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(74) Agent: **BARKER BRETTELL**; 138 Hagley Road, Edgbaston, Birmingham B16 9PW (GB).

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(71) Applicant (for all designated States except US): **THE BABRAHAM INSTITUTE** [GB/GB]; Babraham Hall, Babraham, Cambridge CB2 4AT (GB).

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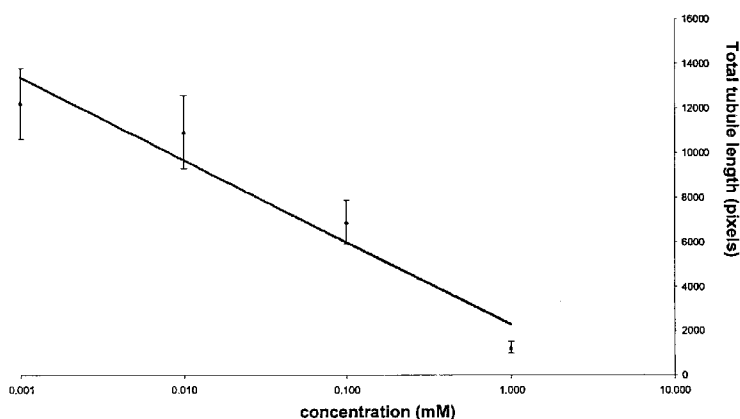
(72) Inventors; and

(75) Inventors/Applicants (for US only): **JONES, Robert** [GB/GB]; 14 Thornton Close, Girton, Cambridge CB3 0NQ (GB). **CLARK, Jonathan** [GB/GB]; 7 Burrell Way, Balsham, Cambridge CB21 4DY (GB). **GABORIAU, David, Claude, Andre** [FR/GB]; 28 Enniskillen Road, Cambridge CB4 1SQ (GB).

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(57) Abstract: A compound having: a bicyclic ring structure that comprises two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the bicyclic ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the bicyclic ring structure being substituted with an optionally substituted pendent aromatic ring, wherein the compound includes one or more acidic substituent group selected from sulfate, sulfonate, phosphate and carboxylate; and pharmaceutically acceptable salts, solvates and pro-drugs thereof. The compounds, pharmaceutically acceptable salts, solutes or pro-drugs thereof, may be used for the treatment of inappropriate angiogenesis, for example in the treatment of cancer, diabetic retinopathy, arthritis, psoriasis, atherosclerosis, macular degeneration, or hemangioma.

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COMPOUNDS

This invention relates to compounds and pharmaceutically acceptable salts, solvates and pro-drugs thereof. The present compounds are useful
5 as anti-angiogenic, in particular anti-cancer, agents. Methods of preparing these compounds and the medical use of these compounds are also provided.

Angiogenesis is the process whereby growth of new blood vessels is
10 stimulated. This in particular occurs in cancer, where tumours stimulate the blood vessel growth in order to enable the tumour to expand and spread throughout the body. The increased blood flow to the tumour allows for continued growth, and also metastasis, because successful metastasis requires the presence of blood vessels to allow for the tumour
15 cells to enter the circulation. Various growth factors, such as vascular endothelial growth factor (VEGF) are required for this process.

Angiogenesis normally occurs only during embryonic and post-embryonic development, reproductive cycle and wound repair. However,
20 angiogenesis is induced in many disease states, including cancer, diabetic retinopathy, arthritis, psoriasis, atherosclerosis, macular degeneration, and hemangioma.

Inhibition of angiogenesis is a known route for chemotherapeutic
25 treatments and a variety of anti-angiogenic agents are known and form part of cancer therapy. Known anti-angiogenic agents used in cancer treatment include both natural products (e.g. chemokines, gluco-galactan sulphate) and synthetic products (e.g. suramin, pentosan polysulfate), and may be directed against the growth factor or its receptor or both. The
30 end result is stabilisation of the tumour or its shrinkage.

The problem with known anti-cancer agents, including anti-angiogenic agents, is the side effects associated with efficacious treatments. For example, suramin is very effective in preventing blood vessel growth but its cytotoxicity is considerable.

5

There is therefore a need for chemical compounds that have good activity as anti-angiogenic agents but that have fewer side effects than known anti-angiogenic agents with good efficacy.

10 The present invention provides, in a first aspect, a compound, and pharmaceutically acceptable salts, solvates and pro-drugs thereof, the compound having:

a bicyclic ring structure that comprises two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one
15 carbon in the bicyclic ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the bicyclic ring structure being substituted with an optionally substituted pendent aromatic ring;

wherein the compound includes one or more acidic substituent group selected from sulfate, sulfonate, phosphate and carboxylate.

20

These novel compounds have been found to have an anti-angiogenesis effect.

It is believed that these compounds act as angiogenesis inhibitors by
25 blocking the important biological receptor-ligand interactions that occur during angiogenesis.

Surprisingly, these compounds that include one or more acidic substituent group selected from sulfate, sulfonate, phosphate and carboxylate are less
30 toxic than known anti-angiogenic agents with good efficacy.

In particular, these compounds do not cause damage to cell integrity of the human or animal being treated.

5 The compound may include two or more acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate, for example three or more, such as four or more, e.g. five, six, seven or eight or more acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate.

10 When there are two or more acidic substituent groups, the acidic substituent groups may be the same or different.

The acidic substituent groups may be sulfate or sulfonate groups, for example sulfate groups.

15

In one embodiment the compound includes one or more sulphate group.

In one embodiment there are two or more acidic substituent groups, which are all sulfate groups. For example there may be three, four, five,
20 six, seven, eight or more acidic substituent groups, which are all sulfate groups.

One or more positions on the ring structure and the pendent aromatic ring may be substituted with an acidic substituent group selected from sulfate,
25 sulfonate, phosphate and carboxylate.

Alternatively, or additionally, the ring structure or the pendent aromatic ring may have a substituent group that itself is substituted with one or more acidic substituent group selected from sulfate, sulfonate, phosphate
30 and carboxylate.

Two or more positions on the ring structure and the pendent aromatic ring may be substituted with an acidic group selected from sulfate, sulfonate, phosphate and carboxylate, for example three or more positions, such as four or more positions, e.g. five, six, seven or eight or more positions.

- 5 The acidic group substitutions may all be at positions on the bicyclic ring structure, all at positions on the pendent aromatic ring, or one or more at positions on the bicyclic ring structure and one or more at positions on the pendent aromatic ring.
- 10 The positions on the ring structure and the pendent aromatic ring that are not substituted with an acidic group selected from sulfate, sulfonate, phosphate and carboxylate may optionally be substituted. In particular, the substituent groups, which may be the same or different, may, for example, be selected from carbonyl, hydroxyl, aliphatic ring groups (e.g.
- 15 saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), and nitrile. In particular, these groups may be carbonyl, hydroxyl, or saccharide groups.

- When one carbon in the bicyclic ring structure is replaced with nitrogen, sulfur or oxygen, this nitrogen, sulfur or oxygen group may be
- 20 substituted or unsubstituted. This may be a position that is substituted with an acidic group selected from sulfate, sulfonate, phosphate and carboxylate. This may alternatively be the position substituted with a pendent aromatic ring. It may alternatively be a position that is
- 25 substituted with a different group, which may, for example, be selected from hydrogen, carbonyl, hydroxyl, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), and nitrile.

- 30 In one embodiment, the compound includes one or more aliphatic ring group. The bicyclic ring structure may be substituted with an aliphatic

ring group. An aliphatic ring group may alternatively, or additionally, be provided on the pendent aromatic ring.

5 The aliphatic ring group may in particular be a five or six membered ring group.

The aliphatic ring group may be carbocyclic or heterocyclic. In one embodiment, the ring is a heterocyclic ring with one oxygen, nitrogen or sulfur in the ring and the remaining ring members being carbon. In one
10 such embodiment, the ring is a heterocyclic ring with one oxygen group in the ring and the remaining ring members being carbon.

The aliphatic ring group may be substituted or unsubstituted. When one or more positions on the ring group are substituted, the substituent
15 groups, which may be the same or different, may be selected from carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), carboxylate and nitrile.

20

In one embodiment, the aliphatic ring group may be a saccharide group. The saccharide group may be a monosaccharide, disaccharide or trisaccharide.

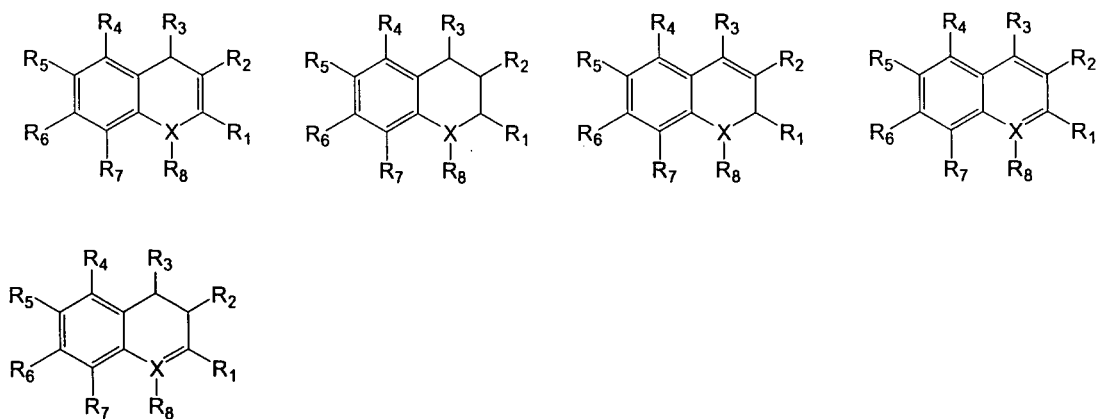
25 The saccharide group may have one or more of its OH groups substituted. The substituent groups, which may be the same or different, may be selected from carbonyl, sulfate, sulfonate, phosphate, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl),
30 carboxylate and nitrile.

