



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2504999 A1 2004/05/21

(21) **2 504 999**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2003/11/06
(87) Date publication PCT/PCT Publication Date: 2004/05/21
(85) Entrée phase nationale/National Entry: 2005/05/04
(86) N° demande PCT/PCT Application No.: AU 2003/001463
(87) N° publication PCT/PCT Publication No.: 2004/041812
(30) Priorité/Priority: 2002/11/08 (10/291,863) US

(51) Cl.Int.⁷/Int.Cl.⁷ C07D 407/12, A61K 31/357, A61P 9/10,
A61P 35/00, A61P 17/06, A61P 19/02

(71) Demandeur/Applicant:
THE GOVERNMENT OF THE STATE OF SARAWAK,
MALAYSIA, MY

(72) Inventeurs/Inventors:
MEURER-GRIMES, BARBARA MARTHA, AU;
YU, JIN, AU;
VAIRO, GINO LUIGI, AU

(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : COMPOSES ET METHODES THERAPEUTIQUES
(54) Title: THERAPEUTIC COMPOUNDS AND METHODS

(57) **Abrégé/Abstract:**

The present invention provides compounds having a cyclopentabenzofuran core and the use of such compounds in therapy as well as compositions comprising said compounds.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/041812 A1

(51) International Patent Classification⁷: **C07D 407/12**,
A61K 31/357, A61P 35/00, 9/10, 17/06, 19/02

Gino, Luigi [AU/AU]; 23 Tanner Grove, Northcote,
Victoria 3070 (AU).

(21) International Application Number:
PCT/AU2003/001463

(74) Agents: **CAINE, Michael, J.** et al.; Davies Collison Cave,
1 Little Collins Street, Melbourne, Victoria 3000 (AU).

(22) International Filing Date:
6 November 2003 (06.11.2003)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,
CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN,
MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU,
SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/291,863 8 November 2002 (08.11.2002) US

(84) Designated States (*regional*): ARIPO patent (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): **THE
GOVERNMENT OF THE STATE OF SARAWAK,
MALAYSIA** [MY/MY]; Tingkat 17, Wisma Baba
Malaysia, Petra Jaya, 93502 Kuching, Sarawak (MY).

Published:
— *with international search report*

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **MEURER-
GRIMES, Barbara, Martha** [DE/AU]; 10 Omerod
Court, Gisborne, Victoria 3437 (AU). **YU, Jin** [AU/AU];
47A Stroud Street, Balwyn, Victoria 3103 (AU). **VAIRO,**

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: THERAPEUTIC COMPOUNDS AND METHODS

(57) Abstract: The present invention provides compounds having a cyclopentabenzofuran core and the use of such compounds in therapy as well as compositions comprising said compounds.

WO 2004/041812 A1

- 1 -

THERAPEUTIC COMPOUNDS AND METHODS

FIELD OF THE INVENTION

5 The present invention relates generally to compounds having a
cyclopentabenzofuran core. More particularly, the present invention relates to
cyclopentabenzofuran compounds having a bulky substituent at the 6-oxy position, such as
where the cyclopentabenzofuran core is substituted by a dioxanyloxy moiety. The
invention also relates to the use of these compounds in therapy and compositions
10 comprising said compounds.

BACKGROUND

Aglaia is a large genus of the family Meliaceae comprising over 100 (mostly
15 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).

20

 More recently, a number of 1*H*-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
25 and Bohnenstengel *et al*, *Z. Naturforsch.*, 1999, 54c (12):1075-83, Xu, Y. J., *et al*, 2000, *J.*
Nat. Prod., 63, 4732-76, the entire contents of which are incorporated herein by
reference). 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).
30 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.

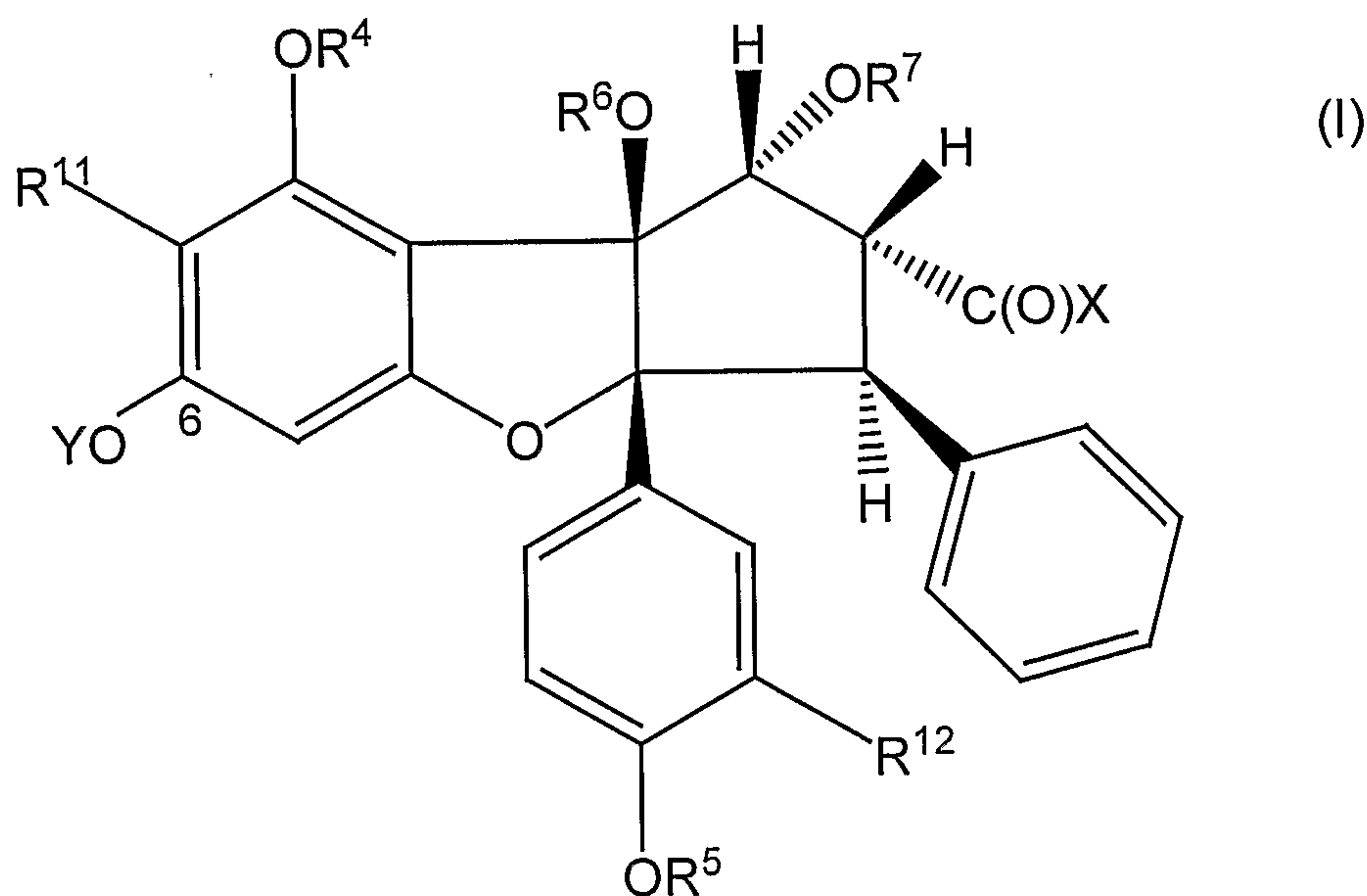
- 2 -

New compounds (Compounds **A** and **B**, as described herein) have now been isolated from *Aglaia leptantha*, Miq. (Meliaceae) which uniquely possess a dioxanyloxy group at the 6-position of 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 and derivatives thereof, may be useful as therapeutic agents in the treatment of cancer and cancerous conditions or other diseases associated with cellular hyperproliferation.

10 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 of Formula (I) or a salt or prodrug thereof.



wherein

- 3 -

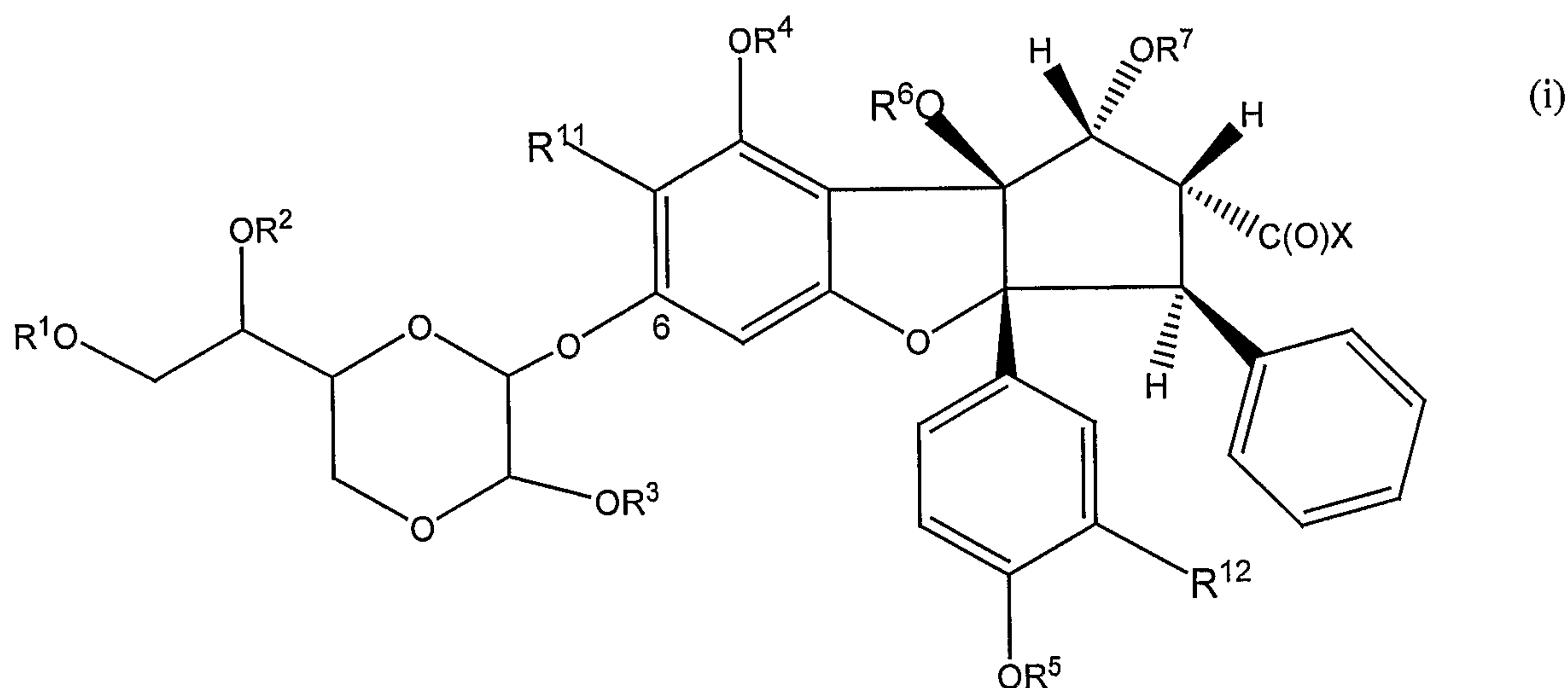
each R^4 - R^{10} is independently selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted acyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted cycloalkylalkyl, optionally substituted arylacyl, optionally substituted cycloalkylacyl and a C-1 linked saccharide;

5 X is OR^8 or NR^9R^{10} ;

R^{11} and R^{12} are preferably each independently hydrogen or, alternatively, OR^4 and R^{11} , and/or OR^5 and R^{12} together form a methylenedioxy group; and

Y is selected from the group consisting of optionally substituted phenyl, optionally substituted benzyl, optionally substituted benzoyl, optionally substituted C_3 - C_8 cycloalkyl, (preferably optionally substituted C_5 - C_6 cycloalkyl) optionally substituted CH_2 -(C_3 - C_8 cycloalkyl) (preferably optionally substituted CH_2 -(C_5 - C_6 cycloalkyl), optionally substituted 5-6 membered heterocyclyl, and optionally substituted CH_2 -(5-6 membered heterocyclyl).

15 In a preferred embodiment, the invention relates to compounds (including stereoisomers within the dioxanyl group) of formula (i) or a salt or prodrug thereof.



wherein

20 and each R^1 - R^{10} is independently selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted acyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted cycloalkylalkyl, optionally substituted arylacyl, optionally substituted cycloalkylacyl and a C-1 linked saccharide; and

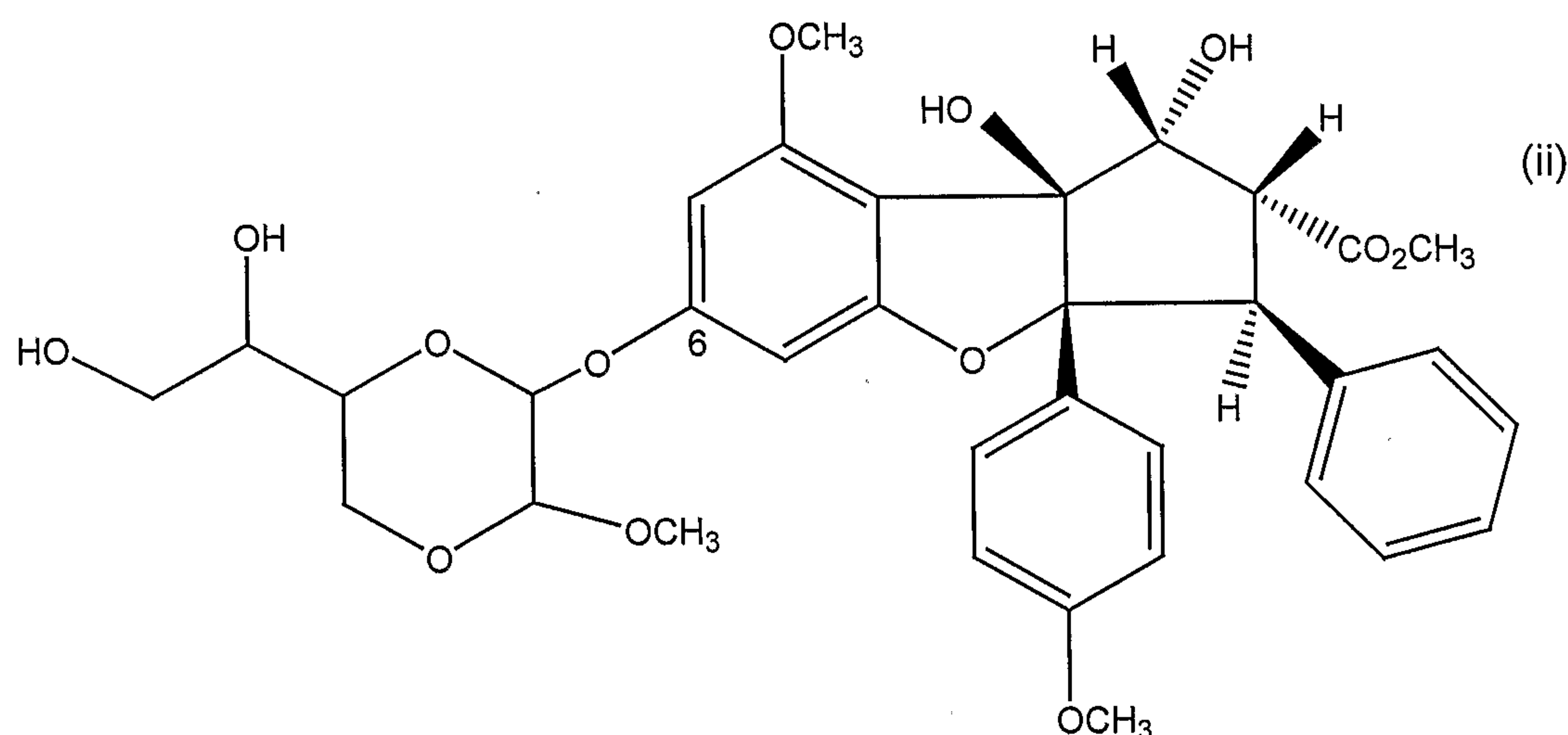
- 4 -

X is OR^8 or NR^9R^{10} ;

R^{11} and R^{12} are each independently hydrogen or, OR^4 and R^{11} , and/or OR^5 and R^{12} together form a methylenedioxy group. In one preferred embodiment, R^{11} and R^{12} are both hydrogen.

