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SUBSTITUTED CHROMAN DERIVATIVES, MEDICAMENTS AND USE IN THERAPY

Field of the Invention

- 5 The present invention relates to certain novel chroman derivatives and their salts and derivatives, compositions containing same, methods for their preparation and uses thereof as therapeutic agents particularly as anti-cancer and chemotherapeutic selective agents.

Background of the Invention

- 10 Over 700 different naturally occurring isoflavones are known some of which have biological properties with potential therapeutic benefit.

US 5,726,202 generically discloses certain isoflavan compounds, particularly 3,4-diarylchroman and centchroman for the treatment of benign prostatic hypertrophy.

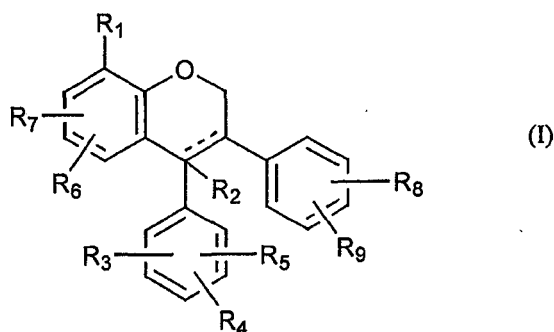
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WO 01/17986 also discloses certain isoflavan compounds.

Summary of the Invention

- Surprisingly, the present inventors have found a novel group of compounds of the general
20 formula (I) which exhibit important therapeutic activities including strong anti-cancer activity, chemotherapeutic selectivity and radiosensitisation of cancers.

Thus according to an aspect of the present invention there is provided a compound of the general formula (I):



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or a salt or derivative thereof wherein:

- R_1 is hydrogen, hydroxy, halo, $NR_{10}R_{11}$, C_{3-6} cycloalkyl, C_{1-6} alkoxy, C_{2-6} alkenyl, C_{1-6} fluoroalkyl or C_{1-6} alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$ groups;
- 5 the drawing "==" and R_2 together represent a double bond or the drawing "-" represents a single bond and R_2 is hydrogen, hydroxy, $NR_{10}R_{11}$, C_{1-3} alkoxy, C_{1-3} fluoroalkyl, halo or C_{1-3} alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$ groups;
- R_3 is hydrogen, hydroxy, halo, $NR_{10}R_{11}$, C_{3-6} cycloalkyl, C_{1-6} alkoxy, C_{1-6} fluoroalkyl, C_{2-6} alkenyl, $COOR_{12}$, COR_{13} , $(O)_nC_{1-4}$ alkylene $NR_{14}R_{15}$ or C_{1-6} alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$ groups;
- 10 R_4 , R_5 , R_6 , R_7 , R_8 and R_9 are independently hydrogen, hydroxy, halo, $NR_{10}R_{11}$, C_{3-6} cycloalkyl, C_{1-6} alkoxy, C_{1-6} fluoroalkyl, C_{2-6} alkenyl, $COOR_{12}$, COR_{13} or C_{1-6} alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$;
- 15 R_{10} , R_{11} and R_{12} are independently hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl or trialkyl silyl; R_{13} is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl or $NR_{10}R_{11}$;
- n represents 0 or 1; and R_{14} and R_{15} independently represent hydrogen or C_{1-6} alkyl or $NR_{14}R_{15}$ when taken together represents a 5 or 6 member heteroaromatic or heterocyclic;
- 20 and pharmaceutically acceptable salts thereof, with the proviso that when R_1 represents hydrogen and "-" is a single bond then R_2 does not represent hydrogen.

According to another aspect of the present invention there is provided a process for the
25 preparation of a compound of formula (I).

According to another aspect of the present invention there is provided a pharmaceutical composition which comprises one or more compounds of formula (I) or a pharmaceutically acceptable salt or derivative thereof in association with one or more pharmaceutical
30 carriers, excipients, auxiliaries and/or diluents.

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Thus, according to another aspect of the present invention there is provided the use of a compound of formula (I) in therapy, particularly chemotherapy and as radiosensitising agents.

- 5 According to another aspect of the present invention there is provided a method for the treatment, prevention or amelioration of a disease or disorder, which comprises administering to a subject an effective amount of one or more compounds of the formula (I) or a pharmaceutically acceptable salt or derivative thereof optionally in association with a carrier and/or excipient.

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According to another aspect of the present invention there is provided an agent for the treatment, prophylaxis or amelioration of a disease or disorder which agent comprises one or more compounds of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

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Brief Description of the Figure

Figure 1. Shows pharmacokinetics and distribution of compound No. 1 identified below in serum, faeces and urine. Average values are presented for free and total concentrations of the compound ($\pm$ SEM) as a semi-log plot in part A and as a standard linear plot in part

20

B.

Detailed Description of the Invention

The present inventors have found that compounds of the general formula (I) show surprising and unexpected biological and pharmaceutical properties.

25

The compounds of formula (I) of the invention are believed to have favourable toxicity profiles with normal cells and good bioavailability. Surprisingly the compounds of the invention exhibit anti-cancer activity, significantly better than or at least comparable to known cancer treatments.

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The compounds of formula (I) are cytostatic and cytotoxic against a broad range of cancer cells of human and animal origin. By cancer cells, it is meant cells that display malignant characteristics and which are distinguished from non-cancer cells by unregulated growth and behaviour which usually ultimately is life-threatening unless successfully treated.

5 The cancer cells that have been found to be responsive to compounds of formula (I) are of epithelial origin (for example, prostate, ovarian, cervical, breast, gall-bladder, pancreatic, colorectal, renal, and non-small lung cancer cells), of mesenchymal origin (for example, melanoma, mesothelioma and sarcoma cancer cells), and of neural origin (for example glioma cancer cells).

10

It is highly unusual and surprising to find a related group of compounds that display such potent cytotoxicity against cancer cells. Furthermore it is thought that the compounds according to the invention also have low toxicity against non-cancer cells such as keratinocytes derived from human foreskin. Such cancer cell selectivity is highly unusual
15 and unexpected.

Advantageously the compounds of formula (I) show cytotoxicity against cancer cells that are well recognised for being poorly sensitive to standard anti-cancer drugs. It is highly unusual and unexpected to find such potent activity against cancers, for example,
20 cholangiocarcinoma, pancreatic adenocarcinoma and melanoma, that are highly resistant to known anti-cancer drugs.

Advantageously the compounds of formula (I) also seem to display an ability to radio-sensitise cancer cells, by which it is meant that these compounds either lower the amount
25 of gamma-irradiation that is required to kill the cells, or they convert cancer cells from a state of radio-resistance to radio-sensitivity.

Additionally the compounds of formula (I) are thought to possess chemo-sensitising activity, that is they increase the cytotoxicity of chemotherapeutic agents, especially to
30 cancer cells, and/or convert cancerous cells from a state of chemo-resistance to a chemo-sensitive state.

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Compounds of the invention may also provide chemo and/or radio-protective properties for non-cancerous cells. This has significant therapeutic implications because the traumatic side-effects of chemotherapy and radiotherapy are caused by the toxicity of the traditional treatments to non-cancerous cells.

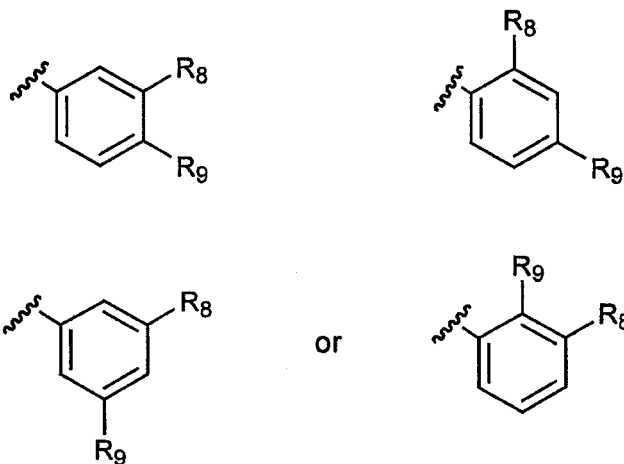
The properties described above offer significant clinical advantages.

The radio and/or chemo-protective properties of the compounds of the invention may be employed to protect healthy individuals from the effects of radiation and/or chemical toxins, or lessen the effects of the same.

The properties described above offer significant clinical advantages.

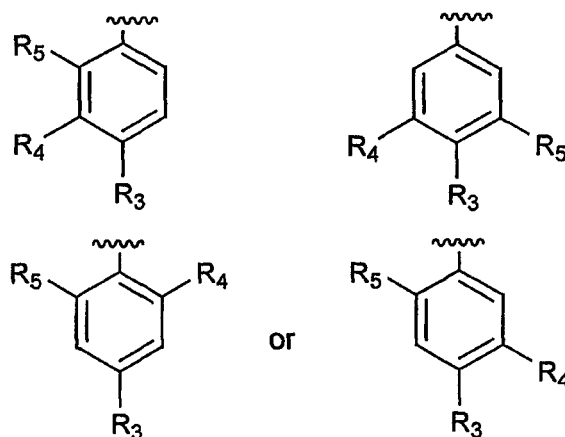
The invention also provides the use of compounds of formula (I) to treat patients with cancer by either reducing the rate of growth of such tumours or by reducing the size of such tumours through therapy with such compounds alone, and/or in combination with each other, and/or in combination with other anti-cancer agents, and/or in combination with radiotherapy.

Generally in compounds of formula (I) according to the invention the substituents R_8 and R_9 will be distributed as shown below:



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Generally in compounds of formula (I) according to the invention the substituents R_3 , R_4 and R_5 will be distributed as shown below:



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Preferably in compounds of formula (I) the drawing "—" represents a single bond.

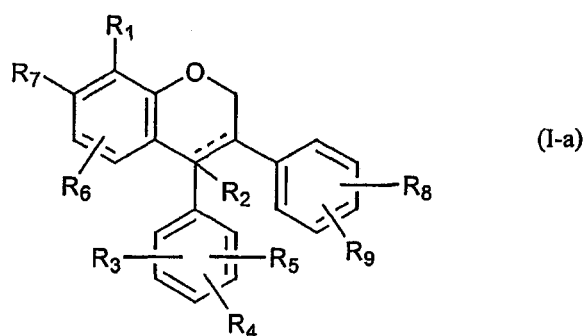
Preferably in compounds of the invention, including compounds of formula (I) R_3 will be in the *para*-position.

10

In compounds of the invention, including compounds of formula (I) when R_3 represents $(O)_n C_{1-4}$ alkylene $NR_{14}R_{15}$ preferably it represents $-OC_2$ alkylene $NR_{14}R_{15}$ wherein $NR_{14}R_{15}$ represents pyrrolidinyl.

15

According to the invention there is provided compounds of formula (I-a):



(I-a)

or a salt or derivative thereof wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are as defined above for compounds of formula (I)

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with the proviso that when R₁ represents hydrogen and "—" is a single bond then R₂ does not represent hydrogen.

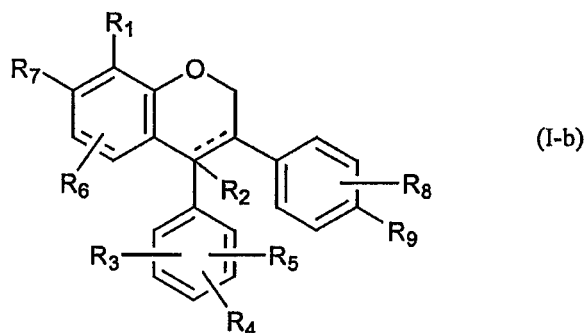
The positions of R₃, R₄ and R₅ shown above for compounds of formula (I) apply equally to
5 compounds of formula (I-a).

The position of R₈ and R₉ shown above for compounds of formula (I) equally applies to compounds of formula (I-a).

10 In compounds of formula (I-a) R₇ preferably represents C₁₋₆ alkoxy or hydroxy, especially methoxy or hydroxy.

In compounds of formula (I-a) preferably "—" represents a single bond.

15 In another preferred aspect the invention there is provided compounds of formula (I-b):



or a salt or derivative thereof wherein

R₁ represents hydroxy, halo, NR₁₀R₁₁, C₃₋₆ cycloalkyl, C₆ alkoxy, C₂₋₆ alkenyl, C₁₋₆
20 fluoroalkyl, C₁₋₆ alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or NR₁₀R₁₁ groups; and

R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are defined above for compounds of formula (I).

The positions of R₃, R₄ and R₅ shown above for compounds of formula (I) apply equally to
25 compounds of formula (I-b).

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The position of R₈ and R₉ shown above for compounds of formula (I) apply equally to compounds of formula (I-b).

- 5 Preferably in compounds of formula (I-b) R₁ represents hydroxy, C₁₋₆ alkoxy, C₁₋₆ fluoroalkyl, C₁₋₆ alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or NR₁₀R₁₁ groups, especially C₁₋₆ alkyl, particularly methyl.

- 10 Preferably in compounds of formula (I-b) R₃ represents hydroxy, C₁₋₆ alkoxy or C₁₋₆ alkyl optionally substituted by one or more hydroxy, chloro, bromo, iodo or NR₁₀R₁₁ groups, especially C₁₋₆ alkoxy or hydroxy, particularly methoxy.

Preferably in compounds of formula (I-b) R₃ is in the para position.

- 15 Preferably in compounds of formula (I-b) R₄, R₅ and R₆ independently represents hydrogen.

Preferably in compounds of formula (I-b) R₇ represents hydroxy or C₁₋₆ alkoxy, especially hydroxy or methoxy.

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Preferably in compound of formula (I-b) R₈ represents hydrogen, hydroxy or C₁₋₆ alkoxy, especially hydrogen, hydroxy or methoxy, particularly hydrogen.

Preferably in compounds of formula (I-b) R₈ is in the 3 position.

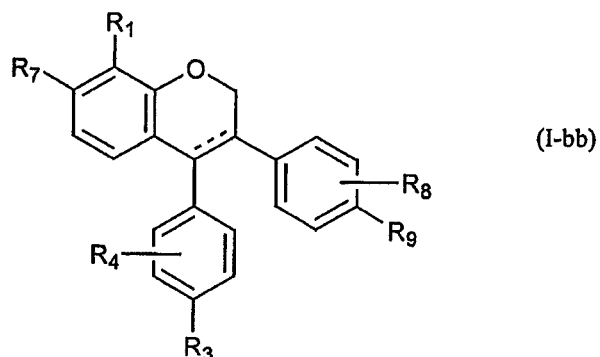
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Preferably R₉ in compounds of formula (I-b) represents hydrogen hydroxy or C₁₋₆ alkoxy, especially hydroxy or C₁₋₆ alkoxy, particularly hydroxy or methoxy.

Thus, in another aspect the invention provides compounds of the formula (I-bb):

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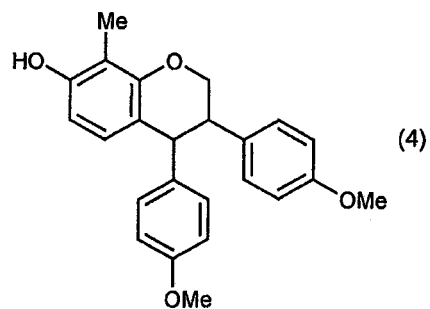
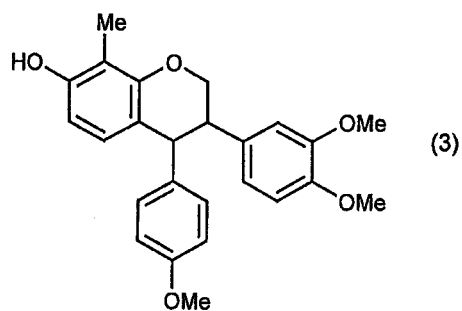
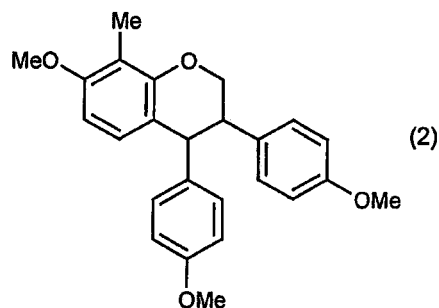
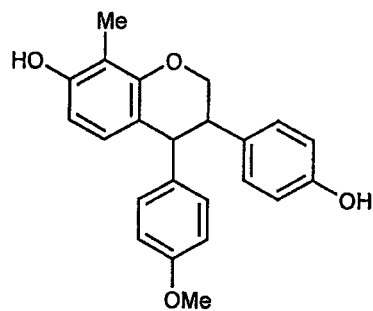
or a salt or derivative thereof wherein

R₁, R₃, R₄, R₇, R₈ and R₉ are as defined above for compounds of formula (I-b).