In particular, the saccharide group may have one or more of its OH groups substituted with an acidic substituent group selected from sulfate, sulfonate, phosphate and carboxylate.

- 5 The bicyclic ring structure may comprise two aromatic rings. Alternatively, one ring may be aromatic and the other ring may be non-aromatic. The non-aromatic ring may be saturated or unsaturated.

- 10 The pendent aromatic ring group may be a carbocyclic or heterocyclic aromatic group. The pendent aromatic ring group may, for example, be substituted or unsubstituted benzene or naphthalene, or a heterocyclic derivative thereof.

- 15 The compounds of the first aspect may have one of the following general formulae:



wherein X is O, S, N or C,

- 20 one of the groups R1-R8 is an optionally substituted pendent aromatic group,

and the remaining groups R1-R8, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups (e.g. saccharides), halide,

alkyl (e.g. C1-12 alkyl, such as C1-6 alkyl), alkyl ether (e.g. C1-12 alkyl ether, such as C1-6 alkyl ether), carboxylate and nitrile,

with the proviso that:

when X is O, S or N, R8 need not present, and

- 5 one or more of the R1-R8 groups are acidic groups selected from sulfate, sulfonate, phosphate and carboxylate, or comprise acidic groups selected from sulfate, sulfonate, phosphate and carboxylate.

When any of the groups R1-R8 are aliphatic ring groups, these may, in
10 one embodiment, be 5 or 6 membered aliphatic ring groups. The aliphatic ring groups may optionally be substituted. The aliphatic ring groups, when substituted, may have one or more substituents selected from carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether
15 (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), carboxylate and nitrile.

The pendent aromatic group, when substituted, may have one or more substituents selected from carbonyl, hydroxyl, sulfate, sulfonate,
20 phosphate, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), carboxylate and nitrile.

In one embodiment, X is O. In one aspect of this embodiment, R8 is not
25 present.

In one embodiment, group R1 is the pendent aromatic group. This group may be substituted, for example with one or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate.

In one embodiment, group R2 is an aliphatic ring group, e.g. a saccharide, such as a monosaccharide or disaccharide. This group may be substituted with one or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate.

5

In one embodiment, group R3 is hydrogen or carbonyl.

In one embodiment, group R4 is not hydrogen. For example, it may be hydroxyl, sulfate, sulfonate, phosphate or carboxylate.

10

In one embodiment, group R5 is hydrogen or hydroxyl.

In one embodiment, group R6 is not hydrogen. For example, it may be hydroxyl, sulfate, sulfonate, phosphate or carboxylate.

15

In one embodiment, group R7 is hydrogen.

In a preferred embodiment, group R1 is the pendent aromatic group, which may or may not be substituted with one or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate group; R2 is an aliphatic ring group, e.g. a saccharide group, which may or may not be substituted with one or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate group; group R3 is hydrogen or carbonyl; group R4 is not hydrogen, for example, it may be hydroxyl, sulfate, sulfonate, phosphate or carboxylate; group R5 is hydrogen or hydroxyl; group R6 is not hydrogen, example, it may be hydroxyl, sulfate, sulfonate, phosphate or carboxylate; and group R7 is hydrogen.

20

In one embodiment, the R1-R8 groups are such that there are a total of two or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate present in the compound.

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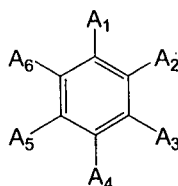
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In one such embodiment, the R1-R8 groups are such that there are a total of two or more sulphate groups present in the compound.

- 5 In one embodiment, the R1-R8 groups are such that there are a total of three or more, such as four or more, e.g. five, six, seven or eight or more, acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate present in the compound.
- 10 In one such embodiment, the R1-R8 groups are such that there are a total of three or more sulphate groups present in the compound, such as four or more, e.g. five, six, seven or eight or more.

The pendent aromatic group may be of the following general formula:

15



- wherein A1 attaches the pendent aromatic group to the bicyclic ring structure, and may be a bond (i.e. the aromatic group is directly attached to the bicyclic ring structure) or may be an oxygen, sulphur, nitrogen or carbon group,
- 20

- and A2-A6, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups (e.g. saccharides), halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), carboxylate and nitrile.
- 25

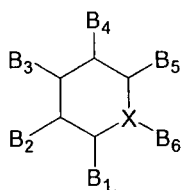
In one embodiment, the pendent aromatic group is substituted or unsubstituted benzene. When substituted, there may be one or more, for

example two, substituent groups. The substituent groups, which may be the same or different, may, for example, be selected from hydroxyl, alkyl ether (e.g. C1-12 alkyl ether), sulfate, sulfonate, phosphate, and carboxylate. In one embodiment, there is one or more sulfate, sulfonate, phosphate or carboxylate substituent group.

An aliphatic ring group may, in particular, be present in the compound. This group may be a substituent on the bicyclic ring structure, but may alternatively be a substituent on the pendent aromatic group.

10

The aliphatic ring group may be of the following general formula:



wherein B1 is an anomeric oxygen group which attaches the aliphatic ring group to the bicyclic ring structure,

X is selected from O, S, N and C,

B2-B6, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, saccharide, halide, alkyl (e.g. C1-12 alkyl), alkyl ether (e.g. C1-12 alkyl ether), ROH where R is alkyl (e.g. C1-12 alkyl), carboxylate and nitrile,

with the proviso that when X is O or S, B6 is not present.

In one embodiment, there is one or more sulfate, sulfonate, phosphate or carboxylate substituent group.

The aliphatic ring group may in particular be a monosaccharide, disaccharide or trisaccharide.

In one embodiment, X is O (and therefore B6 is not present) and B2-B5, which may be the same or different, are each selected from hydroxyl, saccharide, ROH where R is alkyl (e.g. C1-12 alkyl), sulfate, sulfonate, phosphate and carboxylate.

Compounds in accordance with the present invention can be readily made starting from commercially available compounds, which have a bicyclic ring structure comprising two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the ring structure being substituted with an optionally substituted pendent aromatic ring. These products may have the required acidic groups incorporated by standard techniques. Specifically, conventional sulfation, sulfonation, phosphorylation or carboxylation routes may be used.

Equally, the skilled man may prepare a compound, which has a bicyclic ring structure comprising two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the ring structure being substituted with an optionally substituted pendent aromatic ring, using conventional organic synthesis techniques. This product may then have the required acidic groups incorporated by conventional sulfation, sulfonation, phosphorylation or carboxylation techniques.

25

Accordingly, in a second aspect, the present invention provides a method of manufacturing a compound in accordance with the first aspect, the method comprising:

(a) providing a compound, which has a bicyclic ring structure comprising two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the ring structure may

30

optionally be replaced with a nitrogen, sulfur or oxygen, the ring structure being substituted with an optionally substituted pendent aromatic ring;

- 5 (b) incorporating one or more acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate, using sulfation, sulfonation, phosphorylation or carboxylation techniques.

In one embodiment the method of manufacturing a compound in accordance with the first aspect comprises:

- 10 (a) providing a compound, which has a bicyclic ring structure comprising two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the ring structure being substituted with an optionally substituted pendent aromatic
15 ring, using conventional organic synthesis techniques;

(b) incorporating one or more acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate, using sulfation, sulfonation, phosphorylation or carboxylation techniques.

- 20 In one embodiment, commercially available products may be readily sulfated using standard sulfation techniques, for example using sulfur trioxide. Desired levels of sulfation can be achieved by controlling the sulfation, or by introducing differential protecting groups, which can be removed after the sulfation step.

25

In one embodiment, the starting material has one or more hydroxyl group and the sulfation is controlled so as to sulfate one or more of the hydroxyl groups, such as two or more, three or more, or four or more of the hydroxyl groups.

30

In one embodiment, the starting material has one or more hydroxyl group and the sulfation is controlled so as to sulfate all the hydroxyl groups.

The compound provided in step (a) may be the same as the compound of
5 the first aspect defined above but without any acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate.

Examples of commercially available materials suitable for use as starting materials in this regard include, but are not limited to, catechin,
10 (-)-epicatechin, apigenin, hesperidin, naringin, quercetin, quercetin-3-D-galactoside, quercetin-3-beta-D-glucoside, rutin and baicalin.