5

In one preferred embodiment, compounds of the present invention have the Formula (ii):



10 or a salt or prodrug thereof.

Formula (ii) has 4 chiral centres in the dioxanyl moiety. Two isomers (isomeric in the dioxanyl group) of Formula (ii) have now been isolated – Compounds **A** and **B** as described in Example 1.

15

In another aspect, the invention provides a composition comprising a compound of Formula (I), such as Formula (i), or a salt or prodrug thereof, together with a pharmaceutically acceptable carrier, excipient or diluent.

20

In still a further aspect, the present invention provides a method for the treatment of cancer or a cancerous condition or a disease state or condition associated with cellular hyperproliferation comprising the administration of a treatment effective amount of a compound of Formula (I), such as Formula (i), or a salt, derivative or prodrug thereof, to a

- 5 -

subject in need thereof. Some particular cancerous conditions which may be treated by the compounds of the invention may include lung, prostate, colon, brain, melanoma, ovarian, renal and breast tumours and leukemia. Disease states or conditions associated with cellular hyperproliferation which may be treated by compounds of the invention may include atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, psoriasis, peridontal disease or virally induced cellular hyperproliferation.

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).

15

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 indicted 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.

30

- 6 -

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 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.

- 7 -

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 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

- 8 -

post inoculation of PC3 cells) the mice were sacrificed, the tumours excised and then weighed. The data represents mean tumour weight $\pm$ SEM.

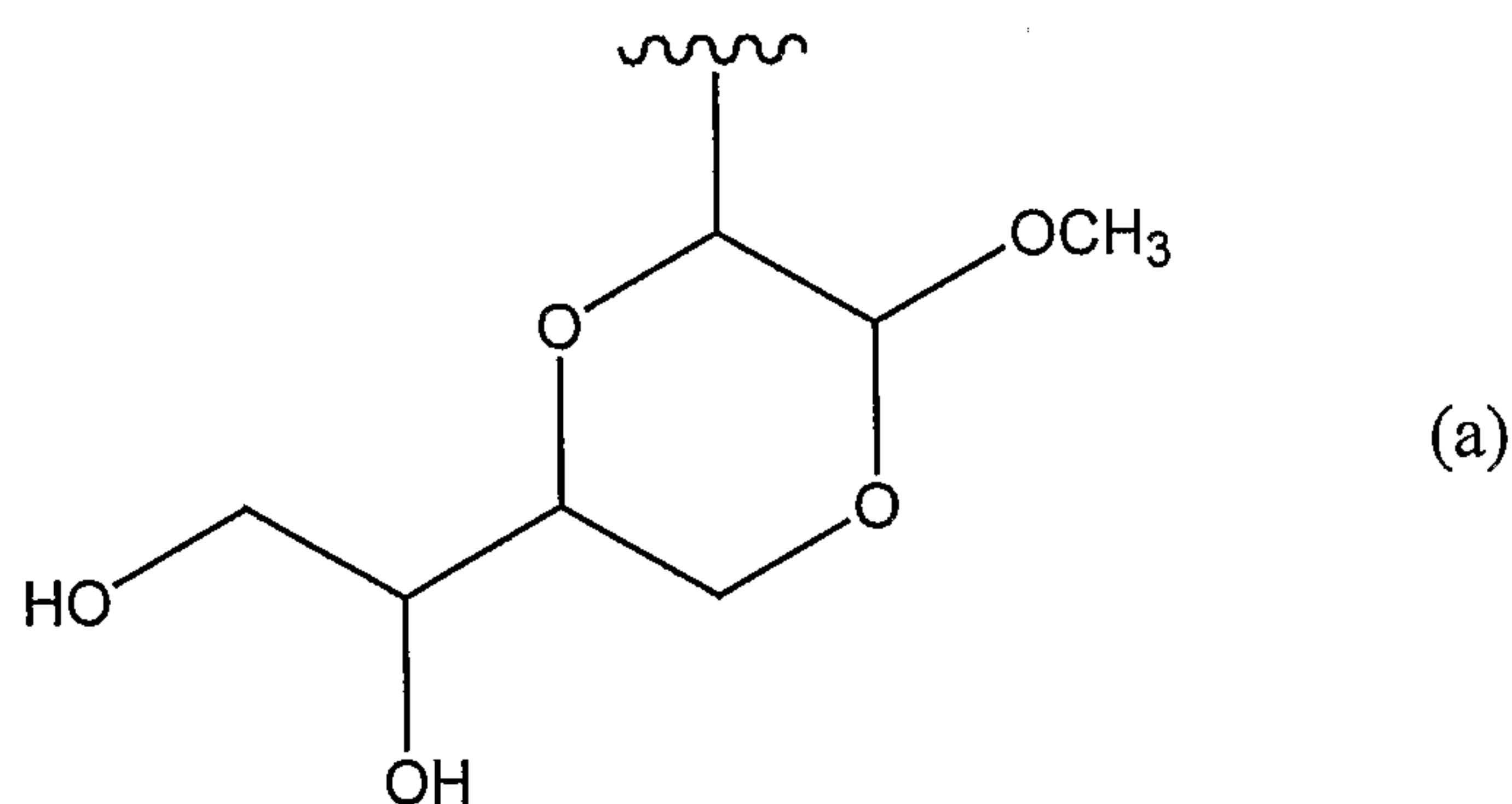
DETAILED DESCRIPTION OF THE INVENTION

5

Cyclopenta[*b*]benzofurans previously reported carry a methoxy group or similar small substituent (Greger *et al*, 2001, *Phytochemistry*, 57, (1); 57-64) at the 6- or 8-positions. In contrast, the compounds of the present invention carry a sterically bulky group at the 6-oxy-position, in particular, a dioxanyl group. The dioxanyl group of

10 Formula (ii) (depicted below as sub-Formula (a)) has not previously been reported from a natural source. Without intending to limit the invention by theory, it is believed that the presence at the 6-oxy-position 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.

15



The invention includes within its scope pharmaceutically acceptable salts, derivatives, or prodrugs of compounds of Formula (I), particularly of Formula (i), such as

20 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, for example, by chemical or

25 *in vivo* enzymatic or hydrolytic degradation) a compound of the invention as described herein.

- 9 -

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 are 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 solvation will be known to those skilled in the art.

Prodrugs of compounds of formula (I) are also within the scope of the invention. The term "prodrug" includes derivatives that are converted *in vivo* to the compounds of the invention and include for example, ester (eg acetate) and glycoside derivatives of free hydroxy groups, which may undergo *in vivo* degradation to release a compound of the invention. Other suitable prodrugs may include esters of free carboxylic acid groups. The preparation of suitable prodrugs is further described in *Design of Prodrugs*, H. Bundgaard, Elsevier, 1985, the contents of which is incorporated by reference.

It will also be recognised that certain Y groups of Formula (I), in particular the dioxanyl groups of compounds as depicted in Formula (i) and (ii), may possess asymmetric centres and are 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

- 10 -

more asymmetric (chiral) centres 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.

5

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. 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.

30

The term "aryl", when used alone or in compound words such as "arylalkyl", denotes single, polynuclear, conjugated or fused residues of aromatic hydrocarbons or

- 11 -

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 any carbon containing moiety such as an optionally alkyl or substituted 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, phenylisobutylyl, phenylpentanoyl and phenylhexanoyl) and naphthylalkanoyl (e.g. naphthylacetyl, 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, C_{1-6} alkyl (eg methyl, ethyl, propyl (*n*- and *i*- isomers)), C_{1-6} alkoxy (eg

- 12 -

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. Furthermore, optional substituents for Y (phenyl, benzyl, benzoyl, C₃-C₈ cycloalkyl, CH₂-(C₃-C₈ cycloalkyl), 5-6 membered-heterocyclyl and CH₂-(5-6 membered-heterocyclyl)) include, as well as the substituents above, alkyl substituted with one or more of hydroxy C₁₋₆alkyloxy, C₁₋₆acyloxy, aryloxy, arylC₁₋₆alkyloxy, C₁₋₆cycloalkylC₁₋₆alkyloxy, arylC₁₋₆acyloxy, C₁₋₆cycloalkylC₁₋₆acyloxy and C1-linked saccharidoxy.

The term "arylalkyl" and "cycloalkylalkyl" refer to an alkyl group (preferably straight chain) substituted (preferably terminally) by an aryl and a cycloalkyl group, respectively. Similarly, the terms "arylacyl" and "cycloalkylacyl" refer to an acyl group (preferably where R is straight chain alkyl) substituted (for example, terminally substituted) by an aryl and a cycloalkyl group, respectively.

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 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.

A 5-6 membered heterocyclyl group includes aromatic 5-6-membered heterocyclic aryl groups (heteroaryl) as described above and non aromatic 5-6-membered heterocyclic groups containing one or more heteroatoms (preferably 1 or 2) independently selected from O, N, S and Se. Examples thereof include dioxanyl, pyranyl, tetrahydrofuranyl, piperidyl, morpholino, piperazinyl, thiomorpholino and saccharides, for example, those described above.

- 13 -

In one embodiment of Formula (I) or Formula (i) of the invention, each of R^4 - R^7 and R^1 - R^7 respectively may independently be selected from the group consisting of hydrogen, methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *t*-butyl, cyclopropylmethyl (or cyclopropylethyl), cyclobutylmethyl (or -ethyl), cyclopentylmethyl (or -ethyl), cyclohexylmethyl (or -ethyl), phenyl, benzyl, acetyl and C-1 linked saccharide.

In another embodiment of Formula (I), (i) or (ii) of the invention, R^8 of $X=OR^8$ is selected from the group of hydrogen, C_{1-6} alkyl, phenyl, benzyl and C-1 linked saccharide.

In another embodiment of Formula (I), (i) or (ii) of the invention R^9 and R^{10} of $X=NR^9R^{10}$ are independently selected from hydrogen, C_{1-6} alkyl, phenyl or benzyl.

The derivatisation of hydroxy groups of Compounds **A** and **B** to form compounds of Formula (i), (ie where any one of R^1 - R^7 is not hydrogen) 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 (the entire contents of which are incorporated herein by reference). 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 treatment with appropriate carboxylic acids, acid halides or 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.

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

- 14 -

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 glycocyltransferase
5 (SIGMA).

The skilled person will recognise that in order to selectively install any one or more of the R^1 - R^{10} groups as defined herein (eg where R^1 - R^7 are not hydrogen), this may require the judicious protection and/or deprotection, of one or more of the oxy and/or carboxy
10 groups. 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; ie. where X is OR^8
15 to an amide (X is NR^9R^{10}) 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).

20 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
25 *Protective Groups in Organic Synthesis (supra)*. Suitable protecting groups for hydroxy include alkyl, (such as C_1 - C_6 alkyl), acyl (such as $C(O)C_1$ - C_6 alkyl, 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
30 certain conditions is understood by the skilled person and is further exemplified in *Protective Groups in Organic Synthesis (supra)*.

- 15 -

It will be appreciated that these protected compounds may be useful as intermediates in the preparation of certain compounds of Formula (I) and therefore, these also form a further aspect of the invention.

5

It will also be recognised that some groups, eg alkyl, acyl or arylalkyl, (such as methyl, ethyl, propyl, acetyl, benzyl etc) may serve as either a temporary protecting group or as a non-hydrogen R^1 - R^8 group in Formula (I).

10 The dioxanyl group may be cleaved from the 6-oxy position of the cyclopentabenzofuran core using known methods to afford a dioxane compound. The resulting dioxane compound could be used to substitute other compounds, such as oxy-substituted compounds, including the corresponding 6-oxy position, or other oxy positions, on other cyclopentabenzofuran compounds such as those described in the references
15 herein.

It will also be understood that cyclopentabenzofuran compounds, having a methoxy substituent at the 6-position, such as those described in the references cited herein (incorporated herein by reference) eg Reference Compounds 1-3 (as described in Example
20 4), can, where appropriate be 6-demethylated, and the resulting 6-hydroxy group reacted with a suitable Y precursor to form an 6-OY group. Methods therefor are known in the art, for example, one method may involve reacting the 6-OH group with a Y-halogen compound where halogen includes Cl, Br and I. Alternatively, access to the cyclopentabenzofuran core, incorporation of the Y group can be achieved via synthetic
25 methods analogous to that described in Trost *et al*, *J. Am. Chem. Soc.*, 1990, 112, 9022-9024. Such 6-OY compounds form a further aspect of the invention.

In some preferred embodiments of the present invention, one or more of the following definitions apply:

30

$-R^1$ and R^2 are both hydrogen.

- 16 -

-R¹ and R² are hydrogen, and R³ is methyl.

-at least one of R³-R⁵ is methyl, ethyl or propyl, preferably methyl.

5

-at least two of R³-R⁵ are methyl, ethyl or propyl, preferably methyl.

-all of R³-R⁵ are methyl, ethyl or propyl, preferably methyl.

10

-R⁶ and R⁷ are both hydrogen.

-at least one of R¹¹ and R¹² is hydrogen, preferably R¹¹ and R¹² are both hydrogen.

15

-X is OR⁸ where R⁸ is selected from hydrogen, methyl, ethyl or propyl, preferably, methyl.

-X is NR⁹R¹⁰ where R⁹ and R¹⁰ are both hydrogen or methyl; or R⁹ and R¹⁰ are different but at least one of R⁹ or R¹⁰ is hydrogen and the other is C₁₋₆ alkyl, such as methyl, ethyl or propyl.

20

-Y is an optionally substituted 5-6 membered heterocyclyl group or an optionally substituted C₅-C₆ cycloalkyl group.

Particularly preferred forms of Formula (ii) are Compounds **A** and **B**.

25

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), companion animals, (eg. dogs, cats, rabbits, guinea pigs), laboratory test animals, (eg, rats,

30

- 17 -

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,
5 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
10 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
15 the present invention include leukemia and breast, colon, bladder, pancreatic, endometrial, head and neck, mesothelioma, myeloma, oesophagal/oral, testicular, thyroid, uterine, prostate, renal, lung, ovarian, cervical brain, skin, liver, bone, bowel and stomach cancers, sarcomas, tumours and melanomas. Examples of benign hyperplasias include those of vascular (eg hemangioma), prostate, renal, adrenal, hepatic, colon (eg colonic crypt),
20 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
25 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.

