properties, they may also have potential use as therapeutic agents in the suppression of the growth of target populations of cells other than cancer or tumour cells, for example disease states or conditions associated with cellular hyperproliferation. Such conditions may include atherosclerosis and restinosis (neointimal hyperplasia) and hyperproliferation due to or accompanying an inflammatory response, eg arthritis, (including rheumatoid arthritis, osteoarthritis and inflammatory arthritis), psoriasis and periodontal disease, or cellular hyperproliferation due to the viral infection of cells such as human papilloma virus.

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The compounds of the invention, eg Compounds A and B, may be used in therapy in conjunction with other therapeutic compounds, such as anti-cancer compounds, including paclitaxel, camptothecin, vinblastin and doxorubicin.

Thus, another aspect of the invention relates to a method for the treatment of cancer or a cancerous condition comprising the administration of an effective amount of a compound of Formula (I) and a further therapeutic agent to a subject in need thereof, and the use of said compound in the manufacture of a medicament for use in conjunction with other therapeutic agents.

The compounds of the invention and the further therapeutic agent may be administered simultaneously, as a single composition or as discrete compositions, or may be administered separately, ie, one after the other at suitable intervals as determined by the attending physician. Thus, the invention also provides a kit comprising a compound of Formula (I) together with a further therapeutic agent.

As used herein, the term "effective amount" of a compound relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired therapeutic activity. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. Suitable dosages lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 μ g to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1 μ g to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg body weight per dosage. The dosing regime for each subject may be dependent upon the age, weight, health and medical history of the subject and

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the extent and progress of the condition to be treated, and can be determined by the attending physician.

The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parental (including subcutaneous, intramuscular, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g inert diluent, preservative disintegrant such as sodium starch

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glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Compositions for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter, gelatin, polyethylene glycol.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for

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injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants may include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents may include glyceryl monostearate or glyceryl distearate.

One or more embodiments of the present invention may also provide methods, compositions agents or compounds which have an advantage over (or avoid a disadvantage) associated with known methods, compositions, agents or compounds used in the chemotherapeutic treatment of cancerous conditions or conditions associated with the hyperproliferation of cells. Such advantages may include one or more of: increased therapeutic activity, reduced side effects, reduced cytotoxicity to non-cancerous or non-

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proliferative cells, improved solubility or dispersibility for formulation into pharmaceutical compositions, improved stability or a more readily available means of obtaining said compounds, eg. by simpler, cheaper or higher yielding synthetic or isolation processes.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that prior art forms part of the common general knowledge in Australia.

The invention will now be described with reference to the following Examples which are included for the purpose of illustrating embodiments of the invention and not to be construed as limiting the generality hereinbefore described.

EXAMPLES

Example 1

*Isolation of Compounds A and B from *Aglaia leptantha**

Compounds A and B were isolated using the following procedure:

- (a) Treat a sample of ground bark from the tree species *Aglaia leptantha* with methanol.
- (b) Filter the extract and concentrate the resulting solution under vacuum.

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- (c) Fractionate the extract via solid-phase extraction on a C-18 Varian[™] extraction column (10 g) using 0.1% formic acid in acetonitrile/water with increasing acetonitrile concentrations.
- (d) Collect the eluate obtained with an acetonitrile/water ratio of 7:20. Compounds A and B have a UV absorption maximum of 200, 273 nm (acetonitrile/water/0.1% formic acid) and a HPLC retention times of approximately 30.67 (Compound A) and 31.05 minutes (Compound B) under the following conditions:
C-8 Symmetry column (WATERS[™]), 4.6 x 250 mm, 5µm 1 ml/min, linear gradient from 0% to 90% acetonitrile in water in 40 minutes with 0.1% formic acid.
- (e) Concentrate fraction obtained under step (d).
- (f) Chromatograph the concentrate obtained under step (e) on a C-18 preparative column (WATERS[™], Nova-Pak[™] C-18, 6 micron, 2.5 x 25 cm) at a flow rate of 20 ml/min using a linear gradient from 25% to 45% acetonitrile in water in 30 minutes with 0.1% formic acid.
- (g) Collect and concentrate the eluates with the chromatographic and spectroscopic characteristics outlined in step (d) at approximately 22 minutes.
- (h) Chromatograph each eluate obtained under (g) on a Sephadex[™] LH20 column using methanol as a solvent. Collect and concentrate the fractions with spectral characteristics outlined in (d).
- (i) (i) Alternatively to steps (b), (c) and (d), the methanol extract obtained under (a) may be partitioned with equal volumes of water and dichloromethane. The dichloromethane phase is then processed according to steps (e) to (h).

The compounds thus obtained have the following spectroscopic and physical characteristics;

UV/Vis absorption maxima: 223, 275 nm (in MeCN/H₂O, 0.1% HCOOH).

MS: Mass spectra were obtained on a Finnigan LCQ iontrap mass spectrometer using the ESI source in the positive ion mode. The sample was dissolved in 0.1%FA in MeOH and introduced into the source by infusion with a syringe pump at rate of 3 µL/min. For

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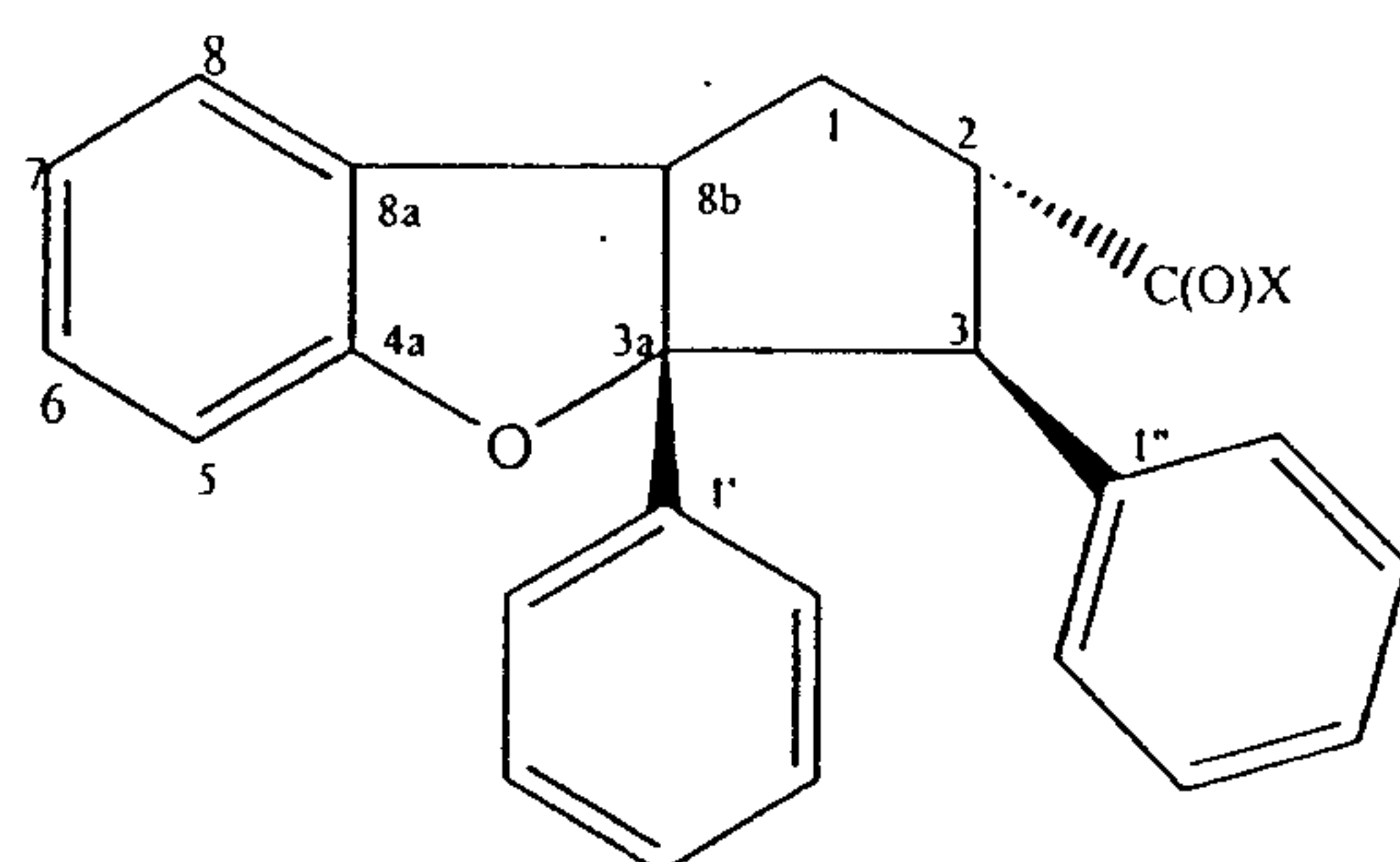
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Compound A, signals were observed at m/z 677 $[M+Na]^+$; MS^2 yielded m/z 659 $[M+Na-H_2O]^+$; MS^3 yielded m/z 627 (loss of 32 amu); MS^4 yielded m/z 595 (loss of another 32 amu) and m/z 451 (loss of 176 amu, equivalent to the dioxane sidechain). For compound B signals were observed in the positive ion mode at m/z 677.2 $[M+Na]^+$; MS^2 yielded daughter ions at m/z 627.2 and m/z 659.2. Further fragmentation of the signal at m/z 627.2 yielded a daughter ion at m/z 595.3.

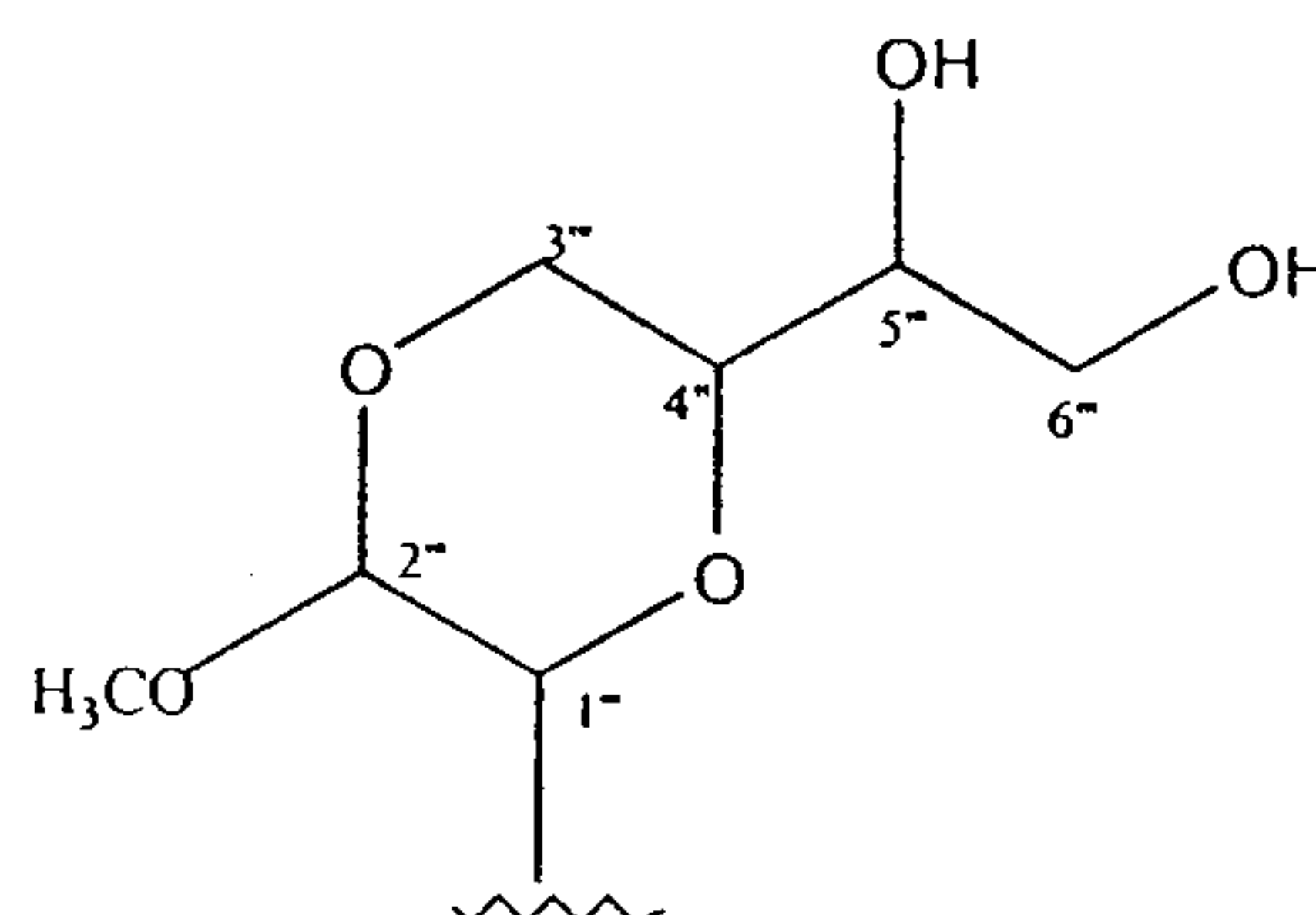
Accurate mass spectra for Compound A were obtained on the BrukerTM 47e Fourier Transform – Ion Cyclotron Resonance Mass Spectrometer (FTMS) fitted with an Analytica Electrospray Source (ESI). The sample was dissolved in MeOH and introduced into the source by direct infusion with a syringe pump at a rate of 60 μ L/min. The source was operated with capillary voltage of 100v. One signal was observed at m/z 677.2194 $[M+Na]^+$ meas.; $C_{34}H_{38}O_{13}Na^+$ requires 677.2204.

NMR

The NMR spectra of Compounds A and B were acquired on 400 and 500 MHz Varian INOVATM NMR spectrometers, in CD_3OD and $CDCl_3$, respectively. The following experiments were conducted: 1H , ^{13}C , DEPT, HMQC, HMBC, COSY. The 1H NMR chemical shifts (obtained in $CDCl_3$) and the ^{13}C NMR chemical shifts are listed in Table 1.