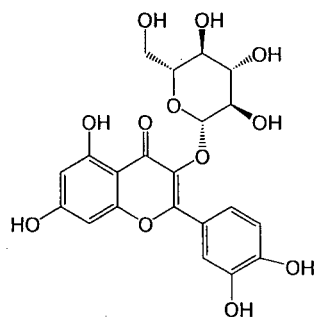
Surprisingly, these compounds can be reacted so that they are provided with one or more acidic groups selected from sulfate, sulfonate,
15 phosphate and carboxylate, to result in novel compounds that have anti-angiogenic effects.

The present invention therefore provides compounds in accordance with the first aspect which are catechin, (-)-epicatechin, apigenin, hesperidin,
20 naringin, quercetin, quercetin-3-D-galactoside, quercetin-3-beta-D-glucoside, rutin or baicalin, when reacted so that they are provided with one or more acidic groups selected from sulfate, sulfonate, phosphate and carboxylate.

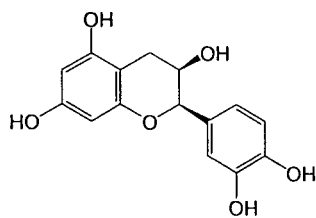
25 In one embodiment, they are provided with two or more, such as three or more, or four or more, acidic groups selected from sulfate, sulfonate, phosphate and carboxylate.

In one embodiment, they are provided with one or more, e.g. two or
30 more, such as three or more, or four or more, sulphate groups.

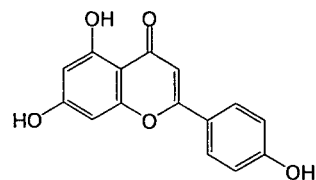
The structures of these commercially available starting materials are shown below:



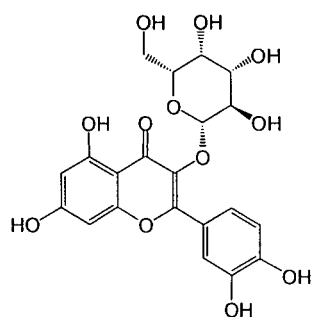
Quercetin-3-D-glucoside



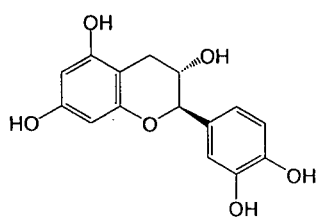
(-)-epicatechin



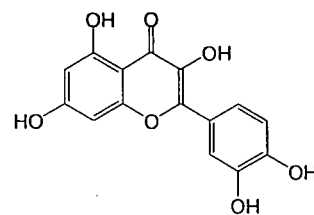
Apigenin



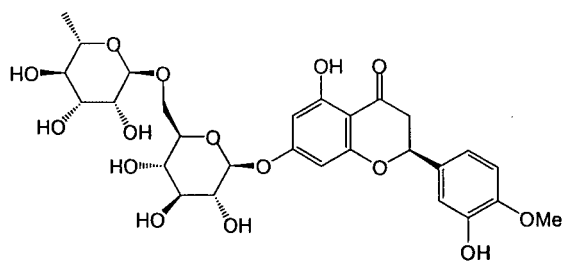
Quercetin-3-D-galactose



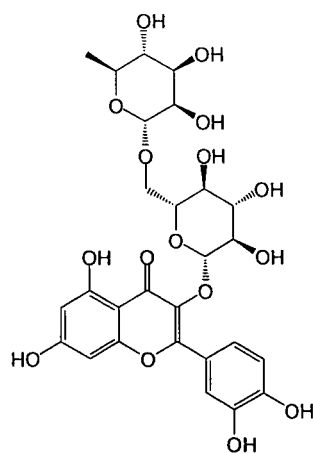
Catechin



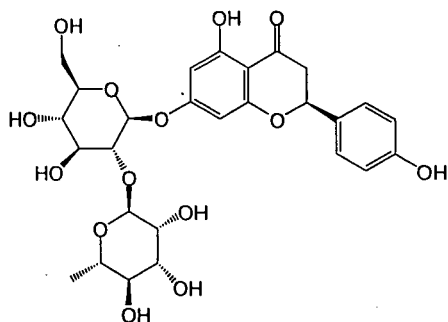
Quercetin



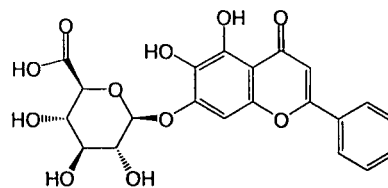
Hesperidin



Rutin trihydrate



Naringin



Baicalin

The present invention also provides, in a third aspect, a compound of the first aspect, or a pharmaceutically acceptable salt, solute or pro-drug thereof, for use in medicine.

5

In one embodiment, the invention provides a compound of the first aspect, or a pharmaceutically acceptable salt, solute or pro-drug thereof, for use in the treatment of inappropriate angiogenesis. In particular, the use may be in the treatment of cancer (including, but not limited to, leukemia, lung cancer, colon cancer, CNS cancer, skin cancer, ovarian cancer, renal cancer, prostate cancer, stomach cancer, breast cancer, pancreatic cancer, bladder cancer), diabetic retinopathy, arthritis (including, but not limited to, rheumatoid arthritis), psoriasis, atherosclerosis, macular degeneration, or hemangioma.

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The present invention also provides, in a fourth aspect, use of a compound of the first aspect, or a pharmaceutically acceptable salt, solute or pro-drug thereof, in the manufacture of a medicament for the treatment of inappropriate angiogenesis.

20

In particular, the use may be in the treatment of cancer (including, but not limited to, leukemia, lung cancer, colon cancer, CNS cancer, skin cancer, ovarian cancer, renal cancer, prostate cancer, stomach cancer, breast cancer, pancreatic cancer, bladder cancer), diabetic retinopathy, arthritis (including, but not limited to, rheumatoid arthritis), psoriasis, atherosclerosis, macular degeneration, or hemangioma.

25

Treatment refers to both therapeutic and prophylactic treatment.

30 The compound of the first aspect, or pharmaceutically acceptable salt, solute or pro-drug thereof, may be used at a concentration of 0.01mM or

higher, such as 0.05mM or higher; preferably 0.1mM or higher, e.g. 0.5mM or higher; most preferably 0.75mM or higher, such as 1mM or higher.

- 5 The present invention also provides, in a fifth aspect, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the first aspect, or a pharmaceutically acceptable salt, solute or pro-drug thereof, together with a pharmaceutically acceptable carrier, excipient or diluent.

10

The carrier, diluent or excipient must be acceptable in the sense of being compatible with the other components of the composition and not being deleterious to the recipient thereof. The carrier, diluent or excipient may, for example, be selected from water, aqueous solutions, ethanol, glycerol, and edible carbohydrates (e.g. starch or mannitol).

15

The pharmaceutical composition may be in unit dose form, containing a predetermined amount of active ingredient per unit dose. Such a unit may contain, for example, 0.5mg to 1g, such as 1 mg to 750mg, such as 5mg to 500mg, e.g. 10mg to 100mg, of the compound of the first aspect or of a pharmaceutically acceptable salt, solute or pro-drug thereof. The unit dose amount will of course depend on the condition being treated, the route of administration and the age, weight and condition of the patient.

20

- 25 Preferred unit dosage compositions are those containing a daily dose, or an appropriate fraction thereof, of the active ingredient.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the composition may be adapted for oral, rectal, nasal, topical, vaginal, subcutaneous, intramuscular, or parenteral administration.

30

The pharmaceutical composition may be in the form of a capsule, tablet, lozenge, pastille, powder, granules, solution or suspension in aqueous or non-aqueous liquids, emulsion, ointment, cream, lotion, paste, gel, spray, aerosol, oil, suppository, or enema.

Conventional additives, such as flavouring, preservative, dispersing agent, colouring agent, binder, or lubricant, can also be present.

A therapeutically effective amount of a compound of the present invention will depend upon a number of factors including, for example, the age and weight of the recipient, the precise condition requiring treatment and its severity, the nature of the formulation, and the route of administration. However, an effective amount will generally be in the range of 0.1 to 100 mg/kg body weight of recipient per day, e.g. in the range of 1 to 10 mg/kg body weight per day.

The present invention also provides, in a sixth aspect, a method of treating a disorder in a human or animal, said disorder being mediated by inappropriate angiogenesis, the method comprising administering to said human or animal a therapeutically effective amount of a compound of the first aspect, or a pharmaceutically acceptable salt, solute or pro-drug thereof.

In one embodiment, the method is a method of treating a disorder in a human or animal, said disorder being mediated by inappropriate angiogenesis, wherein the method does not cause damage to cell integrity of the human or animal.

The method may be a method for treating a disorder in a mammal.

The compound of the first aspect, or pharmaceutically acceptable salt, solute or pro-drug thereof, may be administered in the form of a composition according to the fifth aspect.

- 5 The therapeutically effective amount of a compound of the present invention will depend upon a number of factors including, for example, the age and weight of the recipient, the precise condition requiring treatment and its severity, the nature of the formulation, and the route of administration. However, an effective amount will generally be in the
10 range of 0.1 to 100 mg/kg body weight of recipient per day, e.g. in the range of 1 to 10 mg/kg body weight per day.