30

- 18 -

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.

5 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.

10

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
15 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,
20 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
25 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 the extent and progress of the condition to be
30 treated, and can be determined by the attending physician.

- 19 -

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.

5 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
10 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
15 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
20 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
25 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 glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding
30 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

- 20 -

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.

5

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
10 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.

15

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.

20

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
25 freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

- 21 -

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.

5 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,
10 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
15 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.

20

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
25 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-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.

30

- 22 -

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 references and citations disclosed within this specification are taken to be incorporated herein in their entirety.

10

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.

15 **EXAMPLES**

Example 1

Isolation of Compounds A and B from *Aglaia leptantha*

20 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.
- (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:

30

- 23 -

C-8 Symmetry column (WATERS), 4.6 x 250 mm, 5 μ m, 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
5 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.
- 10 (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). These samples were used for the structural elucidation of Compounds **A** and **B**.
- (i) Alternatively to steps (b), (c) and (d), the methanol extract obtained under (a) may
15 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;

20

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
25 introduced into the source by infusion with a syringe pump at rate of 3 μ L/min. For Compounds **A**, signals were observed at m/z 677 [M+Na]⁺; MS² yielded m/z 659 [M+Na-H₂O]⁺; MS³ yielded m/z 627 (loss of 32 amu); MS⁴ 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]⁺; MS² yielded product
30 ions at m/z 627.2 and m/z 659.2. Further fragmentation of the signal at m/z 627.2 yielded a product ion at m/z 595.3.

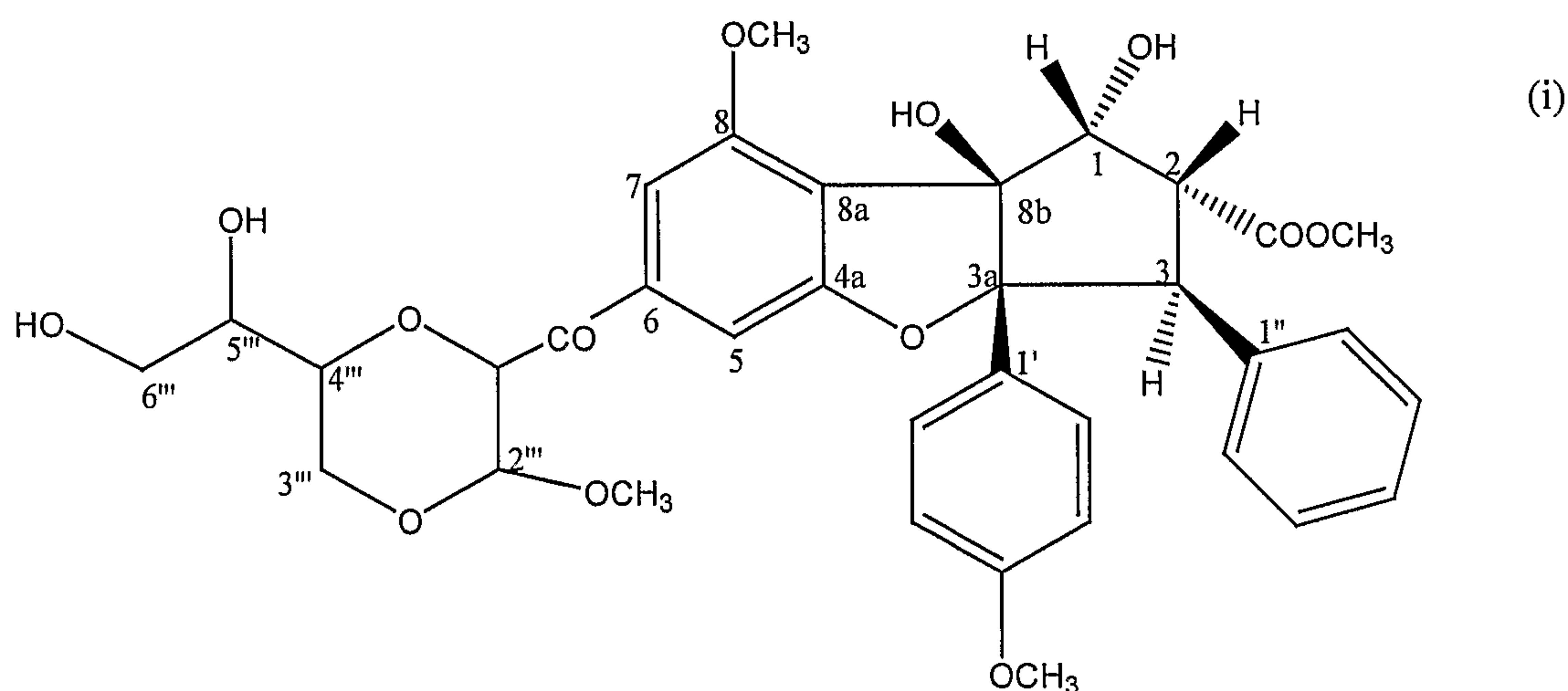
- 24 -

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

NMR

10

The NMR spectra of Compounds **A** and **B** (see Formula (ii) below) were acquired on 400 and 500 MHz Varian INOVA 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
 15 in Table 1.



20

Compounds **A** and **B**

- 25 -

Table 1: ^1H and ^{13}C NMR shifts for Compounds A and B
(Preliminary Position Assignments)

Position	Assignments	Compound A		Compound B	
		^1H NMR (ppm)	^{13}C NMR (ppm)	^1H 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
6	C		160		159.8
	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
8	C		157.1		157.1
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
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
3'''		4.13, t, 11.2 Hz, 1H	59	4.02, t, 11.2 Hz, 1H	59.6
	CH ₂	3.56, dd, 11.7, 2 Hz, 1H		3.78, dd, 11.7, 2.4 Hz, 1H	
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	

- 26 -

Example 2*Determination of the substitution position of the dioxanyl sidechain on the cyclopentabenzofurane core of Compounds A and B (Acetylation of Compounds A and B)*

5

The objective of this experiment was to unambiguously determine the attachment position of the dioxanyl sidechain to the cyclopentabenzofuran core in Compounds A and B.

Compounds A and B were dissolved in anhydrous pyridine (A: 4.2 mg in 280 μ L; B: 3 mg in 400 μ L) and acetic anhydride was added (A: 140 μ L, B: 200 μ L). The reaction mixtures were stirred under an argon atmosphere for 14 (A) and 22 (B) hrs, respectively. The solvents were removed under reduced pressure to afford the diacetates as an orange residue (A: 5.8 mg; B: 3 mg). Purification of the crude residues was achieved by silica gel column chromatography eluting with 60% ethylacetate/petrol. The diacetate of Compound A, Compound A' (Formula (iii)), was obtained in 68% yield (3.2 mg), and the diacetate of Compound B, Compound B' (Formula (iii)), was obtained in 41% yield (1.4 mg).

The purity of the two reaction products was assessed by reversed phase HPLC using the same instrumentation as outlined in Example 1 (column: Xterra C-18, 1 mL/min, gradient: from 0 to 100% MeCN in 40 mins). The structures of compounds A' and B' were elucidated by electrospray MS and 1D and 2D NMR experiments using the same conditions as described in Example 1. NMR spectra of Compounds A' and B' were obtained in CDCl₃ with 500 and 400 MHz Varian INOVA instruments.

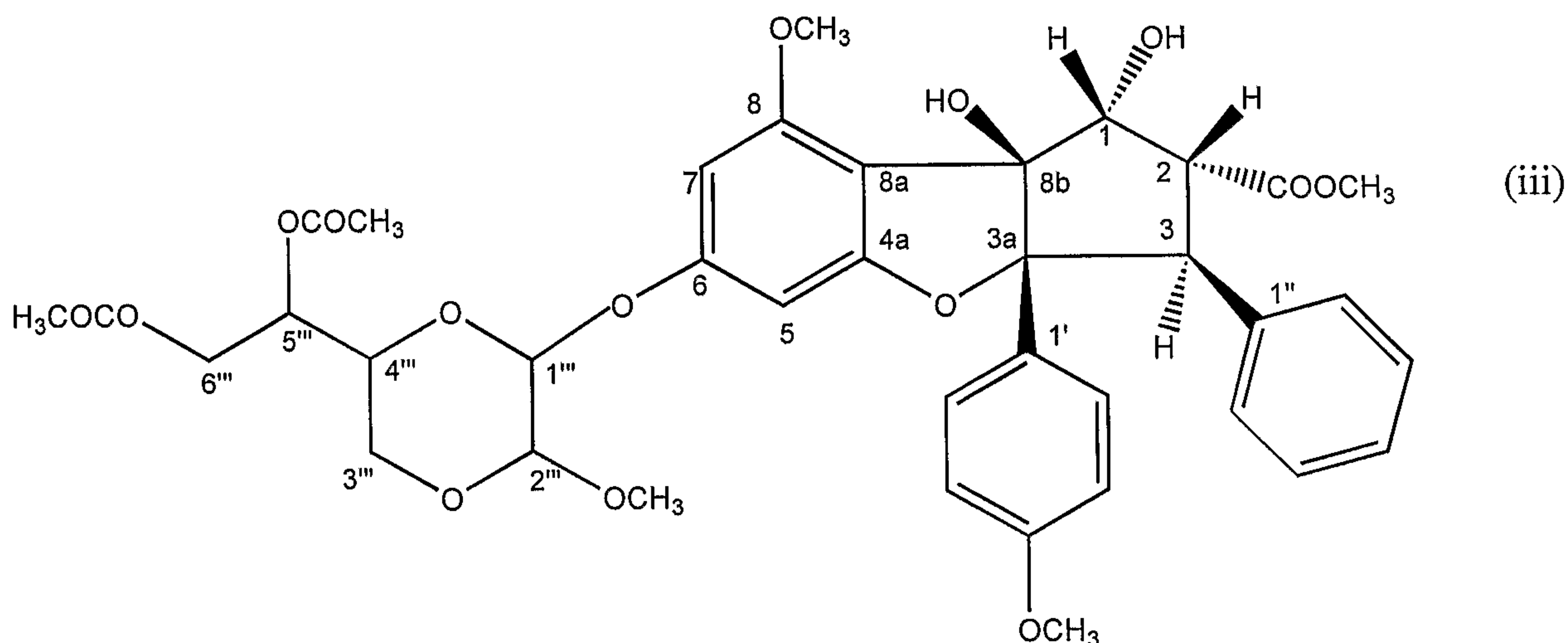
Both compounds yielded a single peak in the HPLC analysis with retention times of 26.3 mins for Compound A', and 27.7 mins for Compound B'. Compounds A' and B' showed positive molecular ions at m/z 761 [M+Na]⁺ and 1499 [2M+Na]⁺ indicative of a molecular formula C₃₈H₄₂O₁₅. 1 and 2 D NMR experiments (¹H, HMQC and HMBC, NOESY) revealed that the two hydroxyl functions on the dioxanyl sidechain were acetylated. The ¹H and ¹³C NMR chemical shifts are summarized in Table 2, and the NOESY spectra in Table 3.

- 27 -

The HMBC experiments of both diacetates show clear correlations of the proton signals of H-5, H-7 and H-1''' to the carbon 6 of the aromatic ring. The proton signals of H-7 and a methoxy group are correlated to the carbon 8. This clearly indicates that the dioxanyl sidechain is attached at the position C-6 of the cyclopentabenzofuran core.

5

Further support for the position of the dioxanyl sidechain was derived from the NOESY spectra of both compounds. The NOE signals are observed from both H-5 and H-7 to H-1''' of the dioxanyl side chain, and only H-7 shows a NOE signal to the C-8 methoxy signal. The NOE signals observed in the dioxanyl ring systems of the two compounds clearly indicate that they differ in regard to the stereochemistry of the di-hydroxyethane sidechain. The NOE signals for the cyclopentabenzofuran core are in agreement with published data and confirm the stereochemistry depicted in Tables 1 and 2.



15

Compounds A' and B' (Diacetates of Compounds A and B)

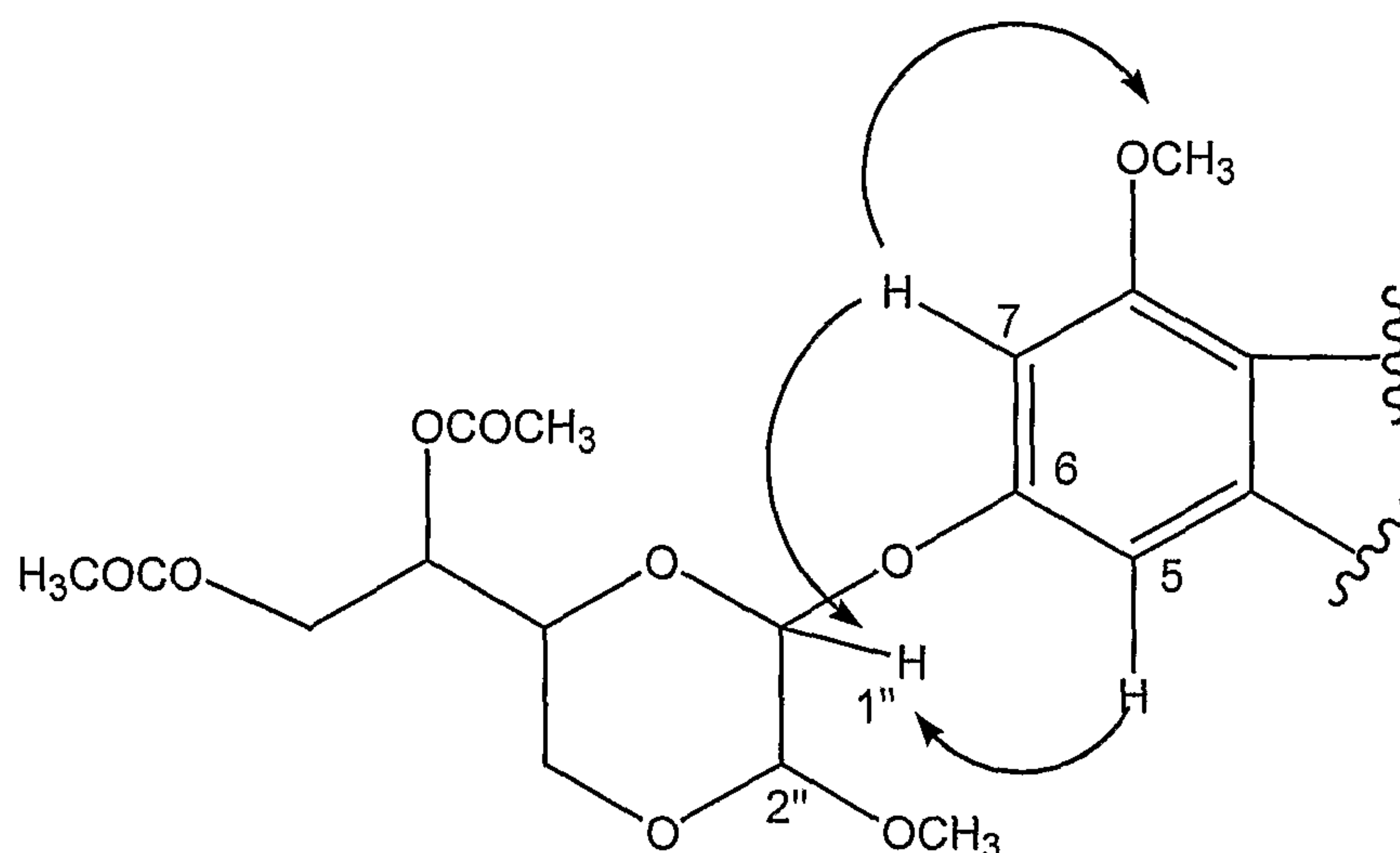
20

- 28 -

Table 2: ^1H and ^{13}C NMR chemical shifts for Compounds A' and B'