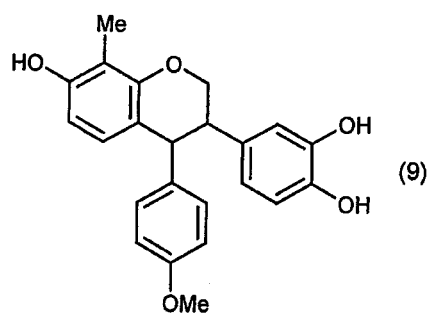
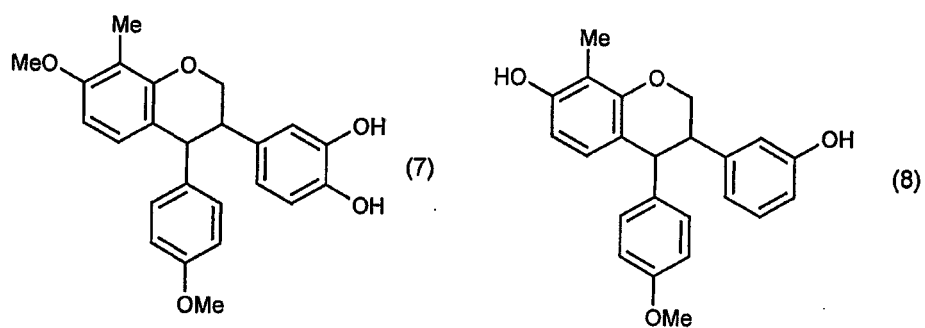
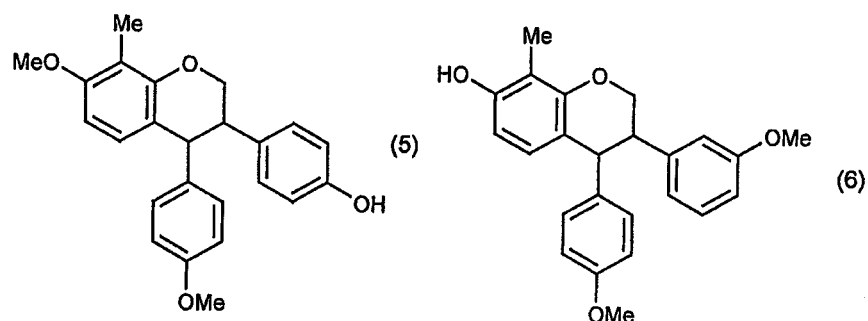
- 5 In a highly preferred embodiment, "----" represents a single bond.

The preferences expressed above for compounds of formula (I-b) apply equally to compounds of formula (I-bb).

- 10 Specific compounds within the scope of this first aspect of the invention are as follows:



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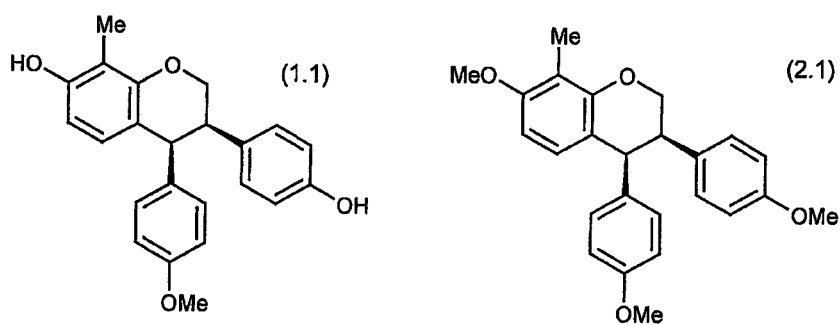


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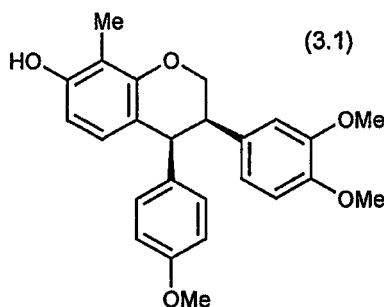
or a salt or derivative thereof.

Most preferably the compounds of formula (I-bb) have the following structure:

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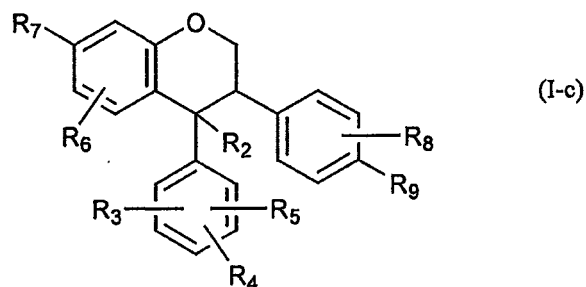


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or salts or derivatives thereof.

5 In another preferred aspect the invention provides a compound of formula (I-c):



or a salt or derivative thereof wherein

R_2 represents hydroxy, halo, $NR_{10}R_{11}$, C_{1-3} alkoxy, C_{1-3} fluoroalkyl, C_{1-3} alkyl optionally
 10 substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$ groups; and R_3 , R_4 ,
 R_5 , R_6 , R_7 , R_8 and R_9 are as defined above for compounds of formula (I).

The positions of R_3 , R_4 and R_5 shown above for compounds of formula (I) apply equally to
 compounds of formula (I-b).

15

The position of R_8 and R_9 shown above for compounds of formula (I), where R_9 is in the
 para position apply equally to compounds of formula (I-b).

$NR_{10}R_{11}$ in compounds of formula (I-c) preferably represents hydrogen or C_{1-3} alkyl,
 20 especially hydrogen or methyl.

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Preferably in compounds of formula (I-c) R_2 represents hydroxy, methoxy, methyl or trifluoromethyl.

- Preferably in compounds of formula (I-c) R_3 represents hydroxy, C_{1-6} alkoxy or C_{1-6} alkyl
 5 optionally substituted by one or more hydroxy, chloro, bromo, iodo or $NR_{10}R_{11}$ groups, especially C_{1-6} alkoxy, such as methoxy, particularly methoxy.

Preferably in compounds of formula (I-c) R_4 , R_5 and R_6 independently represent hydrogen.

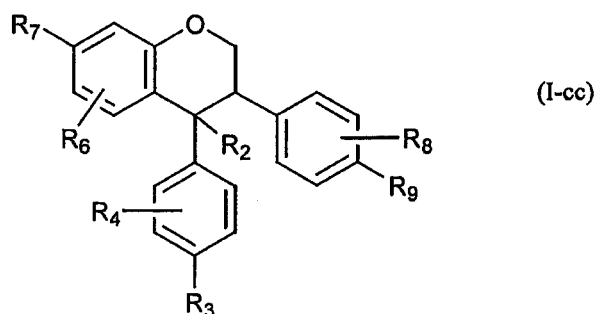
- 10 Preferably in compounds of formula (I-c) R_8 represents hydrogen, hydroxy or C_{1-6} alkoxy, more preferably hydrogen or methoxy, especially hydrogen.

Preferably in compounds of formula (I-c) R_8 is situated in the 3 position.

- 15 Preferably in compounds of formula (I-c) R_9 represents, hydrogen, hydroxy or C_{1-6} alkoxy, especially hydroxy or C_{1-6} alkoxy, particularly hydroxy or methoxy.

More preferably in this second aspect the invention provides a compound of formula (I-cc):

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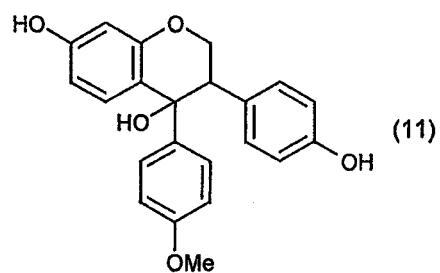
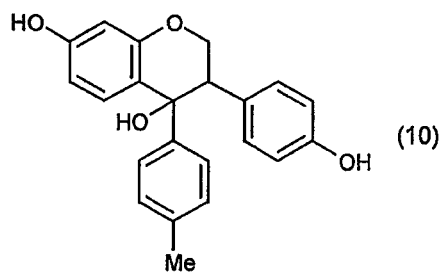
or a salt or a derivative thereof wherein:

R_2 , R_3 , R_4 , R_6 , R_7 , R_8 and R_9 are defined above for compounds of formula (I-c).

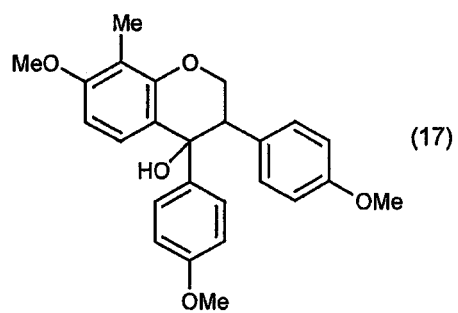
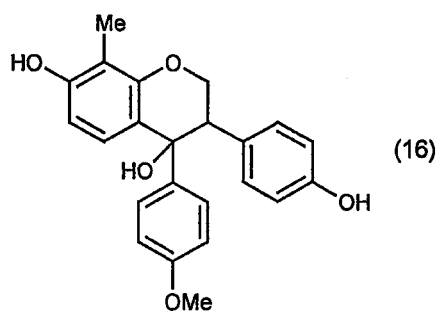
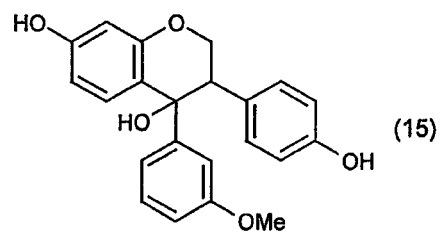
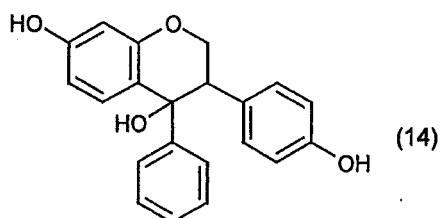
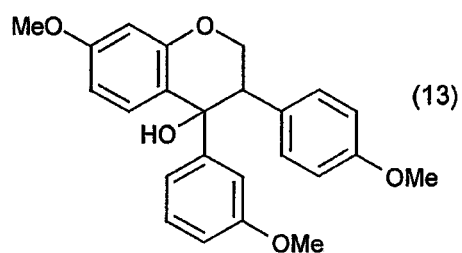
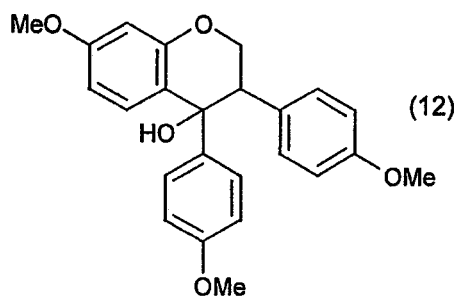
- 25 Preference expressed above for compounds of formula (I-c) apply equally to compound of formula (I-cc).

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Specific compounds of formula (I-cc) are shown below:

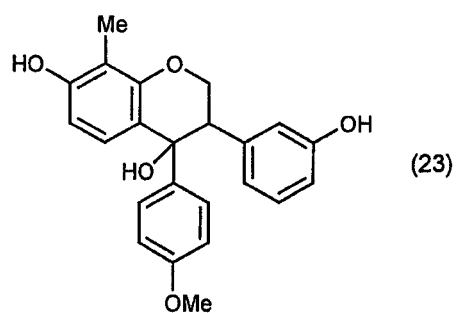
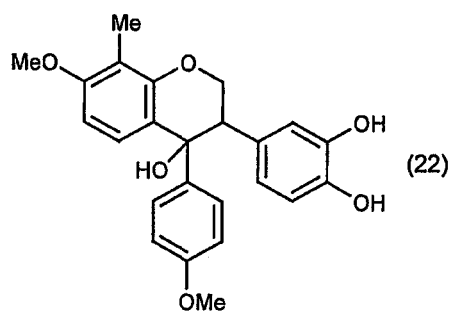
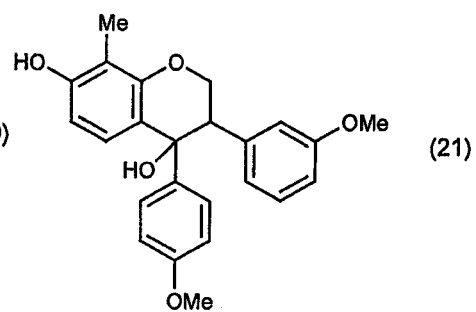
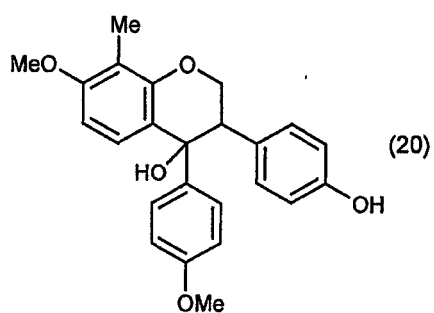
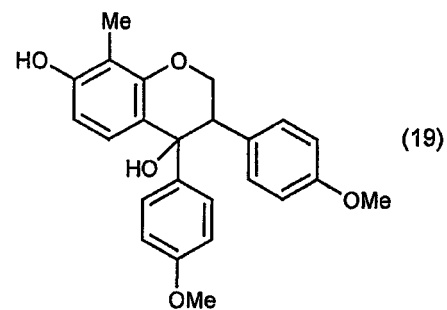
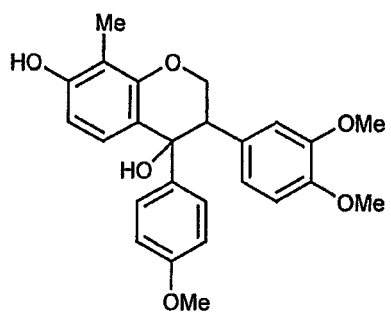


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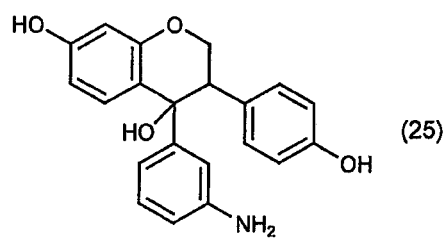
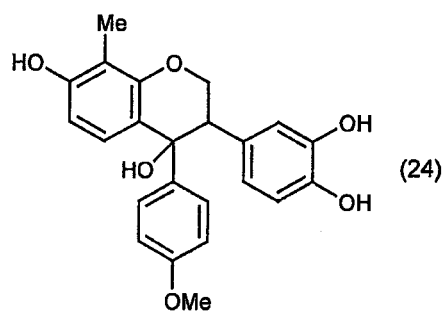


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or a salt or a derivative thereof.

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The compounds of formula (I) according to the invention include two chiral centres. The present invention includes all the enantiomers and diastereoisomers as well as mixtures thereof in any proportions. The invention also extends to isolated enantiomers or pairs of enantiomers. Methods of separating enantiomers and diastereoisomers are well known to
5 person skilled in the art.

It will be clear to persons skilled in the art that in the compounds according to the invention the aryl substituents on the heterocyclic ring can be *cis* or *trans* relative to each other. Preferably in the compounds according to the invention of formula (I) these
10 substituents will be *cis*.

A particularly preferred compound of the present invention is the *cis*-isomer of compound labelled compound No. 1 above.

15 Likewise, particularly preferred compounds are compound Nos. (2) to (9) in the *cis*-conformation.

Preferably, the salts of compounds according to the invention will be pharmaceutically acceptable salts.
20

The term alkyl is taken to include straight chain and branched chain saturated alkyl groups of 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tertiary butyl, pentyl and the like. The alkyl group more preferably contains preferably from 1 to 4 carbon atoms, especially methyl, ethyl, propyl or isopropyl.

25 Cycloalkyl includes C₃₋₆ cycloalkyl such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The alkyl group or cycloalkyl group may optionally be substituted by one or more of
30 fluorine, chlorine, bromine, iodine, carboxyl, C₁₋₄ alkoxy carbonyl, C₁₋₄ alkylamino-

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carbonyl, di-(C₁₋₄ alkyl)-amino-carbonyl, hydroxyl, C₁₋₄ alkoxy, formyloxy, C₁₋₄ alkyl-carbonyloxy, C₁₋₄ alkylthio, C₃₋₆ cycloalkyl or phenyl.

Preferably the alkyl group does not bear any substituents.

5

The term C₁₋₆ alkoxy includes groups wherein the alkyl portion therein is a straight chain or branched chain alkyl moiety. C₁₋₆ alkoxy groups include: methoxy, ethoxy, propoxy, isopropoxy, butoxy, tertiary butoxy and sec-butoxy. Preferably the C₁₋₆ alkoxy substituents will be methoxy or ethoxy, especially methoxy.

10

The term fluoroalkyl includes "alkyl" wherein one or more such as 1, 2, 3, 4 or 5 of the hydrogens have been replaced by fluoro. The fluoroalkyl may be a straight chain or branched chain "alkyl" unit. Preferred fluoroalkyl groups include trifluoromethyl and pentafluoromethyl.

15

The term aryl is taken to include phenyl, benzyl, biphenyl and naphthyl and may be optionally substituted by one or more C₁₋₄ alkyl, hydroxy, C₁₋₄ alkoxy, carbonyl, C₁₋₄ alkoxycarbonyl, C₁₋₄ alkylcarbonyloxy, nitro or halo.

20 The term "halo" is taken to include fluoro, chloro, bromo and iodo, preferably fluoro, chloro.

5 or 6 membered heterocyclic and heteroaromatic rings include: pyrrole, pyrroline, pyrrolidine, oxazoline, thiazole, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, isoxazole, isothiazole, oxadiazole, furazan, triazole, thiadiazole, pyridine, piperidine, morpholine, thiomorpholine, pyridazine, pyrimidine, pyrazine, piperazine, triazine, thiadiazine and dithiazine each of which may be optionally substituted by one or more of fluorine, chlorine, bromine, iodine, carboxyl, C₁₋₄ alkoxycarbonyl, C₁₋₄ alkylamino-carbonyl, di-(C₁₋₄ alkyl)-amino-carbonyl, hydroxyl, C₁₋₄ alkoxy, formyloxy, C₁₋₄ alkyl-carbonyloxy, C₁₋₄ alkylthio or C₃₋₆ cycloalkyl.