The present invention will now be further described with reference to the following, non-limiting, examples:

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Preparation Example 1

Preparation of Quercetin 3- β -D-glucoside-sulfate

Quercetin 3- β -D-glucoside (25mg, 0.054mmol) was added to sulfur
20 trioxide trimethylamine complex (59.9mg, 0.43mmol, 1 equivalent for each OH) followed by 400ul dry *N,N*-dimethylformamide. The mixture was left at room temperature under argon for 2 hours after which time the compounds were fully in solution. The reaction was then heated for 20 hours at 55 to 60°C and then allowed to cool to room temperature.

25

4ml diethyl ether was then added and the sample left to stand until the product had fully separated, either as a solid or as an oil, at the bottom of the reaction vessel. The diethyl ether layer was then removed and the sample washed with another 4ml diethyl ether. The product was dried
30 under vacuum over night. It was then dissolved in a small amount of water and freeze-dried.

Control Preparation Example

Unsulphated quercetin 3- β -D-glucoside, used as control treatment, was diluted in DMSO.

5

Preparation Examples 2-9

The method of **Preparation Example 1** was followed for starting materials of: catechin, (-)-epicatechin, apigenin, hesperidin, naringin, quercetin, quercetin-3-D-galactoside, quercetin 3-rutinoside and baicalin respectively, with adjustments made to the amount of sulfur trioxide trimethylamine complex used to match the number of free OH groups.

10

Preparation Example 10 - Sulphation of Quercetin 3- β -D-glucoside to different levels

15

Quercetin 3- β -D-glucoside was reacted with a range of concentrations of sulphating reagent (sulfur trioxide @ 1eq, 2eq, 3eq, 4eq, 5eq, 6eq, 7eq, 8eq, 16eq, 32eq, and 8eq SO₃ with 4eq triethylamine) in order to produce a range of sulphated quercetin 3- β -D-glucosides.

20

a. Method

Twenty-five mg quercetin 3- β -D-glucoside sulphation reactions were set up in 0.4ml DMF solvent (**Table 1**) and heated overnight at 55-60°C. Solvent was removed by adding 4ml diethyl ether to precipitate the product and removed under vacuum overnight. Samples were dissolved in 30% sodium acetate and precipitated with ethanol, filtered and dried.

25

30

Reaction product Code	Eq	Amount SO ₃ .NMe ₃ /mg
S1	1	7.49
S2	2	14.98
S3	3	22.48
S4	4	29.97
S5	5	37.46
S6	6	44.95
S7	7	52.45
S8	8	59.94
S9	16	119.88
S10	32	239.75
47ppt	8 (+ 4eq NEth)	59.94

Table 1

b. Results

From the sulphated compounds, two were chosen to be evaluated for anti-angiogenic properties, based on varying degrees of sulphation as determined from the MS profiles. These compounds and their levels of sulphation are shown in Table 2.

Reaction Code	Eq SO ₃	Level of Sulphation (%)		
		Mono	Di	Tetra
S8	8	0	100	0
47ppt	8 (+ 4eq NEth)	0	0	100

Table 2

The conditions for Reaction code 47ppt were also repeated using quercetin-3-rutinoside as starting material, to produce sulphated quercetin 3-rutinoside (Reaction code 48-5ppt).

Example A – Evaluation of the angiogenic activity of test compounds in the TCS Human Angiogenesis Kit (AngioKit) assays by TCS CellWorks Ltd

5

1. Materials

a. Test Compounds

- 10 The test compound is tetra-sulphated quercetin 3- β -D-glucoside material produced in **Preparation Example 1** (Reaction code 47ppt).

The samples were provided as concentrates dissolved in water. Samples were diluted on the day of their addition to the AngioKit plates.

15

The control is the unsulphated quercetin 3- β -D-glucoside produced in the **Control Preparation Example**. This unsulphated quercetin 3- β -D-glucoside was provided as a concentrated stock dissolved in DMSO.

20 **b. AngioKit Components**

The AngioKit plate ZHA-1000 (#26967T) was used in these tests.

Plate Serial Numbers	Compounds Tested
25 C060901_7	sulphated quercetin 3- β -D-glucoside (26.9mM in distilled water) and quercetin 3- β -D-glucoside (107.6mM in DMSO)
30 Also used: CD31 Staining Kit, ZHA-1225 (Batch 26709T) Control Reagent Kit, ZHA-1300 (Batch 26843T)	

2. Methods

The AngioKit were prepared according to TCS Cellworks SOP 110.

5 Briefly, 24 well plates were seeded with cells on day 0 and medium was changed on days 3, 4, 7, 10 and 12 in accordance with the standard AngioKit procedure.

10 Test and control compounds at the appropriate dilutions were included in the medium changes on days 4, 7, 10 and 12. All test samples were diluted in medium to their final concentration on the day that they were added to the appropriate wells in duplicate.

15 Two 'untreated' control wells were included in each plate as were duplicate wells containing 0.1% DMSO, 4% distilled water, 20 μ M suramin (negative control), and 2ng/ml VEGF (positive control). All AngioKits were then fixed and stained on day 14, using the CD31 Staining Kit according to the standard AngioKit procedure.

20 a. Image Recording & Analysis of Results

Comparison of tubule development was conducted using the "AngioSys" image analysis system developed specifically for the analysis of images produced using the AngioKit. Four images taken from predetermined
25 positions within each well were recorded. Each concentration of test compound therefore yielded 4 images for analysis in duplicate.

Images were always taken from as close to the centre of each quadrant as possible. The areas from which images were taken are shown in **Figure 1**.

Four tubule parameters were measured: total tubule length, total tubule area, number of branch points and number of tubules formed.

Figure 2 shows the order in which wells were recorded in the AngioKit.

5

b. Statistical analysis

The total tubule length of capillaries formed in the AngioKit has been found to be the most reliable indicator of pro and angiogenic effects generated in the model system. As a result comparisons between the different treatments have been made in terms of total tubule length, except where specifically stated otherwise. In order to calculate results that were measurably altered all treatments were compared with the appropriate control.

15

All statistical analyses were carried out using the Stat 100 programme from BIOSOFT Ltd. using ANOVA and Duncan's Multiple Comparison Test to measure differences between the test compounds with the DMSO control values. Alpha was always 0.05 unless otherwise stipulated.

20

3. Results

Tubule length

Control levels of total capillary length in the AngioKit plate used are shown in Table 1 and are consistent with the predicted response. In the experiment conducted, there was no significant difference between untreated control, water control and DMSO control.

There was a significant decrease in total capillary length following addition of 20µM suramin and a significant increase in capillary length

30

elicited following addition of 2ng/mL VEGF when compared to the untreated control (Table 1).

These values are within the predicted response range for the AngioKit
5 model system.

	C060901 7	C060901 7	mean	SD
VEGF	14430	13909	14169	368
Control	12940	12719	12830	157
suramin	7198	6670	6934	374
water control	12118	11380	11749	1529
DMSO control	12006	11823	11914	1457

Table 1 - Control values across the experiment (total tubule length)

- 10 Individual test article results are presented in Figure 3A, which shows the inhibition results for various doses of the sulphated test compound. As can be seen, at concentrations of 0.1mM and 1.0mM, the result values are significantly lower than the control.
- 15 Results obtained for the unsulphated quercetin 3- β -D-glucoside (control) are presented in Figure 3B.

These results are summarised in Figure 4A and 4B.

- 20 Figure 4A is a dose response curve for inhibition compared to the appropriate control for the four categories of treatment for each of the tested concentrations. $y = -1601.2\ln(x) + 2279.1$; $R^2 = 0.9338$ in Figure 4A.
- 25 Figure 4B is a dose response curve for inhibition which represents the values obtained following treatment with unsulphated quercetin 3- β -D-glucoside.

As can be seen, a dose dependent inhibition of total tubule length was observed in the assays for the sulphated compound: sulphated quercetin 3- β -D-glucoside. There was a significant decrease in angiogenesis even at 0.1mM.

5

Images generated from wells treated with unsulphated quercetin 3- β -D-glucoside showed that the underlayer of feeder cells necessary to support the assay that had been considerably disrupted.

10 4. Conclusions

The AngioKits performed well and generated results for all control treatments that were within the expected range and similar to values seen in previous experiments with AngioKit assays. This allowed accurate
15 quantification of the treatment effects.

The treatments with the sulphated quercetin 3- β -D-glucoside compound inhibited the angiogenic response in a dose dependent manner.

20 The control compound, unsulphated quercetin 3- β -D-glucoside, appears to disrupt the underlayer of feeder cells.

Example B – Evaluation by ELISA of the anti-angiogenic properties of sulphated quercetin 3- β -D-glucosides using the cell based commercial kit, AngioKit from TCS CellWorks Ltd.

5 Test compounds:

- Sulphated quercetin 3 β -D-glucoside material (47ppt produced in Preparation Example 10)
- Sulphated quercetin -3-rutinoside, prepared using the same method, (48-5ppt produced in Preparation Example 10)

10

1. Methods

Dried synthesized sulphated quercetin 3 β -D-glucoside material (47ppt) and sulphated quercetin 3-rutinoside (48-5ppt) were pre-weighed in 2ml plastic tubes so that upon addition of 2ml of tissue culture medium (pre-equilibrated in a humidified TC incubator at 37°C, 8% CO₂ for 30 min), a 1mM solution would be achieved.