(i)



(ii)

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Table 1

Position Assignments		Compound A		Compound B	
		¹ H NMR (ppm)	¹³ C NMR (ppm)	¹ H NMR (ppm)	¹³ C NMR (ppm)
1	CH	5.03, d, 6.7 Hz, 1H	79.6	5.04, d, 6.8 Hz, 1H	79.6
2	CH	3.89, dd, 14.2, 6.7 Hz, 1H	50.03	3.9, dd, 14, 6.8 Hz, 1H	50
	COOCH ₃		170.6		170.7
	COOCH ₃	3.65, s, 3H	52.06	3.66, s, 3H	52
3	CH	4.28, d, 14.2 Hz, 1H	55.03	4.28, d, 14 Hz, 1H	55
3a	C		101.9		101.8
4a	C		160.6		160.2
5	CH	6.43, d, 2 Hz, 1H	92.8	6.45, d, 2 Hz, 1H	92.8
	C		157.1		157.1
	OCH ₃	3.87, s, 3H	55.9	3.86, s, 3H	55.8
7	CH	6.28 d, 2 Hz, 1H	93.9	6.29 d, 2 Hz, 1H	94.3
	C		160		159.8
8a	C		109.6		109.4
8b	C		93.4		
1'	C		126.2		126.2
2', 6'	2xCH	7.10, br d, 9 Hz, 2H	128.9	7.10, br d, 9 Hz, 2H	128.9
3', 5'	2xCH	6.68, br d, 9 Hz, 2H	112.7	6.69, br d, 9 Hz, 2H	112.8
4'	C		158.8		158.8
	OCH ₃	3.71, s, 3H	55.05	3.72, s, 3H	55
1"	C		136.7		136.6
2", 6"	2xCH	6.84, m, 2H	127.8	6.86, m, 2H	127.5
3", 5"	2xCH	7.06, m, 2H	127.8	7.06, m, 2H	127.5
4"	CH	7.06, m, 1H	126.6	7.06, m, 1H	126.6

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1'''	CH	5.28, s, 1H	94	5.26, s, 1H	93.4
2'''	CH	4.59, s, 1H	95.2	4.60, s, 1H	95.2
	OCH ₃	3.49, s, 3H	55.1	3.5, s, 3H	55
		4.13, t, 11.2 Hz, 1H		4.02, t, 11.2 Hz, 1H	
3'''	CH ₂	3.56, dd, 11.7, 2 Hz, 1H	59	3.78, dd, 11.7, 2.4 Hz, 1H	59.6
4'''	CH	4.23, br t, 11.3 Hz, 1H	68.3	4.12, ddd, 11, 6.8, 2.8 Hz, 1H	67.6
5'''	CH	3.61, m, 1H	70.6	3.66, m, 1H	71.4
6'''	CH ₂	3.61, m, 2H	63.3	3.61, dd, 10.4, 4.4 Hz, 1H	62.5
				3.72, m, 1H	

Example 2***Compounds A and B are cytostatic and cytotoxic for human tumour cell lines***

Compounds A and B were identified from a bark sample of *Aglaia lephantha* through their ability to inhibit production of Tumour Necrosis Factor- α (TNF- α) by THP-1 human promonocytic leukemia cells (Tsuchiya, *et al*, *Int. J. Cancer*, 1980, 26(2):171-6) activated with lipopolysaccharide (LPS). Table 2 summarises the results comparing the activity of Compounds A and B for inhibition of TNF- α production to their effects on general cell metabolism measured using WST-1 reduction, DNA synthesis and protein synthesis assays for THP-1 cells. Compounds A and B potently inhibited TNF- α production at broadly similar concentrations that were active in the WST-1 reduction, DNA and protein synthesis assays. For comparison, the effects of Compounds A and B on A549 lung epithelial carcinoma cells (Leiber *et al*, *Int. J. Cancer*, 1976, 17(1):62-70) were also measured and the data is also included in Table 2. Compounds A and B are significantly less potent for inhibition of interleukin-1 (IL-1)-induced Intercellular Adhesion Molecule-1 (ICAM-1) expression by A549 cells even though in these cells the protein and DNA synthesis inhibition occur at broadly similar concentrations as for THP-1 cells.

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Table 2 : Comparison of the effects of Compounds A and B in THP-1 and A549 Cells.

Purified Compound A or Compound B solubilized in DMSO were tested over a range of concentrations in parallel for inhibitory activity in the various assays in both THP-1 and A549 cells. The concentration that resulted in a 50% inhibition of the relevant response (IC_{50}) is shown. Production of $TNF\alpha$ by THP-1 cells was measured as that released into the culture supernatant over 18 hours by sandwich enzyme-linked immunosorbent assay (ELISA) using the following mouse anti- $TNF\alpha$ monoclonal antibodies (capture antibody, MAB610; detection antibody, biotinylated MAB210; both from R & D Systems, Minneapolis MN, USA). Surface expression of ICAM-1 by A549 cells was assayed after 24 hours of culture by direct antibody binding using a europium-labelled mouse anti-ICAM-1 monoclonal antibody (R&D Systems Cat No. BBA3) and measured by time-resolved fluorescence using DelfiaTM assay (EG&G Wallac, Turku, Finland). Reduction of WST-1 (Roche, Cat. No. 1644807) by THP-1 cells was measured after 18 hours of culture according to the manufacturer's instructions. Protein synthesis was measured as the uptake of [^{14}C]-leucine (0.5 μ Ci/mL) after 48 hours for THP-1 cells and 72 hours for A549 cells cultured in growth medium (RPMI-1640, 10% FBS) containing 10% the usual L-leucine concentration (5 mg/mL). DNA synthesis was measured as the uptake of [^{14}C]-thymidine (0.5 μ Ci/mL) after 48 hours for THP-1 cells and 72 hours for A549 cells in normal growth medium.

Compound	IC_{50} (μ M)						
	THP-1 cells				A549 cells		
	<i>TNF-α</i> <i>Production</i>	<i>WST-1</i> <i>Reduction</i>	<i>Protein</i> <i>Synthesis</i>	<i>DNA</i> <i>Synthesis</i>	<i>ICAM-1</i> <i>Production</i>	<i>Protein</i> <i>Synthesis</i>	<i>DNA</i> <i>Synthesis</i>
Compound A	0.06	0.03	0.06	0.015	2	0.02	0.007
Compound B	0.015	0.04	0.003	0.003	5	0.01	0.004

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Compound A was assessed for cytotoxic and cytostatic activity against a panel of cell lines derived from a variety of human tumour types in addition to THP-1 and A549 cells (Table 3). These included K562 leukemic cells (Lozzio and Lozzio, 1975, *Blood* 45:321-34), PC3 prostate tumour cells (Kaighn et al., 1979, *Invest. Urol.* 17:16-23) and SF268 glioblastoma cells (Westphal et al, 1985, *Biochem. Biophys. Res. Commun.*, 132:284-9). Compound A exhibited potent cytostatic activity in nearly all cell lines tested with GI₅₀ values ranging between 1-7 nM. Compound A also exhibited potent cytotoxic effects against the various tumour cell lines. Interestingly, the THP-1 and PC3 cells proved the most rapidly killed with little difference in LC₅₀ values obtained after 3 or 6 days of culture. However, the cytotoxic potency of Compound A increased dramatically after 6 days of culture for the K562, A549 and SF268 cells. It should be noted that the concentration of Compound A required to inhibit cell proliferation were significantly lower than those required to elicit a cytotoxic response. Hence, the cytostatic effect of Compound A is biochemically distinguishable from its ability to induce cell death. Table 4 shows that Compound B exhibited cytotoxic effects against the various tumour cell lines with comparable potency to that observed with Compound A.

Table 3: Compound A has potent cytostatic and cytotoxic activity in various human tumour cell lines in vitro.

Purified Compound A was tested over a range of concentrations up to a maximum of 1×10^{-6} M (1000 nM) for cytostatic and cytotoxic activity against a panel of cell lines derived from various human tumour types as indicated. The GI₅₀ value represents the concentration of compound that inhibited the cell number increase (relative to untreated cells) by 50% after 3 days of culture. Relative cell number was determined by measuring cellular DNA using a fluorescent DNA-binding dye (YOYO-1) after lysing the cells with digitonin (Becker et al., *Anal Biochem*, 1994, 221(1):78-84). The LC₅₀ value represents the concentration of compound that killed 50% of the cells. Cell death was measured as the proportion of dead cells exhibiting sub-diploid DNA content determined by flow cytometry after staining with propidium iodide (Nicoletti et al., *J. Immunol. Methods*, 1991, 139:271-79).

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Tumour Source	Tumour Cell Line	Compound A		
		GI ₅₀ (nM) (3 day cultures)	LC ₅₀ (nM) (3 day cultures)	LC ₅₀ (nM) (6 day cultures)
Leukemia	THP-1	-	36	24
	K562	1	>1000	10
Lung	A549	7	914	21
Prostate	PC3	5	18	12
Brain	SF268	3	461	29

Table 4: Compounds A and B exhibit similar cytotoxic activity

The cytotoxic activity of Compounds A and B were compared for the various tumour cell lines as described in Table 3.

Tumour Source	Tumour Cell Line	LC ₅₀ (nM) (6 day cultures)	
		Compound A	Compound B
Leukemia	THP-1	11	15
	K562	12	15
Lung	A549	15	12
Prostate	PC3	12	12
Brain	SF268	12	22

Example 3

Cytotoxic activity of Compound A is not shared by other known related compounds lacking the novel dioxanyloxy substitution.

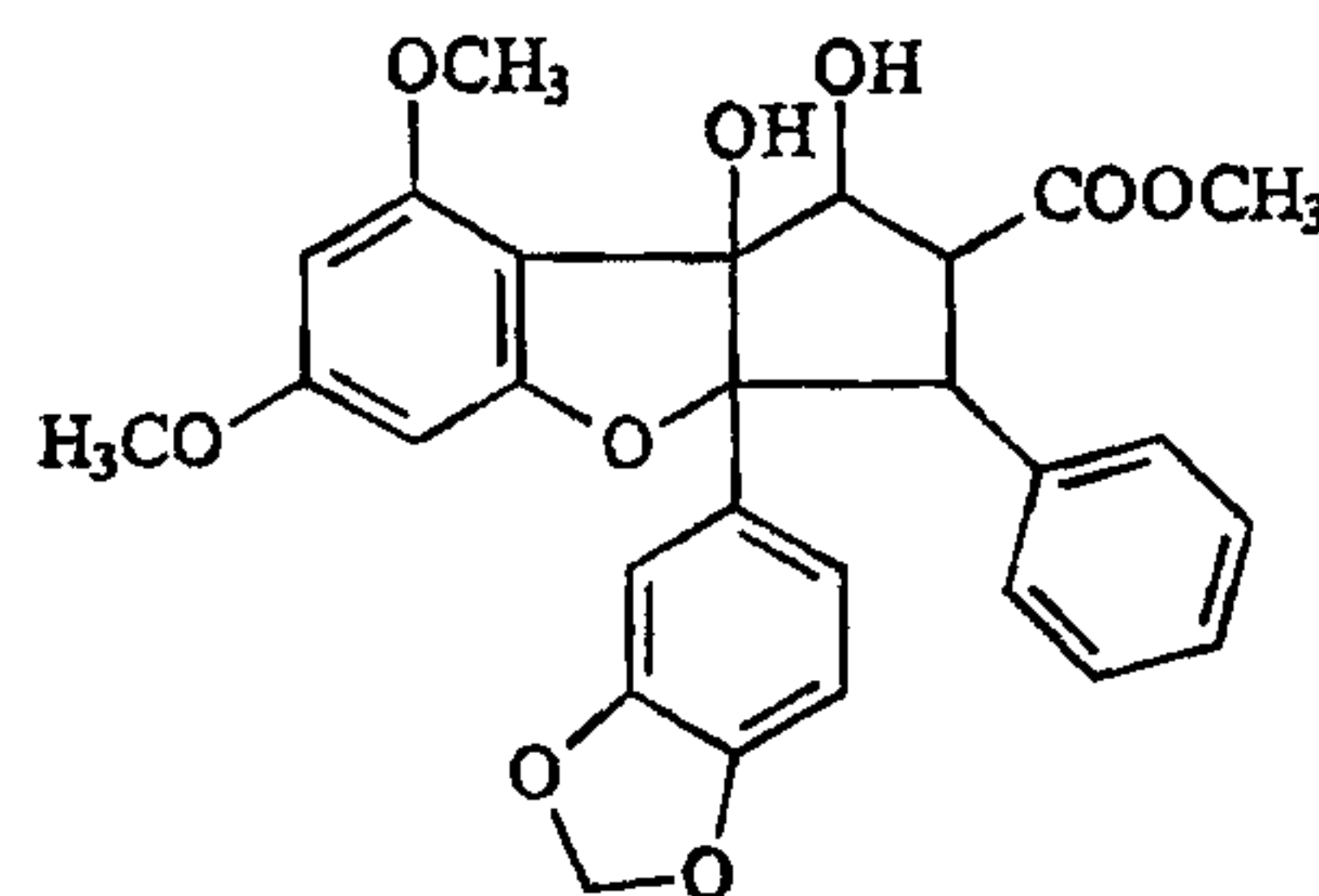
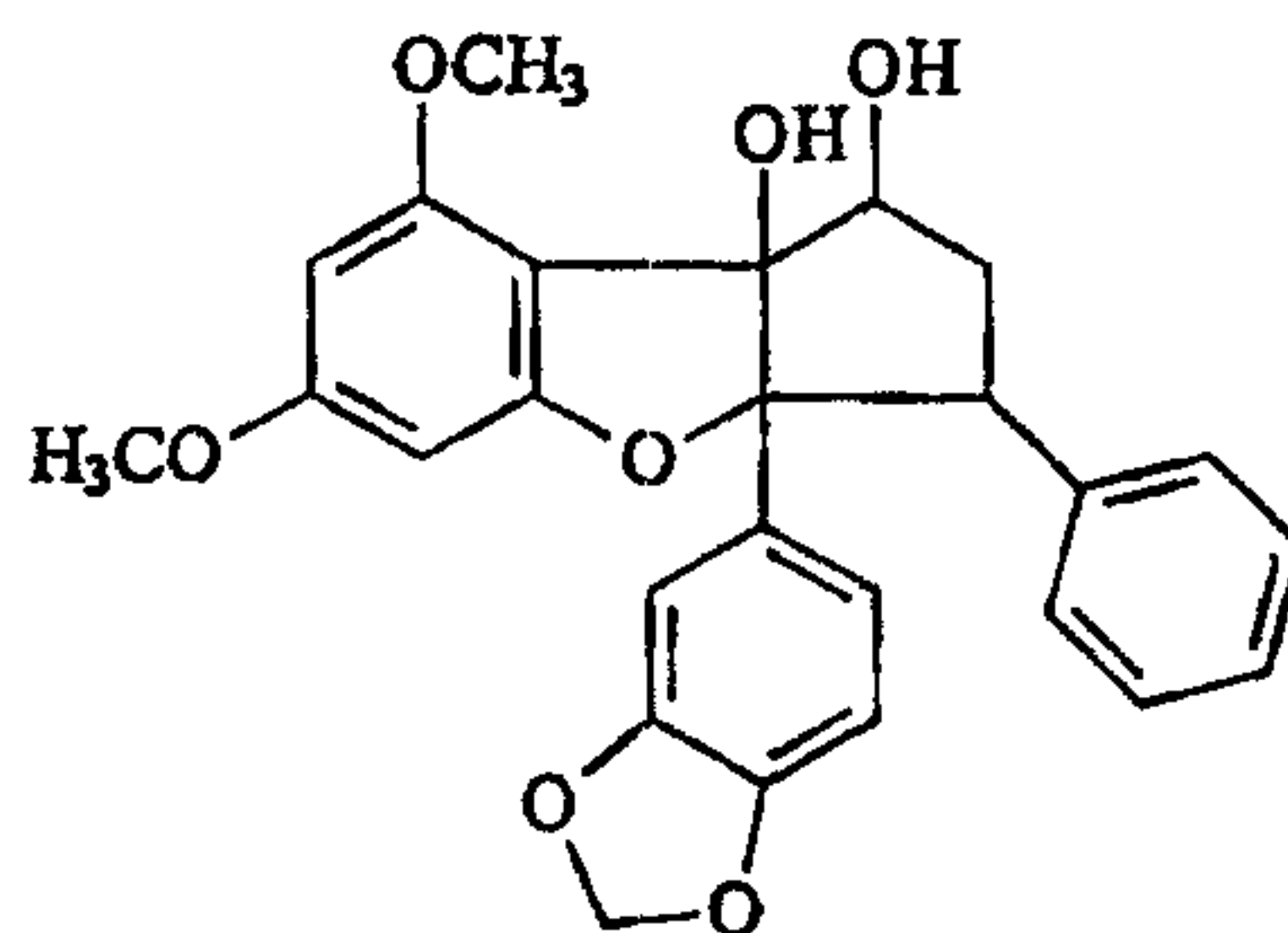
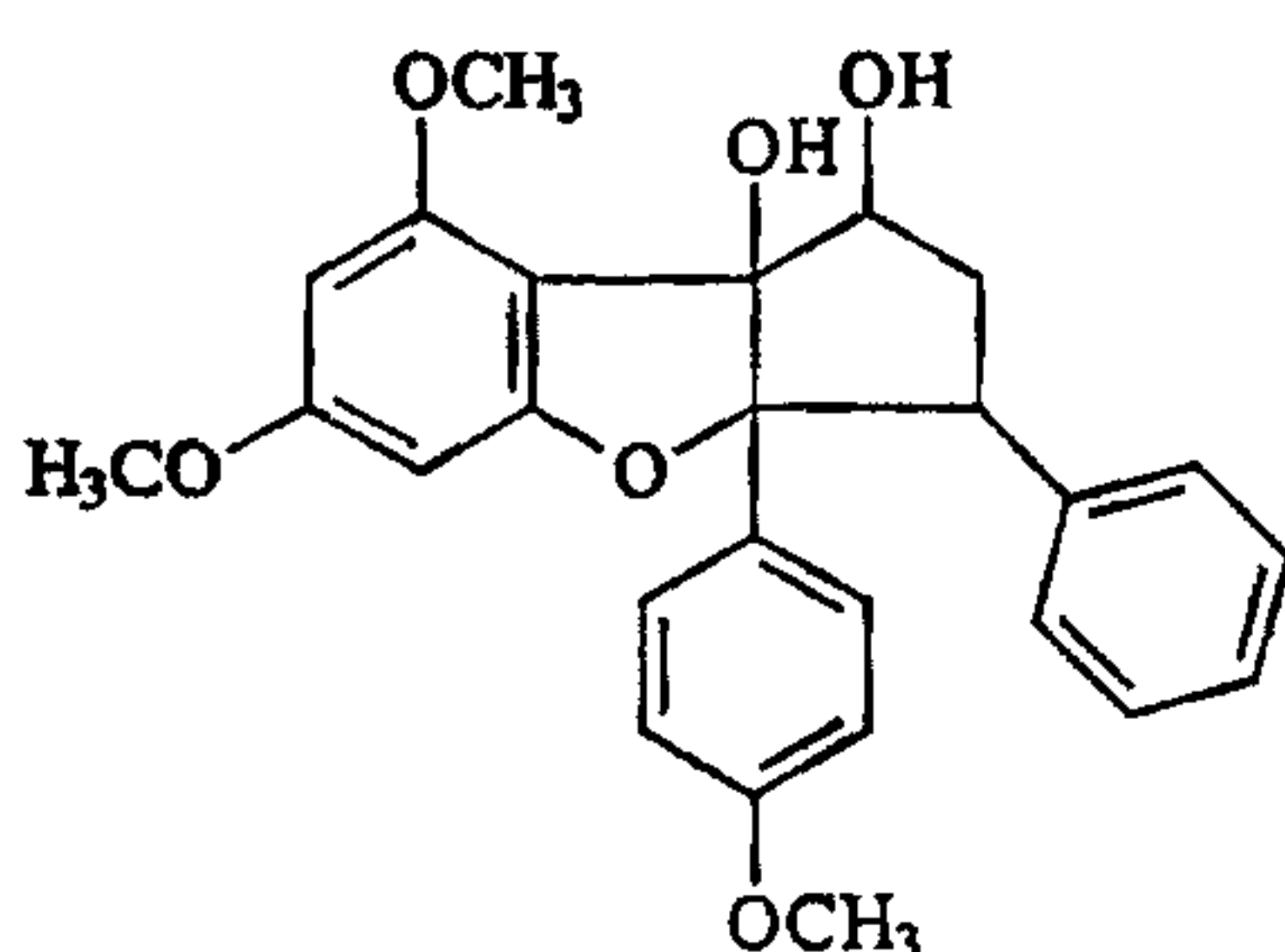
Table 5 compares the cytostatic and cytotoxic effects of Compound A to three previously identified 1H-cyclopenta[*b*]benzofuran lignans that lack the novel dioxanyloxy group. The