Position	Assignment	Compound A' (Diacetate of Compound A)		Compound B' (Diacetate of Compound B)	
		^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1	CH	5.06, d, 8 Hz	79.8	5.07, d, 8 Hz	79.8
2	CH	3.89, under OCH_3 -8	50.4	3.87, under OCH_3 -8	50
2	COOCH_3		170.4		170.4
2	COOCH_3	3.64, s	51.9	3.63, s	51.8
3	CH	4.26, d, 14 Hz	55	4.27, d, 14.4 Hz	54.8
3a	C		101.9		102
4a	C		160.6		160.5
5	CH	6.43, d, 2 Hz	93.3	6.45, d, 2 Hz	93.2
6	C		159.6		159.7
7	CH	6.27 d, 2 Hz	93.2	6.29, d, 2 Hz	93
8	C		157		156.9
8	OCH_3	3.89, s	56	3.88, s	55.8
8a	C		109.8		109.6
8b	C		93.5		93.4
8b	OH	2.35, s			2.33
1'	C		126.2		126.4
2', 6'	2 x CH	7.10, br d, 9 Hz	129.1	7.10, br d, 9 Hz	129
3', 5'	2 x CH	6.67, br d, 9 Hz	112.7	6.68, br d, 9 Hz	112.6
4'	C		158.8		158.6
4'	OCH_3	3.72, s	55	3.72, s	55
1''	C		136.8		136.6
2'', 6''	2 x CH	6.83, m	127.7	6.86, m	127.8
3'', 5''	2 x CH	7.05, m	127.1	7.05, m	127.8
4''	CH	7.05, m	126.6	7.05, m	126.6
1'''	CH	5.38, s	93.7	5.31, s	93.2
2'''	CH	4.61, s	95.2	4.62, s	95.2
2'''	OCH_3	3.50, s	55.2	3.5, s, 3H	55
3'''	CH_2	3.94, t, 11.2 Hz	58.8	3.93, t, 12 Hz	59.5
		3.54, dd, 11.2, 3 Hz		3.59, dd, 12, 2.5 Hz,	
4'''	CH	4.38, dt, 11, 3 Hz	66.3	4.37, td, 9, 2.5 Hz	64.9
5'''	CH	5.12, td, 6, 3 Hz	69.1	5.00, ddd, 9, 4, 2.5 Hz	70.2
6'''	CH_2	4.22, dd, 11.2, 6 Hz	61.3	4.21, dd, 12.4, 4 Hz,	61.5
		3.88, ? under OCH_3 -8		4.12, dd, 12.4, 2.5 Hz	
5'''	COCH_3		170.3		169.9
5'''	COCH_3	2.14, s	20.8	2.07	20.8
6'''	COCH_3		170.7		170.8
6'''	COCH_3	1.79	20.4	1.74	20.1

- 29 -



5

Table 3: Comparison of NOESY Spectra of Compounds A' and B'

Compound A'		Compound B'	
1H NMR	NOEs	1H NMR	NOEs
6.43, d, H-5	5.38, s, H-1''' 4.38, dt, H-4'''	6.45, d, H-5	5.31, s, H-1''' 4.37, td, H-4''' 4.21, dd, H-6'''-1 1.74, s, COCH ₃ -6'''
6.27, d, H-7	5.38, s, H-1''' 3.89, s, OCH ₃ -8	6.29, d, H-7	5.31, s, H-1''' 4.21, dd, H-6''-1 3.88, s, OCH ₃ -8
5.38, s, H-1'''	4.38, dt, H-4''' 3.89, s, OCH ₃ -8 3.50, s, OCH ₃ -2'''	5.31, s, H-1'''	4.37, td, H-4''' 3.88, s, OCH ₃ -8 3.5, s, OCH ₃ -2'''

10 Example 3

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

- (a) Compounds A and B were identified from a bark sample of *Aglaia leptantha* 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)

- 30 -

activated with lipopolysaccharide (LPS). Table 4 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 4. 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.

Table 4 : Comparison of the effects of Compounds A and B in THP-1 and A549 Cells*

Compound	IC ₅₀ (μ M)						
	THP-1 cells				A549 cells		
	TNF- α Production	WST-1 Reduction	Protein Synthesis	DNA Synthesis	ICAM-1 Production	Protein Synthesis	DNA Synthesis
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

* 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 (IC₅₀) is shown. Production of TNF α 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 α monoclonal antibodies (capture antibody, MAB610; detection antibody, biotinylated MAB210; both from R & D Systems,

- 31 -

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 Delfia 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.

(b) 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 5). 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 GI_{50} 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 LC_{50} 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 6 shows that Compound B exhibited cytotoxic effects against the various tumour cell lines with comparable potency to that observed with Compound A.

- 32 -

Table 5: Compound A has potent cytostatic and cytotoxic activity in various human tumour cell lines in vitro*

Tumour Source	Tumour Cell Line	Compound A		
		GI₅₀ (nM) (3 day cultures)	LC₅₀ (nM) (3 day cultures)	LC₅₀ (nM) (6 day cultures)
<i>Leukemia</i>	<i>THP-1</i>	-	36	24
	<i>K562</i>	1	>1000	10
<i>Lung</i>	<i>A549</i>	7	914	21
<i>Prostate</i>	<i>PC3</i>	5	18	12
<i>Brain</i>	<i>SF268</i>	3	461	29

* 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).

15

- 33 -

Table 6: Compounds A and B exhibit similar cytotoxic activity*

<i>Tumour Source</i>	<i>Tumour Cell Line</i>	<i>LC₅₀ (nM)</i> <i>(6 day cultures)</i>	
		<i>Compound A</i>	<i>Compound B</i>
<i>Leukemia</i>	<i>THP-1</i>	<i>11</i>	<i>15</i>
	<i>K562</i>	<i>12</i>	<i>15</i>
<i>Lung</i>	<i>A549</i>	<i>15</i>	<i>12</i>
<i>Prostate</i>	<i>PC3</i>	<i>12</i>	<i>12</i>
<i>Brain</i>	<i>SF268</i>	<i>12</i>	<i>22</i>

* The cytotoxic activity of Compounds A and B were compared for the various tumour
5 cell lines as described in Table 5.

(c) Testing of Compound A against a much larger cell line panel in the NCI *in vitro*
anticancer screen (Table 7) confirmed the results described above. Using a different assay
methodology based on measurement of total cellular protein the results confirm that
10 Compound A had broad and potent cytostatic effects with all of the cell lines exhibiting
maximal inhibition of cell growth even at the lowest dose tested (10nM). Consistent with
the data in Table 5 the cytotoxic effects measured after 2 days of culture were more varied
with LC₅₀ values ranging from 10 nM for COLO-205 colon tumour cells to ~90 µM for
786-0 renal tumour cells. These data indicate that Compound A had potent *in vitro* activity
15 against a wide range of tumour cell lines representing a variety of different major types of
cancer including leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate and
breast tumours.

- 34 -

Table 7: Activity of Compound A measured in the NCI *in vitro* anticancer drug discovery screen*

Tumour Source	Tumour Cell Line	GI ₅₀ (nM)	LC ₅₀ (nM)
Lung	EKVX	<10	23
Lung	NCI-H226	<10	193
Lung	NCI-H460	<10	38,019
Lung	NCI-H522	<10	2,399
Colon	COLO-205	<10	10
Colon	HT29	<10	1,000
Brain	SF-268	<10	1,000
Brain	SF-295	<10	1,230
Brain	SF-539	<10	1,096
Brain	SNB-75	<10	54
Melanoma	LOX IMVI	<10	1,000
Melanoma	MALME-3M	<10	23,988
Melanoma	M14	<10	51
Melanoma	SK-MEL-2	<10	19,055
Melanoma	SK-MEL-28	<10	2,661
Melanoma	SK-MEL-5	<10	67
Melanoma	UACC-62	<10	30
Ovarian	IGROV1	<10	2,600
Ovarian	OVCAR-4	<10	11
Ovarian	OVCAR-5	<10	1,000
Ovarian	OVCAR-8	<10	21,878
Ovarian	SK-OV-3	<10	82,224
Renal	786-0	<10	91,201
Renal	A498	<10	18,621
Renal	ACHN	<10	31,623
Renal	RXF 393	<10	1,641
Breast	MCF7	<10	1,000
Breast	MDA-MB-231/ATCC	<10	25,410
Breast	MDA-MB-435	<10	45
Breast	MDA-N	<10	543
Breast	BT-549	<10	32,734

- 35 -

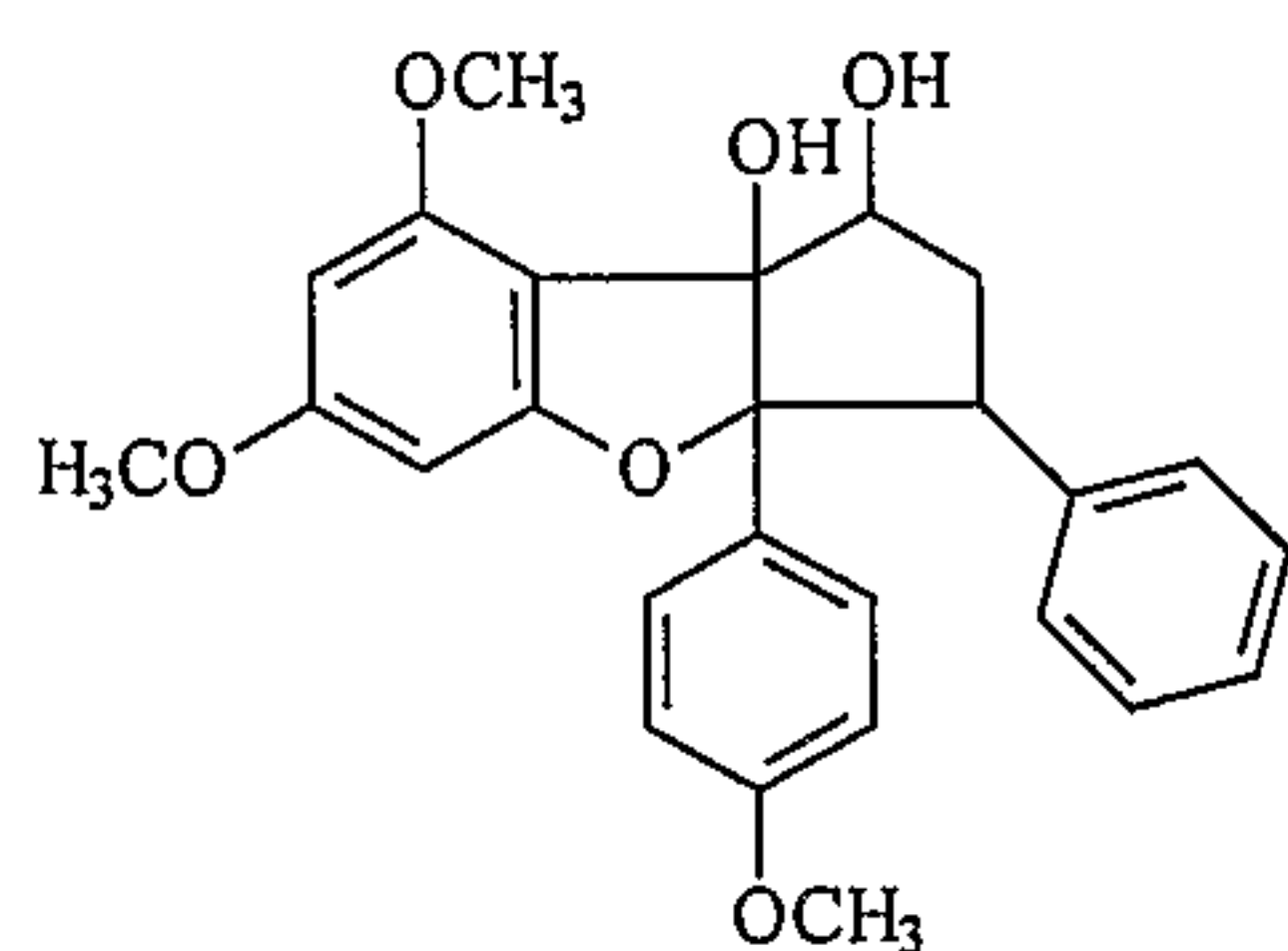
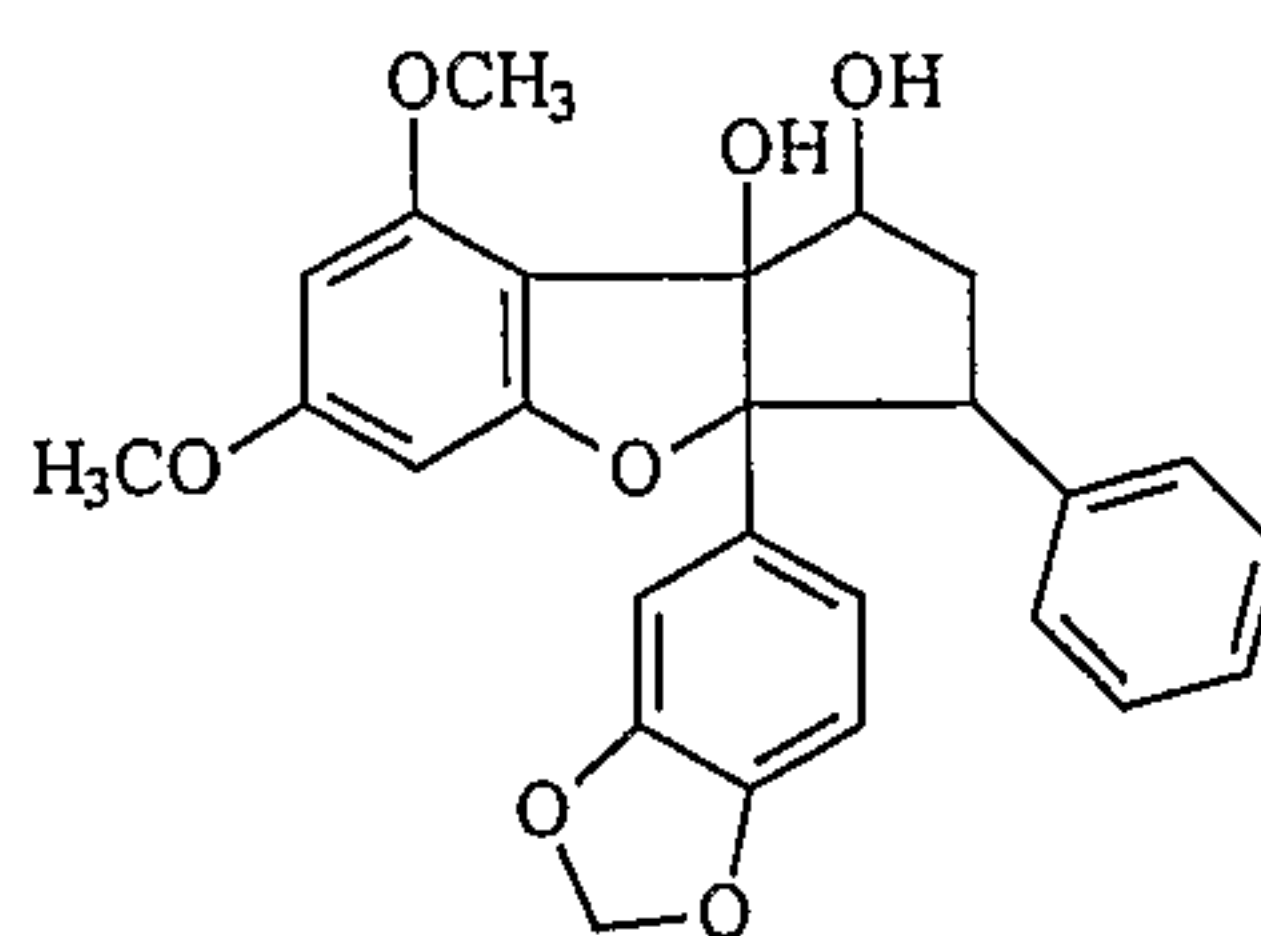
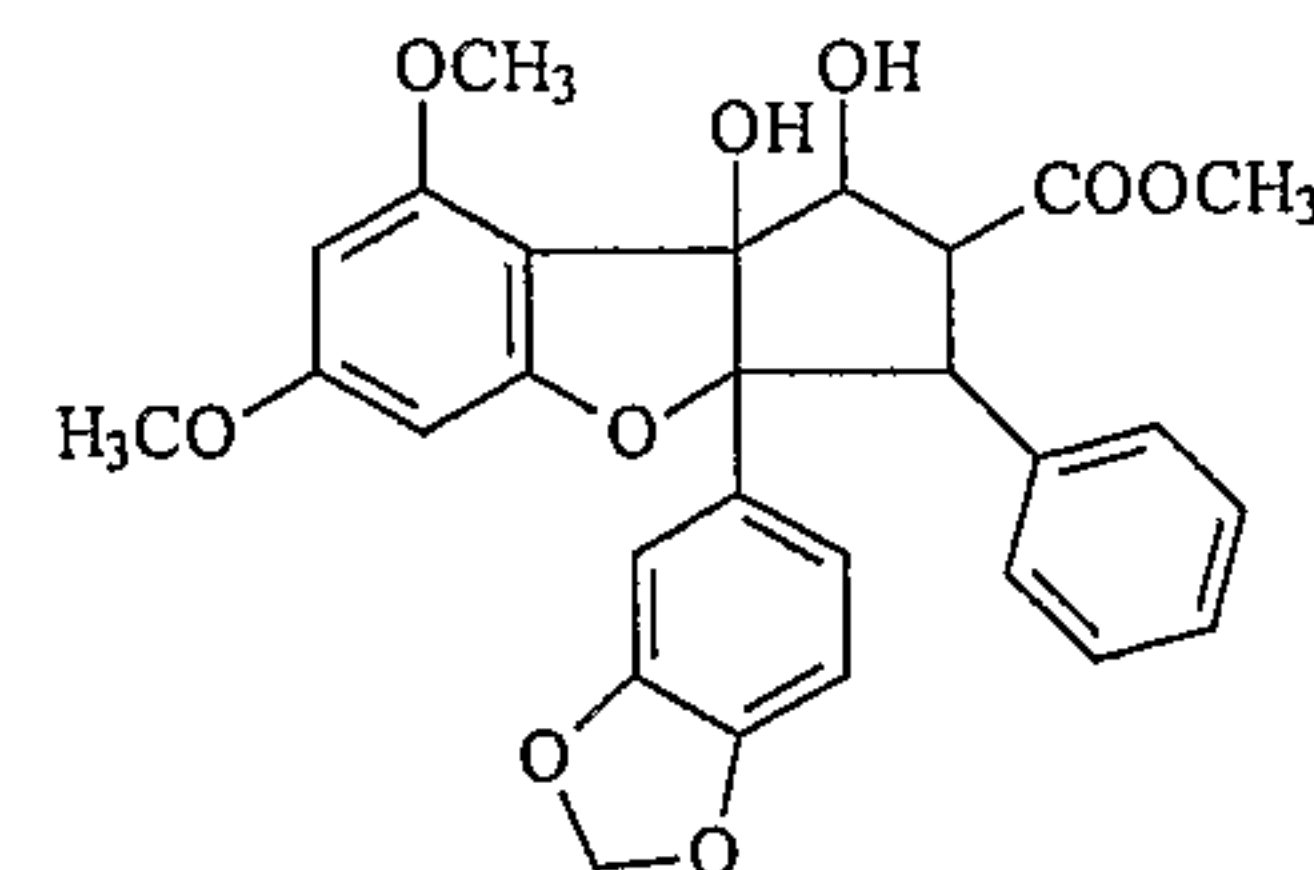
* Compound A was tested for activity in the National Cancer Institute *in vitro* anticancer drug discovery screen. For this Compound A was tested at five 10-fold dilutions ranging from 10^{-4} M to 10^{-8} M against a panel of different human tumour cell lines representing major types of cancer as described by Boyd and Paull, *Drug Development Research*, 1995, 34:91-109. Briefly, this involved a 48 hr incubation of the cells with Compound A prior to measuring the relative cell number by staining with sulforhodamine B. GI₅₀ values represent the concentration of Compound A that inhibited net growth of the cells by 50% compared to untreated controls. LC₅₀ values represent the concentration of Compound A that resulted in a net 50% loss (killing) of the cells relative to the start of the experiment. The data represent the average values from two such experiments conducted.