Solutions were sterilized by passing through a 0.2 μ m filter and used immediately in the AngioKit protocol, which was carried out in a Class II sterile laminar flow hood (HERAsafe, Kendro). Serial dilution with medium (0.2ml added to 1.8ml) was carried out to achieve a four point dose response curve containing the drug concentrations: 1, 0.1, 0.01 and 0.001mM.

25 All manipulations of the AngioKit test plate were carried out under sterile tissue culture (TC) conditions with strict adherence to aseptic technique.

On receiving the AngioKit test plates #C070727_08 and #C070727_10 (4 x 6 wells containing small islands of human endothelial cells within a co-culture matrix of other human cells) on day 1 of the experiment, silicone well seals were removed using a blunt forceps and replaced with a sterile

30

plate lid. The plate was pre-equilibrated in a humidified TC incubator at 37°C, 8% CO₂ for 30 min.

Storage medium was aspirated carefully from the wells using a P1000
5 Pipetman and replaced with 0.5ml fresh pre-equilibrated medium containing the treatment. The plate was returned to TC incubator.

Medium containing dilution of the sulphated compounds was changed on days 4, 7 and 9 and the wells assayed for tubule formation on day 11.
10

All samples were tested in duplicate and three controls were included:

- No treatment control: cells incubated only in medium. This measures the normal rate of angiogenesis in the endothelial cells and all other wells will be compared to this control.
- 15 -Positive control: Vascular endothelial growth factor (VEGF) at 2ng/ml of medium.
- Negative control: Suramin at 20µM in growth medium.

On day 11 the assay wells were tested for the presence of CD31
20 (PECAM-1) protein, a biomarker for endothelial tubule formation.

The following steps were not performed under sterile conditions.

Medium was aspirated from wells, washed with 1ml of Washing Buffer (1
25 X PBS) and fixed with 1ml of Fixative (70% ethanol straight from -20°C fridge) for 30min at room temperature. The fixative was removed and washed with 1ml Blocking Buffer (1 X PBS containing 1% BSA).

The primary antibody (mouse anti-human CD31) was diluted 1:400 in
30 Blocking Buffer and 0.5ml added per well for 1hr at 37°C.

The primary antibody solution was removed and washed 3 times for 10min each with 1ml Blocking Buffer.

The secondary antibody (goat anti-mouse IgG alkaline phosphatase conjugate) was diluted 1:500 with Blocking Buffer and added at 0.5ml per well for 1hr at 37°C.

The secondary antibody solution was removed and washed 3 times for 10min each with 1ml purified water.

10

Bound secondary antibody alkaline phosphatase activity was quantified by an ELISA method:

One soluble substrate p-nitrophenol phosphate tablet and one Tris buffer tablet were dissolved in 20ml purified water and used within one hour.

15

0.3ml ELISA substrate was added to each well and incubated at 37°C for 20min.

Two 100ul aliquots were removed from each well and each pipetted into a well of a 96 well plate containing 25ul 3M NaOH. The plate was read in a plate reader at 405nm and wells containing 25ul 3M NaOH and 100ul ELISA substrate served as reagent blanks.

20

The remaining ELISA substrate in the AngioKit wells was removed and washed 3 times for 10min each with 1ml purified water.

25

The insoluble alkaline phosphatase substrate was prepared by dissolving 2 BCIP/NBT tablets in 20ml water and filtering through a 0.2µm filter.

30

One 0.5ml of substrate was added to each well and incubated at 37°C for 5-15min until a dark purple colour developed in the positive control wells.

- 5 Development was also monitored using an inverted brightfield microscope.

Development was stopped by removal of substrate solution and washing 3 times with 1ml purified water. The final wash was removed and wells
10 allowed to dry.

For data handling, duplicate OD405 spectrophotometric readings were averaged and values presented $\pm$ standard error of the mean (SE). Each compound concentration was assayed in two AngioKit wells and two
15 OD405 values were averaged for each well in the CD31 ELISA. Results were expressed as a % of the untreated control value and subtracted from a figure of 100% to produce "% inhibition w.r.t. untreated control".

2. Results

20

Control wells in each plate produced the expected result when analysed by ELISA. Application of 2ng/ml VEGF or 20 μ M suramin produced a 150% increase and 50% decrease respectively in CD31 immunoreactivity, respectively when compared to untreated control wells.

25

Angiogenesis inhibition levels as monitored by CD31 ELISA can be seen in **Figure 5** for sulphated quercetin 3- β -D-glucoside material (47ppt) and in **Figure 6** for sulphated quercetin 3-rutinoside (48-5ppt).

Both sulphated quercetin 3- β -D-glucoside (47ppt) and sulphated quercetin 3-rutinoside (48-5ppt) produced significant tubule growth inhibition at 1mM and 0.1mM concentration and this inhibition was dose dependent.

- 5 No feeder cell disruption or cytotoxic effects were observed in the treated wells.

3. Conclusions

- 10 Both sulphated querceptin 3- β -D-glucoside (47ppt) and quercetin 3-rutinoside (48-5ppt) reactions were shown to inhibit angiogenesis by up to 60% at the two highest concentrations used (1mM and 0.1mM).

- Indeed, the sulphated quercetin 3-rutinoside demonstrated inhibition at
15 levels of 0.01mM.

Example C – Evaluation of the anti-angiogenic properties of sulphated quercetin 3- β -D-glucoside using a commercial *in vitro* angiogenesis kit, AngioKit.

5 Test compounds:

- Sulphated quercetin 3- β -D-glucoside material (47ppt produced in Preparation Example 10)
- Sulphated quercetin-3-rutinoside, prepared using the same method, (48-5ppt produced in Preparation Example 10)

10

A four point dose response curve ranging from 1mM to 1 μ M was carried out to test the efficiency of tetra-sulphated quercetin 3- β -D-glucoside and tri-sulphated quercetin-3-rutinoside.

- 15 AngioKit wells containing fixed immunostained tubules from Example B were photomicrographed and analysed using AngioSys software that automatically extracted useful parameters such as total tubule length, total tubule area, number of branch points and number of tubules formed.

20 1. Methods

Dried CD31 immunostained cells were photomicrographed using an Olympus CellR inverted microscope in brightfield mode using a 4X objective. The second highest image resolution was used and images were
25 stored as TIF files.

The centre of each well was marked and four separate centre images recorded per well.

A new AngioSys project database was created and named after the plate ID. Next individual well names were added (A1 – D6) and then the four image files recorded from each well added.

- 5 The first image from well A1 was opened and visualized. The image can be checked for even illumination by pressing "*Brightness Check*" which should produce a pseudocoloured image with the same hue of colour across the entire background of the image. Uneven hue can complicate further analysis.

10

To start on the process of creating a simplified "binary" image, the TIF image was smoothed, by clicking "Smoothing" and then "*Gauss*".

- 15 Thresholding is the data reduction operation where a new binary image is created based on the intensity levels of the underlying image. If the underlying pixel is within the specified range, the binary image will contain a pixel at that location. From the "*Image*" menu, "*Thresholding*" was selected and manual adjustment of the high and low sliders on the Threshold Image dialog box was carried out so all the darkly coloured
20 tubules were highlighted. Pressing "enter" created the binary image.

The total area under the tubules can be determined using "*Measure areas*" to give the Field Area parameter.

- 25 The binary file was skeletonised by clicking "*Skeletonise*": layers of pixels are progressively stripped off until each tubule is rendered as a line of single pixels. The single pixel lines can be detected, measured, and split to locate junction points. This allowed determination of Junction Number, Tubule Number, Total Tubule Length and Mean Tubule Length.

30

The skeletonised images were cleaned by removing any lines containing 10 pixels or less by clicking "*Clean*".

Finally the parameter values were extracted by clicking "*Measure tubules*".

All settings from the above procedure were saved in a script file by clicking "*Scripts*" and "*Configure*". This file was used as a template to automatically analyse every other image from the AngioPlate that were stored in this project database. Click "*Scripts*" and "*Process Experiment*".

To export parameter data, "*Results*" and "*Save Summary*" were selected, and data was saved as an Excel file.

For data handling, the 8 values of a single parameter recorded per treatment were averaged and expressed +/- standard error of the mean (SE). Statistical analysis comprised of an unpaired two-tailed Student's t-test comparison.

20

2. Results

Control wells in each plate produced the expected result when analysed by AngioSys for the parameters total tubule area, number of branch points and number of tubules formed and total tubule length. Application of 25 2ng/ml VEGF or 20µM suramin produced a 300 - 600% increase or 50% decrease in the various parameters, respectively, when compared to untreated control wells.

Comparison of control wells parameter values determined from the two separate plates produced similar and reproducible data as there were no significant differences between two sets of values ($p = 0.06 - 0.93$).

- 5 **Figure 7A** shows the Dose Response Curve: Field Area for Sulphated quercetin 3- β -D-glucoside (47ppt);
Figure 7B shows the Dose Response Curve: No. of Junctions for sulphated quercetin 3- β -D-glucoside (47ppt);
Figure 7C shows the Dose Response Curve: No. of Tubules for sulphated
10 quercetin 3- β -D-glucoside (47ppt); and
Figure 7D shows the Dose Response Curve: Total Tubule for sulphated quercetin 3- β -D-glucoside (47ppt).