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reference compounds are: Rocaglaol (Reference Compound 1) (Ohse et al., *J Nat Prod*, 1996, 59(7):650-52); 4'-Demethoxy-3',4'-methylenedioxyrocaglaol (Reference Compound 2); Methyl 4'-demethoxy-3',4'-methylenedioxyrocaglate (Reference Compound 3) (Lee et al., *Chem Biol Interact*, 1998, 115(3):215-28). All four compounds exhibited detectable cytostatic activity in A549 cells with Compound A being the most potent followed in decreasing order by Reference Compounds 3, 2 and 1 respectively. Importantly, of the compounds tested, other than Compound A none of the Reference Compounds exhibited any appreciable cytotoxicity in either THP-1 or A549 cells at doses up to 5000 nM over the 3 day assay. Without intending to limit the invention by theory, it is suggested that the novel dioxanyloxy substitution is important for the cytotoxic activity exhibited by Compound A and distinguishes it from any other previously identified 1H-cyclopenta[*b*]benzofuran lignans.

Table 5: Related 1H-cyclopenta[*b*]benzofuran lignans lacking the novel dioxanyloxy side chain do not exhibit cytotoxic activity.

A549 and THP-1 cells were treated with increasing concentrations of the various compounds up to a maximum of 5×10^{-6} M (5000 nM) and the effects on cell proliferation and cell viability were determined after 3 days of culture. GI₅₀ values were determined by measuring relative changes in cell number using YOYO-1 as described for Table 3. LC₅₀ values were determined by measuring cell death as a function of loss of membrane integrity using YOYO-1 uptake (Becker et al., *Anal Biochem*, 1994, 221(1):78-84). The structures of the reference compounds are also shown.



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*Reference Compound 1**Reference Compound 2**Reference Compound 3*

Compound	A549 cells		THP-1 cells
	GI₅₀ (nM)	LC₅₀ (nM)	LC₅₀ (nM)
<i>Compound A</i>	13	514	15
<i>Reference Compound 1</i>	3980	>5000	>5000
<i>Reference Compound 2</i>	389	>5000	>5000
<i>Reference Compound 3</i>	56	>5000	>5000

Example 4***Compound A has acute protein synthesis inhibitory activity***

Compound A was also examined to determine whether it could rapidly inhibit general protein biosynthesis. Using [¹⁴C] leucine incorporation into insoluble cellular material as an assay for general protein biosynthesis, Table 6 shows that Compound A had an inhibitory effect evident within 3 hrs after addition to THP-1 cells with an IC₅₀ of ~ 30 nM. DNA synthesis measured over the same time was also inhibited, but less potently (IC₅₀ ~ 70 nM) and may be secondary to protein synthesis inhibition. Cycloheximide, a known protein synthesis inhibitor (Obrig et al, 1971, *J. Biol. Chem.* 246(1), 174-181), also inhibited both protein and DNA synthesis with Compound A being significantly more potent than cycloheximide in its effects. Table 6 shows that Compound A also inhibited general protein synthesis in A549 cells with an IC₅₀ of ~30 nM which is similar to that observed in the THP-1 cells.

Table 6: Compound A inhibits general protein biosynthesis.

THP-1 cells and A549 cells were pretreated with the indicated concentrations of Compound A for 1 hour prior to the addition of (1 µCi/mL) [¹⁴C] leucine (protein synthesis) or [¹⁴C] thymidine (DNA synthesis) for a further 2 hours. The IC₅₀ values represent the concentration of compound A required to inhibit incorporation of isotope by 50% relative to untreated control cell cultures.

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Compound	IC ₅₀ (nM)		
	THP-1 cells		A549 cells
	Protein synthesis	DNA Synthesis	Protein synthesis
Compound A	27	72	32
Cycloheximide	263	303	238

Example 5***Compound A induces differentiation of human leukemic cell lines.***

In the experiments with the THP-1 monocytic leukemia cells, which normally grow unattached in suspension, we noticed that prolonged exposure of the cells to 10 nM Compound A resulted in accumulation of cells that adhered to the plastic and exhibited numerous pseudopodia (Figure 1). This is a morphology highly characteristic of mature macrophages and similar morphological effects were observed when the cells were treated with other known inducers of macrophage differentiation including interferon- γ (IFN γ) or phorbol 12-myristate 13-acetate (PMA). To investigate this further the effects of Compound A on HL60 human promyelocytic leukemic cells (Collins, *et al*, *Nature*, 1977, 270:347-9) were examined (Table 7). This widely used cell line is well characterised as a model of human myelomonocytic differentiation (Collins, *Blood*, 1987, 70(5):1233-44). In this experiment monocytic differentiation was quantitated by measuring CD14 surface antigen expression by flow cytometric analysis. CD14, an LPS-binding protein, is expressed on the surface of cells of the myelomonocytic lineage and is normally expressed at very low levels in undifferentiated HL60 cells (Ferrero et al., *Blood*, 1983, 61(1):171-9). Consistent with the THP-1 data above, Table 7 shows that Compound A at doses greater than 10 nM significantly enhanced CD14 expression in the viable HL60 cells remaining after 4 days of culture. Taken together these data strongly indicate that Compound A has the ability to induce differentiation of human leukemic cell lines.

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Table 7: Compound A promotes monocytic differentiation of HL60 leukemic cells.

HL60 cells were cultured for 4 days with the indicated concentration of Compound A then collected and fixed in 70% ethanol. Cells were then stained with mouse monoclonal anti CD14 antibody (OKM1) and this was measured using FITC-conjugated goat anti-mouse IgG1 as a secondary antibody. Stained cells were visualised by flow cytometry and analysis was restricted to cells judged viable at the time of fixing based on their forward and side light-scatter characteristics. Non specific staining of cells was controlled for by incubating with secondary antibody only.

Compound A concentration (nM)	% cells expressing CD14
0	1.3 %
5	3.3 %
10	5.7 %
25	46.0 %
50	43.0 %

Example 6***Cytostatic activity of Compound A is associated with a general inhibition of cell cycle progression in A549 cells***

DNA content analysis of THP-1 cells treated with varying concentrations of Compound A (Figure 2) demonstrated that at 10 nM it was only weakly cytotoxic (increased accumulation of dead cells from 7% to 17%) and under these conditions caused cells to accumulate in the G0/G1 phases of the cell cycle. This indicates that Compound A also has cytostatic activity in THP-1 cells. For comparison, Figure 2 shows that the microtubule destabilising drug paclitaxel (Sorger et al., *Curr Opin Cell Biol*, 1997, 9(6):807-14) which also induced THP-1 cell death, caused cells to accumulate in the G2/M phases of the cell cycle.

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The cytostatic effect of Compound A on the proliferation of A549 cells was confirmed by directly counting the number of cells at intervals over a nine day period (Figure 3). When compared to untreated cells 10 nM Compound A prevented the increase in cell number by more than 95% with fewer than 10% dead cells observed at this time (measured by trypan blue exclusion). Thus, under these conditions the decreased cell number can not simply be accounted for by increased cell death. A significant inhibition of cell number was seen within 2 days indicating that Compound A acts in a rapid manner. At the higher concentrations of 50 nM and 250 nM Compound A had cytotoxic effects and increased cell death to 86% and 100% respectively after 9 days and accounts for the decline in cell number to levels below the original starting number at this time. At the non-cytotoxic concentration of 10 nM, Compound A has a rapid and potent cytostatic effect on A549 cells.

To help identify a potential mechanism for the effects of Compound A, DNA content analysis was performed to determine where in the cell cycle it exerted its effect (Figure 4). Cell cycle analysis of A549 cells treated with Compound A for 6 days showed that at 10 nM, where no obvious cytotoxicity was evident, there was a minor decline in the proportion of cells in the G0/G1 phases of the cell cycle with a concomitant increase in cells in the G2/M phases. Taken together with the growth curve data in Figure 3 above, these data indicate that 10 nM Compound A results in a general lengthening of all phases of the cell cycle with perhaps a slightly more pronounced elongation of the G2/M phases. This contrasts to the effects of paclitaxel a drug known to act selectively at the G2/M phases of the cell cycle (Figure 4). As the concentration of Compound A was increased and its cytotoxic effects became evident the proportion of cells in the S and G2/M phases decreased with a corresponding rise in cells in G0/G1 phases. Although there was little difference in the number of dead cells between 50 nM and 250 nM the higher dose resulted in a greater accumulation of cells in the G0/G1 phases of the cell cycle. Thus, compared to THP-1 cells (see Figure 2) higher concentrations of Compound A are required to inhibit progression through the G0/G1 phases of the cell cycle in A549 cells.

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K562 leukemic cells treated with 10-15 nM Compounds A or B exhibited a characteristic accumulation of cells in G2/M phases of the cell cycle (Figure 5). This occurred over a narrow range of concentrations since Compounds A or B at less than 5-8 nM or more than 25 nM did not cause a G2/M phase accumulation. These data indicate that different cell lines can vary in their sensitivity and responses to Compounds A and B for cell cycle phase-specific effects.

Example 7

The cytostatic effect of Compound A is reversible in A549 cells

The reversibility of the effects of Compound A were determined. For this, A549 cells remained untreated or were cultured in the presence of various concentrations of Compound A or with paclitaxel for 5 days prior to removal of the compounds and the cells cultured for a further 4 days prior to determining cell number (Figure 6). 10 nM of Compound A significantly suppressed the increased cell number for up to 9 days without significant cytotoxicity. However, for these cultures when Compound A was removed after 5 days there was over a five-fold increase in cell number over the subsequent 4 days of culture, representing 2 –3 population doublings. The effects of treatments which were deleterious to the cells, such as higher concentrations of Compound A or the presence of paclitaxel, were not reversed upon their removal.