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The compounds of the invention include all salts, such as acid addition salts, anionic salts and zwitterionic salts, and in particular include pharmaceutically acceptable salts as would be known to those skilled in the art. The term "pharmaceutically acceptable salt" refers to an organic or inorganic moiety that carries a charge and that can be administered in association with a pharmaceutical agent, for example, as a counter-cation or counter-anion in a salt. Pharmaceutically acceptable cations are known to those of skilled in the art, and include but are not limited to sodium, potassium, calcium, zinc and quaternary amine. Pharmaceutically acceptable anions are known to those of skill in the art, and include but are not limited to chloride, acetate, tosylate, citrate, bicarbonate and carbonate.

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Pharmaceutically acceptable salts include those formed from: acetic, ascorbic, aspartic, benzoic, benzenesulphonic, citric, cinnamic, ethanesulphonic, fumaric, glutamic, glutaric, gluconic, hydrochloric, hydrobromic, lactic, maleic, malic, methanesulphonic, naphthoic, hydroxynaphthoic, naphthalenesulphonic, naphthalenedisulphonic, naphthaleneacrylic, oleic, oxalic, oxaloacetic, phosphoric, pyruvic, para-toluenesulphonic, tartaric, trifluoroacetic, triphenylacetic, tricarballic, salicylic, sulphuric, sulphamic, sulphanilic and succinic acid.

15

The term "pharmaceutically acceptable derivative" or "prodrug" refers to a derivative of the active compound that upon administration to the recipient is capable of providing directly or indirectly, the parent compound or metabolite, or that exhibits activity itself and includes for example phosphate derivatives and sulphonate derivatives. Thus, derivatives include solvates, pharmaceutically active esters, prodrugs or the like.

20

The preferred compounds of the present invention also include all derivatives with physiologically cleavable leaving groups that can be cleaved *in vivo* to provide the compounds of the invention or their active moiety. The leaving groups may include acyl, phosphate, sulfate, sulfonate, and preferably are mono-, di- and per-acyl oxy-substituted compounds, where one or more of the pendant hydroxy groups are protected by an acyl group, preferably an acetyl group. Typically acyloxy substituted compounds of the invention are readily cleavable to the corresponding hydroxy substituted compounds.

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Chemical functional group protection, deprotection, synthons and other techniques known to those skilled in the art may be used where appropriate to aid in the synthesis of the compounds of the present invention, and their starting materials.

5

The protection of functional groups on the compounds and derivatives of the present invention can be carried out by well established methods in the art, for example as described in T. W. Greene, *Protective Groups in Organic Synthesis*, John Wiley & Sons, New York, 1981.

10

Hydroxyl protecting agroups include but are not limited to carboxylic acid esters, eg acetate esters, aryl esters such as benzoate, acetals/ketals such as acetonide and benzylidene, ethers such as *ortho*-benzyl and *para*-methoxy benzyl ether, tetrahydropyranyl ether and silyl ethers such as *tert*-butyldimethyl silyl ether.

15

Protecting groups can be removed by, for example, acid or base catalysed hydrolysis or reduction, for example, hydrogenation. Silyl ethers may require hydrogen fluoride or tetrabutylammonium fluoride to be cleaved.

20 It will be clear to persons skilled in the art of medicinal chemistry that compounds of formula (I) may be converted into other compounds of formula (I), for example, where a compound of formula (I) bears one or more hydroxyl substituents then one or more of these substituents can be converted in to a halogen such as bromo, chloro or iodo by treating the alcohol with a halogenating agent, with use of protecting groups as required to
25 protect other functionality in the molecule. Halogenating agents include compounds like NBS, hydrobromic acid and chlorine gas.

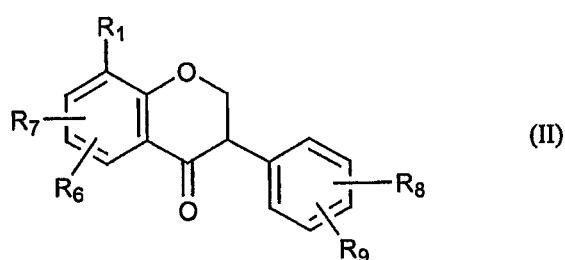
Phenolic type hydroxyls may not be readily convertible to the corresponding halogen compound by treatment with a halogenating agent. However, the desired halogen
30 compound may be prepared by, for example, treating an appropriate aryl amine starting material with NaNO_2 in the presence of HCl under reduced temperature conditions such as

- 19 -

0°C, to form the corresponding azide salt. Subsequent treatment with CuCl, CuBr, KI or HBF₄ may be used to convert the azide into the required halo-compound.

A general process for preparing compounds of formula (I) comprises the steps of:

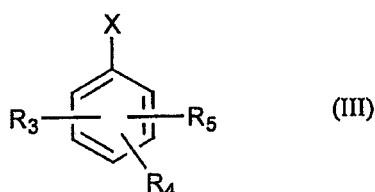
- 5 i) treating a compound of formula (II):



or a protected derivative thereof wherein:

- 10 R₁, is hydrogen, hydroxy, NR₁₀R₁₁, C₃₋₆ cycloalkyl, C₁₋₆ alkoxy, C₂₋₆ alkenyl, C₁₋₆ fluoroalkyl, C₁₋₆ alkyl optionally substituted by one or more hydroxy or NR₁₀R₁₁ groups;

- 15 R₆, R₇, R₈ and R₉ independently represent hydrogen, hydroxy, NR₁₀R₁₁, C₃₋₆ cycloalkyl, C₁₋₆ alkoxy, C₁₋₆ fluoroalkyl, COOR₁₂, COR₁₃ or C₁₋₆ alkyl optionally substituted by one or more hydroxy or NR₁₀R₁₁ groups with a of formula (III):



or a protected derivative thereof wherein:

- 20 R₃ represents represents hydrogen, hydroxy, NR₁₀R₁₁, C₃₋₆ cycloalkyl, C₁₋₆ alkoxy, C₁₋₆ fluoroalkyl, C₂₋₆ alkenyl, COOR₁₂, COR₁₃, (O)_nC₁₋₄ alkyleneNR₁₄R₁₅, or C₁₋₆ alkyl optionally substituted by one or more hydroxy or NR₁₀R₁₁ groups;
- R₄ and R₅ independently represent hydrogen, hydroxy, NR₁₀R₁₁, C₃₋₆ cycloalkyl, C₁₋₆ alkoxy, C₁₋₆ fluoroalkyl, C₂₋₆ alkenyl, COOR₁₂, COR₁₃ or C₁₋₆ alkyl optionally

- 20 -

substituted by one or more hydroxy or $\text{NR}_{10}\text{R}_{11}$ groups; and

X represents a metallohalo moiety; and

- ii) optionally followed by converting the tertiary alcohol group on the heterocyclic ring in the product formed to another substituent, and
- 5 iii) optionally followed by deprotection.

In step i) described above the compound of formula (III) is preferably an organo metallic reagent which is reacted with the ketone compound of formula (II) under anhydrous conditions in an inert atmosphere such as under nitrogen or argon, in an inert solvent such
10 as THF (tetrahydrofuran), at a non-extreme temperature such as room temperature, or reduced temperature, for example, 0°C .

Suitable organometallic reagents include organolithium reagents, organomagnesium reagents and organocopper reagents. More preferably the arylating agent employed is an
15 organomagnesium reagent such as a Grignard reagent, which may be prepared by reacting a compound of formula (III), wherein X represents halo such as bromo with magnesium metal under anhydrous conditions in an inert atmosphere.

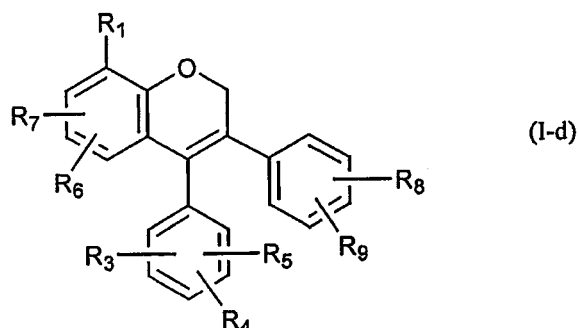
In step ii) described above the tertiary alcohol substituent on the heterocyclic ring in the
20 product formed from the nucleophilic addition reaction may be converted into other R_2 substituents by known methods. For example, treatment with para-toluenesulfonic acid can be used to convert the tertiary alcohol into a good leaving group. This intermediate tosylate may then be treated with a nucleophile such as a hydride source, an alcohol or an amine to provide the required substitution for the R_2 moiety.

25

Alternatively the tertiary hydroxyl may be converted to a halogen by use of a halogenating agent.

In a further aspect of the invention there is the dehydration of the product of said
30 nucleophilic addition reaction to form a compound of the general formula (I-d):

- 21 -



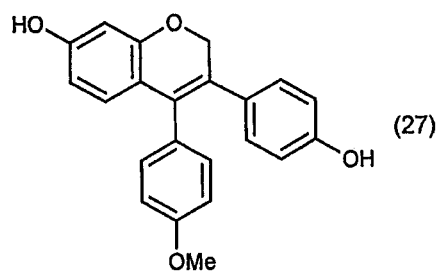
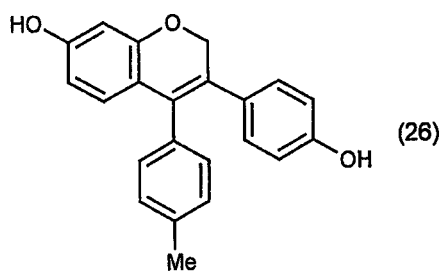
or a protected derivative thereof wherein R₁, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are as defined above for compounds of formula (I-b).

5

Dehydration can, for example, be catalysed by acid, by base or facilitated by conversion of the tertiary alcohol into a better leaving group. Preferably compounds of formula (III) are dehydrated, for example, by treatment with para-toluene sulphonic acid.

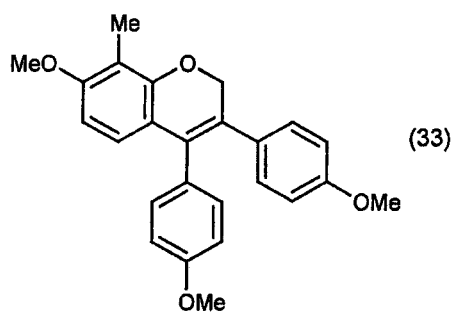
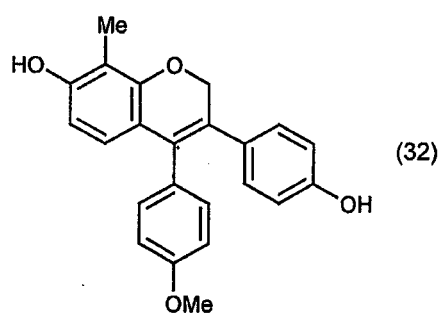
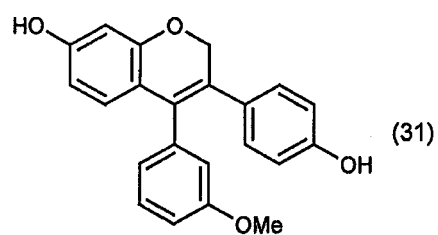
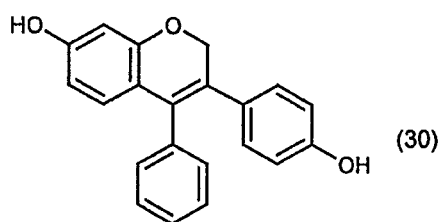
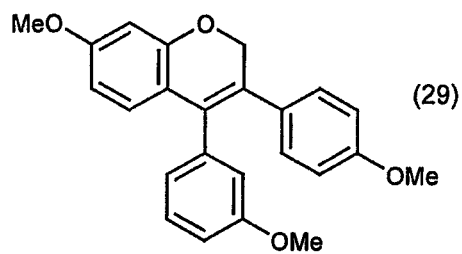
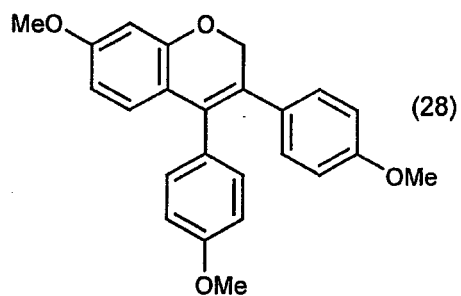
- 10 Preference expressed above for compounds of formula (I-b) apply equally to compound of formula (I-d).

Specific compounds of formula (I-d) are shown below:

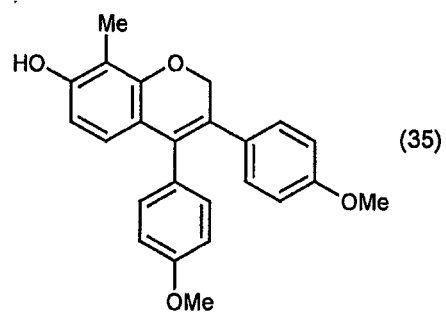
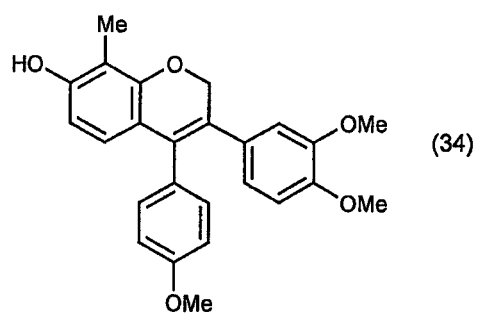


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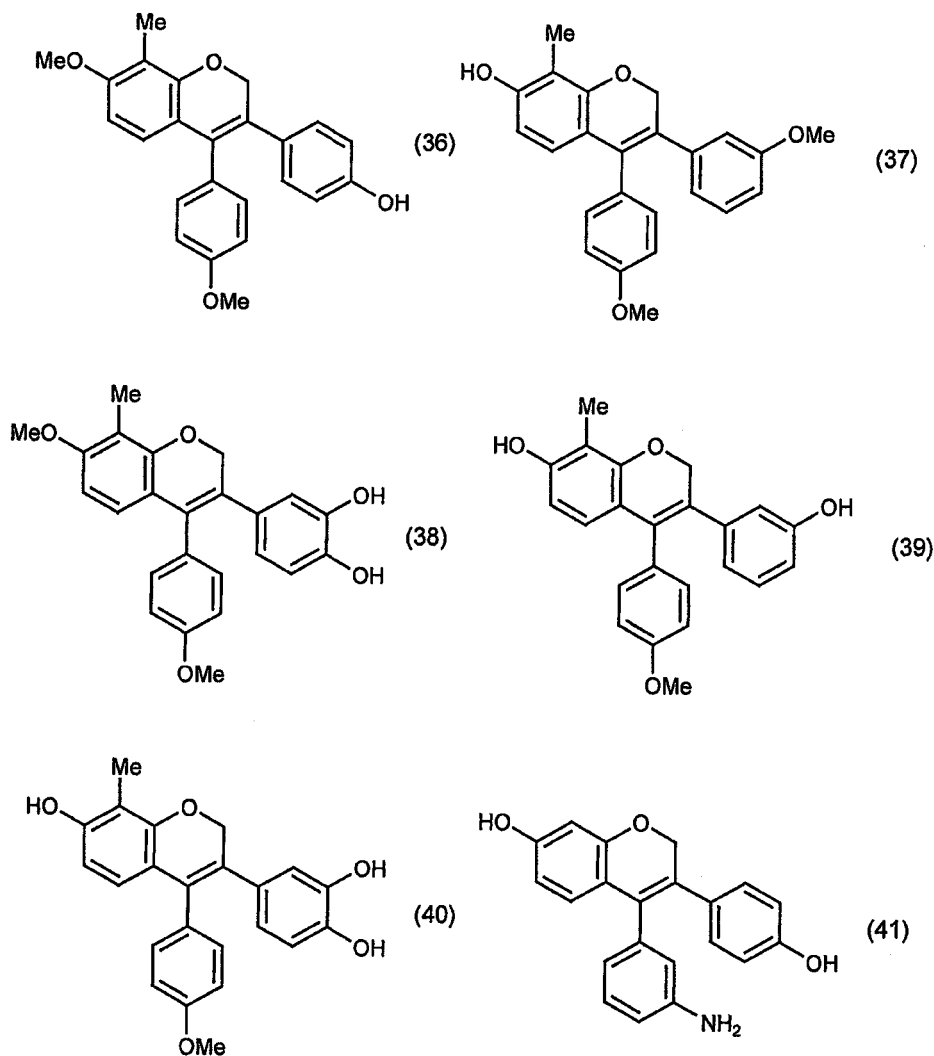
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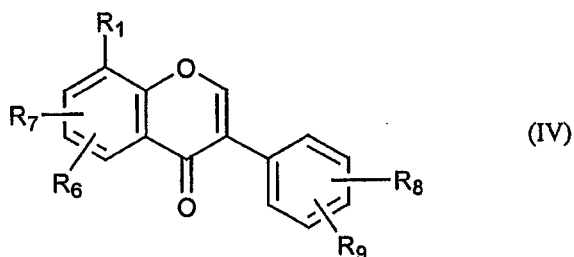
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If required the double bond in the heterocycle in compounds of formula (I-d) can be removed by treatment with a reducing agent to provide other compounds of formula (I). Reducing agents are well known to persons skilled in the art and can include hydride sources like borohydrides and alkali metal borohydrides, but would include hydrogen in catalytic hydrogenation where a suitable catalyst such as palladium on carbon may be used. Other suitable hydride sources include sodium triacetoxyborohydride tetrabutyl ammonium triacetoxyborohydride and sodium cyanoborohydride.