- Figure 8A** shows the Dose Response Curve: Field Area for sulphated
15 quercetin 3-rutinoside (48-5ppt);
Figure 8B shows the Dose Response Curve: No. of Junctions for sulphated quercetin 3-rutinoside (48-5ppt);
Figure 8C shows the Dose Response Curve: No. of Tubules for sulphated quercetin 3-rutinoside (48-5ppt); and
20 **Figure 8D** shows the Dose Response Curve: Total Tubule for sulphated quercetin 3-rutinoside (48-5ppt).

- Cells treated with sulphated quercetin 3- β -D-glucoside (47ppt) or sulphated quercetin 3-rutinoside (48-5ppt) displayed significantly reduced
25 tubule growth parameter values at 1mM; this inhibition was dose dependent although significant inhibition did not occur at concentrations lower than 1mM.

- No feeder cell disruption was seen in the sulphated querceptin 3- β -D-glucoside (47ppt) or sulphated quercetin-3-rutinoside (48-5ppt) treated
30 cells.

3. Conclusions

The AngioSys software analysis successfully extracted useful parameters
5 of tubule growth from photomicrographs of fixed CD31 immunostained
cells in the AngioKit.

Angiogenesis inhibition was observed for sulphated quercetin 3- β -D-
glucoside (47ppt) and sulphated quercetin-3-rutinoside (48-5ppt).

Example D - Visualisation of the Anti-Angiogenic Properties of sulphated quercetin 3- β -D-glucoside using imaging of CD31 immunostained assay plates.

5

Following quantification of angiogenesis in the AngioKit wells by an ELISA using an antibody against CD31 (PECAM1), a biomarker for tubule formation, an insoluble alkaline phosphate substrate, 5-Bromo-4-chloro-3-indolyl Phosphate (BCIP)/Nitroblue Tetrazolium (NBT) was used
10 to visualize immunoreactivity.

Test compounds:

- Positive control : VEGF 2ng/ml
- Negative control: suramin 20 μ M
- 15 - 1 mM quercetin 3- β -D-glucoside (unsulphated) - control compound
- Sulphated quercetin 3- β -D-glucoside reactions from Preparation Example 10 (Table 2):
 - 1mM S8 reaction
 - 1mM 47ppt reaction

20

1. Methods

Plate handling and immunohistochemistry were as described for Examples
25 B and C.

Following BCIP/NBT stain development for 15 – 20 min at 37°C, wells were washed 3 times with purified water and allowed to dry.

30 The plate was stored upside down in tinfoil at room temperature for several weeks prior to image capture.

Photomicrograph images were captured using an Olympus CellR inverted microscope in brightfield mode using a black and white (monochrome) camera.

5

Images were initially saved in an Olympus file format and then converted to a TIF file format.

2. Results

10

The purple insoluble BCIP/NBT stain took between 15-20 min to develop and the reaction was stopped when deep purple tubules could be seen in the positive control. Background staining was very low showing the support matrix cells do not contain any CD31 antigen.

15

Figure 9 shows representative photomicrograph images of CD31 immunostained AngioKit endothelial cells for various compounds tested.

20

Visual inspection of the images in the untreated controls, which measure the normal rate of endothelial cell angiogenesis, showed threadlike structures with few junctions.

25

The positive control (VEGF 2ng/ml) demonstrates a robust increase in tubule growth with considerable junction formation that is referred to as a network of anastomosing tubules.

The negative control (suramin 20 μ M) had less tubule formation compared to the untreated controls and no junction formation.

30

The underlying cell matrix or feeder cells in wells treated with 1mM unsulphated quercetin 3- β -D-glucoside have changed morphology from the

normal adherent cell shape to a rounded shape. This reflects cell distress and cell death. This effect may be due to uncharged quercetin 3- β -D-glucoside passing through the cell membrane and disrupting normal cell function.

5

This effect did not occur in wells treated with the sulphated forms of quercetin 3- β -D-glucoside.

The wells treated with S8 had very little tubule growth and 47ppt were
10 virtually devoid of tubules. These observations mirror the quantitative ELISA data.

3. Conclusions

15 Examination of photomicrographs of TCS CellWorks AngioKit showed the expected network of anastomosing tubules in positive control wells and negative control within the expected ranges.

Sulphated quercetin 3- β -D-glucosides demonstrate anti-angiogenic
20 properties in this assay. The lowest level of angiogenesis was shown in wells treated with 47ppt (tetra-sulphated form), in agreement with our quantitative ELISA data.

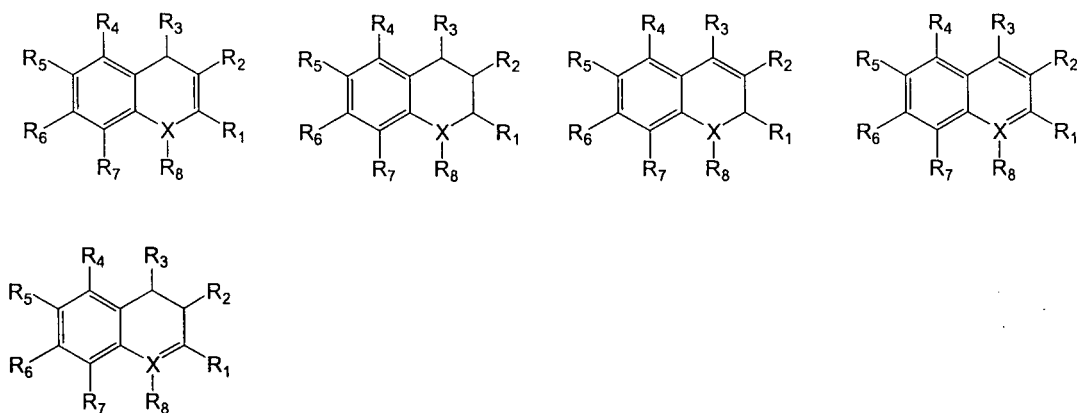
The unsulphated form of the compounds appears to disrupt the underlayer
25 of feeder cells.

CLAIMS

1. A compound having:
 - a bicyclic ring structure that comprises two fused six-membered
 - 5 carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the bicyclic ring structure may optionally be replaced with a nitrogen, sulfur or oxygen, the bicyclic ring structure being substituted with an optionally substituted pendent aromatic ring,
 - wherein the compound includes one or more acidic substituent
 - 10 group selected from sulfate, sulfonate, phosphate and carboxylate;
 - and pharmaceutically acceptable salts, solvates and pro-drugs thereof.
2. The compound of Claim 1, wherein there are two or more acidic
- 15 substituent groups selected from sulfate, sulfonate, phosphate and carboxylate.
3. The compound of Claim 2, wherein there are four or more acidic substituent groups selected from sulfate, sulfonate, phosphate and
- 20 carboxylate.
4. The compound of any one of Claims 1 to 3, wherein the compound includes one or more aliphatic ring group.
- 25 5. The compound of Claim 4, wherein the aliphatic ring group is a five or six membered ring group.
6. The compound of Claim 4 or Claim 5, wherein the aliphatic ring group is a heterocyclic ring with one oxygen, nitrogen or sulfur in the
- 30 ring and the remaining ring members being carbon.

7. The compound of Claim 6, wherein the aliphatic ring group is a monosaccharide, disaccharide or trisaccharide.
8. The compound of Claim 7, wherein the saccharide group has one or more of its OH groups substituted with substituent group(s), which may be the same or different, selected from carbonyl, sulfate, sulfonate, phosphate, aliphatic ring groups, halide, alkyl, alkyl ether, ROH where R is alkyl, carboxylate and nitrile.
9. The compound of any one of Claims 1 to 8 wherein the pendent aromatic group is a carbocyclic aromatic group.
10. The compound of any one of Claims 1 to 9, wherein the compound has one of the following general formulae:

15



wherein X is O, S, N or C,

one of the groups R1-R8 is an optionally substituted pendent aromatic group,

and the remaining groups R1-R8, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups, halide, alkyl, alkyl ether, carboxylate and nitrile,

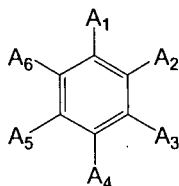
with the proviso that:

when X is O, S or N, R8 need not present, and

one or more of the R1-R8 groups are acidic groups selected from sulfate, sulfonate, phosphate and carboxylate, or comprise acidic groups selected

5 from sulfate, sulfonate, phosphate and carboxylate.

11. The compound of any one of Claims 1 to 10, wherein the pendent aromatic group is of the following general formula:



10

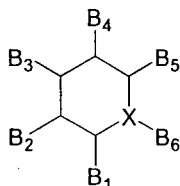
wherein A1 attaches the pendent aromatic group to the bicyclic ring structure, and may be a bond or may be an oxygen, sulphur, nitrogen or carbon group,

15 and A2-A6, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, aliphatic ring groups, halide, alkyl, alkyl ether, carboxylate and nitrile.

12. The compound of Claim 11 wherein the pendent aromatic group is
20 substituted or unsubstituted benzene.

13. The compound of Claim 12 wherein the pendent aromatic group is substituted benzene with substituent group(s), which may be the same or different, selected from hydroxyl, alkyl ether, sulfate, sulfonate,
25 phosphate, and carboxylate.