Example 8

Compound A inhibits cell cycle-dependent cytotoxicity elicited by various anti-cancer agents

To further examine the cell cycle effects of Compound a cytostatic concentration of this compound was combined together with other anti-cancer agents known to act at specific points in the cell cycle to see if Compound A could perturb their cell cycle-dependent effects. Cell viability was assayed after 3 days by measuring exclusion of the fluorescent DNA-binding dye YOYO-1. (Becker et al., *Anal Biochem*, 1994, 221(1):78-84). A549 cells were treated with 10 nM non-cytotoxic dose of Compound A in the presence of increasing

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concentrations of camptothecin and paclitaxel. Camptothecin is an inhibitor of DNA topoisomerase 1, an enzyme required for DNA replication, and results in perturbation of the S phase of the cell cycle with subsequent cell death due to activation of an S phase checkpoint (Darzynkiewicz et al., *Ann N Y Acad Sci*, 1996, 803:93-100). Paclitaxel, as already mentioned, inhibits microtubule function required for formation of the mitotic spindle thereby resulting in activation of an M phase checkpoint and subsequent cell death (Sorger et al., *Curr Opin Cell Biol*, 1997 9(6):807-14). Figure 7 shows that 10 nM Compound A significantly reduced the cytotoxic effects of both camptothecin and paclitaxel even when these drugs were added at up to a 2000-fold excess. Compound A may, in a dominant manner, prevent the cell cycle-dependent cytotoxic effects of camptothecin and paclitaxel.

This was examined in more detail using DNA content analysis to specifically measure cell cycle progression and cell death. In this experiment in addition to camptothecin and paclitaxel cells were also treated with vinblastin (another microtubule inhibitor) (Sorger et al., 1997, *supra*) and staurosporine (a kinase inhibitor) (Gescher, *Crit Rev Oncol Hematol.*, 2000, 34(2):127-35). As previously found, A549 cells treated with 10 nM Compound A showed a minor decrease in cells in G0/G1 with a slight increase in G2/M phase cells with no detectable increase in cell death over the three days of culture (Figure 8). Consistent with its known mechanism of action camptothecin resulted in accumulation of cells in S phase of the cell cycle and also increased the level of dead cells detected as those with a sub-diploid DNA content. Also as expected, both vinblastin and paclitaxel resulted in the majority of cells arresting in the G2/M phases of the cell cycle and increased appearance of sub-diploid dead cells. However, for all of these agents the presence of 10 nM Compound A prevented their characteristic cell cycle arrest and significantly inhibited their cytotoxic effects, dramatically reducing the appearance of sub-diploid dead cells. In contrast, Compound A had little effect on the cytotoxic effects of staurosporine, an agent which appears capable of killing cells at all active phases of the cell cycle.

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Example 9***Cytostatic effects of Compound A do not correlate with a biomarker for replicative senescence.***

The dramatically decreased growth rate of A549 cells cultured in the presence of 10 nM Compound A (see Figure 3) led to the consideration of the possibility that this compound was inducing replicative senescence of these immortal tumour cells. Consistent with this possibility under these conditions A549 cells with a morphology highly suggestive of a senescent phenotype were often observed, being highly flattened with an enlarged surface area compared to their usual appearance (compare for example Figures 9 subpanels a and b). This was evaluated further by measuring senescence-associated β -galactosidase (SA- β -gal) activity, a biomarker previously described to correlate well with senescence of human cells (Dimri et al., *Proc Natl Acad Sci USA* 1995 92(20):9363-7). Recently, it has been found that some anti-cancer agents that act by diverse mechanisms, including doxorubicin, cisplatin, cytarabine, etoposide and paclitaxel, can all induce SA- β -gal activity in a variety of tumour cell lines (Chang et al., *Cancer Res* 1999, 59(15):3761-7). Therefore, in addition to Compound A A549 cells were also treated with doxorubicin as an experimental control. This drug acts by stabilising DNA/topoisomerase II complexes thereby causing DNA damage which results in subsequent S phase cell cycle arrest and/or cell death (Froelich-Ammon and Osheroff, 1995, *J. Biol. Chem.* 270(37):21429-21432). Figure 9 shows that consistent with the earlier report A549 cells treated with 250 nM doxorubicin displayed the flattened enlarged phenotype of senescent cells and exhibited SA- β -gal activity. In contrast, Compound A at various doses from 10-50 nM failed to induce SA- β -gal activity even though the cells exhibited the flattened enlarged morphology. Thus, in contrast to a variety of other anti-cancer drugs the cytostatic effects of Compound A do not correlate with this particular marker of cell senescence.

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Example 10***Compound A inhibits cell proliferation but not increased cell size***

It is well known that cell proliferation and cell growth reflected as increased mass of individual cells are biochemically separable processes (Pardee, *Science*, 1989, 246:603-8). Although at certain concentrations Compound A can inhibit cell proliferation without overt cytotoxicity it was also evaluated whether Compound A also affected cell growth. For these experiments A549 cells were treated with various non-cytotoxic doses of Compound A up to 10 nM and the relative cell size determined after 6 days of culture by measuring forward light scatter using a flow cytometer. The data depicted in Table 8 show that in the presence of Compound A A549 cells exhibited an increase in the mean forward scatter by over 20%. This occurred only at concentrations which are cytostatic for this cell type.

Table 8: Compound A increases cell size

A549 cells cultured for six days with the various non-cytotoxic concentrations of Compound A as indicated were examined by flow cytometry for their forward light scatter characteristics which directly relates to cell size. The % increase in mean cell volume represents the relative change in the mean forward scatter value for the treated versus untreated cell populations.

Compound A concentration (nM)	% increase in mean cell volume
0	-
2.5	10.4 %
5.0	10.7 %
10.0	22.4 %

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Example 11***Compound A inhibits growth of human tumour cell lines in a mouse xenograft tumour model.***

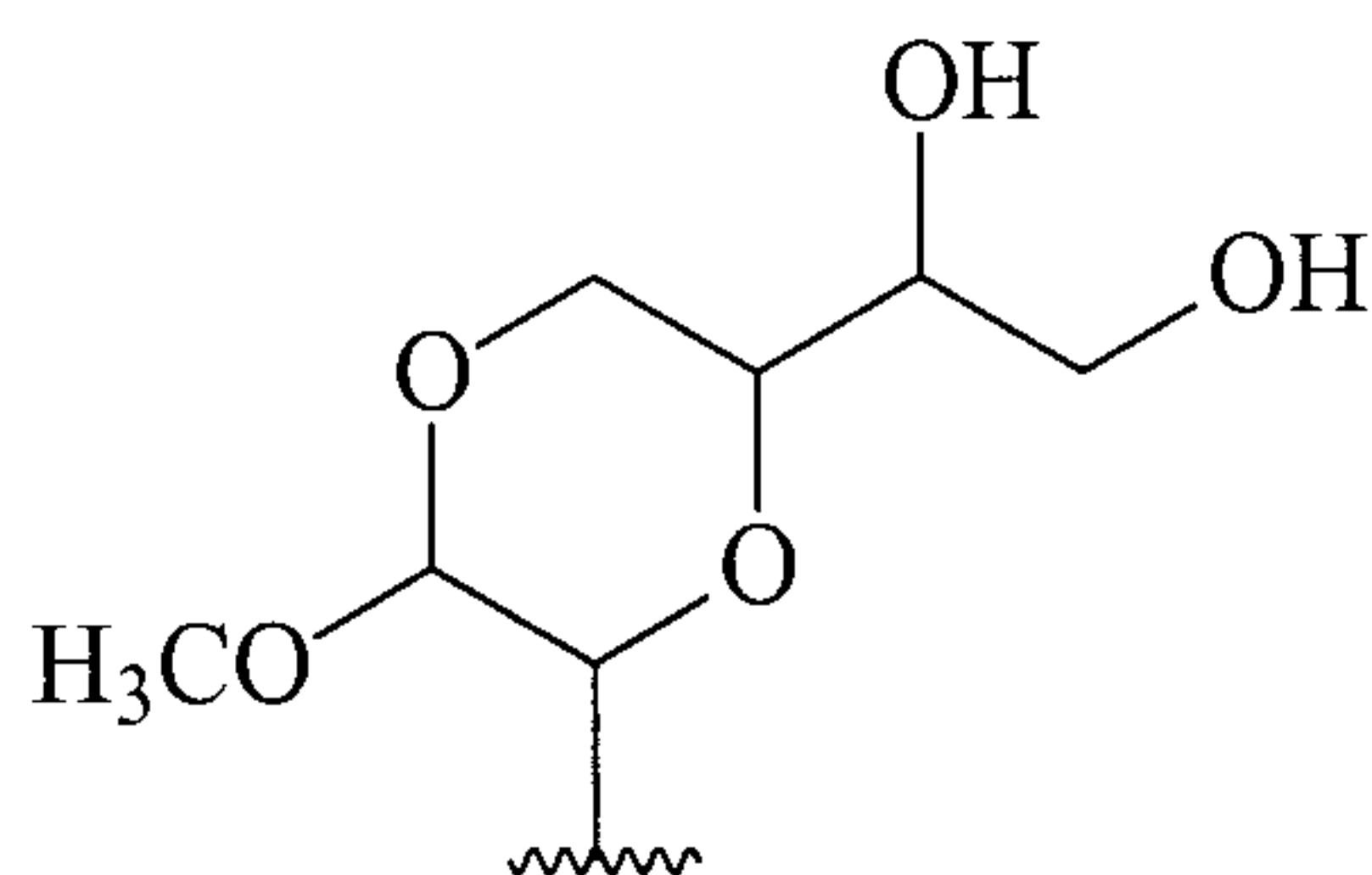
The ability of Compound A to inhibit growth of human tumour cells *in vivo* was assessed using male athymic mice injected subcutaneously in the dorsal flank region with 2×10^6 PC3 human prostate tumour cells. Compound A administration (3 mg/kg) by intraperitoneal injection commenced after eight days once the PC3 tumour was palpable and continued three times a week until 29 days after the initial inoculation of the tumour cells. At this time all mice were killed and tumours excised and weighed. Figure 10A shows that compared to the control animals treated with vehicle alone the mice treated with Compound A displayed a greatly reduced increase in mean tumour volume over the course of the experiment. This was confirmed at the end of the experiment when tumours were excised and weighed it was found that Compound A treatment reduced the mean tumour weight by ~ 60% (Figure 10B). Body weight was unaffected with both control and treated groups exhibiting a similar ~12% decrease in mean body weight over the duration of the experiment. Thus, Compound A exhibits *in vivo* antitumour activity.

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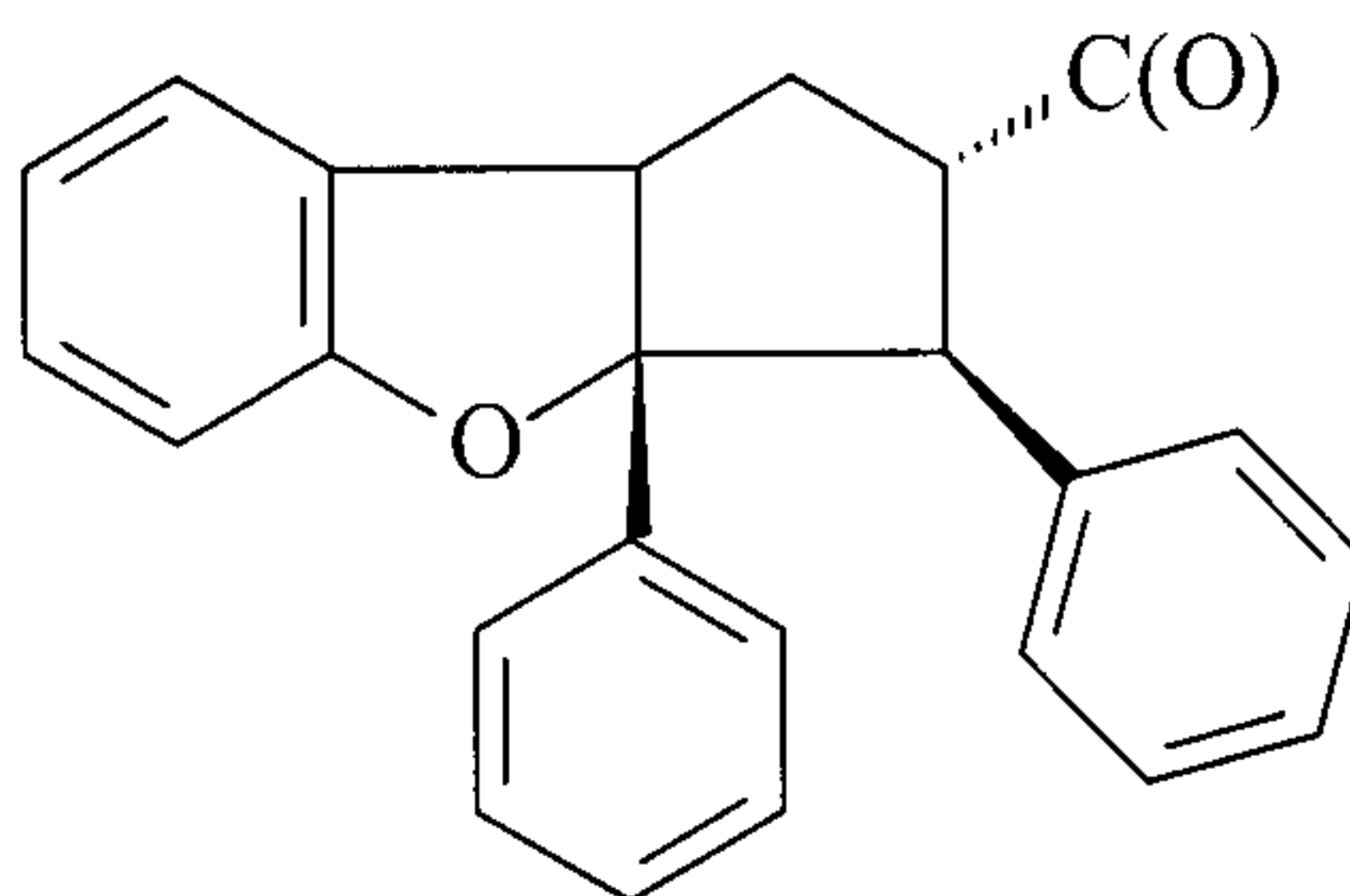
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CLAIMS:

1. A cyclopentabenzofuran compound comprising a group of formula (ii)



5 attached, via an oxygen atom to a cyclopentabenzofuran core of formula (i)



and having the following NMR spectral characteristics:

10 ^1H NMR (CDCl_3) (ppm)

3.49, s, 3H; 3.56, dd, 11.7, 2Hz, 1H; 3.61, m, 1H, 3.61, 2H; 3.65, s, 3H; 3.71, s, 3H; 3.87, s, 3H; 3.89, dd, 14.2, 6.7 Hz, 1H; 4.13, t, 11.2 Hz, 1H; 4.23, brt, 11.3 Hz, 1H; 4.28, d, 14.2 Hz, 1H; 4.59, s, 1H; 5.03, d, 6.7 Hz, 1H; 15 5.28, s, 1H; 6.28, d, 2Hz, 1H; 6.43, d, 2Hz, 1H; 6.68, brd, 9Hz, 2H; 6.84, m, 2H; 7.06, m, 2H; 7.06, m, 1H; 7.10, brd, 9Hz, 2H;