Example 4

Cytotoxic activity of Compound A is not shared by other known related compounds lacking dioxanyloxy substitution. Compound A' displays cytotoxic activity.

(a) Table 8 compares the cytostatic and cytotoxic effects of Compound A to three previously identified 1*H*-cyclopenta[*b*]benzofuran lignans that lack the dioxanyloxy group at the C6-position. The 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) and 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 at the C6-position is important for the cytotoxic activity exhibited by Compound A and distinguishes it from any other previously identified 1*H*-cyclopenta[*b*]benzofuran lignans.

- 36 -

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

5 **Table 8: Related 1*H*-cyclopenta[*b*]benzofuran lignans lacking the novel dioxanyloxy side chain do not exhibit cytotoxic activity***

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

10 * 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 5. 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
15 the reference compounds are also shown.

(b) Table 9 shows that acetylation of the dioxanyl side chain of Compounds A and B did not reduce their biological activity since Compounds A' and B' inhibited WST-1 reduction of
20 THP-1 leukemic with at least similar potencies to the unmodified compounds. The lower IC₅₀ values for all the compounds depicted in this WST-1 reduction experiment compared to

- 37 -

the values shown in Table 4 reflects the enhanced sensitivity of the cells when treated for 3 days compared to the 18 hr treatment used in the latter assay.

Table 9: Acetylation of the dioxanyl side chain of Compounds A and B does not inhibit their biological activity*

Compound	IC ₅₀ (nM)
Compound A	2.0
Compound A'	0.3
Compound B	1.5
Compound B'	0.7

* Purified compounds solubilized in DMSO were tested over a range of concentrations for their effects on the reduction of WST-1 by THP-1 leukemic cells as described for Table 4 except that the cells were cultured in the presence of the various compounds for 3 days prior to measuring WST-1 reduction.

Example 5

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 9 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 10 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.

- 38 -

Table 10: Compound A inhibits general protein biosynthesis*

Compound	IC₅₀ (nM)		
	THP-1 cells		A549 cells
	Protein synthesis	DNA Synthesis	Protein synthesis
<i>Compound A</i>	27	72	32
<i>Cycloheximide</i>	263	303	238

- 5 * THP-1 cells and A549 cells were pretreated with the indicated concentrations of Compound A for 1 hour prior to the addition of (1 μ Ci/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.

10

Example 6***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 11). 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

20

- 39 -

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 10 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.

Table 11: Compound A promotes monocytic differentiation of HL60 leukemic cells*

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

* 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.

Example 7

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

- 40 -

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
5 cell death, caused cells to accumulate in the G2/M phases of the cell cycle.

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
10 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
15 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
20 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
25 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
30 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

- 41 -

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.

5 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 8

The cytostatic effect of Compound A is reversible in A549 cells

15 The reversibility of the effects of Compound A was 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.

25

Example 9

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

30 To further examine the cell cycle effects of Compound A a cytostatic concentration of this compound was combined together with other anti-cancer agents known to act at specific

- 42 -

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 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.

- 43 -

Example 10

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

5

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.

- 44 -

Example 11***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 11 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 12: Compound A increases cell size*

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

* 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.

- 45 -

Example 12***Compound A inhibits growth of human tumour cell lines in a mouse xenograft tumour model.***

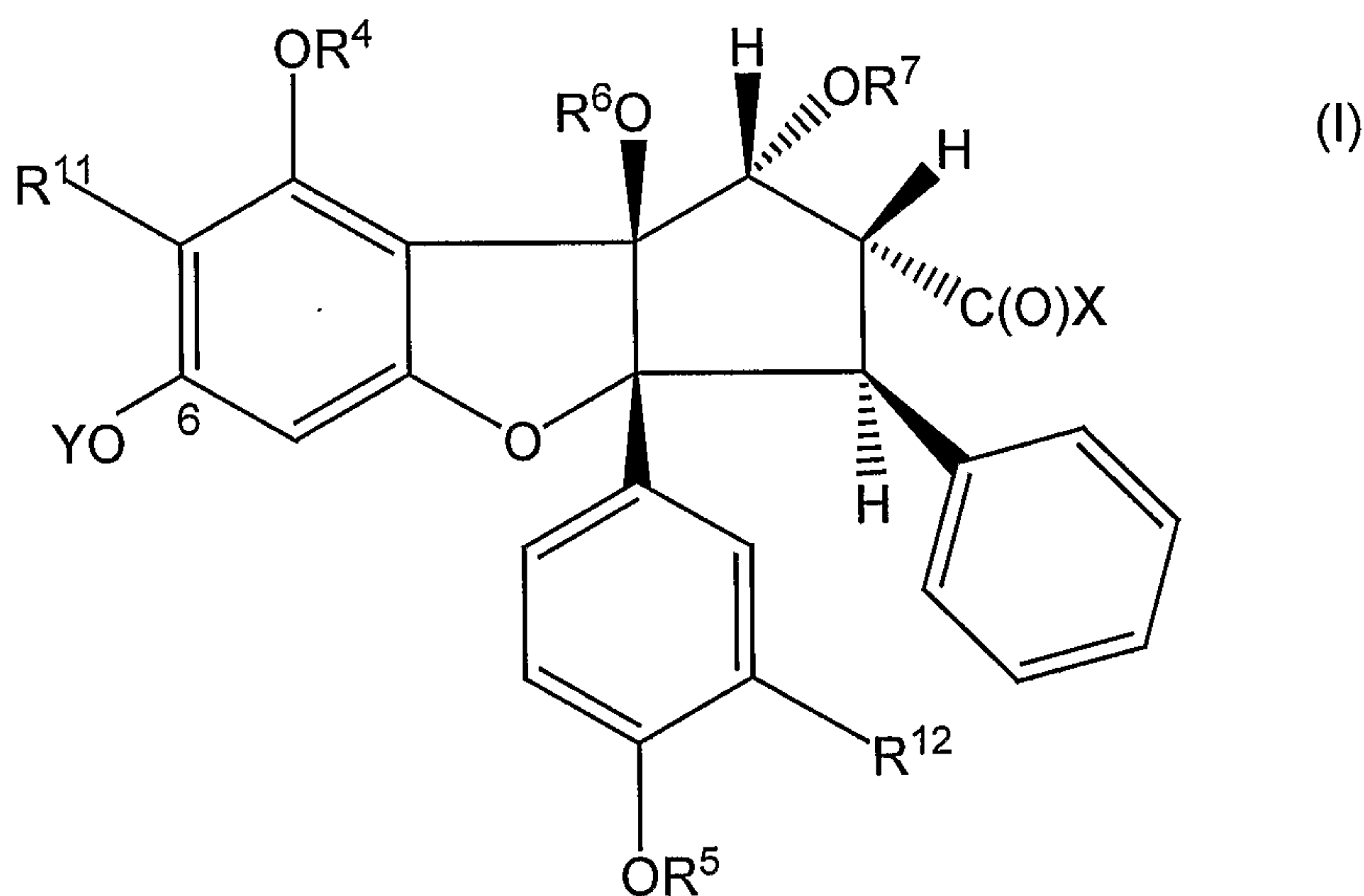
5

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
10 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
15 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.

- 46 -

THE CLAIMS:

1. A compound of Formula (I):



wherein:

each R^4 - R^{10} is independently selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted acyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted cycloalkylalkyl, optionally substituted arylacyl, optionally substituted cycloalkylacyl and a C-1 linked saccharide;

X is OR^8 or NR^9R^{10} ;

R^{11} and R^{12} are each independently hydrogen or, OR^4 and R^{11} , and/or OR^5 and R^{12} together form a methylenedioxy group; and

Y is selected from the group of optionally substituted phenyl, optionally substituted benzyl, optionally substituted benzoyl, optionally substituted C_3 - C_8 cycloalkyl, optionally substituted CH_2 -(C_3 - C_8 cycloalkyl), optionally substituted 5-6 membered heterocyclyl and optionally substituted CH_2 -(5-6 membered heterocyclyl); or a salt or prodrug thereof.