15 Preferably the double bond is reduced by hydrogenation.

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Compounds of formula (II) may be prepared by reducing the double bond, preferably by hydrogenation, in the heterocyclic ring in compounds of formula (IV):

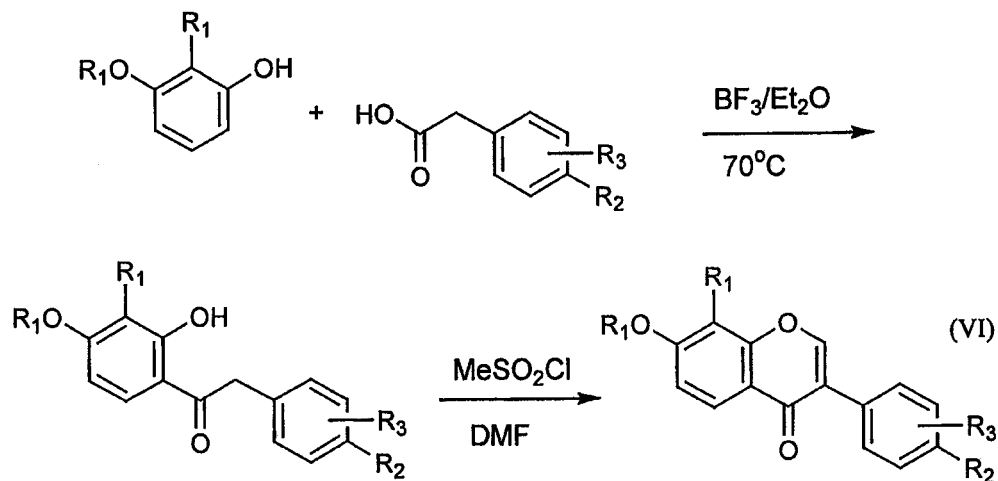


5 or a protected derivative thereof wherein:

R_1 , R_6 , R_7 , R_8 and R_9 are as defined above for compound of formula (II)

Access to compounds of general formula (IV) is available by general synthetic methods as set out in Scheme 1 below and as described in published International application No.

10 WO01/17986, the disclosure of which is incorporated herein by reference. The general synthetic method is set out in Scheme 1.



Scheme 1

15 Compounds for use in the preferred synthetic methods of the present invention may be derived from any number of sources readily identifiable to a person skilled in the art. For

- 25 -

example, daidzein is readily available or can be synthesised by standard methods known in the art. Suitable methods may be found in, for example, published International Patent Applications WO 98/08503 and WO 00/49009, and references cited therein, which are incorporated herein in their entirety by reference.

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Clearly one or more of the above strategies may require the use of a one or more protecting groups in order to protect functionality in other parts of the molecule, when performing a particular treatment or step.

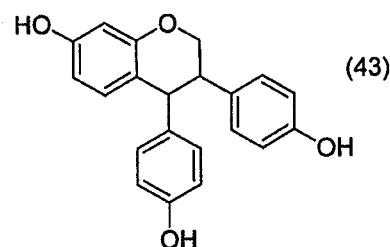
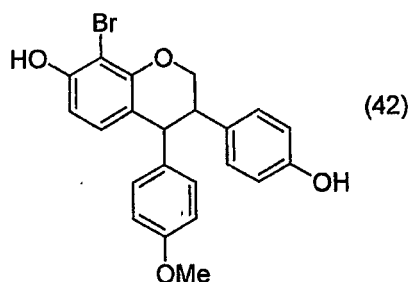
- 10 Preferably any free alcohols, esters or other such reactive groups will be protected, for example, as *t*-butyldimethylsilyl ethers during nucleophilic addition reactions.

Chemical modifications and manipulations may be performed on the compounds of the invention as would be known to one skilled in the art. For example, reaction of compound

- 15 No. 1 with alkylating agents gives ether derivatives at the free phenolic groups.

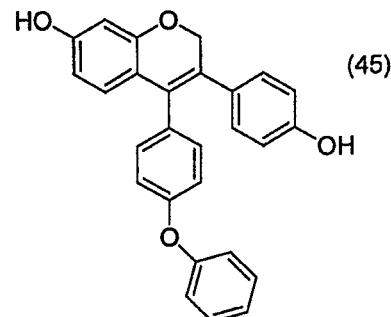
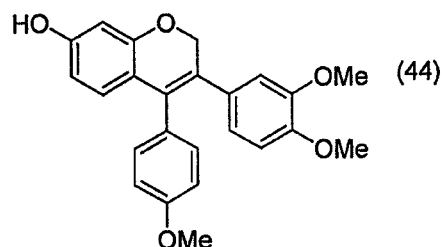
Halogenation of the aromatic rings is also possible and, for example, reaction with N-bromosuccinimide affords the 8-bromo derivative (compound 42) as the main component, with smaller amounts of the 6-bromo isomer. Further reactions can include demethylation

- 20 trihydroxy compound 43.



Additional compounds synthesised by the inventors include:

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As used herein, the terms "treatment", "prophylaxis" or "prevention", "amelioration" and the like are to be considered in their broadest context. In particular, the term "treatment" does not necessarily imply that an animal is treated until total recovery. Accordingly, "treatment" includes amelioration of the symptoms or severity of a particular condition or preventing or otherwise reducing the risk of developing a particular condition.

The amount of one or more compounds according to the invention which is required in a therapeutic treatment will depend upon a number of factors, which include the specific application, the nature of the particular compound used, the condition being treated, the mode of administration and the condition of the patient.

Compounds of formula (I) may be administered in a manner and amount as is conventionally practised. See, for example, Goodman and Gilman, "The pharmacological basis of therapeutics", 7th Edition, (1985). The specific dosage utilised will depend upon the condition being treated, the state of the subject, the route of administration and other well known factors as indicated above. In general, a daily dose per patient may be in the range of 0.1 mg to 5 g; typically from 0.5 mg to 1 g; preferably from 50 mg to 200 mg. The length of dosing may range from a single dose given once every day or two, to twice or thrice daily doses given over the course of from a week to many months to many years as required, depending on the severity of the condition to be treated or alleviated.

It will be further understood that for any particular subject, specific dosage regimens should be adjust over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

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Relatively short-term treatments with the active compounds can be used to cause stabilisation or shrinkage or remission of cancers. Longer-term treatments can be employed to prevent the development of cancers in high-risk patients.

5

The production of pharmaceutical compositions for the treatment of the therapeutic indications herein described are typically prepared by admixture of the compounds of the invention (for convenience hereafter referred to as the "active compounds") with one or more pharmaceutically or veterinary acceptable carriers and/or excipients as are well

10 known in the art.

The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the subject. The carrier or excipient may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose, for example, a tablet, which may contain up to 100% by weight of the active compound, preferably from 0.5% to 59% by weight of the active compound.

15

The preferred concentration of active compound in the drug composition will depend on absorption, distribution, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. One or more active compounds may be incorporated in the formulations of the invention.

20

The formulations of the invention include those suitable for oral, rectal, ocular, buccal (for example, sublingual), parenteral (for example, subcutaneous, intramuscular, intradermal, or intravenous), transdermal administration including mucosal administration via the nose, mouth, vagina or rectum, and as inhalants, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used.

25

Formulation suitable for oral administration may be presented in discrete units, such as capsules, sachets, lozenges, or tablets, each containing a predetermined amount of the

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active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more
5 accessory ingredients as noted above).

In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture such as to form a unit dosage. For
10 example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more other ingredients.

Compressed tablets may be prepared by compressing, in a suitable machine, the compound of the free-flowing, such as a powder or granules optionally mixed with a binder, lubricant,
15 inert diluent, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sublingual) administration include lozenges comprising
20 the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations suitable for ocular administration include liquids, gels and creams
25 comprising the active compound in an ocularly acceptable carrier or diluent.

Compositions of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of the active compounds, which preparations are preferably isotonic with the blood of the intended recipient. These preparations are
30 preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may

- 29 -

conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood. Injectable formulations according to the invention generally contain from 0.1% to 60% w/v of active compound and can be administered at a rate of 0.1 ml/minute/kg.

5

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. Formulations suitable for vaginal administration are preferably presented as unit dose pessaries. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

10

Formulations or compositions suitable for topical administration to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combination of two or more thereof. The active compound is generally present at a concentration of from 0.1% to 5% w/w, more particularly from 0.5% to 2% w/w. Examples of such compositions include cosmetic skin creams.

15

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to the said active compound. See for example Brown, L., *et al.* (1998).

20

Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, Panchagnula R, *et al.*, 2000) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations comprise citrate or Bis/Tris buffer (pH 6) or ethanol/water and contain from 0.1 M to 0.2 M active ingredient.

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- 30 -

Formulations suitable for inhalation may be delivered as a spray composition in the form of a solution, suspension or emulsion. The inhalation spray composition may further comprise a pharmaceutically acceptable propellant such as a hydrogen containing fluorocarbon such as 1,1,1,2-tetrafluoroethane or 1,1,1,2,3,3,3-heptafluoro-n-propane.

5

The active compounds may be provided in the form of food stuffs, such as being added to, admixed into, coated, combined or otherwise added to a food stuff. The term food stuff is used in its widest possible sense and includes liquid formulations such as drinks including dairy products and other foods, such as health bars, desserts, etc. Food formulations
10 containing compounds of the invention can be readily prepared according to standard practices.

In a preferred aspect the invention provides a method of treating humans by administering an effective amount of one or more compounds according to the invention or a
15 composition containing the same.

The active compound or pharmaceutically acceptable derivatives prodrugs or salts thereof can also be co-administered with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics,
20 antifungals, antiinflammatories, or antiviral compounds. The active agent can comprise two or more isoflavones or derivatives thereof in combination or synergistic mixture. The active compounds can also be administered with lipid lowering agents such as probucol and nicotinic acid; platelet aggregation inhibitors such as aspirin; antithrombotic agents such as coumadin; calcium channel blockers such as verapamil, diltiazem, and nifedipine;
25 angiotensin converting enzyme (ACE) inhibitors such as captopril and enalapril, and β -blockers such as propranolol, terbutalol, and labetalol. The compounds can also be administered in combination with nonsteroidal antiinflammatories such as ibuprofen, indomethacin, aspirin, fenoprofen, mefenamic acid, flufenamic acid and sulindac or an antiemetic such as zofran®. The compounds can also be administered with corticosteroids.

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- 31 -

Compounds of formula (I) seem to be particularly suitable for co-administration with other anti-cancer drugs such as cisplatin and/or dehydroequol and/or taxol. This may result in improved effects in the treatment in comparison to when only one of the medicaments is employed.

5

The co-administration may be simultaneous or sequential. Simultaneous administration may be effected by the compounds being in the same unit dose, or in individual and discrete unit doses administered at the same or similar time. Sequential administration may be in any order as required and typically will require an ongoing physiological effect of the first or initial active agent to be current when the second or later active agent is administered, especially where a cumulative or synergistic effect is desired.

10

The invention also extends to novel intermediates employed in the preparation of compounds according to the invention.

15

The compounds of the invention are useful in the treatment, prevention or amelioration of diseases associated with aberrant cell survival, aberrant cell proliferation, abnormal cellular migration, abnormal angiogenesis, abnormal estrogen/androgen balance, dysfunctional or abnormal steroid genesis, degeneration including degenerative changes within blood vessel walls, inflammation, and immunological imbalance.

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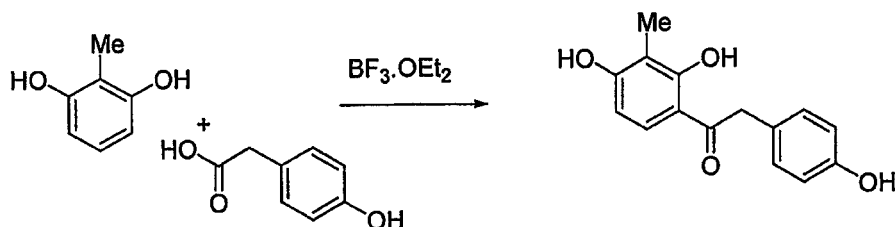
The invention is further illustrated by the following non-limiting Examples and accompanying drawings.

25 Examples

Example 1 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-3,4-dihydro-2H-chromen-7-ol

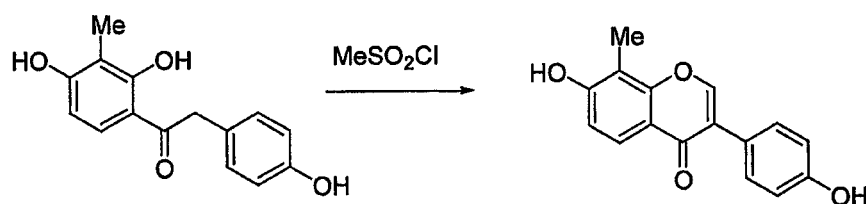
Step1 1-(2,4-Dihydroxy-3-methyl-phenyl)-2-(4-hydroxy-phenyl)-ethanone

- 32 -



2-Methylresorcinol (4.00g, 1 equivalent) and 4-Hydroxyphenylacetic acid (5.00g, 1 equivalent) were added to a round bottom flask. The round bottom flask was attached to a condenser and placed in an oil bath, the whole system was kept under nitrogen. Distilled $\text{BF}_3 \cdot \text{OEt}_2$ (20ml, 5 equiv.) was added to the mixture while stirring. The mixture was refluxed (110°C). A yellow solid formed at 20 minutes indicating that the reaction had gone to completion. The reaction was left on heat for a further 10 minutes and then cooled to room temperature. The yellow solid was collected by suction filtration and washed with distilled water (200ml) to remove any excess $\text{BF}_3 \cdot \text{OEt}_2$ present. ^1H NMR in *d*-DMSO indicated the yellow solid was 1-(2,4-Dihydroxy-3-methyl-phenyl)-2-(4-hydroxy-phenyl)-ethanone in >95% purity. The solid was dried on a freeze dryer for 24hours (8.93g, 99%).

Step 2 7-Hydroxy-3-(4-hydroxy-phenyl)-8-methyl-chromen-4-one



1-(2,4-Dihydroxy-3-methyl-phenyl)-2-(4-hydroxy-phenyl)-ethanone (3.99g) and N,N-DMF (115ml) were added to a 500ml 2-neck round bottom flask, the flask was attached to a condenser and placed in an oil bath. A dropping funnel was attached to the round bottom flask, and the whole system was kept under nitrogen. The flask was heated and maintained at 50°C . $\text{BF}_3 \cdot \text{OEt}_2$ (57ml, 29 equiv.) was added drop wise to the solution over a period of 15 minutes, producing fumes. Methane sulfonyl chloride (MeSO_2Cl) (14ml, 12 equiv.) was added to N,N-DMF (14ml) in the dropping funnel. This mixture was then added drop wise to the round bottom flask over a period of 10 minutes. Once addition was complete, the temperature was increased to reflux (110°C). The reaction was monitored by HPLC

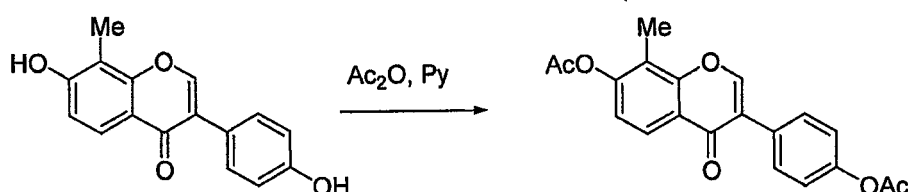
- 33 -

(NV06_R&D.m) and was completed at 1hr and 44 mins. The mixture was cooled to room temperature and poured into chilled stirred distilled water (4L). A bright yellow floccular precipitate was immediately produced and the mixture was left stirring in the cold room overnight. The mixture was then filtered through a buchner funnel, to give a yellow solid.

5 ¹HNMR of the solid in *d*-DMSO indicated it was 7-Hydroxy-3-(4-hydroxy-phenyl)-8-methyl-chromen-4-one with >95% purity. The solid was dried on a freeze dryer for 24 hours. When dry, the solid was weighed (2.73g, 66%).