^{13}C NMR (CDCl_3) (ppm)

50.03, 52.06, 55.03, 55.05, 55.1, 55.9, 59, 63.3, 20 68.3, 70.6, 79.6, 92.8, 93.4, 93.9, 94, 95.2, 101.9, 109.6,

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112.7, 126.2, 126.6, 127.8. 127.8, 128.9, 136.7, 157.1,
158.8, 160, 160.6, 170.6;

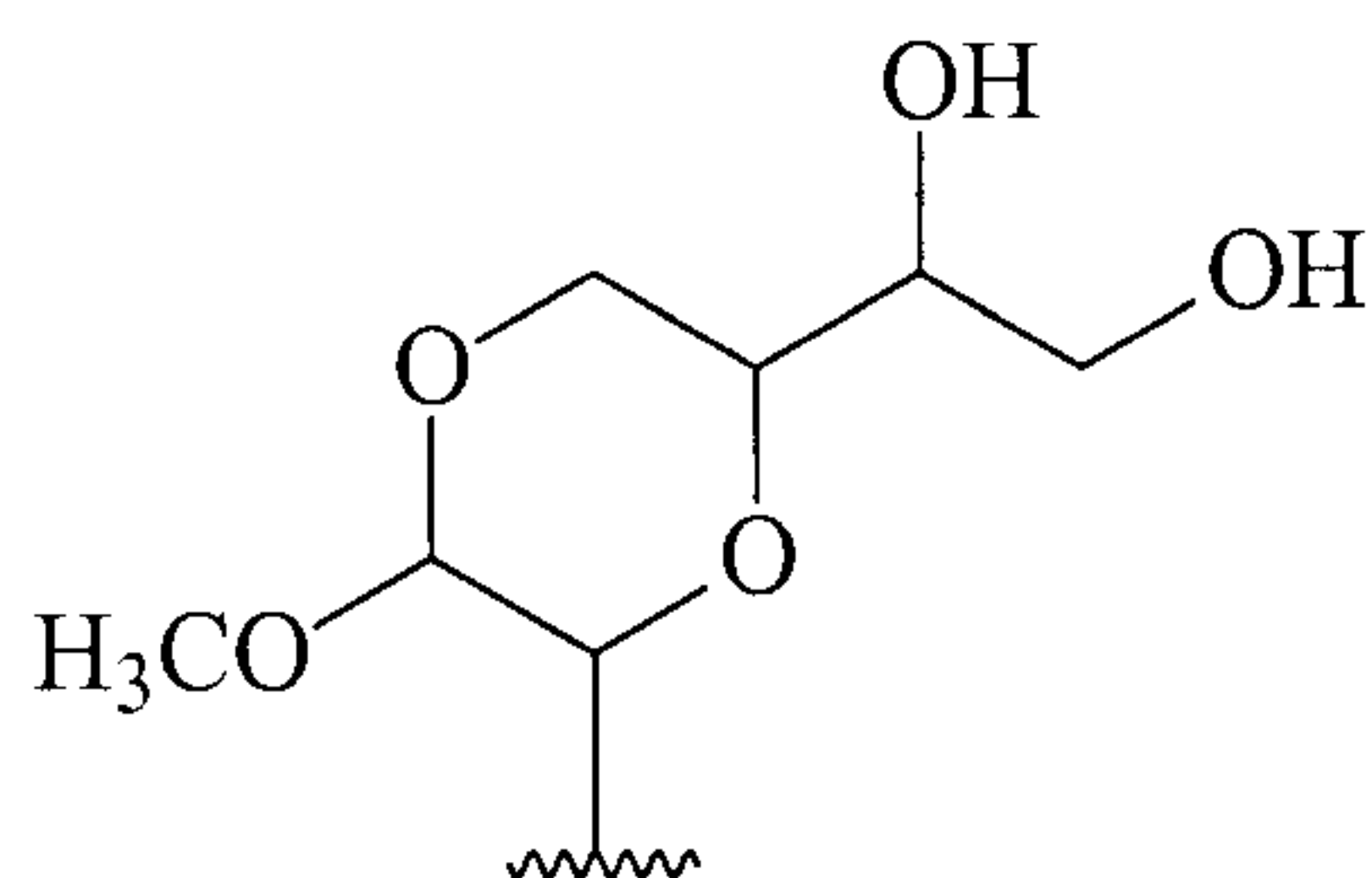
or a salt, derivative or prodrug thereof, wherein
the prodrug or derivative is an ester, glycoside, optionally
5 substituted alkyloxy, optionally substituted cyclic alkoxy,
optionally substituted alkenyloxy, optionally substituted
cyclic alkenyloxy, optionally substituted alkynyloxy,
optionally substituted cyclic alkynyloxy, optionally
substituted acyloxy of formula -O-C(O)-R, where R is an
10 alkyl, cyclic alkyl, alkenyl, cyclic alkenyl, alkynyl,
cyclic alkynyl, heteroaryl or aryl group, or optionally
substituted aryloxy or optionally substituted heteroaryloxy;

and the optional substituents for said optionally
substituted alkyloxy, optionally substituted cyclic alkoxy,
15 optionally substituted alkenyloxy, optionally substituted
cyclic alkenyloxy, optionally substituted alkynyloxy,
optionally substituted cyclic alkynyloxy and optionally
substituted acyloxy are selected from one or more of halo,
hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino, C₁₋₆alkylamino,
20 C₁₋₆dialkylamino, halomethyl, halomethoxy, acetyl, aryl,
heteroaryl and a cyclic alkyl group and the optional
substituents for said optionally substituted aryloxy and
optionally substituted heteroaryloxy are selected from one
or more of halo, hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino,
25 C₁₋₆alkylamino, C₁₋₆dialkylamino, halomethyl, halomethoxy, and
acetyl.

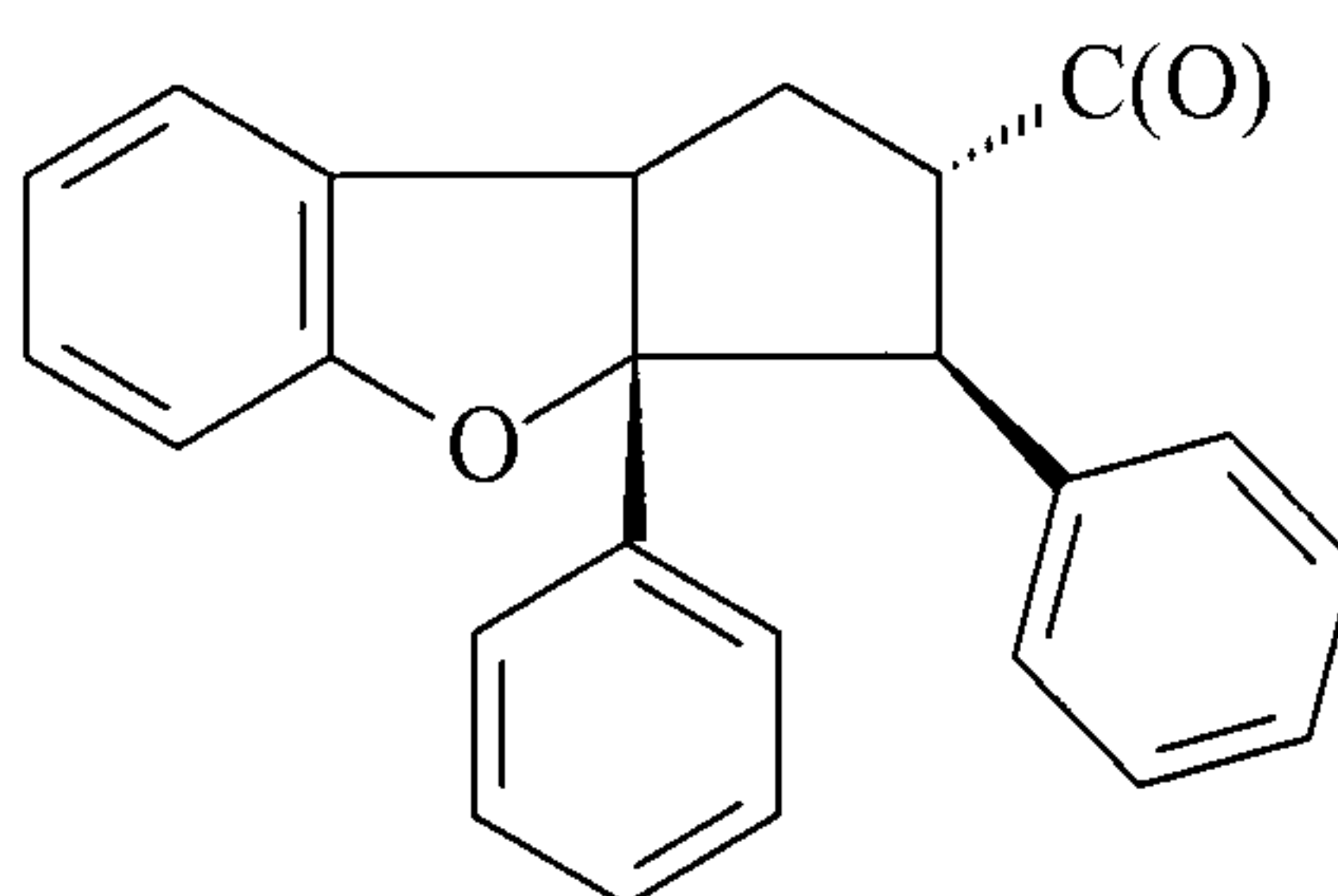
2. A cyclopentabenzofuran compound comprising a group
of formula (ii)

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attached, via an oxygen atom to a
cyclopentabenzofuran core of formula (i)



5 and having the following NMR spectral
characteristics:

^1H NMR (CDCl_3) (ppm)

3.5, s, 3H; 3.61, dd, 10.4, 4.4Hz, 1H; 3.66, m,
1H; 3.66, s, 3H; 3.72, m; 3.72, s, 3H; 3.78, dd, 11.7,
10 2.4 Hz, 1H; 3.86, s, 3H; 3.9, dd, 14, 6.8 Hz, 1H; 4.02, t,
11.2 Hz, 1H; 4.12, ddd, 11, 6.8, 2-8Hz, 1H; 4.28, d, 14Hz,
1H; 4.60, s, 1H; 5.04, d, 6.8Hz, 1H; 5.26, s, 1H; 6.29, d,
2Hz, 1H; 6.45, d, 2Hz, 1H; 6.69, brd, 9Hz, 2H; 6.86, m, 2H;
7.06, m, 2H; 7.06, m, 1H; 7.10, brd, 9Hz, 2H;

15 ^{13}C NMR (CDCl_3) (ppm)

50, 52, 55, 55, 55, 55.8, 59.6, 62.5, 67.6, 71.4,
79.6, 92.8, 93.4, 94.3, 95.2, 101.8, 109.4, 112.8, 126.2,
126.6, 127.5, 127.5, 128.9, 136.6, 157.1, 158.8, 159.8,
160.2, 170.7;

20 or a pharmaceutically acceptable salt, derivative
or prodrug thereof, wherein the prodrug or derivative is an

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ester, glycoside, optionally substituted alkyloxy, optionally substituted cyclic alkoxy, optionally substituted alkenyloxy, optionally substituted cyclicalkenyloxy, optionally substituted alkynyloxy, optionally substituted cyclicalkenynyloxy, optionally substituted acyloxy of formula -O-C(O)-R, where R is an alkyl, cyclic alkyl, alkenyl, cyclic alkenyl, alkynyl, cyclic alkynyl, heteroaryl or aryl group, or optionally substituted aryloxy or optionally substituted heteroaryloxy;

10 and the optional substituents for said optionally substituted alkyloxy, optionally substituted cyclic alkoxy, optionally substituted alkenyloxy, optionally substituted cyclic alkenyloxy, optionally substituted alkynyloxy, optionally substituted cyclic alkynyloxy and optionally substituted acyloxy are selected from one or more of halo, 15 hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino, C₁₋₆alkylamino, C₁₋₆dialkylamino, halomethyl, halomethoxy, acetyl, aryl, heteroaryl and a cyclic alkyl group and the optional substituents for said optionally substituted aryloxy and 20 optionally substituted heteroaryloxy are selected from one or more of halo, hydroxy, C₁₋₆alkyl, C₁₋₆alkoxy, nitro, amino, C₁₋₆alkylamino, C₁₋₆dialkylamino, halomethyl, halomethoxy, and acetyl.

3. A pharmaceutical composition comprising a 25 compound, salt, derivative or prodrug as defined in claim 1 or 2, and a pharmaceutically acceptable carrier; excipient or diluent.

4. A use of a compound, salt, derivative or prodrug as defined in claim 1 or 2, in manufacture of a medicament 30 for treatment of cancer or a cancerous condition.

5. A use according to claim 4, wherein the cancer or cancerous condition is selected from leukemia; breast,

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colon, uterus, prostate, brain, lung, ovarian, skin, liver and bowel and stomach cancers; tumours; and melanomas.

6. A use of a compound, salt, derivative or prodrug as defined in claim 1 or 2, in manufacture of a medicament
5 for treatment of a disease state or condition associated with cellular hyperproliferation.

7. A use according to claim 6, wherein the disease state or condition is atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory
10 arthritis, psoriasis, periodontal disease or virally induced cellular hyperproliferation.

8. A use of a compound, salt, derivative or prodrug as defined in claim 1 or 2, for treatment of cancer or a cancerous condition.

15 9. A use according to claim 8, wherein the cancer or cancerous condition is selected from leukemia; breast, colon, uterus, prostate, brain, lung, ovarian, skin, liver and bowel and stomach cancers; tumours; and melanomas.

10. A use of a compound, salt, derivative or prodrug
20 as defined in claim 1 or 2, for treatment of a disease state or condition associated with cellular hyperproliferation.

11. A use according to claim 10, wherein the disease state or condition is atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory
25 arthritis, psoriasis, periodontal disease or virally induced cellular hyperproliferation.

12. A compound, salt, derivative or prodrug as defined in claim 1 or 2, for treatment of cancer or a cancerous condition.

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13. A compound, salt, derivative or prodrug according to claim 12, wherein the cancer or cancerous condition is selected from leukemia; breast, colon, uterus, prostate, brain, lung, ovarian, skin, liver and bowel and stomach
5 cancers; tumours; and melanomas.

14. A compound, salt, derivative or prodrug as defined in claim 1 or 2, for treatment of a disease state or condition associated with cellular hyperproliferation.

15 15. A compound, salt, derivative or prodrug according to claim 14, wherein the disease state or condition is atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, psoriasis, peridontal disease or virally induced cellular
hyperproliferation.

15 16. A pharmaceutical composition according to claim 3 for treatment of cancer or a cancerous condition.

17. A pharmaceutical composition according to claim 16, wherein the cancer or the cancerous condition is selected from leukemia; breast, colon, uterus, prostate,
20 brain, lung, ovarian, skin, liver and bowel and stomach cancers; tumours; and melanomas.