14. The compound of any one of Claims 1 to 13 wherein the compound includes one or more aliphatic ring group of the following general formula:



5

wherein B1 is an anomeric oxygen group which attaches the aliphatic ring group to the bicyclic ring structure,

X is selected from O, S, N and C,

10 B2-B6, which may be the same or different, are each selected from hydrogen, carbonyl, hydroxyl, sulfate, sulfonate, phosphate, saccharide, halide, alkyl, alkyl ether, ROH where R is alkyl, carboxylate and nitrile, with the proviso that when X is O or S, B6 is not present.

15 15. A compound according to Claim 14, wherein X is O and B2-B5, which may be the same or different, are each selected from hydroxyl, saccharide, ROH where R is alkyl, sulfate, sulfonate, phosphate and carboxylate.

20 16. A method of manufacturing a compound in accordance with any one of Claims 1 to 15, the method comprising:

(a) providing a compound, which has a bicyclic ring structure comprising two fused six-membered carbon rings, wherein one of the rings is aromatic, and wherein one carbon in the ring structure may
25 optionally be replaced with a nitrogen, sulfur or oxygen, the ring structure being substituted with an optionally substituted pendent aromatic ring; and

(b) incorporating one or more acidic substituent groups selected from sulfate, sulfonate, phosphate and carboxylate, using sulfation, sulfonation, phosphorylation or carboxylation techniques.

5 17. The method of Claim 16 wherein the starting material has one or more hydroxyl group and in step (b) sulfation is controlled so as to sulfate all the hydroxyl groups.

10 18. The method of Claim 16 or Claim 17 wherein the compound provided in step (a) is selected from catechin, (-)-epicatechin, apigenin, hesperidin, naringin, quercetin, quercetin-3-D-galactoside, quercetin-3-beta-D-glucoside, quercetin 3-rutinoside and baicalin.

15 19. A compound in accordance with any one of Claims 1 to 15, or a pharmaceutically acceptable salt, solute or pro-drug thereof, for use in medicine.

20 20. Use of a compound in accordance with any one of Claims 1 to 15, or a pharmaceutically acceptable salt, solute or pro-drug thereof, in the manufacture of a medicament for the treatment of inappropriate angiogenesis.

25 21. The use of Claim 20, wherein the use is in the treatment of cancer, diabetic retinopathy, arthritis, psoriasis, atherosclerosis, macular degeneration, or hemangioma.

30 22. A pharmaceutical composition comprising a therapeutically effective amount of a compound in accordance with any one of Claims 1 to 15, or a pharmaceutically acceptable salt, solute or pro-drug thereof, together with a pharmaceutically acceptable carrier, excipient or diluent.

23. The pharmaceutical composition of Claim 22, wherein the composition is in unit dose form, containing a predetermined amount of active ingredient per unit dose.
- 5 24. The pharmaceutical composition of Claim 23, wherein the unit dose contain 0.5mg to 1g of the compound in accordance with any one of Claims 1 to 15 or of a pharmaceutically acceptable salt, solute or pro-drug thereof.
- 10 25. The pharmaceutical composition of any one of Claims 22 to 24, wherein the composition is in the form of a capsule, tablet, lozenge, pastille, powder, granules, solution or suspension in aqueous or non-aqueous liquids, emulsion, ointment, cream, lotion, paste, gel, spray, aerosol, oil, suppository, or enema.

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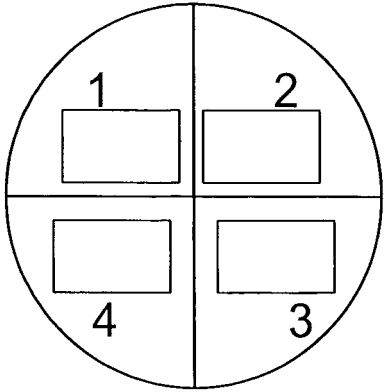


Fig. 1

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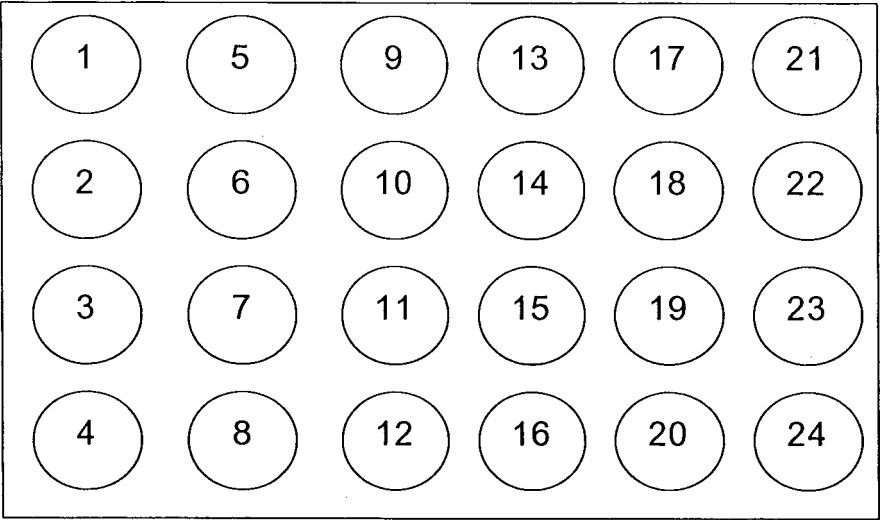


Fig. 2

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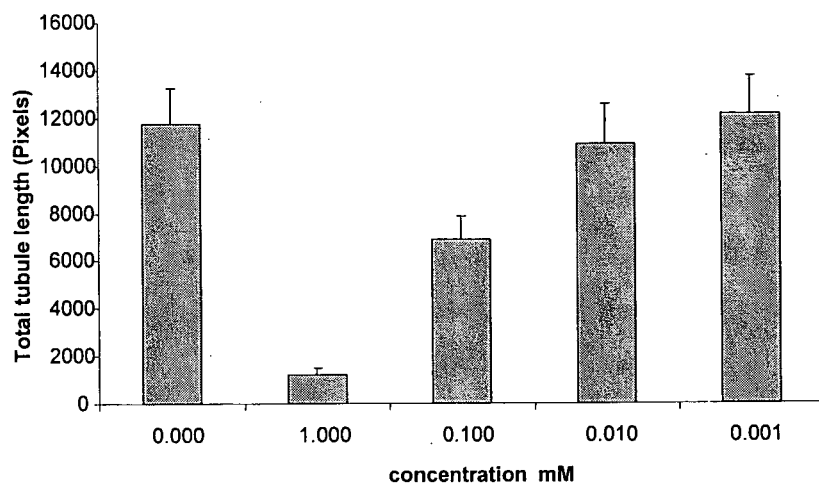