2. A compound according to claim 1 wherein R^4 and Y are independently selected from the group consisting of optionally substituted C_3 - C_8 cycloalkyl, optionally substituted

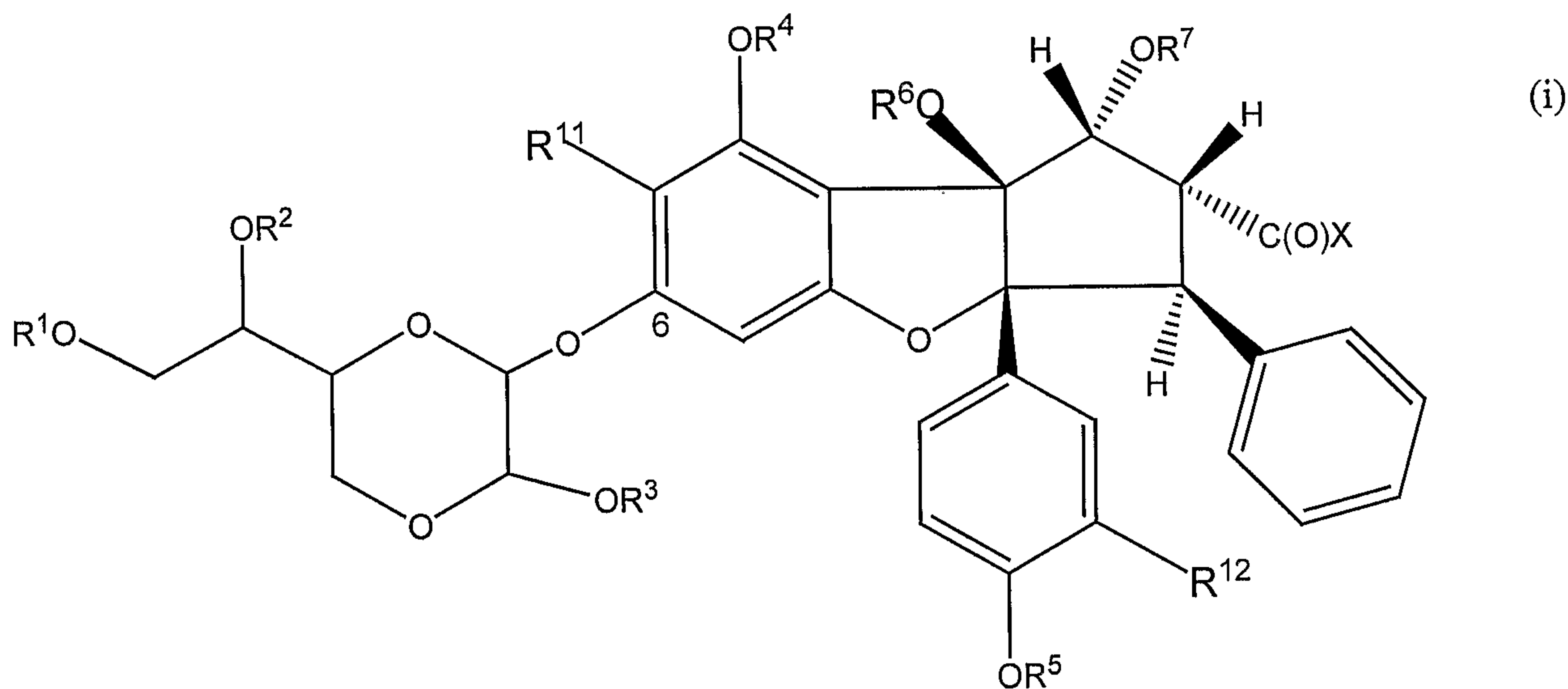
- 47 -

benzoyl, optionally substituted benzyl, optionally substituted CH_2 -(C_3 - C_8 cycloalkyl) and C-1 linked saccharide.

3. A compound according to claim 1 wherein the optionally substituted C_3 - C_8 cycloalkyl group is optionally substituted C_5 - C_6 -cycloalkyl and the optionally substituted CH_2 -(C_3 - C_8 cycloalkyl) group is optionally substituted CH_2 -(C_5 - C_6 cycloalkyl).

4. A compound according to claim 1 wherein Y is an optionally substituted 5-6 membered heterocyclyl group or an optionally substituted C_5 - C_6 cycloalkyl group

5. A compound according to claim 4 having Formula (i)



wherein:

each R^1 - R^{10} is independently selected from the group consisting of hydrogen, optionally substituted alkyl, optionally substituted acyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted cycloalkylalkyl, optionally substituted arylacyl, optionally substituted cycloalkylacyl and a C-1 linked saccharide;

X is OR^8 or NR^9R^{10} ;

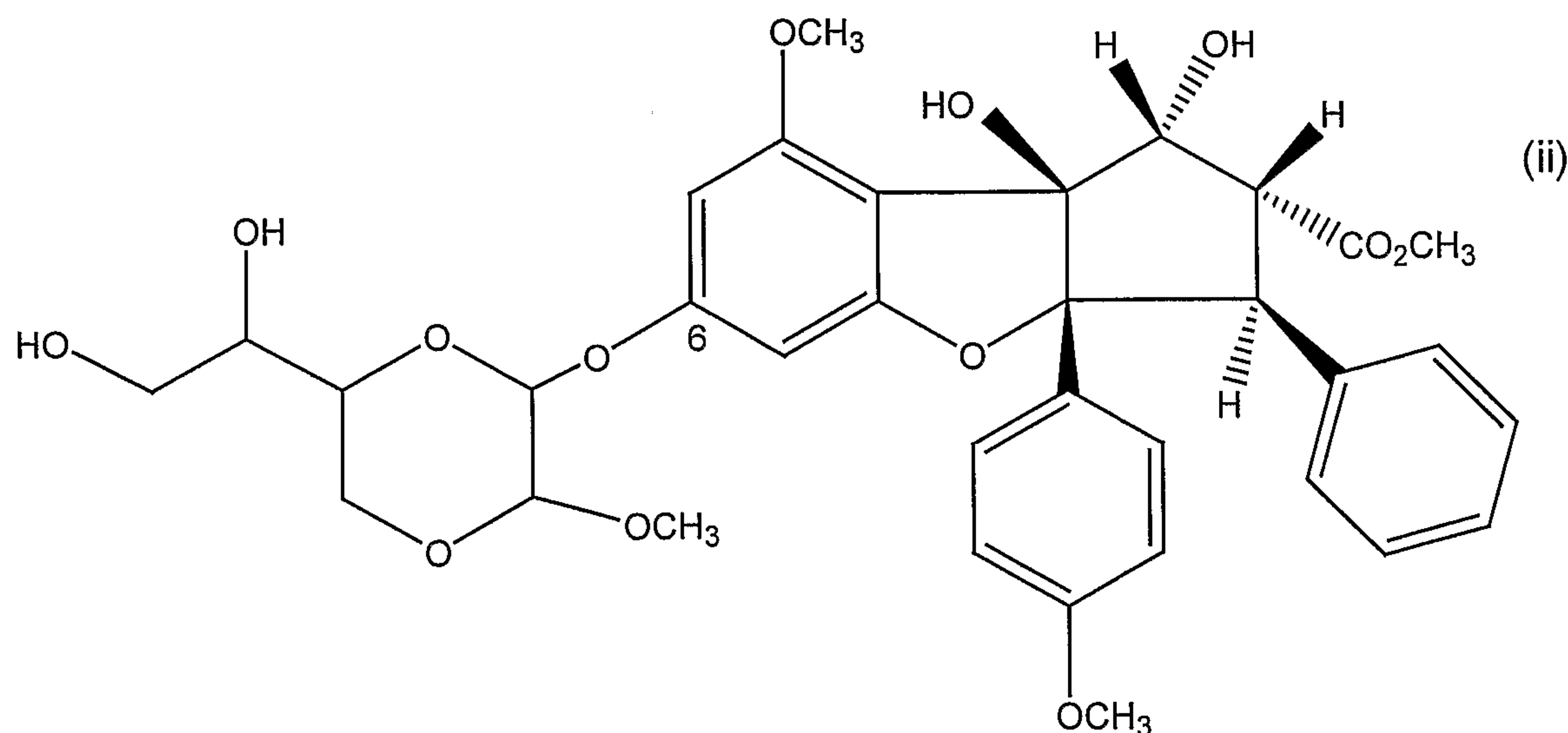
R^{11} and R^{12} are each independently hydrogen or OR^4 and R^{11} , and/or OR^5 and R^{12} together form a methylenedioxy group.

- 48 -

6. A compound according to claim 1 or 5 wherein R^8 is selected from the group consisting of hydrogen, C_{1-6} alkyl, phenyl, benzyl and C-1 linked saccharide.
- 5 7. A compound according to claim 1 or 5 wherein R^9 and R^{10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, phenyl and benzyl.
8. A compound according to claim 1 or 5 wherein R^{11} and R^{12} are both hydrogen.
- 10 9. A compound according to claim 5 wherein each of R^1 - R^7 are independently selected from the group consisting of hydrogen, methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *t*-butyl, cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclobutylethyl, cyclopentylmethyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl, phenyl, benzyl, acetyl and C-1 linked saccharide.
- 15 10. A compound according to claim 5 wherein R^1 and R^2 are both hydrogen.
11. A compound according to claim 5 wherein R^3 is methyl.
- 20 12. A compound according to claim 5 wherein at least one of R^3 - R^5 is methyl, ethyl or propyl.
13. A compound according to claim 12 wherein at least one of R^3 - R^5 is methyl.
- 25 14. A compound according to claim 12 wherein at least two of R^3 - R^5 are methyl, ethyl or propyl.
15. A compound according to claim 14 wherein at least two of R^3 - R^5 are methyl.
- 30 16. A compound according to claim 14 wherein all of R^3 - R^5 are methyl, ethyl or propyl.

- 49 -

17. A compound according to claim 16 wherein all of R^3 - R^5 are methyl.
18. A compound according to claim 1 or 5 wherein R^6 and R^7 are both hydrogen.
- 5 19. A compound according to claim 1 or 5 wherein X is OR^8 and where R^8 is selected from the group consisting of hydrogen, methyl, ethyl and propyl.
20. A compound according to claim 19 wherein R^8 is methyl.
- 10 21. A compound according to claim 1 or 5 wherein X is NR^9R^{10} where R^9 and R^{10} are both hydrogen or methyl; or R^9 and R^{10} are different but at least one of R^9 or R^{10} is hydrogen and the other is C_{1-6} alkyl.
22. A compound according to claim 21 wherein C_{1-6} alkyl is methyl, ethyl or propyl.
- 15 23. A compound according to claim 5 having Formula (ii):



- 20 24. A cyclopentabenzofuran compound having the following NMR spectral characteristics:

- 50 -

¹H NMR (CDCl₃, 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, 11.2Hz, 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; 5.28, s, 1H; 6.28, d, 2Hz, 1H; 6.43, d, 2Hz, 1H; 6.68, 5 brd, 9Hz, 2H; 6.84, m, 2H; 7.06, m, 2H, 7.06, m, 1H; 7.10, brd, 9Hz, 2H

¹³C NMR (CDCl₃), (ppm)

50.03, 52.06, 55.03, 55.05, 55.1, 55.9, 59, 63.3, 68.3, 70.6, 79.6, 92.8, 93.4, 93.9, 94, 95.2, 101.9, 109.6, 112.7, 126.2, 126.6, 127.8, 127.8, 128.9, 136.7, 157.1, 158.8, 160, 160.6, 170.6 10

25. A cyclopentabenzofuran compound having the following NMR spectral characteristics:

¹H NMR (CDCl₃, ppm)

15 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, 2.4 Hz, 1H; 3.86, s, 3H; 3.9, dd, 14, 6.8Hz, 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;

¹³C NMR (CDCl₃), (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.

25 26. A composition comprising a compound of claim 1 together with a pharmaceutically acceptable carrier excipient or diluent.

27. A composition according to claim 26 wherein the compound is of Formula (i) as defined in claim 5 or (ii) as defined in claim 23.

30

- 51 -

28. A method for the treatment of cancer or a cancerous condition comprising the administration of a treatment effective amount of a compound according to claim 1 to a subject in need thereof.

5 29. A method according to claim 28 wherein the compound is of Formula (i) as defined in claim 5 or (ii) as defined in claim 23.

30. A method according to claim 28 or 29 wherein the cancer or cancerous condition is selected from the group consisting of leukemia, sarcoma, breast, colon, bladder, pancreatic,
10 endometrial, head and neck, mesothelioma, myeloma, oesophagal/oral, testicular, thyroid, cervical, bone, renal, uterine, prostate, brain, lung, ovarian, skin, liver and bowel and stomach cancers, tumours and melanomas.

31. A method for the treatment of a disease state or condition associated with cellular
15 hyperproliferation comprising the administration of a treatment effective amount of a compound of claim 1 to a subject in need thereof.

32. A method according to claim 31 wherein the compound is of Formula (i) as defined in claim 5 or (ii) as defined in claim 23.
20

33. A method according to claim 32 wherein the disease state or condition is selected from the group consisting of atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, psoriasis, periodontal disease and virally induced cellular hyperproliferation.

25 34. A composition comprising a cyclopentabenzofuran compound according to claim 24 or 25 together with a pharmaceutically acceptable carrier, excipient or diluent.

30 35. A method for the treatment of cancer or a cancerous condition comprising the administration of a treatment effective amount of a cyclopentabenzofuran compound according to claim 24 or 25 to a subject in need thereof.

- 52 -

36. A method according to claim 35 wherein the cancer or cancerous condition is selected from the group consisting of leukemia, sarcoma, breast, colon, bladder, pancreatic, endometrial, head and neck, mesothelioma, myeloma, oesophagal/oral, testicular, thyroid,
5 cervical, bone, renal, uterine, prostate, brain, lung, ovarian, skin, liver and bowel and stomach cancers, tumours and melanomas.

37. A method for the treatment of a disease state or condition associated with cellular hyperproliferation comprising the administration of a treatment effective amount of a
10 cyclopentabenzofuran compound according to claim 24 or 25 to a subject in need thereof.

38. A method according to claim 37 wherein the disease state or condition is selected from the group consisting of atherosclerosis, restinosis, rheumatoid arthritis, osteoarthritis, inflammatory arthritis, psoriasis, periodontal disease and virally induced cellular
15 hyperproliferation.