Step 3 Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester

10



Hydroxy-3-(4-hydroxy-phenyl)-8-methyl-chromen-4-one (35.18g) were combined into a round bottom flask (1L). Pyridine (38ml, 2 equivalents) and acetic anhydride (576ml, 47 equivalents) were added to the round bottom flask while stirring at room temperature. The

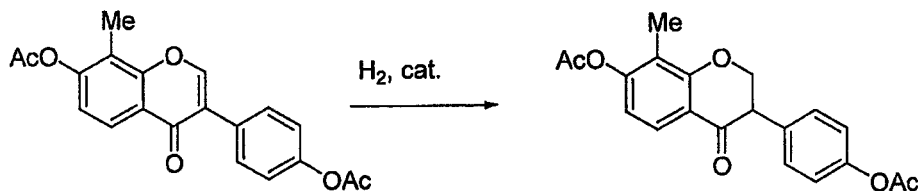
15 reaction was monitored by HPLC and was completed instantaneously. There was a change in colour observed, the reaction mixture was dark brown initially and went bright orange with tan brown floccular particles upon stirring. The reaction mixture was poured into chilled, distilled H₂O (4L) and was left stirring at room temperature for 30 minutes. An off-white solid was collected by suction filtration. ¹HNMR of the solid in *d*-CDCl₃

20 indicated it was Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester with >95% purity The solid was dried on a freeze dryer for 24 hours. When dry, the solid was weighed (31.80g, 69%).

Step 4 Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester

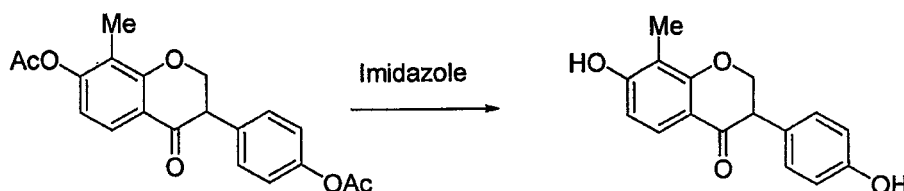
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Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester (26.32g), 10% Pd/Al₂O₃ (12.93g, 50%) and ethyl acetate (EtOAc) (1.5 L) were added to a hydrogenation round bottom flask (2L). The flask was placed on the hydrogenator, evacuated, purged with nitrogen gas (x5) and hydrogen gas (x5). The reaction was monitored by HPLC. 10% Pd/Al₂O₃ (9g, 35%) was added to the round bottom flask at 40 hours, indicated there was no product peak present, only starting material was present. HPLC at 62 hours indicated that the major peak was product peak with starting material peak being half height of product peak. 10% Pd/Al₂O₃ (5.69g, 20%) was added to the round bottom flask to speed up reaction rate. Reaction was completed at 64 hours. The reaction mixture was filtered through celite to remove the Pd/Al₂O₃ catalyst, the celite was rinsed with EtOAc (1L) to ensure majority of product was collected. The EtOAc was evaporated off on a rotary evaporator to give a yellow solid. The solid was re-crystallised in 95% EtOH (650ml) and left in the freezer overnight. Off-white crystals were collected by suction filtration. ¹HNMR in *d*-CDCl₃ indicated the off-white crystals were 8Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester with >95% purity. The crystals were stored in a desiccator for 24 hours, and weighed (18.37g, 69%).

Step 5 7-Hydroxy-3-(4-hydroxy-phenyl)-8-methyl-chroman-4-one

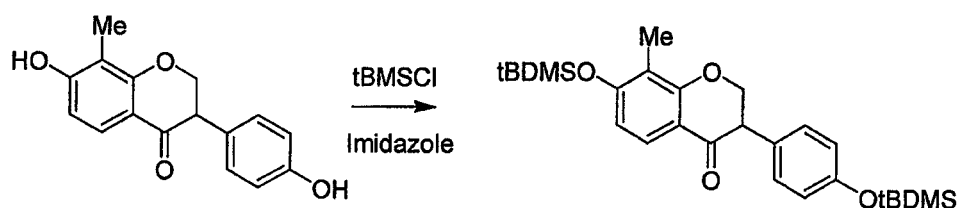


Acetic acid 3-(4-hydroxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester (18.37g), imidazole (21.18g, 6 equivalents) and 100% EtOH (536ml) were added to a round bottom flask (2L). The reaction mixture was refluxed and monitored by HPLC. The reaction was completed

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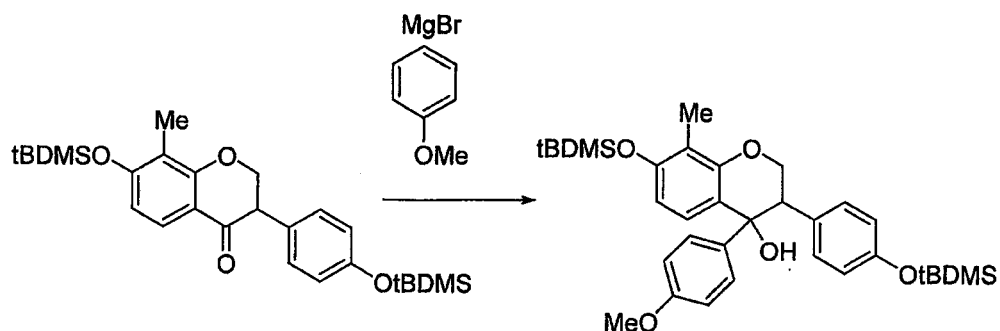
at 8 hours. The reaction mixture was reduced (~130ml) on a rotary evaporator and poured into stirred, chilled distilled water (1.9L). The water crash out was left stirring in the cold room overnight. The pale pink solid was collected by suction filtration. ¹H NMR of the solid indicated it was 7-Hydroxy-3-(4-hydroxy-phenyl)-8-methyl-chroman-4-one with > 95% purity. The solid was dried on the freeze dryer for 3 hours (8.31g, 59%).

10 Step 6 7,4'-Bis *tert*-butyldimethylsiloxy-8-methyl-dihydrodaidzein



8-methyldihydrodaidzein 4.2g, imidazole 13g, *tert*-butyldimethylsilyl chloride 12.7g (70 mmol), and N,N-DMF 50ml were combined in a 250ml round bottom flask and stirred under nitrogen at room temperature for 16 hours. The reaction was quenched with the addition of chilled water (100ml) with the reaction mix cooled in an ice bath. A white solid was filtered off, rinsed with water. Recrystallisation from ethanol afforded white fluffy crystals 3.2g.

20 Step 7 7-(*tert*-butyldimethylsiloxy)-3-(4-(*tert*-butyldimethylsiloxy)phenyl)-4-(4-methoxyphenyl)-8-methyl-3,4-dihydro-2H-chromen-4-ol



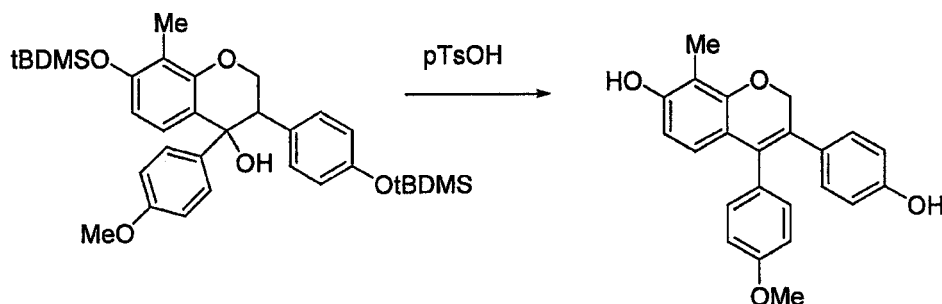
- 36 -

2.5g of the product of step 6 was weighed in a 2-neck round bottom flask, and flushed under nitrogen. Anhydrous THF 10ml was added to the reaction vessel to give a clear slightly yellow solution. A condenser was attached and the reaction vessel placed in an ice bath. Commercial 4-methoxyphenylmagnesium bromide (0.5M solution in THF) 22.5ml was added to the reaction mix dropwise over 10 minutes. The reaction was quenched by the dropwise with wet ether (50:50 H₂O: diethyl ether) while still under nitrogen, with a white precipitate forming as increasing amounts of H₂O added. A further amount of water was added to the reaction mix before extraction with diethyl ether.

The organic layers were combined and washed with water, brine, dried over anhydrous magnesium sulphate and solvent removed *in vacuo* to give clear yellow oil which solidified overnight to give an off-white solid. The oily nature of the material precluded an accurate yield being calculated. There was no further clean up of the product before use in the next reaction. The oily nature of the material precluded an accurate yield being calculated.

15

Step 8 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-2H-chromen-7-ol

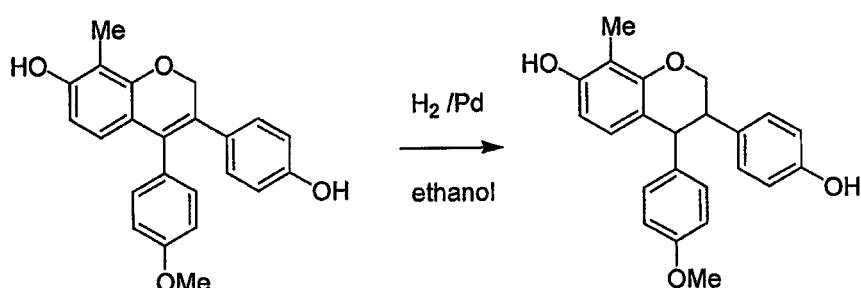


4.2g of the product of step 7, pTsOH (para-toluene sulphoric acid) 4.5g boiling chips and 200ml of ethanol were combined in a 2-neck 500ml round bottom flask with condenser attached. The reaction was heated at reflux for 3 hours. The solvent was concentrated *in vacuo* to ~20ml before being poured into chilled, stirred water(100ml). The mixture was then extracted with ethyl acetate, the combined organic layers washed with water (3 x 100ml), brine (1 x 100ml), dried over anhydrous magnesium sulphate and filtered and solvent removed *in vacuo* to give red/ brown oil. The oil was dissolved in methanol (~15ml) and put in freezer overnight.

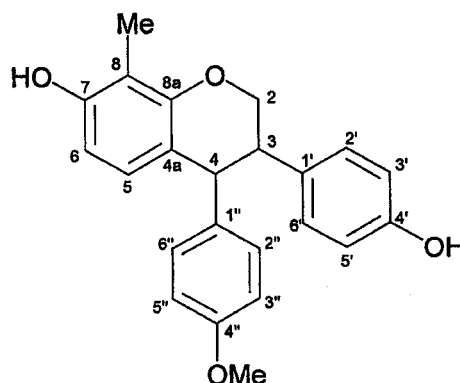
- 37 -

A white precipitate had formed overnight which was filtered off and rinsed with methanol. The filtrate was concentrated in vacuo to give a brown oil.

Step 9 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-3,4-dihydro-2H-chromen-7-ol



2.5g of the product of step 8, 10% Pd/Al₂O₃ 0.4g and 50ml of ethanol were combined in a 2-neck 100ml round bottom flask. The reaction was hydrogenated at low pressure using standard conditions for 3 hours. The reaction was filtered through Celite to remove the catalyst, rinsed through with ethanol (100ml). The filtrate was concentrated to ~15ml before being poured into chilled, stirred water (300mL). A pale orange precipitate formed which then formed a brown oil. The mixture was then extracted with diethyl ether, the combined organic layers washed with water (3 x 100ml), brine (1 x 100ml), dried over anhydrous magnesium sulphate and filtered. The solvent was removed *in vacuo* to give red/ brown oil. The product was recrystallised from diethyl ether (~15ml), to give brown solid which was rinsed with chilled diethyl ether to give off-white crystals. 4 crops of (IV), ~1g. The ¹H NMR spectrum and numbering scheme being shown below.

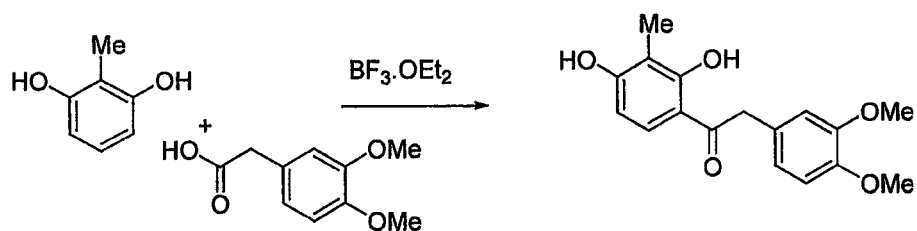


- 38 -

H	ppm	Peak Shape	J Hz	Integrates	Comments
C2equatorial	4.13	m		1	
C2axial	4.25	m		1	
C3	3.31	m		1	Partially obscured by water peak
C4	4.28	m		1	
C5	6.44	d	8.05	1	
C6	6.30	d	8.4	1	
C8-Me	2.0	s	-	3	
C2', C6'	7.5	d	8.7	2	
C3', C5'	6.92	d	8.7	2	
C2'', C6''	6.60	d	8.7	2	
C3'', C5''	6.43	d	8.7	2	
OMe	3.8	s	0	3	

Example 2 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-3',4'-dimethoxy-8-methyl-3,4-dihydro-2H-chromen-7-ol

5 Step 1.1 1-(2,4-dihydroxy-3-methyl-phenyl)-2-(3,4-dimethoxy-phenyl) ethanone

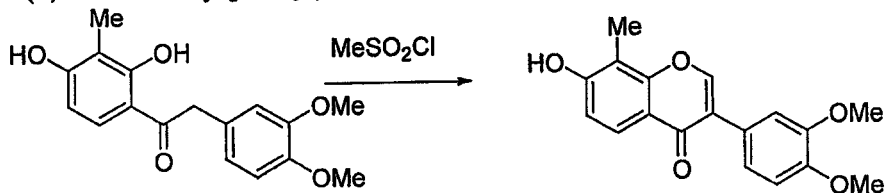


2-Methylresorcinol (6.285g, 1equivalent) and 3,4-dimethoxyphenylacetic acid (9.251g, 1 equivalent) were added to a round bottom flask. The round bottom flask was attached to a condenser and placed in an oil bath, the whole system was kept under nitrogen. Distilled boron trifluoride diethyl etherate, $\text{BF}_3 \cdot \text{OEt}_2$ (42ml, 5 equiv.) was added to the mixture while stirring. The mixture was refluxed (110°C). A yellow solid formed at 75 minutes indicating that the reaction had gone to completion. The reaction was heated for a further 10 minutes and cooled to room temperature. The yellow solid was collected by suction filtration and washed with distilled water (200ml) to remove any excess $\text{BF}_3 \cdot \text{OEt}_2$ present. ^1H NMR in *d*-DMSO indicated the yellow solid was 1-(2,4-dihydroxy-3-methyl-phenyl)-2-

- 39 -

(3,4-dimethoxy-phenyl) ethanone in >95% purity. The solid was dried on a freeze dryer for 24hours (6.303g, 43%).

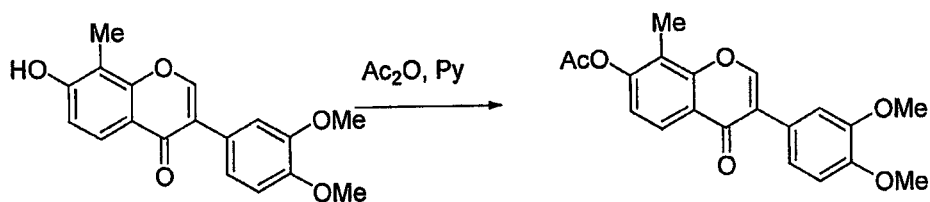
Step 2.1 3-(3,4-dimethoxy-phenyl)-7-hydroxy-8-methyl-chromen-4-one



5

1-(2,4-dihydroxy-3-methyl-phenyl)-2-(3,4-dimethoxy-phenyl) ethanone (1078-1-49; 9.999 g, 34.4 mmol) was dissolved in N,N-DMF (15 mL), dried with MgSO₄. Under an N₂ atmosphere, distilled BF₃-OEt₂ (16.08.04) was added dropwise at r.t. Heat begun after 20 min. After 1 h, methane sulfonyl chloride in DMF (8 mL in 20 mL) was slowly added at 10 50°C. The reaction mixture was heated to reflux for 1.5 h. The dull yellow solution was added to 1.2 L of cold, vigorously stirred water which was left at 4°C overnight. The dull yellow solid was collected by filtration, then placed in water to remove residual BF₃-OEt₂. Solid was collected by filtration and dried using a freeze-dryer overnight. ¹H NMR in *d*-DMSO indicated the yellow solid was 3-(3,4-dimethoxy-phenyl)-7-hydroxy-8-methyl-15 chromen-4-one in 90% purity (8.85 g, 82%).

Step 3.1 acetic acid 3-(3,4-dimethoxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester

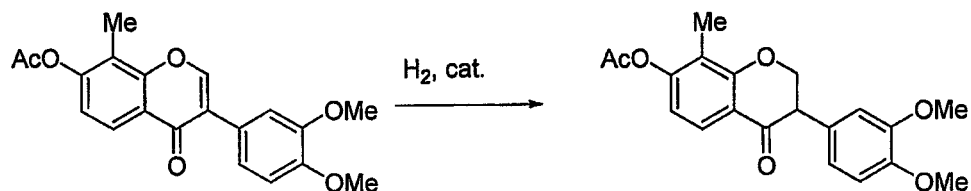


20

3-(3,4-dimethoxy-phenyl)-7-hydroxy-8-methyl-chromen-4-one (9.82g, 31mmol), acetic anhydride (62ml) and pyridine (6.2ml) were combined in a round bottom flask and heated to reflux. The reaction was cooled to room temperature after 3 hours of heating, and a 25 crystalline solid formed. The solid was filtered and rinsed with H₂O (1L). ¹H NMR in *d*-CDCl₃ indicated pale brown crystals was acetic acid 3-(3,4-dimethoxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester in 90% purity (7.214g, 71%).