18. A pharmaceutical composition according to claim 3 for treatment of a disease state or condition associated with hyperproliferation.

25 19. A pharmaceutical composition according to claim 18, wherein the disease state or condition is atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, psoriasis, peridontal disease or virally induced cellular
30 hyperproliferation.

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20. A cyclopentabenzofuran compound as defined in claim 1 or 2 obtained by:

(a) treating a sample of ground bark from tree species *Aglaia leptantha* with methanol;

5 (b) filtering the resulting extract to form a solution and concentrating the resulting solution under vacuum;

(c) fractionating the resulting extract via solid-phase extraction on a C-18 Varian extraction column
10 (10g) using 0.1% formic acid in acetonitrile/water with increasing acetonitrile concentrations;

(d) collecting the eluate obtained in step (c) with an acetonitrile/water ratio of 7:20;

(e) concentrating the fraction from (d);

15 (f) chromatographing the concentrate obtained under step (e) on a C-18 preparative column at a flow rate of 20ml/min using a linear gradient of from 25% to 45% acetonitrile in water in 30 minutes with 0.1% formic acid;

(g) collecting and concentrating the eluates with
20 UV absorption maxima at 200 and 273 nm and HPLC retention times of approximately 30.67 and 31.05 minutes;

(h) chromatographing each eluate obtained in (g) on a Sephadex LH20 column using methanol as a solvent; collecting and concentrating the fractions obtained with
25 spectral characteristics outlined in step (g).

Figure 1

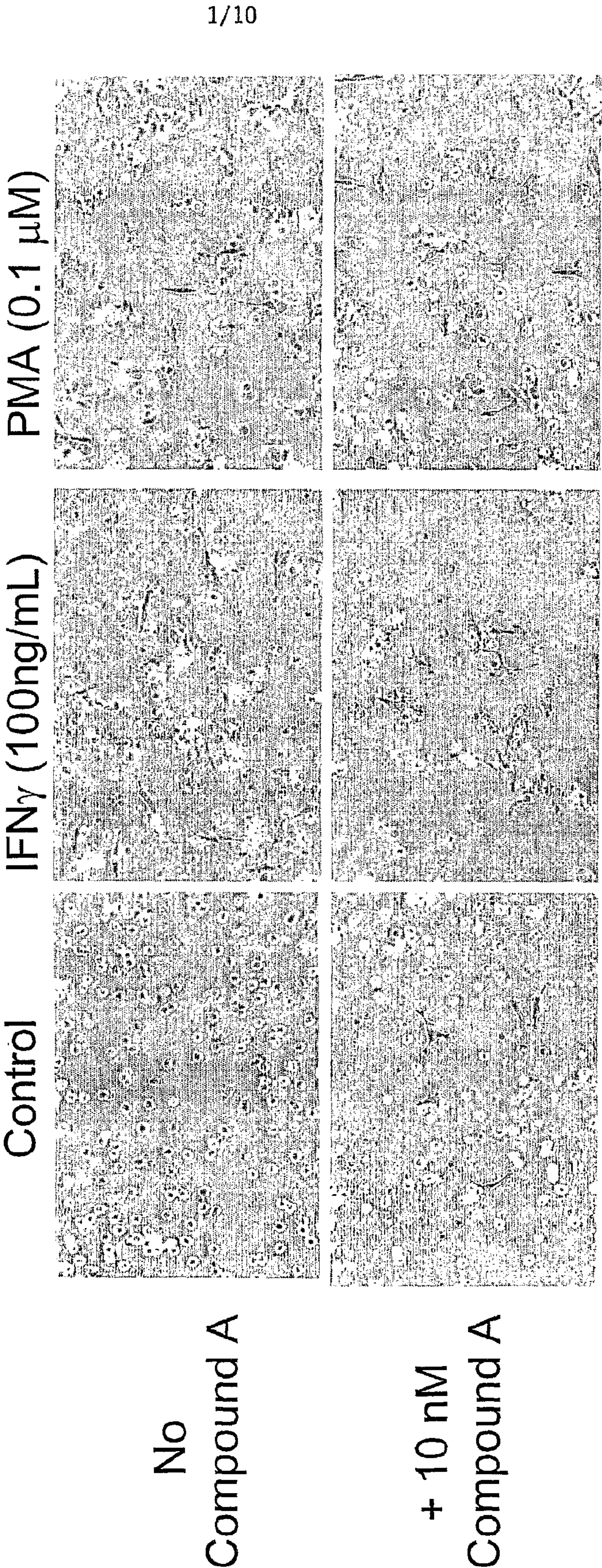
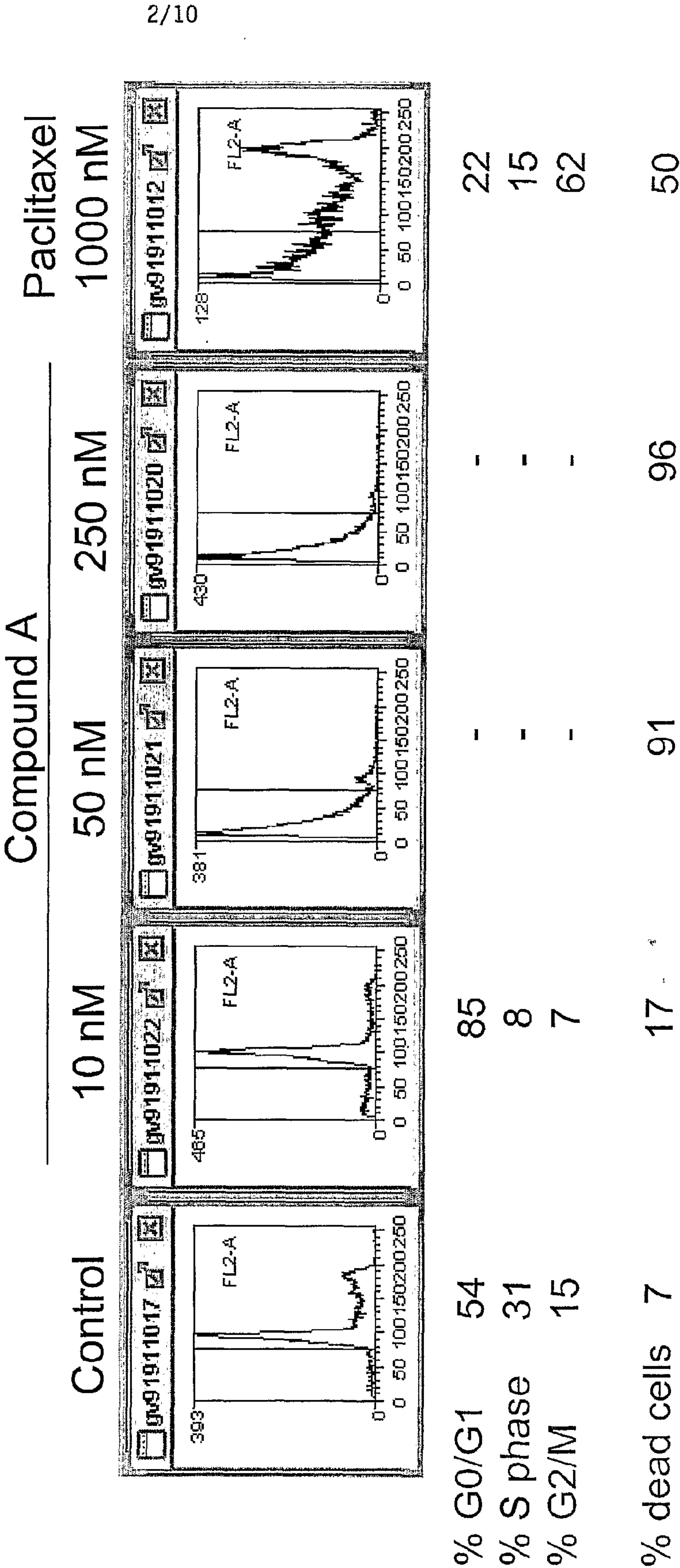


Figure 2



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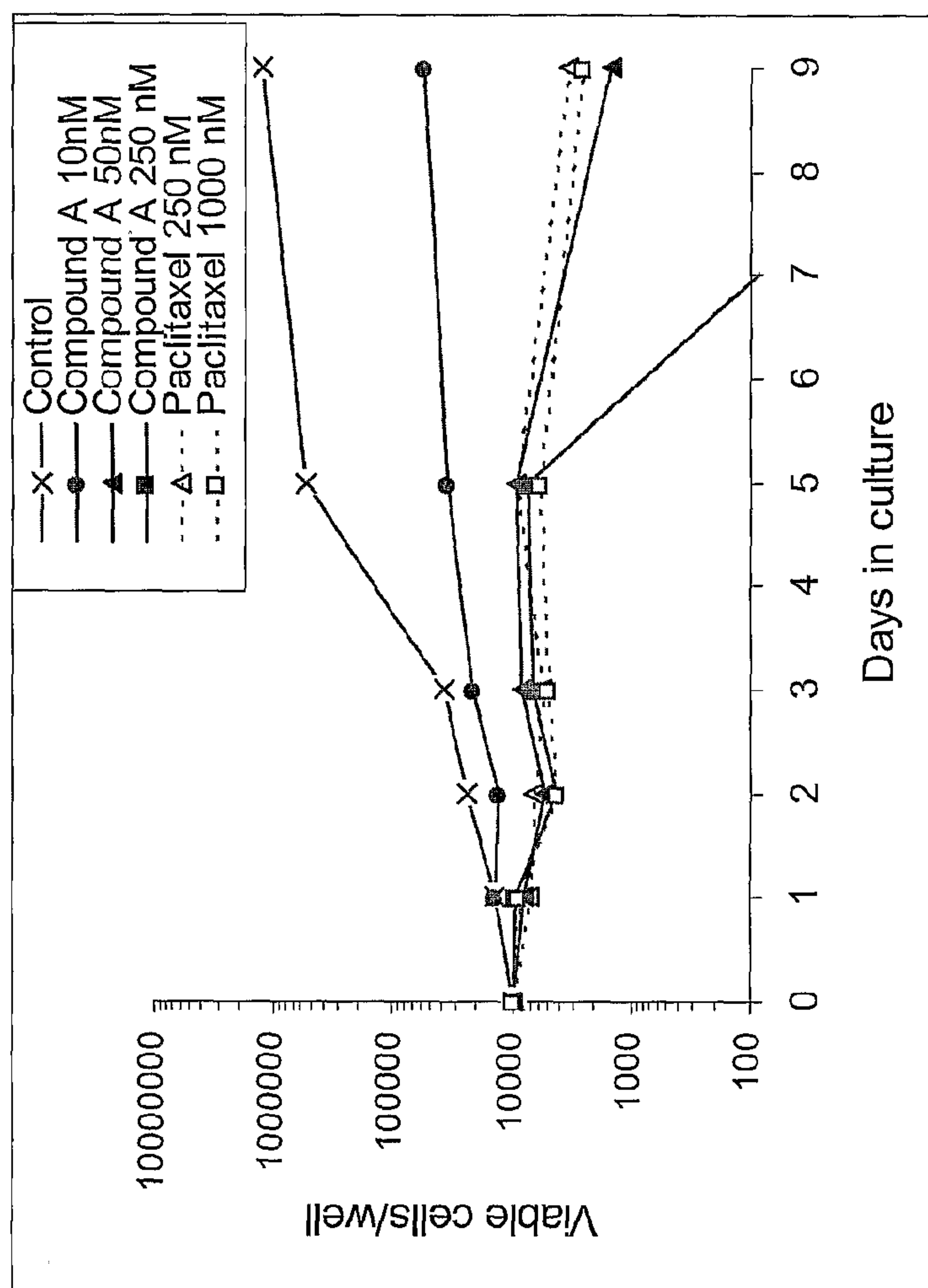
Figure 3