Figure 1

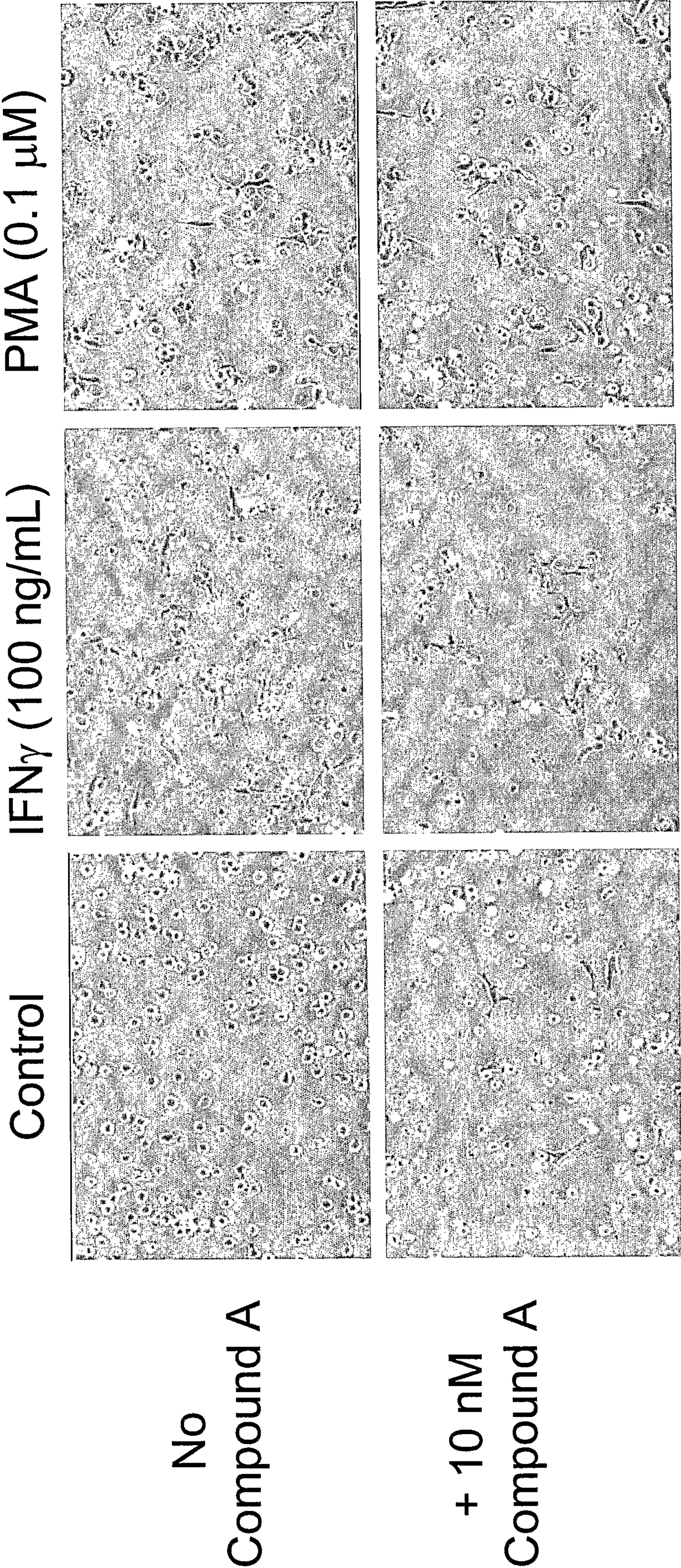


Figure 2

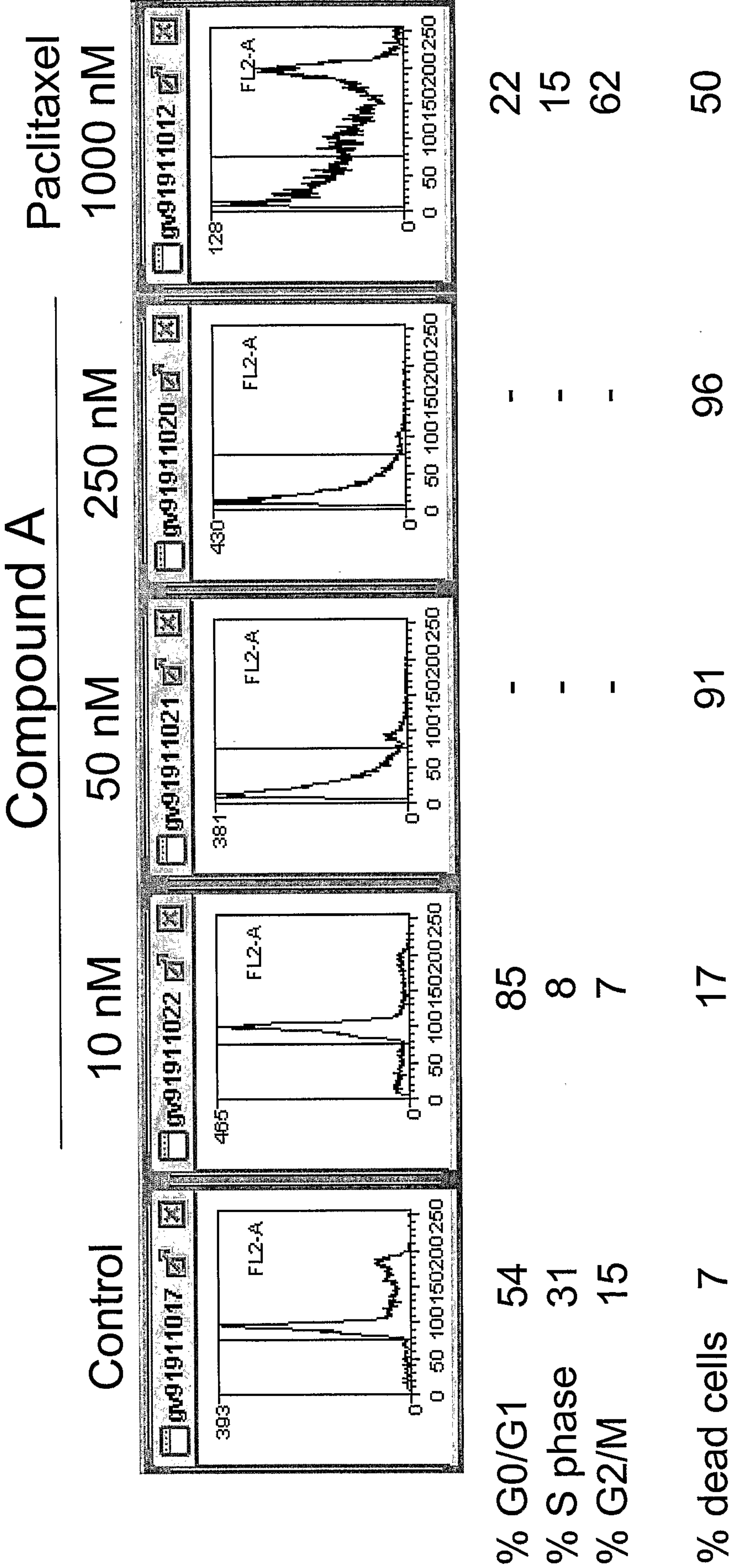


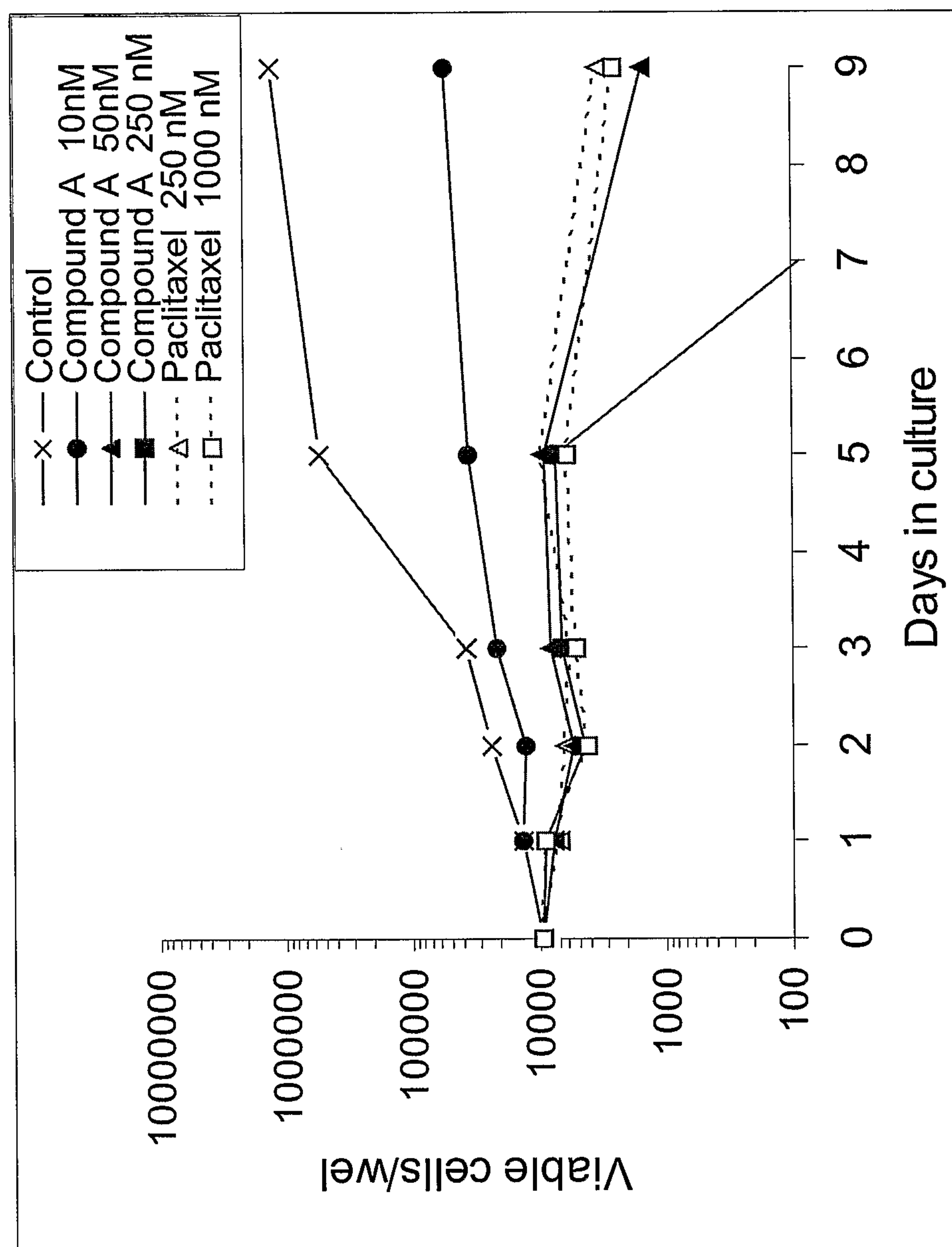
Figure 3

Figure 4

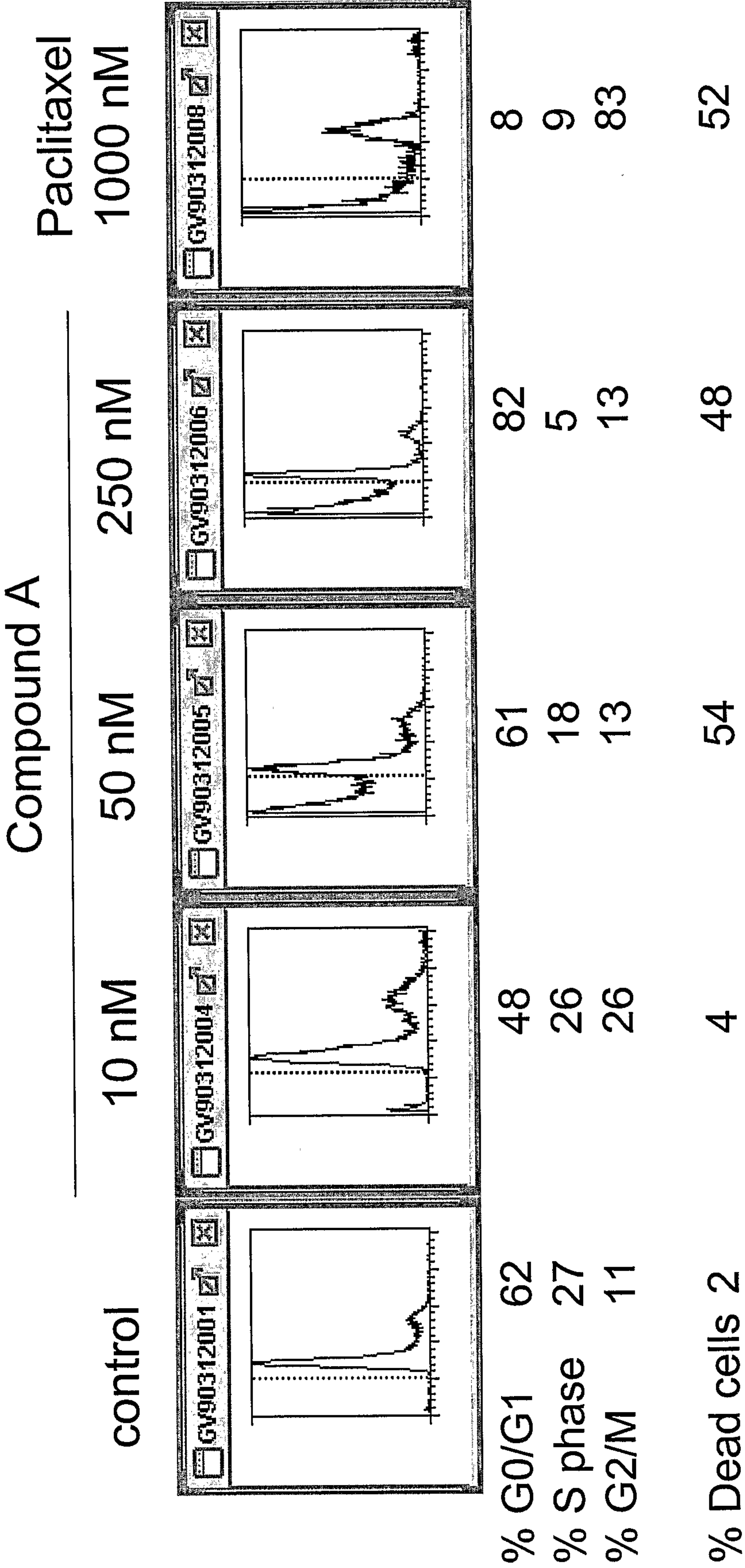


Figure 5

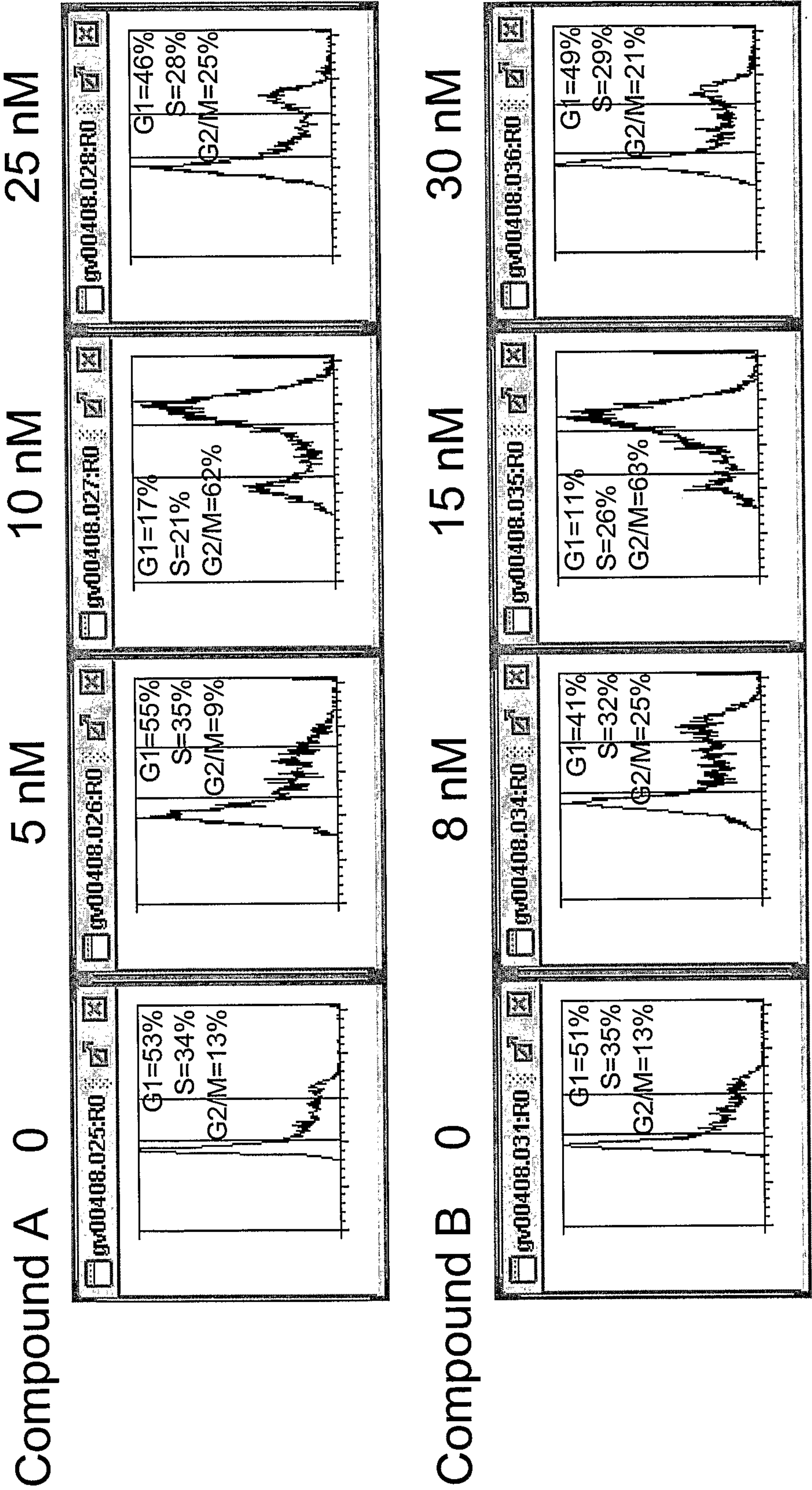


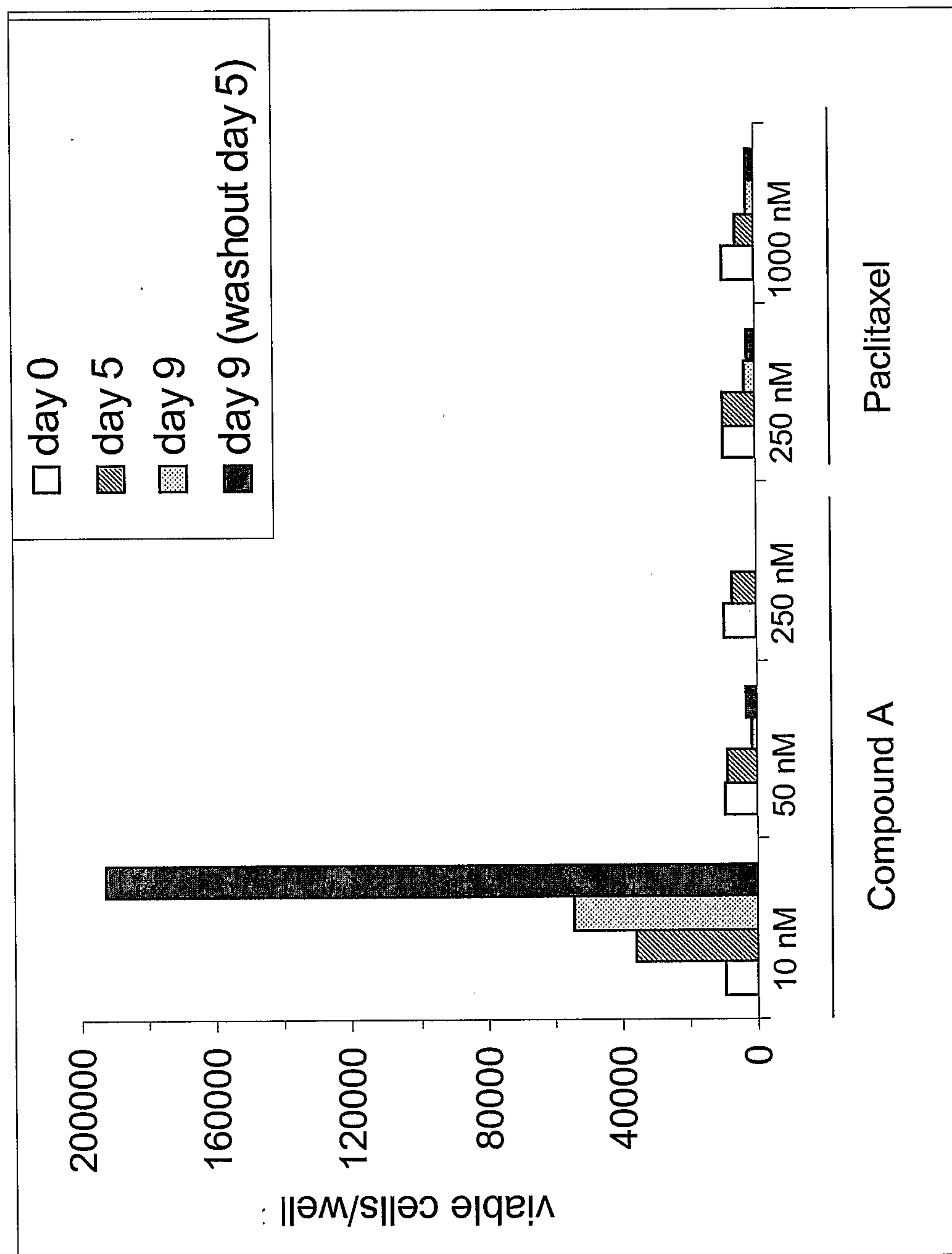