- 40 -

Step 4.1 Acetic acid 3-(3,4-dimethoxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester



- 5 3-(3,4-dimethoxy-phenyl)-8-methyl-4-oxo-4H-chromen-7-yl ester (1.12g, 3mmol), 10% Pd/Al₂O₃ (0.501g, 45% w/w) and dry EtOAc (100ml) were placed in a 2-neck round bottom flask and placed on the hydrogenator. After 4 hours, 1 major product was observed. The reaction was purged and the catalyst filtered off through celite. The filtrate was reduced to give a white solid. ¹H NMR in *d*-CDCl₃ indicated the solid was Acetic acid 3-
- 10 (3,4-dimethoxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester in 85% purity (1.1g).

Step 5.1 3-(3,4-Dimethoxy-phenyl)-7-hydroxy-8-methyl-chroman-4-one

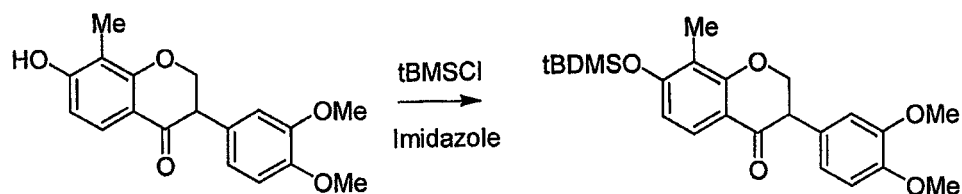


15

- Acetic acid 3-(3,4-dimethoxy-phenyl)-8-methyl-4-oxo-chroman-7-yl ester (1.1g, 3.2mmol) and imidazole (3.2g, 47mmol) were refluxed in EtOH (100ml). The reaction was complete after 90 minutes and allowed to cool to room temperature before pouring into stirred H₂O (800ml). A fine white precipitate was filtered off and ¹H NMR in *d*-CDCl₃ indicated the
- 20 solid was 3-(3,4-Dimethoxy-phenyl)-7-hydroxy-8-methyl-chroman-4-one in >95% purity (0.31g, 30%).

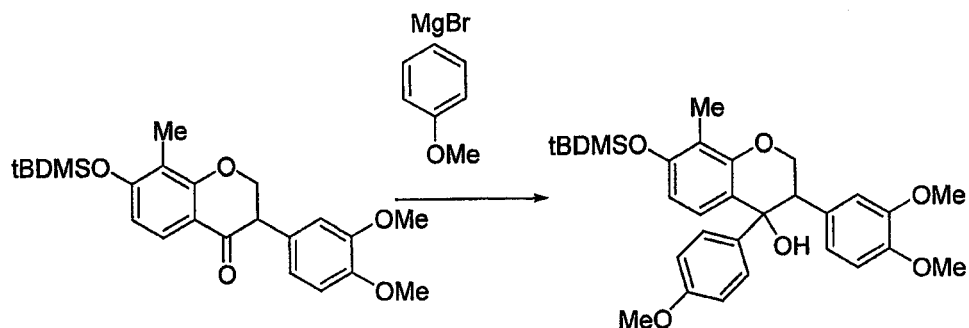
Step 6.1 7,4'-Bis *tert*-butyldimethylsilyloxy-3',4'-dimethoxy-8-methyl-dihydrodaidzein

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3',4'-Dimethoxy-8-methyldihydrodaidzein 2g, imidazole 6.8g, *tert* Butyldimethylsilyl chloride 6.3g, and N,N-DMF 50ml were combined in a 250mL round bottom flask and stirred under nitrogen at room temperature for 16hours. The reaction was quenched with the addition of chilled water (100ml) with the reaction mix cooled in an ice bath. A white solid was filtered off, rinsed with water. Recrystallisation from ethanol afforded white fluffy crystals 2.2g

Step 7.1 7-(*tert*-butyldimethylsilyloxy)-3,3-(4-(*tert*-butyldimethylsilyloxy)phenyl)-4-(4-methoxyphenyl)-3',4'-dimethoxy-8-methyl-3,4-dihydro-2*H*-chromen-4-ol



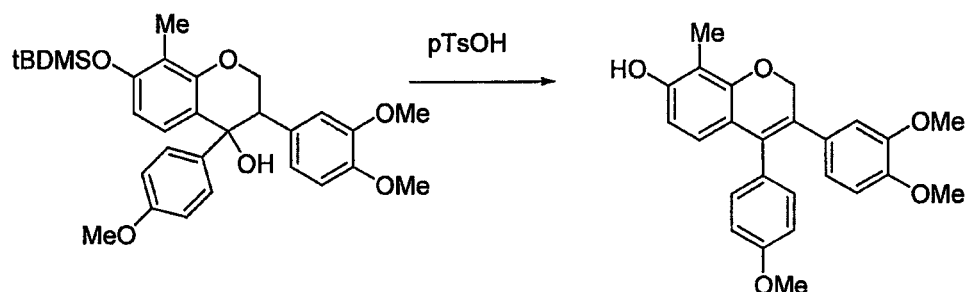
The product of step 6.1 above 2g was weighed in a 2-neck round bottom flask, and flushed under nitrogen. Anhydrous THF 10ml was added to the reaction vessel to give a clear slightly yellow solution. A condenser was attached and the reaction vessel placed in an ice bath. Commercial 4-methoxyphenylmagnesium bromide (0.5M solution in THF) 22.5ml was added to the reaction mix dropwise over 10 minutes. The reaction was quenched by the dropwise with wet ether (50:50 H₂O: diethyl ether) while still under nitrogen, with a white precipitate forming as increasing amounts of H₂O added. A further amount of water was added to the reaction mix before extraction with diethyl ether. The organic layers were combined and washed with water, brine, dried over anhydrous magnesium sulphate and solvent removed in vacuo to give clear yellow oil which

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solidified overnight to give an off-white solid. The oily nature of the material precluded an accurate yield being calculated. There was no further clean up of the product before use in the next reaction. The oily nature of the material precluded an accurate yield being calculated.

5

Step 8.1 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-3',4'-dimethoxy-8-methyl-2H-chromen-7-ol



- 10 2g of the product of step 7.1 pTsOH 4.5g boiling chips and 100ml of ethanol were combined in a 2-neck 500ml round bottom flask with condenser attached. The reaction was heated at reflux for 3 hours. The solvent was concentrated *in vacuo* to ~10ml before being poured into chilled, stirred water (100ml). The mixture was then extracted with ethyl acetate, the combined organic layers washed with water (3 x 100ml), brine (1 x
- 15 100ml), dried over anhydrous magnesium sulphate and filtered and solvent removed *in vacuo* to give red/ brown oil. The oil was dissolved in methanol (~15ml) and put in freezer overnight.

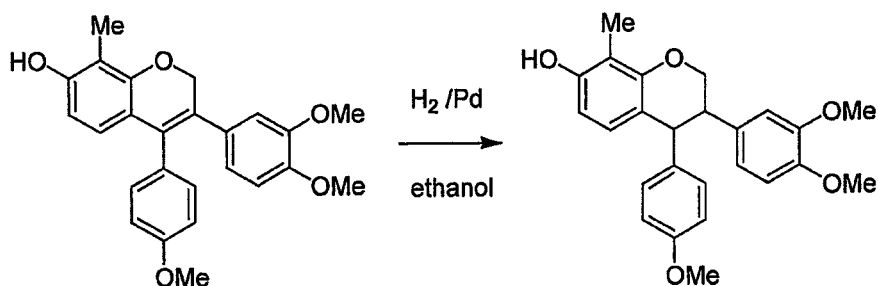
A white precipitate had formed overnight which was filtered off and rinsed with methanol.

- 20 The filtrate was concentrated in vacuo to give a brown oil, which was crashed out into water to give a pale brown solid.

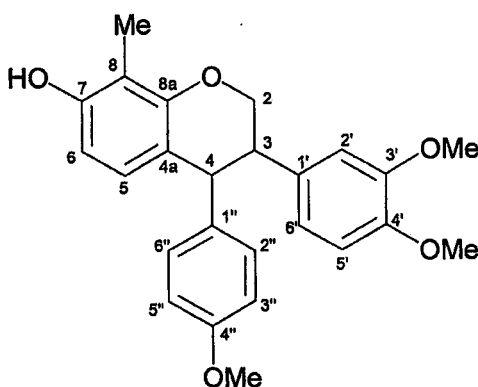
Step 9.1 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-3',4'-dimethoxy-8-methyl-3,4-dihydro-2H-chromen-7-ol

25

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1g of the product of step 8.1 10% Pd/Al₂O₃ 0.2g and 25ml of ethanol were combined in a 2-neck 100ml round bottom flask. The reaction was hydrogenated at low pressure using standard conditions for 3 hours. The reaction was filtered through Celite to remove the catalyst, rinsed through with ethanol (100ml). The filtrate was concentrated to ~5ml before being poured into chilled, stirred water (100mL). A pale orange precipitate formed which then formed a brown oil. The mixture was then extracted with diethyl ether, the combined organic layers washed with water (3 x 100ml), brine (1 x 100ml), dried over anhydrous magnesium sulphate and filtered. The solvent was removed *in vacuo* to give red/ brown oil. The product was recrystallised from diethyl ether (~5ml), to give brown solid which was rinsed with chilled diethyl ether to give off-white crystals. 4 crops of (IV), ~0.2g. The ¹H NMR spectrum and numbering scheme shown below.

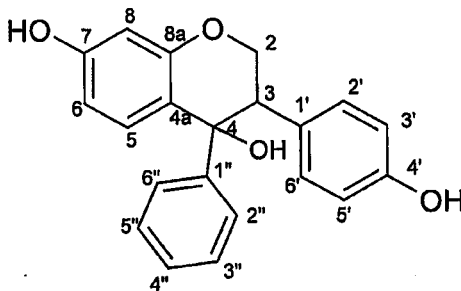


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H	Chemical shift	Peak Shape	J Hz	Integrates	Comments
C2equatorial	4.05	m		1	
C2axial	4.25	m		1	
C3	3.38	m		1	Partially obscured by water peak
C4	4.45	m		1	
C5	6.80	d	8.4	1	
C6	6.38	d	8.4	1	
C8-Me	2.1	s	-	3	
C2'	7.1	d	2.1	1	
C5'	6.95	d	8.4	1	
C6'	6.98	dd	2.1,8.4	1	
C2'', C6''	6.66	d	8.7	2	
C3'', C5''	6.33	d	8.7	2	
OMe x3	3.8,3.82, 3.83	s	0	9	

¹H N.m.r. assignments are provided for the following compounds:

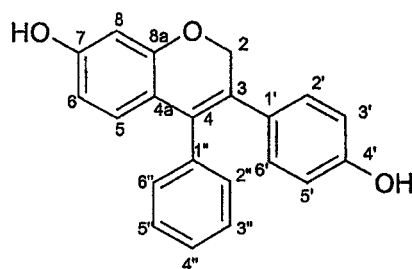
Cpd. 14



5

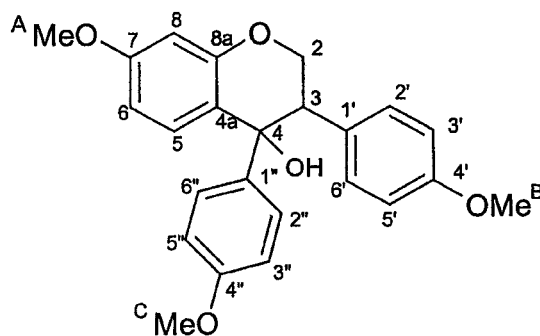
H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	3.30	dd	3.293, 11.709	1	dd is slightly under H ₂ O peak
C2	4.05	dd	3.659, 10.612	1	
C3	4.62	dd	10.612, 12.075	1	dd is overlapped
C5	6.45	d			Under C3', C5' doublet
C6	6.20	dd	2.561, 8.416	1	
C8	6.23	d	2.196	1	Adjacent to O
C2', C6'	6.70	d	8.782	2	
C3', C5'	6.45	d	8.782	2	Integrates for 3 in total including C5
C2'', C6''	7.11	m		2	Overlapping with 4'', 3'', 5'', cannot measure J
C3'', C5''	7.11	m		2	Overlapping with 4'', 2'', 6'', cannot measure J
C4''	7.11	m		1	Overlapping with 3'', 5'', 4'' cannot measure J

- 45 -

Cpd. 30

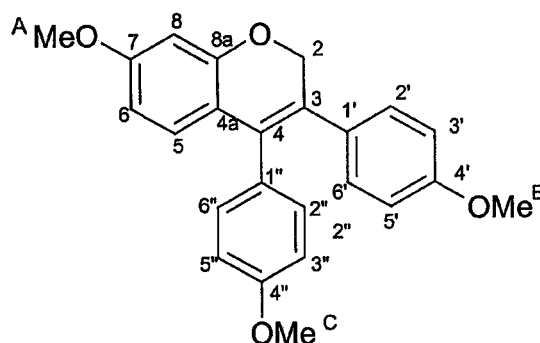
H	δ ppm	Peak Shape	J Hz	Integrates	C	Comments
C2	4.95	s		2	69	
C3	-					
C4	-					
C4a	-				116.5	
C5	6.43	d	8.416	1	130.5	
C6	6.30	dd	2.561, 8.416	1	108	
C7					156	
C8	6.30	d	2.561	1	102	Adjacent to O
C8a					158	
C1'						
C2', C6'	6.79	d	8.416	2	128	
C3', C5'	6.49	d	8.416	2	114.5	
C4'					155	Adjacent to O
C1''						
C2'', C6''	7.04	m		2	130	Overlapping with 4'', cannot measure J
C3'', C5''	7.27	m		2	129	Overlapping with 3'', 5'', cannot measure J
C4''	7.27	m		1	126.5	

5

Cpd. 12

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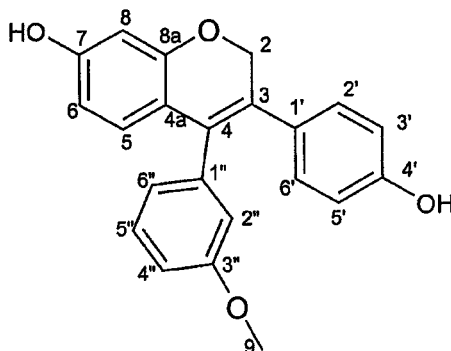
H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2 _{equatorial}	4.30	dd	3.659, 10.612	1	
C2 _{axial}	4.69	dd	10.978	1	dd is overlapped
C3	3.44	dd	3.293, 10.978	1	
C5	6.83	d	8.782	1	
C6	6.43	dd	2.561, 8.416	1	
C8	6.47	d	2.561	1	
C2', C6'	6.83	d	8.782	2	
C3', C5'	6.71	d	8.782	2	
C2'', C6''	7.05	d	8.782	2	
C3'', C5''	6.76	d	8.782	2	
Me A	3.75	s	-	3	Methoxy peaks are interchangeable
Me B	3.79	s	-	3	
Me C	3.79	s	-	3	

Cpd. 28

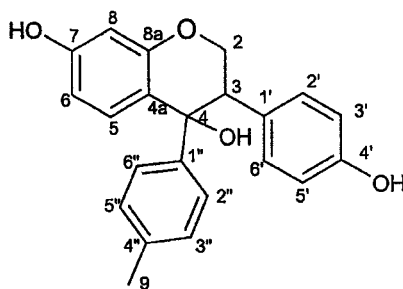
5

H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	5.05	s	-	2	
C5	6.75	d	8.416	1	
C6	6.38	dd	2.561, 8.416	1	
C8	6.50	d	2.651	1	
C2', C6'	6.90	d	8.782	2	
C3', C5'	6.67	d	8.782	2	
C2'', C6''	7.02	d	8.782	2	
C3'', C5''	6.81	d	8.416	2	
Me A	3.74	s	-	3	Methoxy peaks are interchangeable
Me B	3.79	s	-	3	
Me C	3.81	s	-	3	

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Cpd. 31

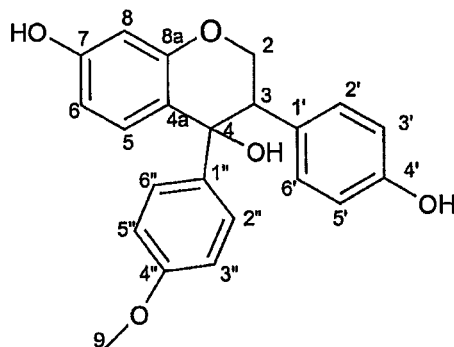
H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	4.93	s		2	
C5	6.28	d	2.196	1	
C6	6.24	dd	2.196, 8.416	1	
C8	6.47	d	8.416	1	
C9	3.63	s		3	
C2', C6'	6.82	d	8.416	2	Overlapping with C2', C6'
C3', C5'	6.51	d	8.416	2	
C2''	6.57			1	
C4''	6.81			1	
C5''	7.20	t	8.050	1	
C6''	6.63			1	