Figure 4

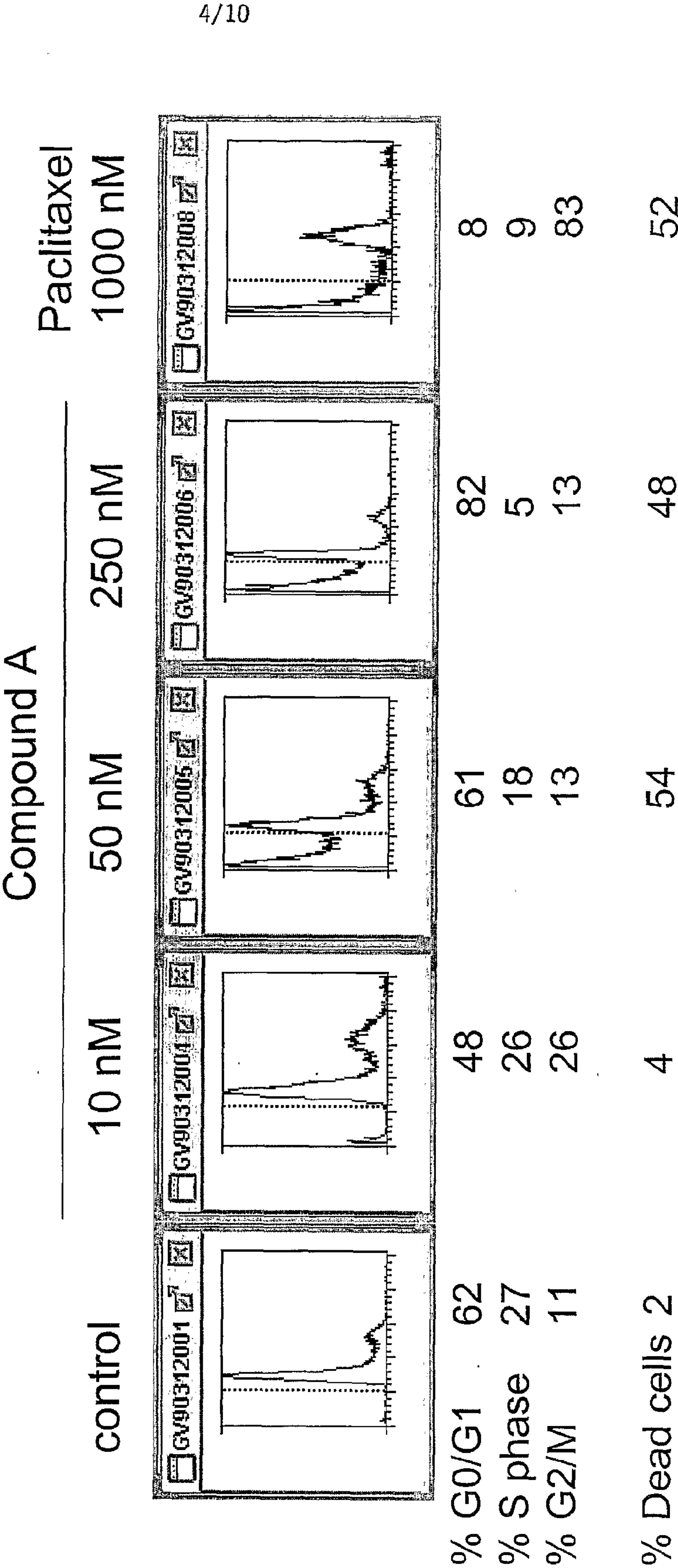
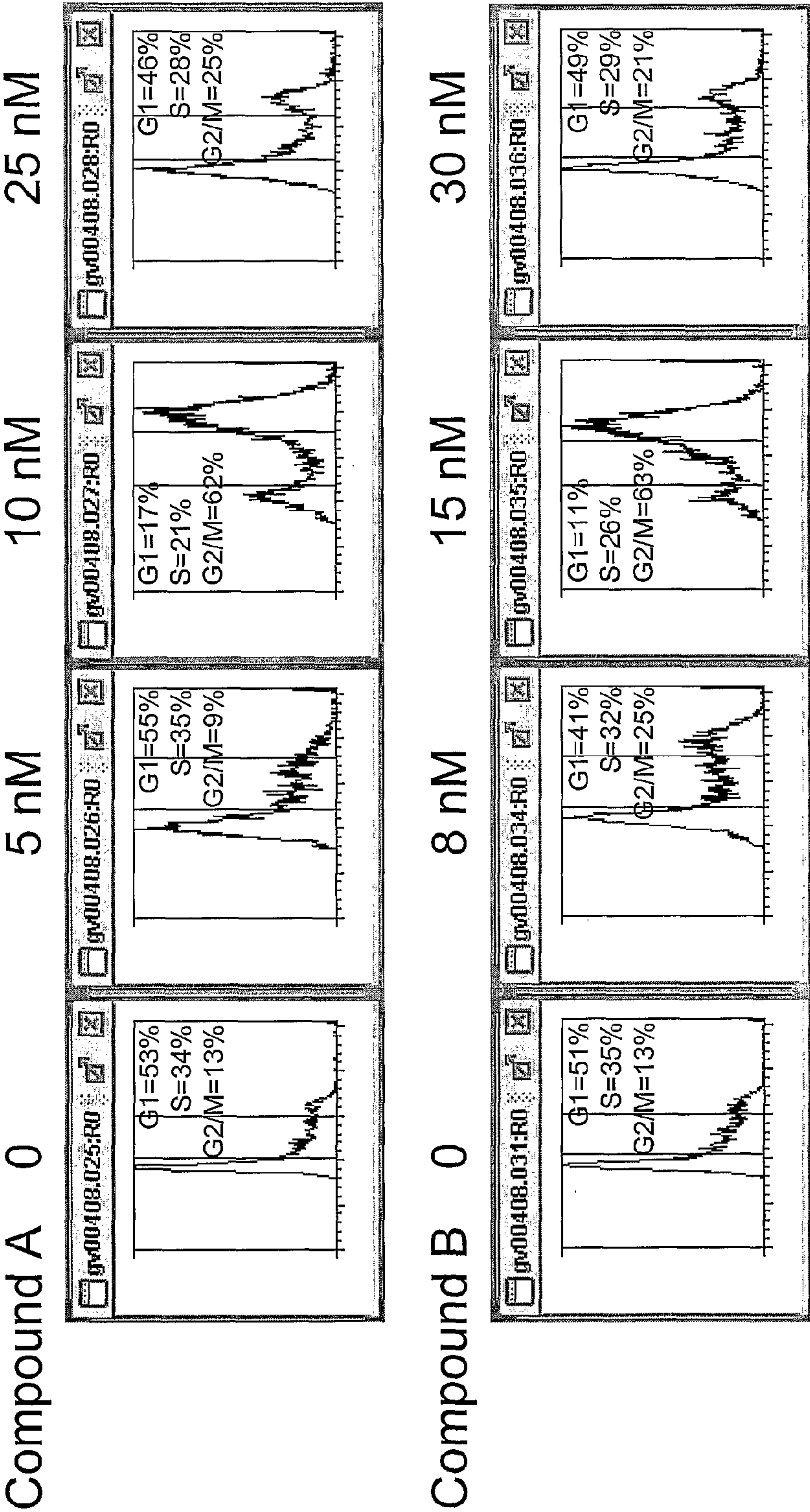
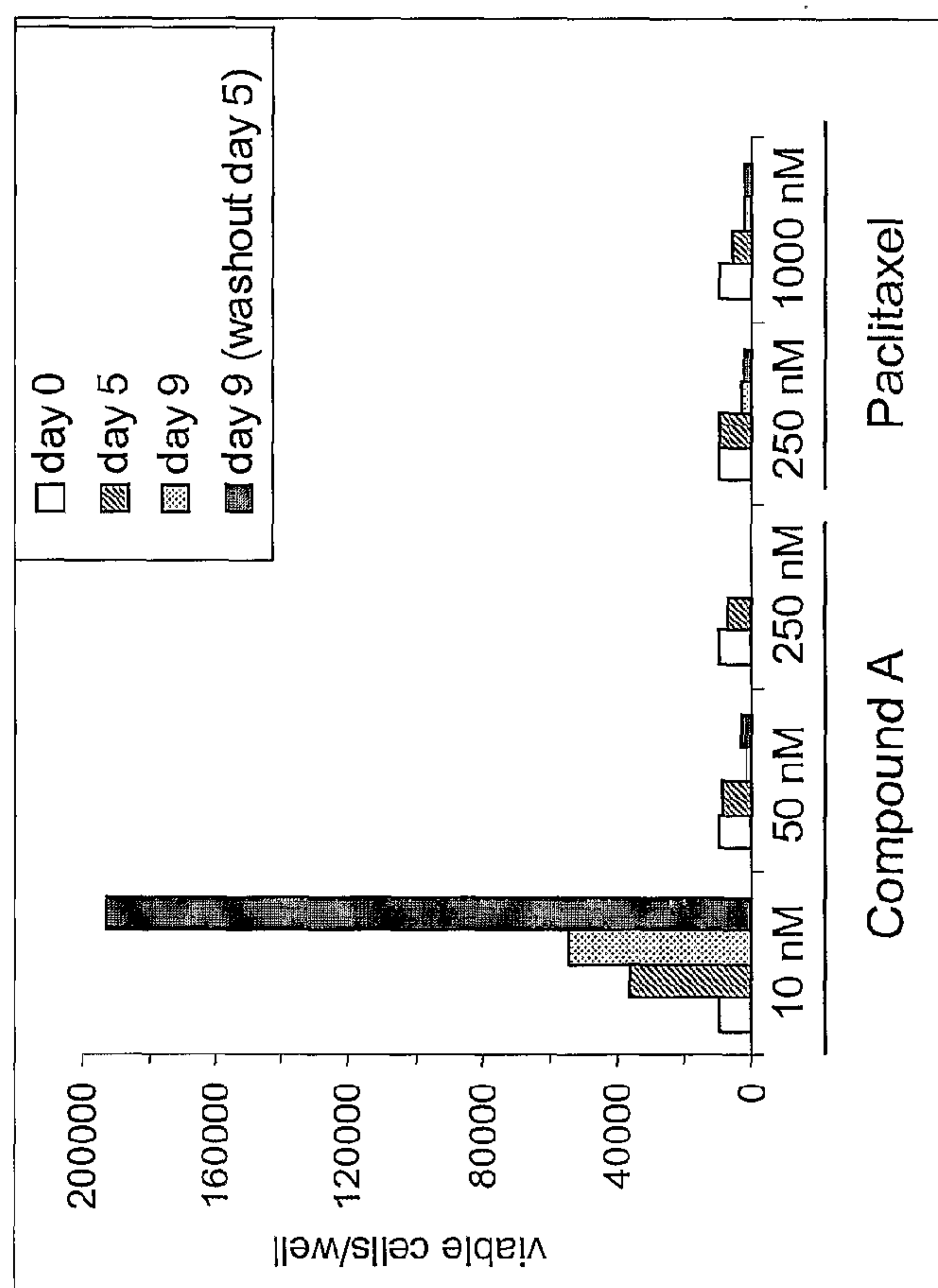


Figure 5



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Figure 6

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Figure 7

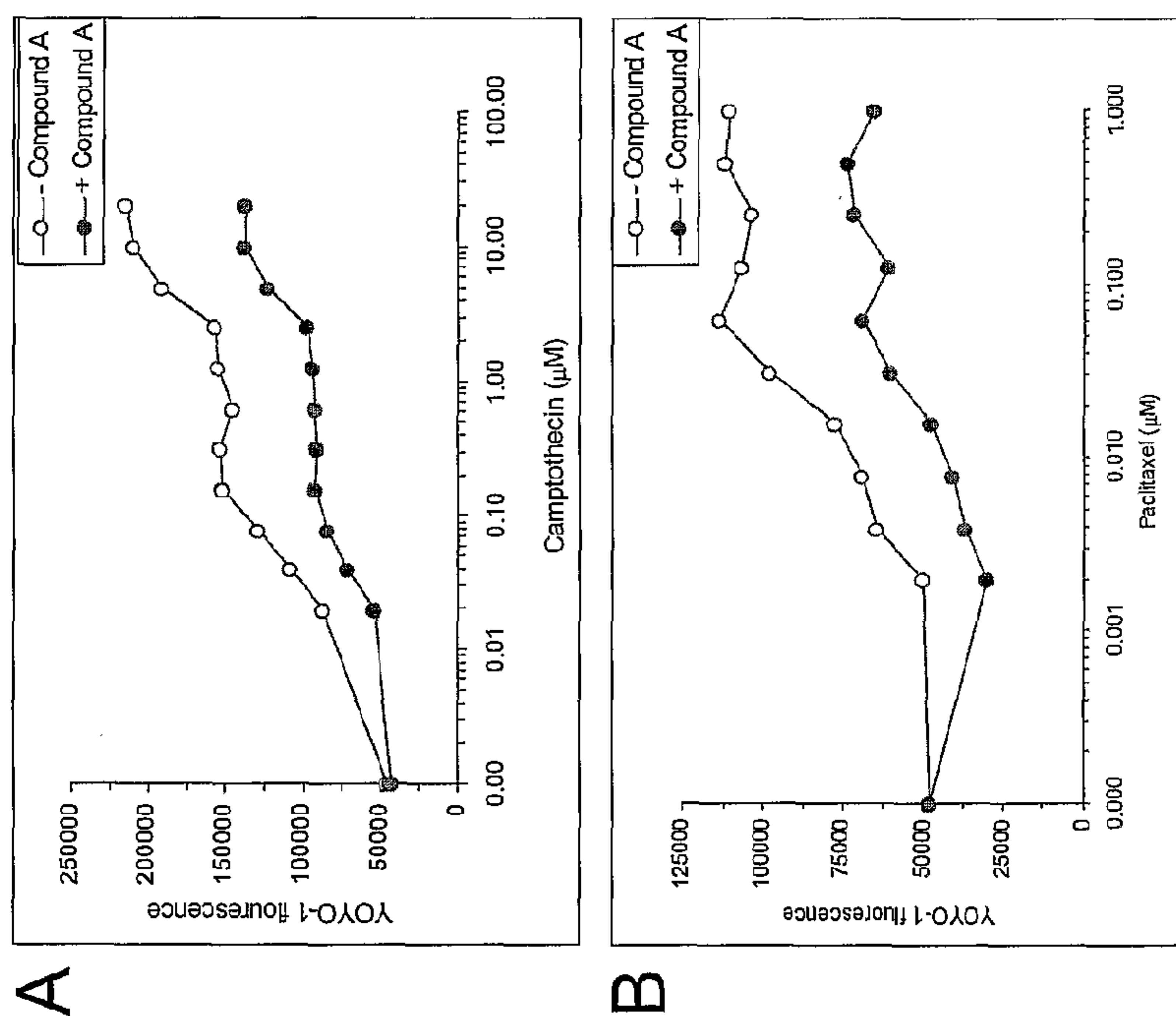
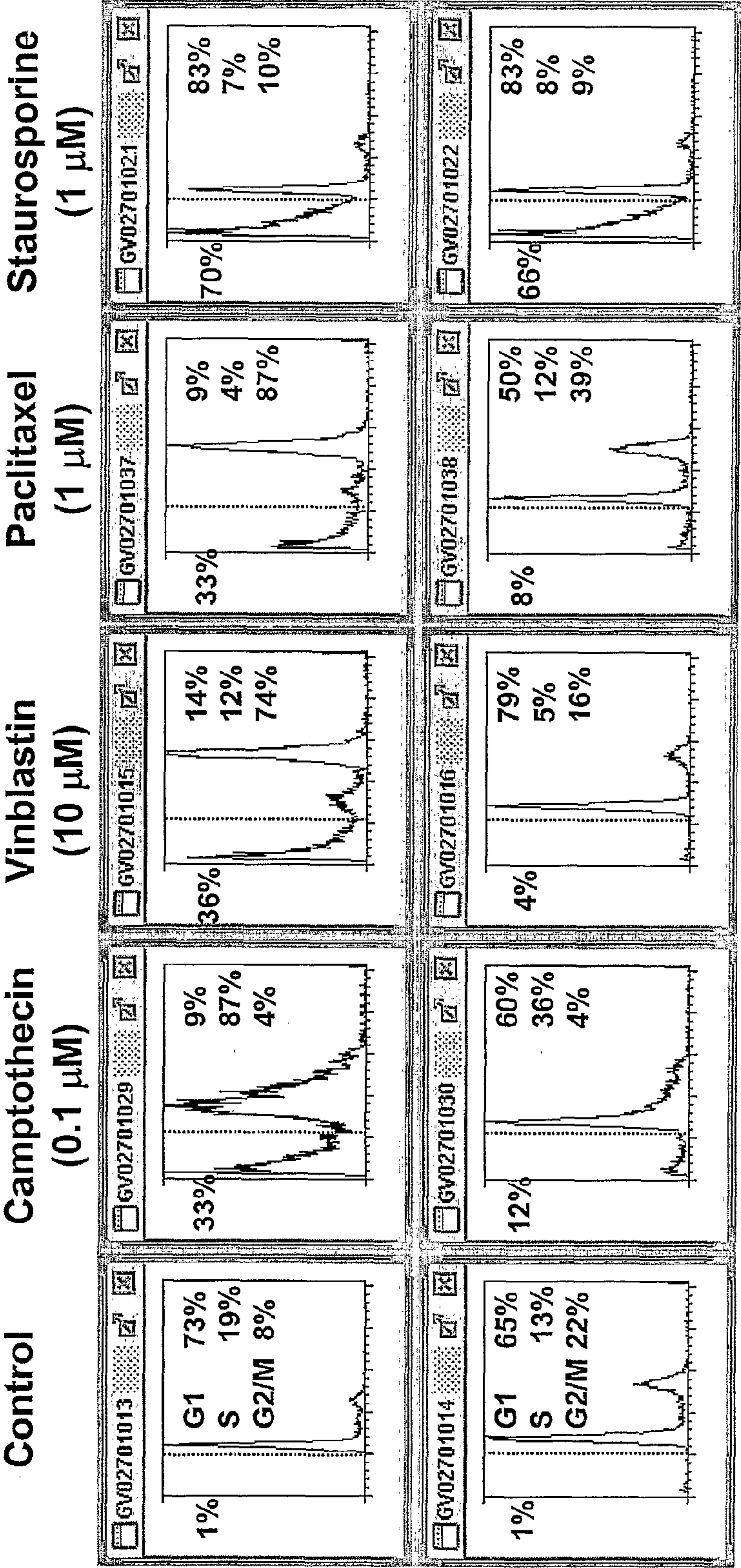