Figure 6

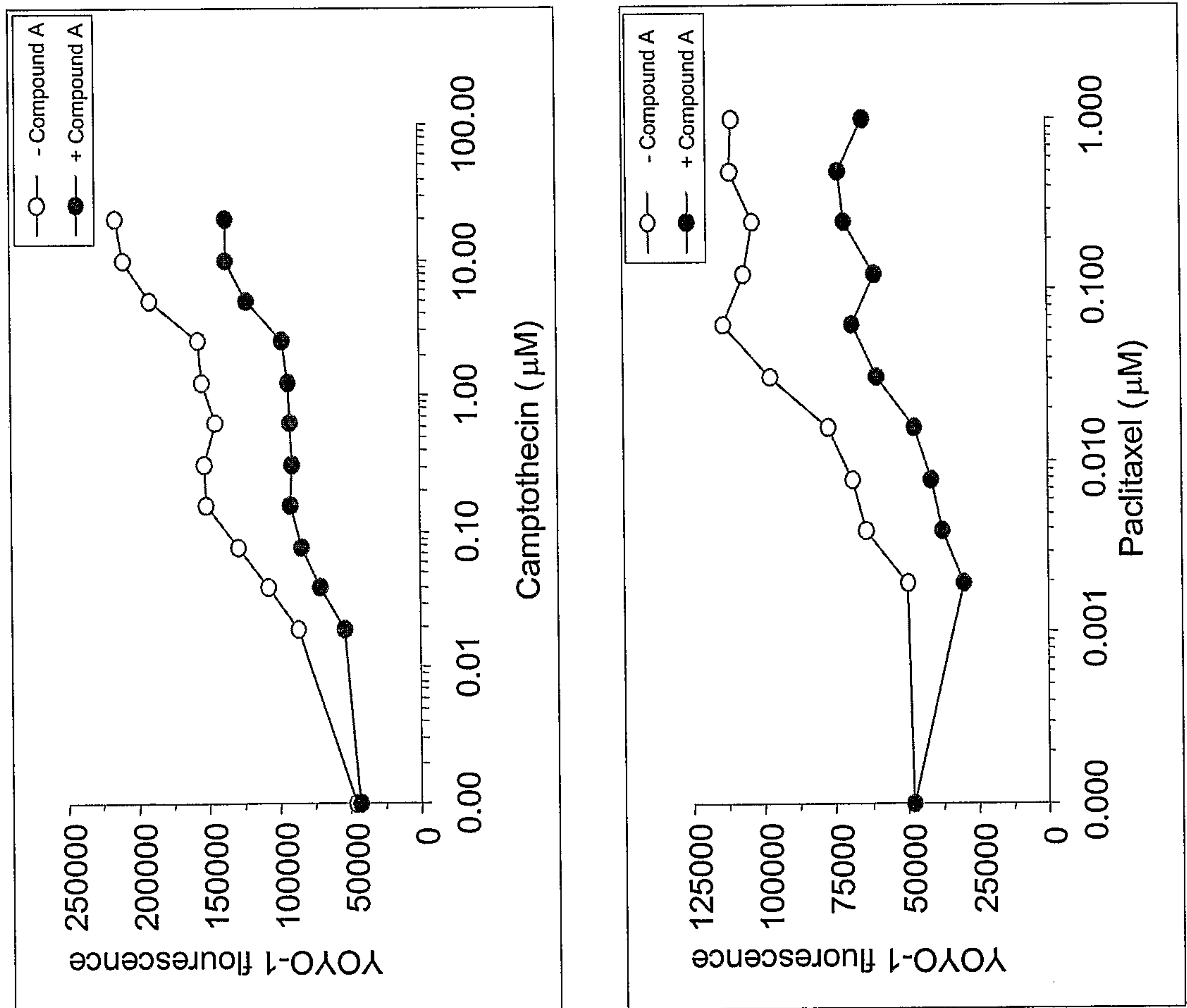
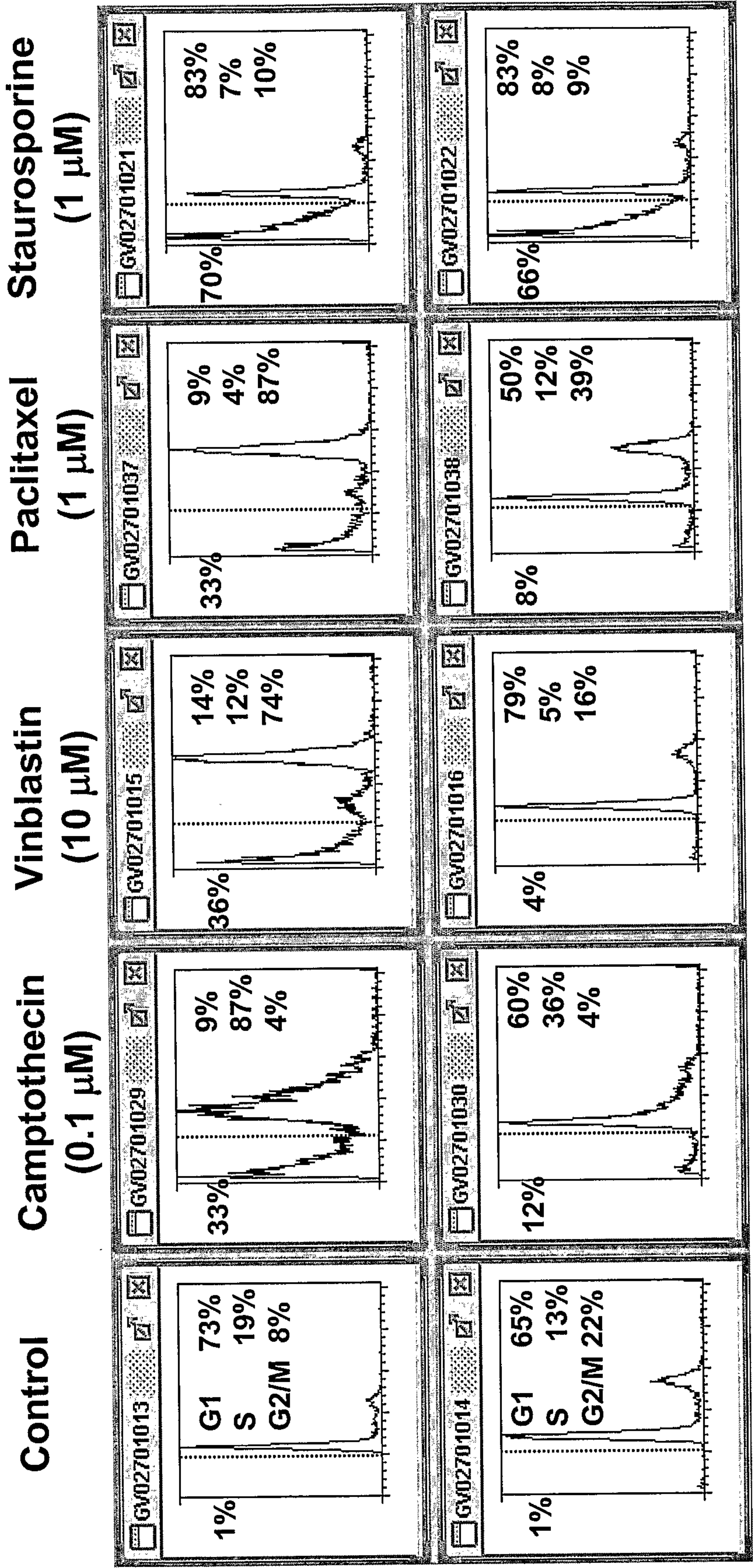
Figure 7

Figure 8



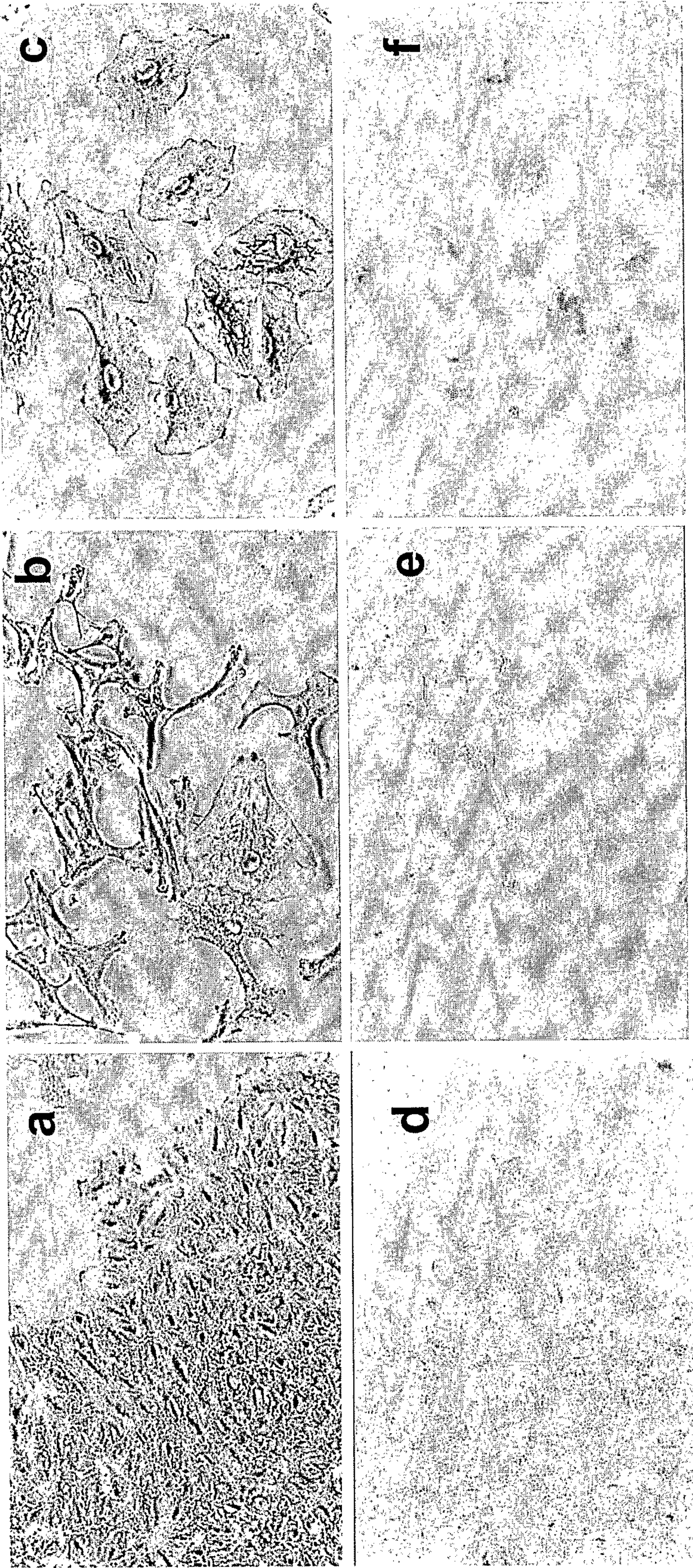
-

+

Compound A
(10 nM)

Figure 9

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



10/10

Figure 10