Cpd. 10

5

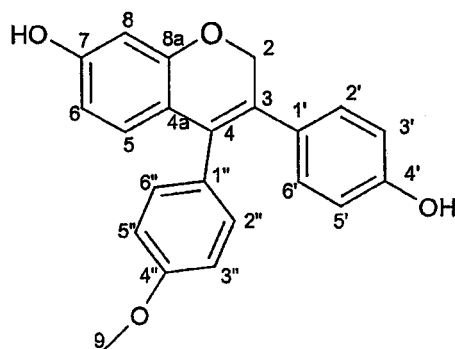
H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	3.30	dd		1	under H ₂ O peak
C2	4.02	dd	4.391, 11.343	1	
C3	4.60	dd	10.612, 12.075	1	dd is overlapped
C5	6.21	d	2.196	1	
C6	6.18	dd	2.561, 8.416	1	
C8	6.42	d	8.416	1	Overlapping with C3', C5'
C9	2.21	s		3	
C2', C6'	6.72	d	8.782	2	
C3', C5'	6.44	d	8.416	2	Overlapping with C8
C2'', C6''	6.94	d	8.050	2	
C3'', C5''	7.01	d	8.050	2	

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Cpd. 11

5

H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	3.30	dd	3.659, 11.709	1	dd is slightly under H ₂ O peak, measured J from previous NMR
C2	4.05	dd	3.659, 10.612	1	
C3	4.62	dd	10.978, 11.709	1	dd is overlapped
C5	6.47	d	8.416	1	Overlapping with C3', C5' doublet
C6	6.20	dd	2.561, 8.416	1	Overlapping with C8 doublet
C8	6.23	d	2.196	1	Overlapping with C6 dd
C9	3.65	s		3	
C2', C6'	6.72	d	8.416	2	
C3', C5'	6.45	d	8.416	2	Integrates for 3 in total including C5
C2'', C6''	6.70	d	8.782	2	
C3'', C5''	7.03	d	8.782	2	

Cpd. 27

10

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H	δ ppm	Peak Shape	J Hz	Integrates	Comments
C2	4.94	s		2	
C5	6.48	d	8.416	1	
C6	6.25	dd	2.561, 8.416	1	
C8	6.30	d	2.561	1	
C9	3.65	s		3	
C2', C6'	6.81	d	8.416	2	C2', C3', C2'', C3'' based on previous ^1H NMR assignment for similar compounds. this assignment.
C3', C5'	6.53	d	8.782	2	
C2'', C6''	6.86	d	8.782	2	
C3'', C5''	6.97	d	8.782	2	

2.0. Materials and Methods

5 2.1. Tissue culture

The human pancreatic cancer cell line, HPAC (CRL-2119) was routinely cultured in 1:1 mixture DMEM (Sigma) plus Ham's F12 (Sigma) medium containing HEPES (15 mM), insulin (0.002 mg/ml), transferrin (0.005 mg/ml), hydrocortisone, (40 ng/ml), epidermal growth factor (10 ng/ml). The ovarian cancer cell lines; CP70 was obtained as a gift from Dr. Gil Mor (Yale University) and cultured in a 1:1 mixture DMEM plus Ham's F12 medium, and SKOV-3 was purchased from ATCC and cultured in McCoy's 5a medium. The breast cancer cell line MDA-MB-468 were cultured in Leibovitz's L-15 medium. The melanoma cell line MM200 was obtained as a gift from Peter Hersey (University of Newcastle) and A2058 was obtained as a gift from Dr Peter Parsons (QIMR). Both were cultured in DMEM medium.

All cultures were supplemented with 10% FCS (CSL, Australia), penicillin (100U/ml), streptomycin (100mg/ml), L-glutamine (2mM) and sodium bicarbonate (1.2 g/L), and cultured at 37°C in a humidified atmosphere of 5% CO₂. All cell lines were purchased from ATCC (Maryland, USA) except where noted.

The normal cell line NFF (neonatal foreskin fibroblasts) was a gift from Dr. Peter Parsons (Queensland Institute of Medical Research). RK (rabbit kidney) cells were obtained from Miller Whalley (Macquarie University). Both cell lines were cultured in RPMI

- 50 -

supplemented with 10% FCS (CSL, Australia), penicillin (100U/ml), streptomycin (100mg/ml), L-glutamine (2mM) and sodium bicarbonate (1.2 g/L), and cultured at 37°C in a humidified atmosphere of 5% CO₂

5 **2.2. Proliferation assays**

IC₅₀ values were determined for each cell line. Cells were seeded in 96-well plates at an appropriate cell density as determined from growth kinetics analysis and cultured for 5 days in the absence and presence of the test compounds. Cell proliferation was assessed after the addition of 20 µl of 3-(4,5-dimethylthiazol-2-yl)-5-(3,4-diphenyl) tetrazolium bromide (MTT, 2.5mg/ml in PBS, Sigma) for 3-4hrs at 37°C according to manufacturer's instructions. IC₅₀ values were calculated from semi-log plots of % of control proliferation on the y-axis against log dose on the x-axis.

2.3. Compound No. 1 Pharmacokinetics - Oral

15 Compound No. 1 labelled above was prepared as homogenous suspensions in 1% CMC(m:v, water). The formulation was delivered orally by gavage to female BALB/c mice at a dosage of 50 mg/kg. Three animals were allocated to each timepoint (15 min, 30 min, 1hr, 4 hr and 24 hr). At each respective timepoint, animals were euthanased by cervical dislocation and blood collected. The concentration of free compound was
20 analysed by mass spectroscopy.

3.0. Results

3.1. Normal cell toxicity.

Duplicate cytotoxicity assays against rabbit kidney cells demonstrated that compound No. 1 has mild toxicity against these cells (Table 1). When compared to cisplatin and phenoxodiol another benchmark against which potential anti-cancer drugs can be tested the degree of toxicity exhibited by compound No. 1 was greater than both the comparator compounds (2 µM compound No.1 vs 9.9 µM cisplatin).

30 **Table 1.** Relative toxicity of compound No. 1 and cisplatin against rabbit kidney cells.

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Tissue/cell type	Designation	Analogue (IC ₅₀ uM)		Antineoplastic (IC ₅₀ uM)
		Phenoxodiol	Compound No.1	Cisplatin
Kidney	Rabbit Kidney	>150	>60	NT
Fibroblast	Neonatal Foreskin Fibroblasts (Human, NFF)	>150	~2	9.85 ± 5

3.2. In vitro efficacy against cancer cells.

When compared the IC₅₀ values of phenoxodiol and compound No. 1, were compared, compound No.1 demonstrated superior activity (~2-10 fold greater) against the ovarian cancer cell line (CP70), the AR negative, p53 Mt prostate cancer cell line (PC3), ER negative (p53 mt) breast cancer cell line (MDA-MB-468 respectively), p53 Mt Glioma (HTB-138), and p53 Mt small cell lung cancer (Table 2). Compound No. 1 exhibited anti-cancer activity comparable to that of phenoxodiol against all other cell lines tested (Table 2) except HT-29 which is a colorectal cell line. Compound No. 1 was also equipotent against the melanoma cell line MM200 (Table 2.1).

Thus, compound No. 1 shows good anticancer activity against a broad range of cancer cell types, including ovarian, prostate, breast, glioma, pancreatic lung, colorectal and melanoma.

Table 2.1. Comparison of compound No. 1 and Phenoxodiol cytotoxicity against cell lines representative of different malignancies

Indication	Cell line	Phenoxodiol	Compound No 1
Ovarian	A2780	1.7 ± 0.61	NT
	CCP70	11.1 ± 23.6	1.7
Prostate	PC3	9.1 ± 8	1.7 ± 0.5
Breast	MDA-MB-468	8.9 ± 4.8	0.6 ± 0.01
Glioma	HTB-138	20.4 ± 17.5	1.1
Pancreatic	HPAC	56.6 ± 16.8	NT
Lung	NCI-H23	8.3 ± 6.7	1.9 ± 0.1
Colorectal	HT-29	52.5 ± 19.5	17,44
Melanoma	MM200	6.2 ± 4.5	1.0 ± 0.01

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A number of cancer cell lines were tested against analogues of compound No. 1 and their IC₅₀ values were compared. The results show that compound No.1 is the best across a broad range of cell lines, whilst compound No. 20 also showed good results against ovarian, prostate breast leukaemia and melanoma cell lines. Compound Nos. 15 and 17 also showed good results against ovarian and melanoma cell lines respectively (Table 2.2).

10

Table 2.2(a). Comparison of compound Nos. 1, 10, 11, 12 and 14 to 20 cytotoxicity against cell lines representative of different malignancies

Indication	Cell line	Compounds (IC ₅₀ uM)					
		Cpd. 14	Cpd. 30	Cpd. 11	Cpd. 15	Cpd. 27	Cpd. 31
Ovarian	CP70	61.3	18	52.7 ± 13	65 ± 1.9	22.4 ± 0.7	53.4
Prostate	PC3	74 ± 4	45 ± 4.9	>100	>100	28 ± 7.3	47 ± 8.4
Breast	MDA-MB-468	>100	>100	NT	NT	NT	NT
Glioma	HTB-138	>100	45.7	86.3	>100	33.7	94.6
Pancreatic	HPAC	>100	41 ± 5.3	>100	>100	27.6 ± 5.3	34.6
leukaemia (ALL)	CCRF-CEM	90.2	66.6	95.4	81.8	16.6	51.5 ± 12
NSC Lung	NCI-H460	>100	43.5 ± 9.2	>100	>100	31.6 ± 4.9	76.3
Colorectal	HT-29	NT	NT	NT	NT	NT	>100
Melanoma	MM200	72.8	33 ± 3	60.4 ± 9.2	NT	14.6	27.2 ± 4.6

15

Table 2.2(b). Comparison of compound Nos. 1, 10, 11, 12 and 14 to 20 cytotoxicity against cell lines representative of different malignancies

Indication	Cell line	Compounds (IC ₅₀ μ M)				
		Cpd. 10	Cpd. 12	Cpd. 28	Cpd. 32	Cpd. 1
Ovarian	CP70	27.5	>100	62 $\pm$ 44	17.7 $\pm$ 5.9	1.9 $\pm$ 0.4
Prostate	PC3	37.5 $\pm$ 0.4	32.8 $\pm$ 4.7	49 $\pm$ 16	13 $\pm$ 0.6	1.6 $\pm$ 0.8
Breast	MDA-MB-468	NT	63.5 $\pm$ 4.6	30.5 $\pm$ 4	9.2 $\pm$ 5.1	1 $\pm$ 0.6
Glioma	HTB-138	60.2	47.7	62.4	29 $\pm$ 2	1.16
Pancreatic	HPAC	50	86.3 $\pm$ 67	50.7 $\pm$ 35	35.8	51.7 $\pm$ 8
leukaemia (ALL)	CCRF-CEM	40.7	>100	>100	15.4	1.6 $\pm$ 1.2
NSC Lung	NCI-H460	39.7	NT	41.6	39.3 $\pm$ 7.7	1.2 $\pm$ 0.45
Colorectal	HT-29	NT	49.5 $\pm$ 32	29.4 $\pm$ 4.4	24.8 $\pm$ 3.9	15.7 $\pm$ 2.4
Melanoma	MM200	NT	>100	64 $\pm$ 74	12.6 $\pm$ 3.7	0.78 $\pm$ 0.23

5

3.3. Compound No. 1 Pharmacokinetics - oral

Oral pharmacokinetics was determined in the female BALB/c mouse. A C_{max} of 27.3 μ M free compound No. 1 was observed in the sera 15 minutes post administrations.

Compound No. 1 was rapidly eliminated from sera with a concentration of 8.2 μ M

10 observed after 30 min and 2.4 μ M after 1 hr making the half life of this agent around 30 minutes (Fig 1 and Table 3). The majority of compound No. 1 in sera was present in its conjugated state with the concentration of total compound No. 1 (free plus conjugate) achieved being $\sim$ 100 μ M 15 min post administration (1:4; free:total). The ratio of free : total decreased over time (1:12 after 30 min and 1:13 after 1 hr). Rapid appearance of
15 compound No. 1 in urine at high concentrations (815 μ M) 15 min post administration provides evidence that compound No. 1 is absorbed from the gastro intestinal tract and excreted via the kidneys.

Compound No. 1 concentrations in urine peaked at 30 min (2640 μ M) with levels
20 declining to $\sim$ 1500 μ M and 545 μ M at 1 and 4 hr respectively post administration. The majority of said compound was present in it's conjugated form with only $\leq$ 2% as the free entity was noted at any time-point. A large proportion of said compound was also excreted in faeces, which was observed at least 1 hr post administration. However, it is not possible to confirm whether hepatic excretion is a mode of removal for this compound due to the

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fact that no liver samples were assessed and because the observation of the compound in faeces may be due to residual compound powder not absorbed from the gastro intestinal tract. It is interesting to note that almost 100% of the compound recovered from faeces was present in it's free form.

5

Table 3. Compound No.1 pharmacokinetics and distribution in serum, faeces and urine. Data are representative of Average $\pm$ SEM.

Time (days)	Compound No. 1 Concentration								
	Serum Free (uM)	Serum Total (uM)	% Free	Faeces Free (uM)	Faeces Total (uM)	% Free	Urine Free (uM)	Urine Total (uM)	% Free
0.25	27.3 $\pm$ 23	103.4 $\pm$ 36.5	26.4				17.1 $\pm$ 7.2	815 $\pm$ 268	2.1
0.5	8.2 $\pm$ 6.5	77.2 $\pm$ 8	10.6				22.1 $\pm$ 1.5	2640 $\pm$ 1190	0.8
1	2.4 $\pm$ 1.6	31.4 $\pm$ 9.4	7.6	1055 $\pm$ 913	1067 $\pm$ 924	98.9	26.3 $\pm$ 2.2	1535 $\pm$ 158	1.7
4	0.3 $\pm$ 0.02	5.2 $\pm$ 0.8	5.8	1792 $\pm$ 625	1861 $\pm$ 630	96.3	1.1 $\pm$ 0.1	545 $\pm$ 203	0.2
24	0.05 $\pm$ 0.02	0.56 $\pm$ 0.5	8.9	800 $\pm$ 670	815 $\pm$ 680	98.2	0.1 $\pm$ 0.1	7.04 $\pm$ 3.6	1.4

- 10 When compared to the oral pharmacokinetic data of phenoxodiol and compound No. 1 would appear to have a similar half life, however 10-20 fold higher free concentrations of compound No. 1 were achieved after 15 and 30 min. Approximately 2-fold higher concentrations were observed after 1 hr.

- 15 **Table 4.** Comparative oral pharmacokinetic data for Compound No. 1 and Phenoxodiol.

Time	Compound No. 1 (uM)		Phenoxodiol (uM)	
	Free	Total	Free	Total
0.25	27.3 $\pm$ 23	103 $\pm$ 36.5	3.3 $\pm$ 0.13	511.5 $\pm$ 99
0.5	8.2 $\pm$ 6.5	77.2 $\pm$ 8	2.9 $\pm$ 0.05	357 $\pm$ 82
1	2.4 $\pm$ 1.6	31.3 $\pm$ 9.4	1.5 $\pm$ 0.11	387 $\pm$ 22.8
4	0.33 $\pm$ 0.02	5.2 $\pm$ 0.7	1.3 $\pm$ 0.07	117.6 $\pm$ 42
24	0.05 $\pm$ 0.02	0.56 $\pm$ 0.49	0.15 $\pm$ 0.04	0.13 $\pm$ 0.1
Dose*	13.8 mg/ml		4.6 mg/ml	

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4.0. Effect on murine macrophages (RAW 264.7) stimulated with LPS

The mouse macrophage cell line RAW 264.7 was cultured in DMEM supplemented with foetal calf serum (FCS), 2mM glutamine and 50U/ml penicillin/streptomycin. Subconfluent cells were detached from the flask by gentle scraping and 24-well plates seeded at 5×10^5 cells per well and allowed to adhere for 1hr. Cells were then treated either test compound at a concentration of $10\mu\text{M}$ (in 0.025% DMSO) or vehicle alone, and incubated for 1hr. LPS 50ng/ml (LPS -Sigma-Aldrich) was then added. After incubation for 16hrs, culture media was collected and stored at -80°C for eicosanoid measurements using enzyme immunometric assays (PGE_2 and TXB_2 - Cayman Chemical).

Table 5: Percentage change in eicosanoid synthesis after incubating test compound at $10\mu\text{M}$ compared with incubation with vehicle alone. Positive values indicate enhanced synthesis; negative values indicate inhibition of synthesis and consequently suggest anti-inflammatory activity.