Figure 8



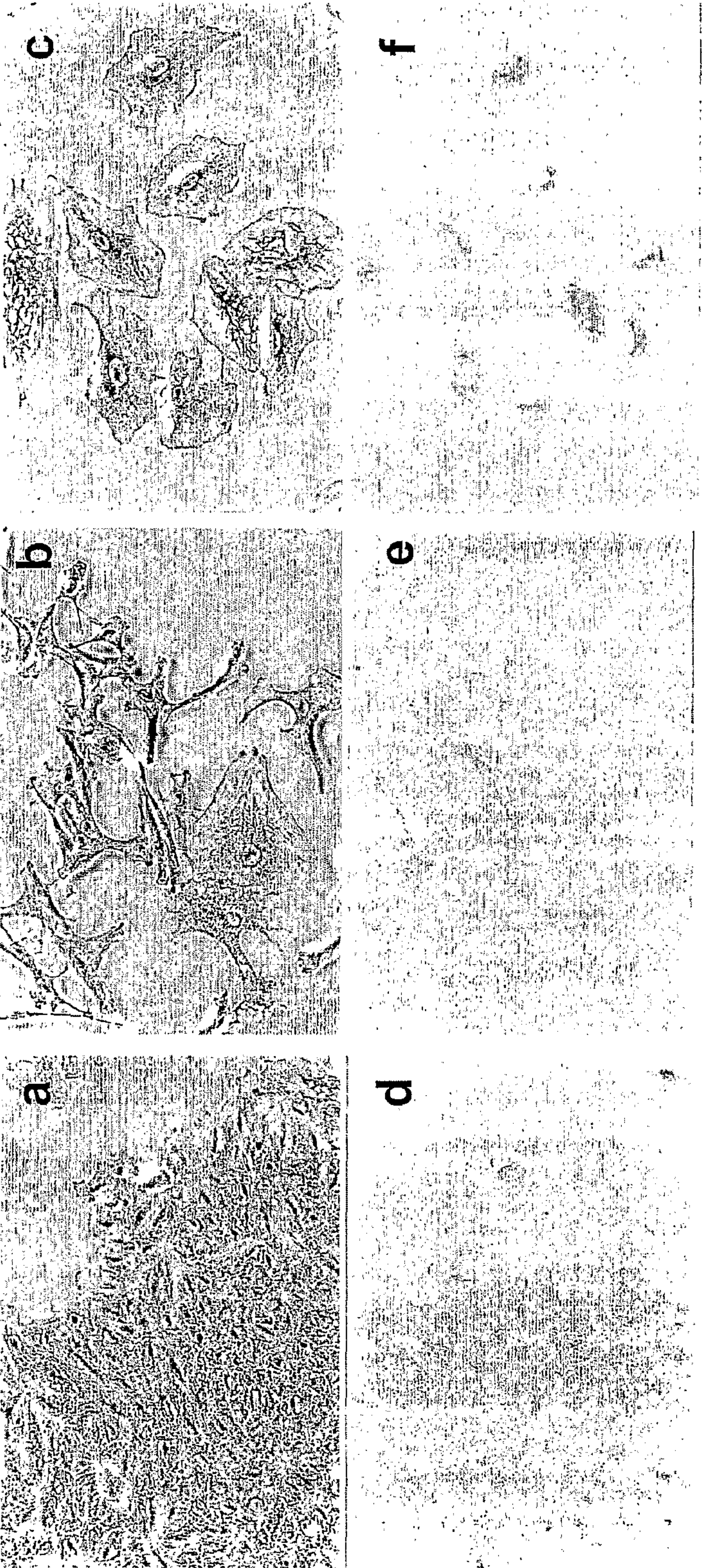
-

+

Compound A
(10 nM)

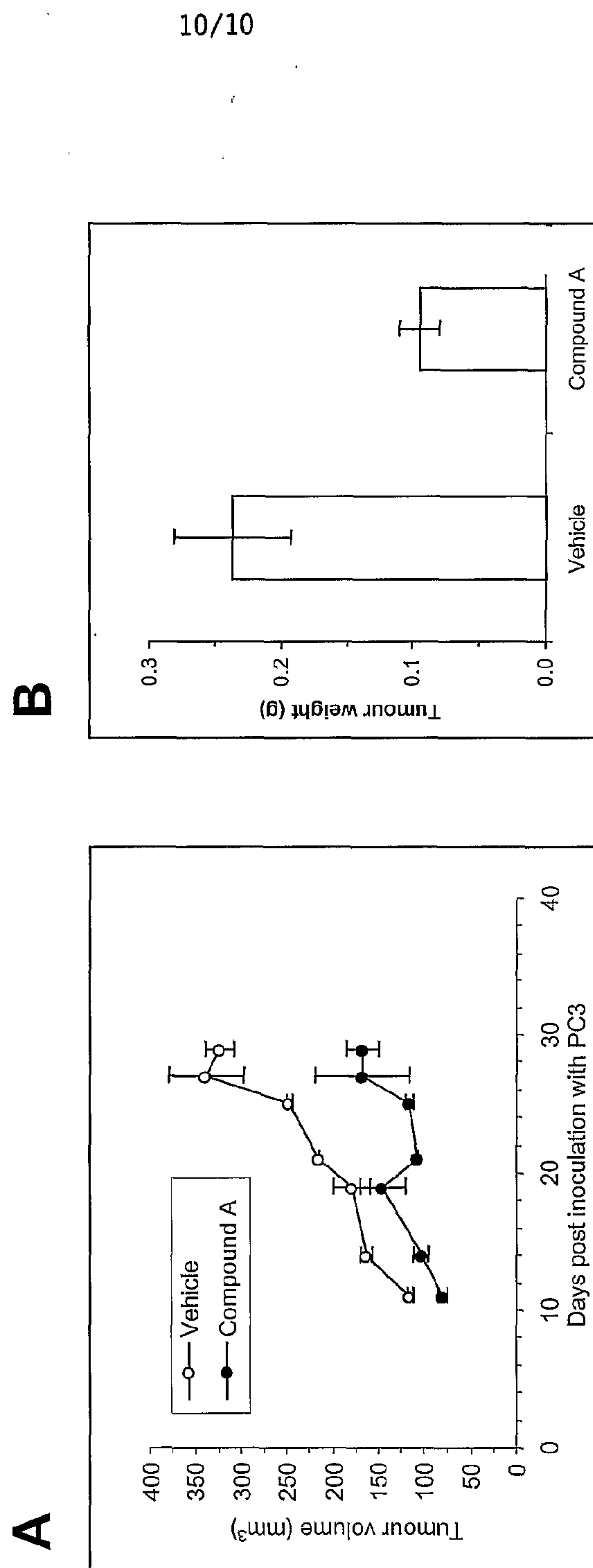
Figure 9

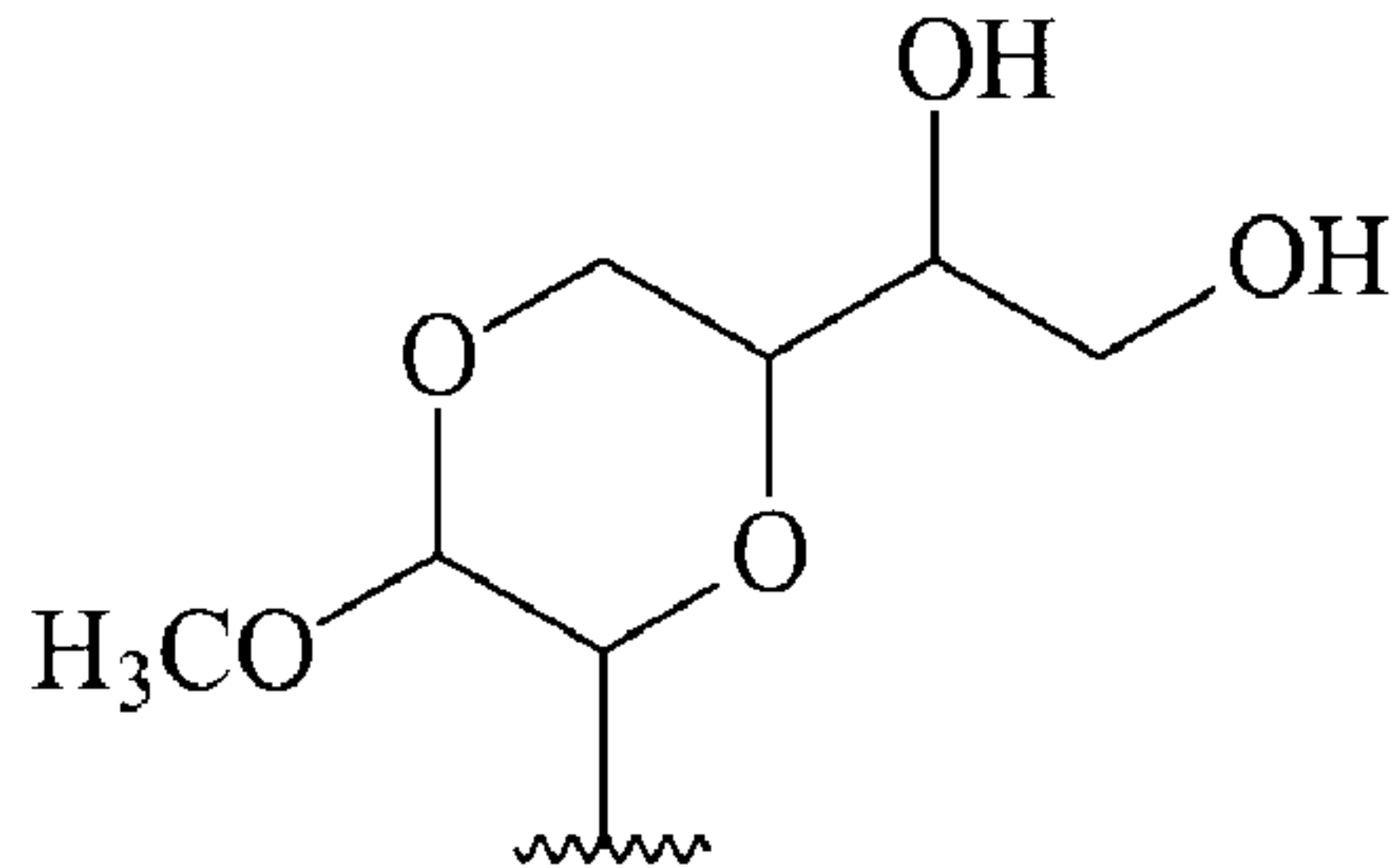
Control Compound A (10 nM) Doxorubicin (250 nM)



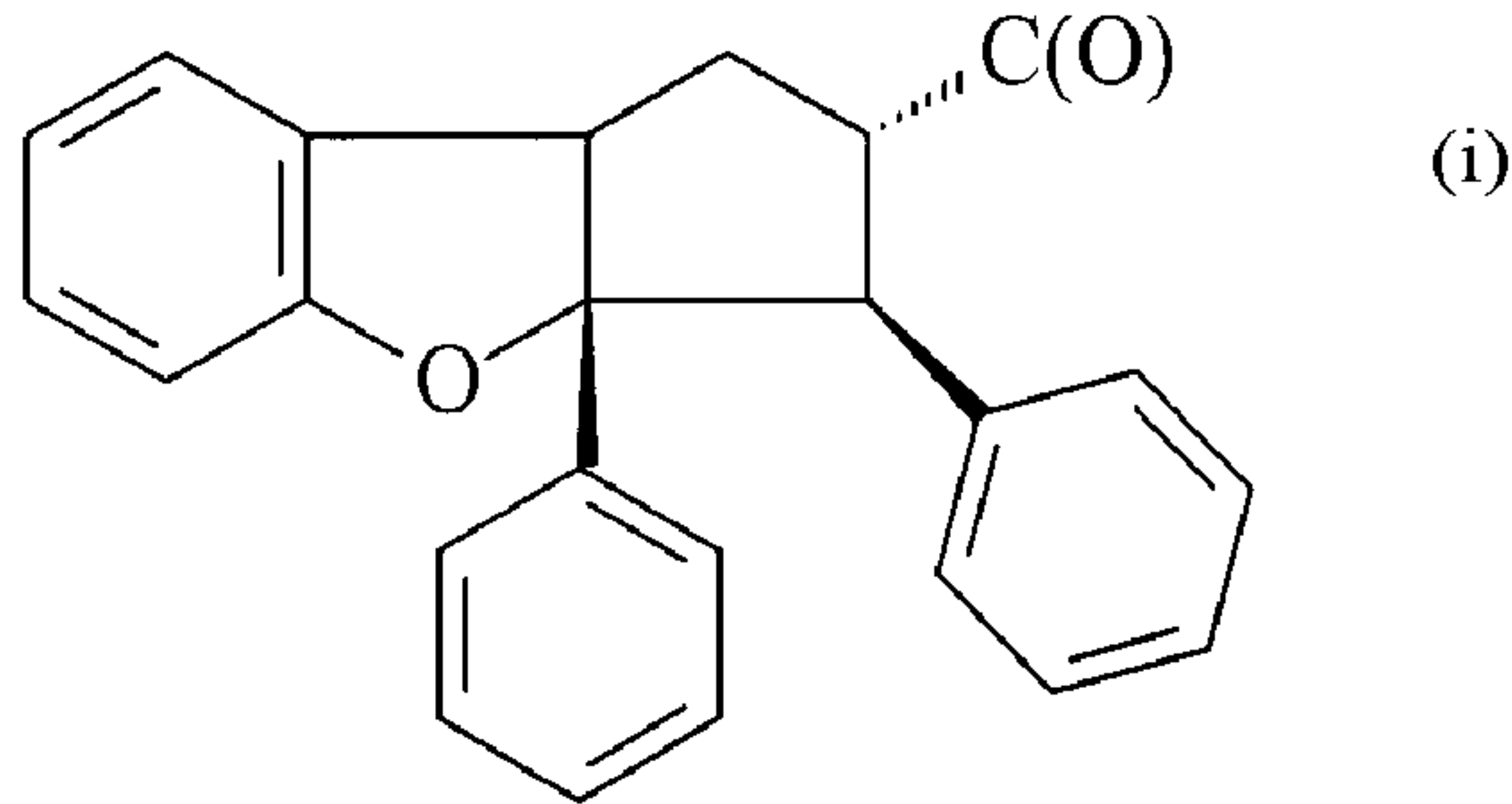
PC

BF

Figure 10



(ii)



(i)