Compound	PGE_2	TXB_2
1	-53.8	-15.9
14	-23.6	-40.9
30	-60.7	-62.6
11	-51.0	-53.2
15	-38.3	-58.4
27	-84.2	-86.1
31	-41.4	-48.3
10	-23.1	-7.1
12	-68.3	-34.0
28	-85.1	-50.1
32	-71.1	-34.4

20

5.0 Conclusions

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Compound No. 1 exhibits marked toxicity toward primary explants of non-transformed neonatal foreskin fibroblasts at concentrations less than cisplatin ($IC_{50} = 2 \mu M$ compound No. 1 vs $9 \mu M$ cisplatin respectively). However, relative mild toxicity was noted against rabbit kidney cells (Compound No. 1 $IC_{50} > 60 \mu M$). Efficacy studies demonstrate that
5 compound No. 1 is active against cell lines representative of melanoma (MM200) and glioma (HTB-128). However, compound seems particularly active against cell lines representative of prostate (PC3), breast (MDA-MB-468) and lung cancer (NCIHH-H23) which traditionally have been cancers which are very difficult to treat.

10 Compound No. 1 was moderately active against the colorectal cell line HT-29.

Pharmacokinetic analysis of compound No. 1 revealed that oral administration of the drug yields markedly higher concentrations of the free form of the drug when compared to similarly administered phenoxodiol at 15 and 30 min post administration. Compound No. 1
15 and phenoxodiol each exhibit similar $t_{1/2}$ (~30 min).

Preliminary formulation studies of compound No. 1 reveal that the molecule has moderate to low solubility in 20% HPBCD (11.2 mg/ml).

20 The invention has been described herein, with reference to certain preferred embodiments, in order to enable the reader to practice the invention without undue experimentation. However, a person having ordinary skill in the art will readily recognise that many of the components and parameters may be varied or modified to a certain extent without departing from the scope of the invention. Furthermore, titles, headings, or the like are
25 provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention.

The entire disclosures of all applications, patents and publications, cited herein, if any, are hereby incorporated by reference.

30

Throughout this specification and the claims which follow, unless the context requires

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otherwise, the word "comprise", and variations such as "comprises" or "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.

- 5 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. 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
10 more of said steps or features.

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

15

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Selected Reference Articles

- Constantinou AI, Mehta R, Husband A. 2003 Phenoxodiol, a novel isoflavone derivative, inhibits dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis in female
5 Sprague-Dawley rats. Eur J Cancer. 39, 1012-8.

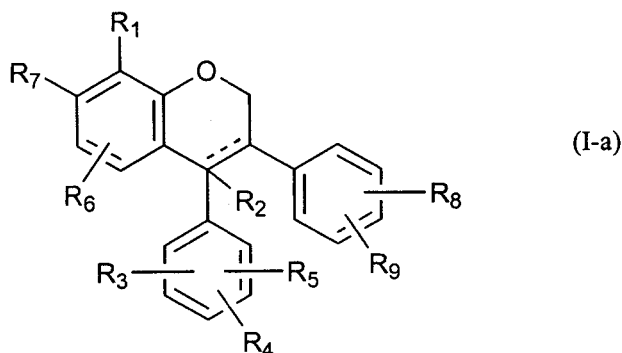
- Constantinou AI, Husband A. 2002 Phenoxodiol (2H-1-benzopyran-7-0,1,3-(4-hydroxyphenyl)), a novel isoflavone derivative, inhibits DNA topoisomerase II by stabilizing the cleavable complex. Anticancer Res. 22, 2581-5.
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- Gamble, JR., Xia, P., Hahn, C., Drew, J., Drogemuller, C., Carter, C., Walker, C., Brown, DM., Vadas, MA. 2003 Phenoxodiol, a derivative of plant flavanoids, shows potent anti-tumour and anti-angiogenic properties. Nature Medicine. Submitted.
- 15 Hersey, P and Zhang, X. D. 2001 How melanoma cells evade Trail-induced apoptosis. Nature reviews, Cancer, 1, 142-150.

- Kamsteeg, M., Rutherford, T., Sapi, E., Hanczaruk, B., Shahabi, S., Flick, M., Brown, D.M and Mor, G. 2003 Phenoxodiol-an isoflavone analogue- induces apoptosis in chemo-
20 resistant ovarian cancer cells. Oncogene, ;22, 2611-20.

Claims

1. A compound of formula (I-a):



wherein

R₁ is hydrogen, hydroxy, halo, C₁₋₆ alkoxy, or C₁₋₆ alkyl;

the drawing "----" and R₂ together represent a double bond or

the drawing "----" represents a single bond and R₂ is hydrogen or hydroxy;

R₃, R₄ and R₅ are independently hydrogen, hydroxy, NR₁₀R₁₁, C₁₋₆ alkoxy or C₁₋₆ alkyl;

R₆ is hydrogen, hydroxy, halo, C₁₋₆ alkoxy or C₁₋₆ alkyl;

R₇ is hydroxy;

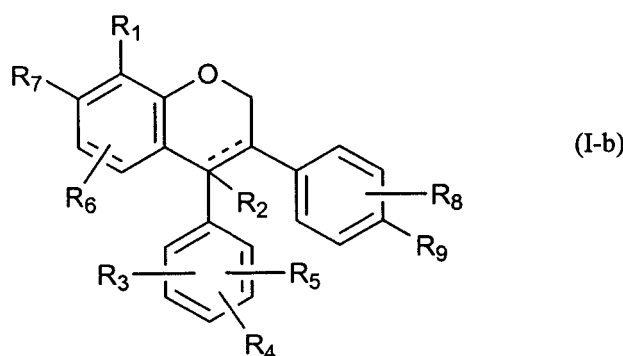
R₈ and R₉ are independently hydrogen, hydroxy, halo, C₁₋₆ alkoxy or C₁₋₆ alkyl; and

R₁₀ and R₁₁ are independently hydrogen, C₁₋₆ alkyl or trialkyl silyl;

or a pharmaceutically acceptable salt thereof,

with the proviso that when R₁ represents hydrogen and "----" is a single bond then R₂ does not represent hydrogen.

2. A compound of claim 1 according to formula (I-b):



wherein

R₁ represents hydroxy, halo, C₁₋₆ alkoxy, or C₁₋₆ alkyl; and

R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are defined in claim 1.

3. A compound of claim 2, wherein:

R₁ is methyl, methoxy or halo;

R₃ is hydrogen, hydroxy, methoxy or amino;

R₄ and R₅ are hydrogen;

R₆ is hydrogen; and

R₈ and R₉ are independently hydrogen, hydroxy, halo, methoxy or methyl.

4. A compound of claim 3, wherein:

R₁ is methyl or bromo;

R₃ is methoxy and is in the 4-position; and

R₈ is hydrogen and R₉ is hydrogen, hydroxy or methoxy, or

R₈ is hydroxy or methoxy and in the 3-position and R₉ is hydrogen, or

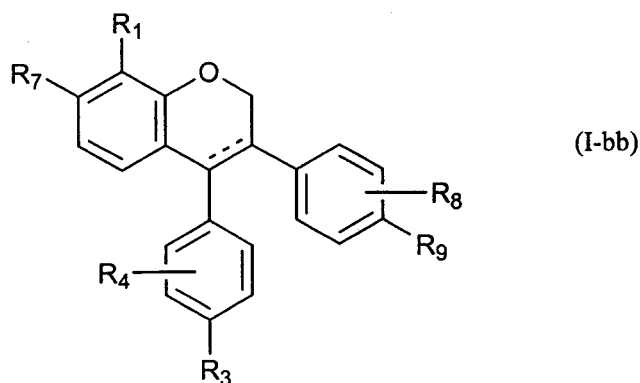
R₈ and R₉ are both hydroxy or methoxy and R₈ is in the 3-position.

5. A compound of claim 4, wherein:

the drawing "—" represents a single bond; and

R₂ is hydrogen and *cis* to the hydrogen at C-3.

6. A compound of claim 1 according to formula (I-bb):



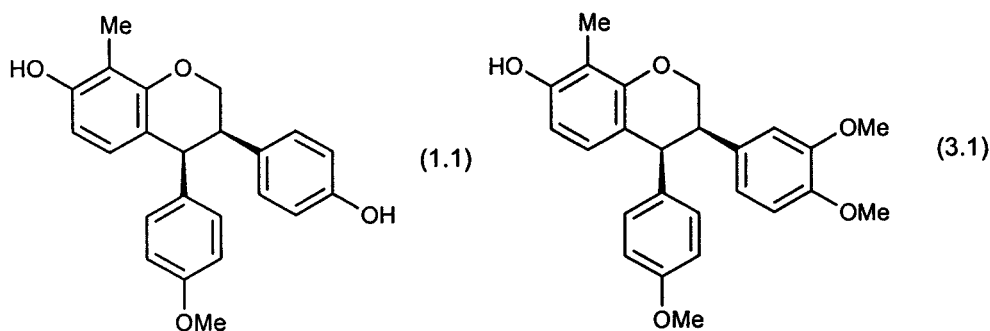
wherein

R_1 , R_3 , R_4 , R_7 , R_8 and R_9 are as defined in claim 2.

7. A compound of claim 1 selected from compound Nos. 1, 3-4, 6, 8-9 and 42:

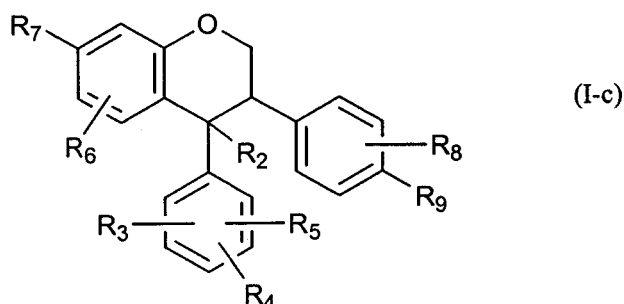
3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (1);
 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (3);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (4);
 3-(3-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (6);
 3-(3-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (8);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-7-ol (9);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-bromo-chroman-7-ol (42);
 or a pharmaceutically acceptable salt thereof.

8. A compound of claim 7 selected from compound Nos. 1.1 and 3.1:



or a pharmaceutically acceptable salt thereof.

9. A compound of claim 1 according to formula (I-c):

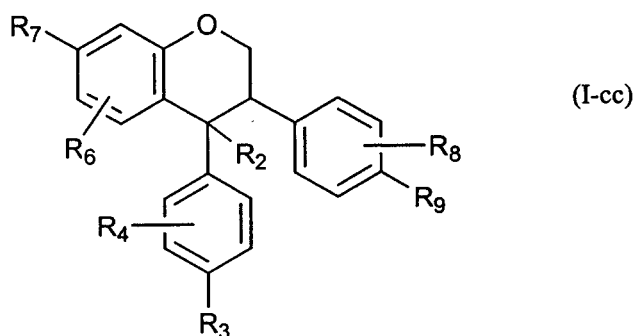


wherein

R₂ represents hydroxy; and

R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are as defined in claim 1.

10. A compound of claim 9 according to formula (I-cc):



wherein

R₂, R₃, R₄, R₆, R₇, R₈ and R₉ are as defined in claim 8.

11. A compound of claim 10 selected from compound Nos. 10-11, 14-16, 18-19, 21 and 23-25:

3-(4-hydroxyphenyl)-4-(4-methylphenyl)-chroman-4,7-diol (10);

3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-chroman-4,7-diol (11);

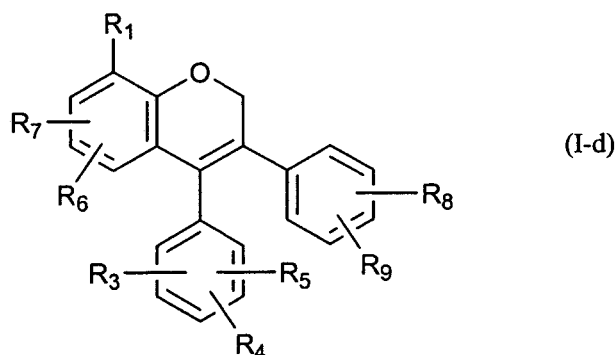
3-(4-hydroxyphenyl)-4-phenyl-chroman-4,7-diol (14);

3-(4-hydroxyphenyl)-4-(3-methoxyphenyl)-chroman-4,7-diol (15);

3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (16);

3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (18);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (19);
 3-(3-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (21);
 3-(3-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (23);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-chroman-4,7-diol (24);
 3-(4-hydroxyphenyl)-4-(3-aminophenyl)-chroman-4,7-diol (25);
 or a pharmaceutically acceptable salt thereof.

12. A compound of claim 1 according to formula (I-d):



wherein

R₁, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ are as defined in claim 1.

13. A compound of claim 12 selected from compound Nos. 26-27, 30-32, 34-35, 37, 39-41 and 44:

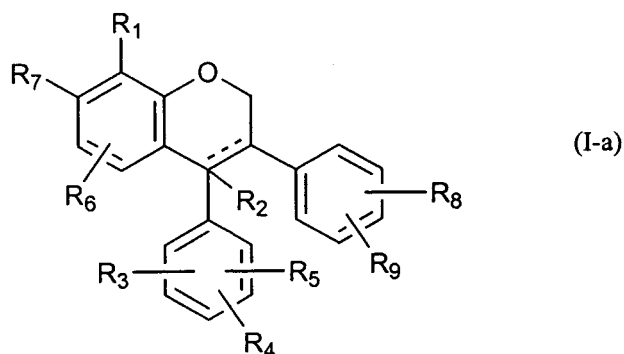
3-(4-hydroxyphenyl)-4-(4-methylphenyl)-2*H*-chromen-7-ol (26);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-2*H*-chromen-7-ol (27);
 3-(4-hydroxyphenyl)-4-phenyl-2*H*-chromen-7-ol (30);
 3-(4-hydroxyphenyl)-4-(3-methoxyphenyl)-2*H*-chromen-7-ol (31);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (32);
 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (34);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (35);
 3-(3-methoxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (37);

3-(3-hydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (39);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-8-methyl-2*H*-chromen-7-ol (40);
 3-(4-hydroxyphenyl)-4-(3-aminophenyl)-2*H*-chromen-7-ol (41);
 3-(3,4-dimethoxyphenyl)-4-(4-methoxyphenyl)-2*H*-chromen-7-ol (44);
 or a pharmaceutically acceptable salt thereof;

14. A compound selected from compound Nos. 2, 5, 7, 12-13, 17, 20, 22, 29, 33, 36, 38 and 45:

3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chromane (2);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chromane (5);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chromane (7);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-chroman-4-ol (12);
 3-(4-methoxyphenyl)-4-(3-methoxyphenyl)-7-methoxy-chroman-4-ol (13);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chroman-4-ol (17);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chroman-4-ol (20);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-chroman-4-ol (22);
 3-(4-methoxyphenyl)-4-(3-methoxyphenyl)-7-methoxy-2*H*-chromene (29);
 3-(4-methoxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-2*H*-chromene (33);
 3-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-2*H*-chromene (36);
 3-(3,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-7-methoxy-8-methyl-2*H*-chromene (38);
 3-(4-hydroxyphenyl)-4-(4-phenoxyphenyl)-2*H*-chromen-7-ol (45);
 or a pharmaceutically acceptable salt thereof.

15. Use of a compound of formula (I-a) for the manufacture of a medicament for chemotherapy or as a radiosensitising or chemosensitising agent, wherein the compound of formula (I-a) is



in which

R_1 is hydrogen, hydroxy, halo, C_{1-6} alkoxy, or C_{1-6} alkyl;

the drawing "---" and R_2 together represent a double bond or

the drawing "___" represents a single bond and R_2 is hydrogen or hydroxy;

R_3 , R_4 and R_5 are independently hydrogen, hydroxy, $NR_{10}R_{11}$, C_{1-6} alkoxy or C_{1-6} alkyl;

R_6 is hydrogen, hydroxy, halo, C_{1-6} alkoxy or C_{1-6} alkyl;

R_7 is hydroxy or methoxy;

R_8 and R_9 are independently hydrogen, hydroxy, halo, C_{1-6} alkoxy or C_{1-6} alkyl; and

R_{10} and R_{11} are independently hydrogen, C_{1-6} alkyl or trialkyl silyl;

or a pharmaceutically acceptable salt thereof,

with the proviso that when R_1 represents hydrogen and "___" is a single bond then R_2 does not represent hydrogen.

16. Use of claim 15, wherein the compound is as defined in any one of claims 1-14.

17. Use of claim 15 or 16, wherein the chemotherapy is for the treatment of cancer or a tumour mass.

18. Use of claim 17, wherein the cancer or tumour mass is of epithelial origin, of mesenchymal origin or of neural origin.

19. Use of claim 18, wherein the cancer is prostate, ovarian, cervical, breast, gall-bladder, pancreatic, colorectal, renal, non-small lung, melanoma, mesothelioma, sarcoma

or glioma cancer.

20. Use according to claim 15 or 16, wherein the one or more compounds is a chemosensitising agent for cisplatin, dehydroequol or taxol.

21. Use according to claim 15 or 16, wherein the chemosensitising agent is for the treatment of ovarian or pancreatic cancer.

22. A method for the manufacture of a pharmaceutical composition including the step of bringing a compound as defined in any one of claims 1-14 into association with a pharmaceutically acceptable carrier or diluent.

23. A pharmaceutical composition which comprises one or more compounds as defined in any one of claims 1-14 or a pharmaceutically acceptable salt thereof in association with one or more pharmaceutical carriers, excipients, auxiliaries and/or diluents.

24. A pharmaceutical composition according to claim 23 which further comprises an additional chemotherapeutic agent.

25. A pharmaceutical composition according to claim 24, wherein the additional chemotherapeutic agent is cisplatin, dehydroequol or taxol.