Fig. 3A

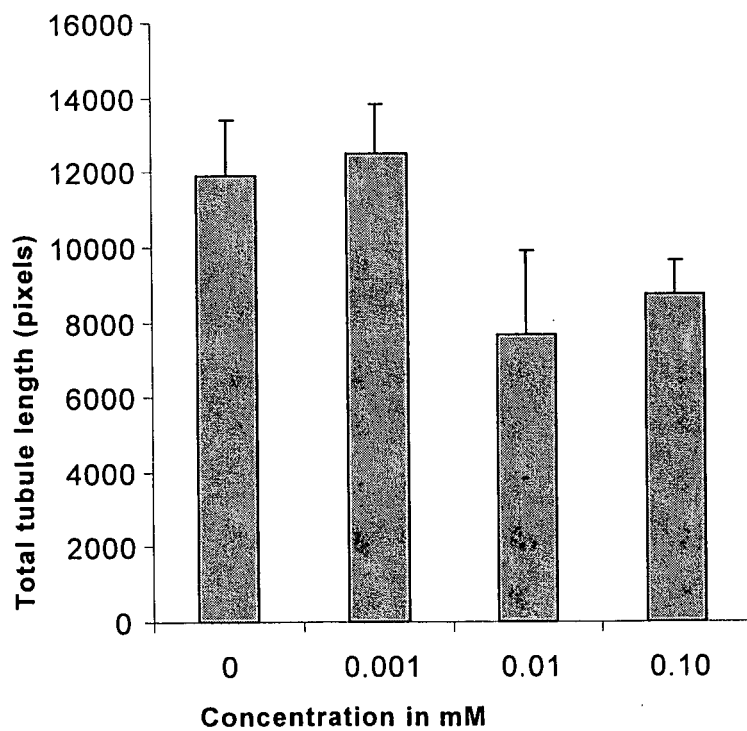


Fig.3B

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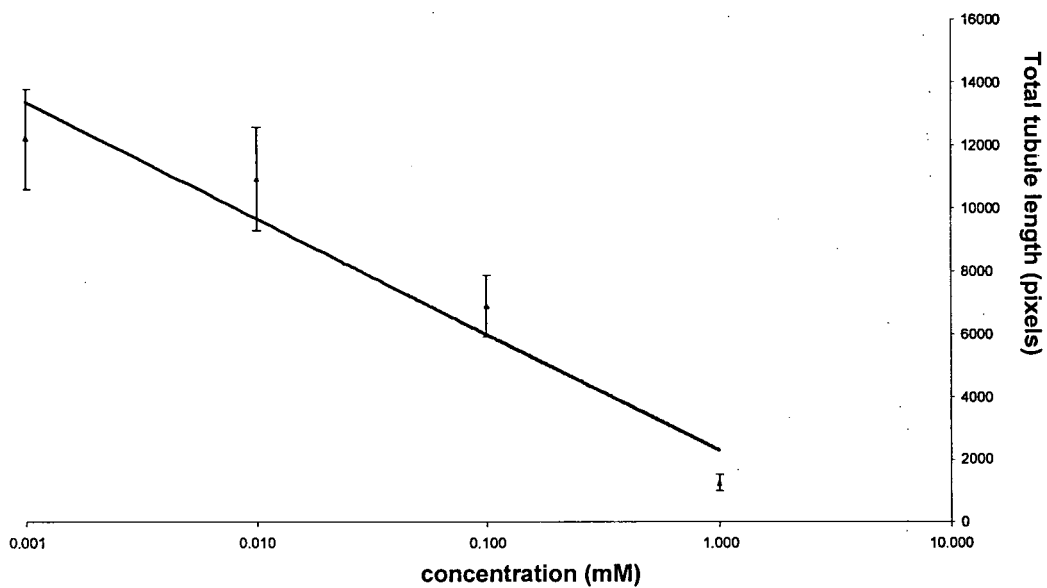


Fig. 4A

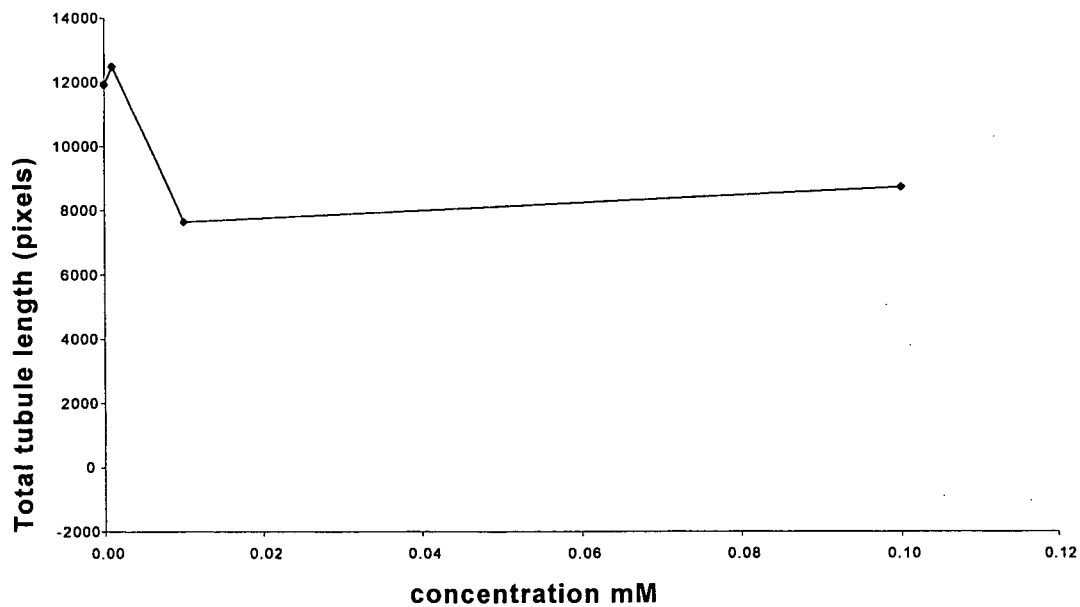


Fig.4B

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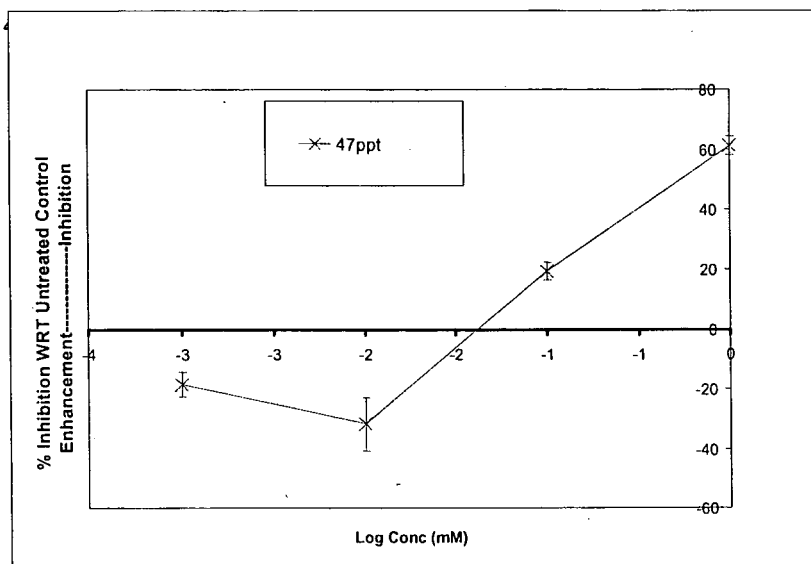


Fig. 5

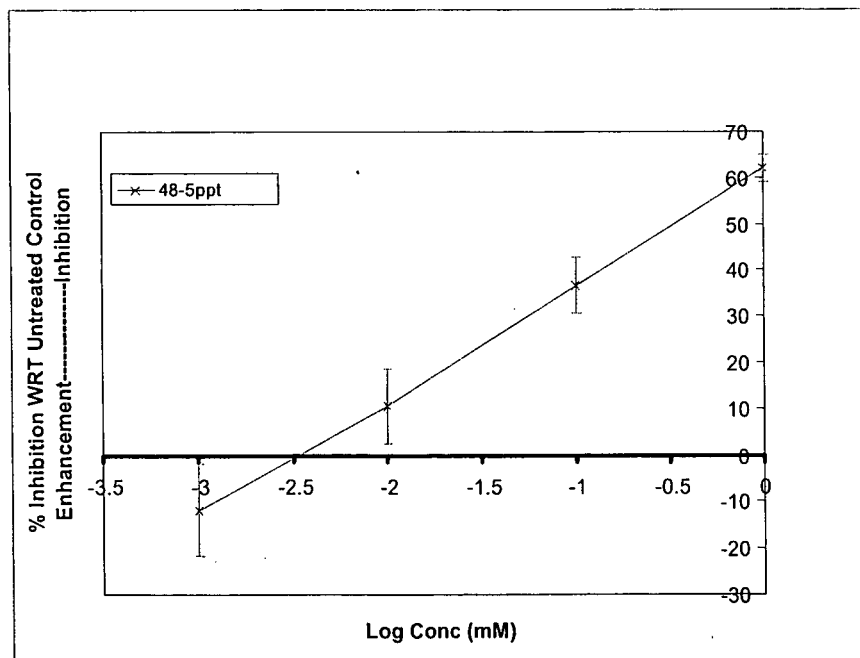


Fig. 6

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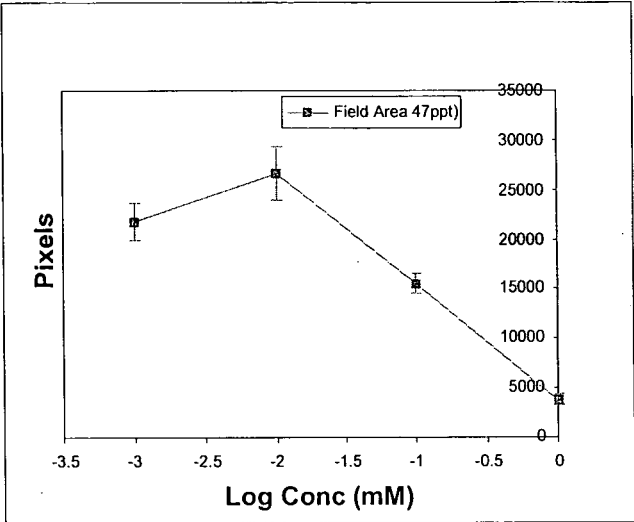


Fig 7A

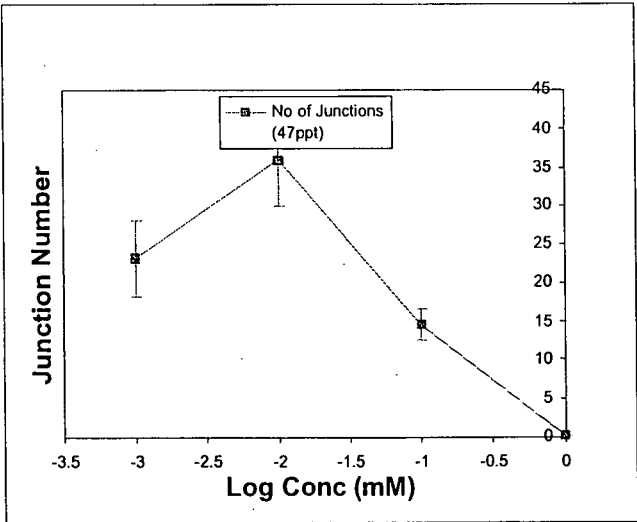


Fig 7B

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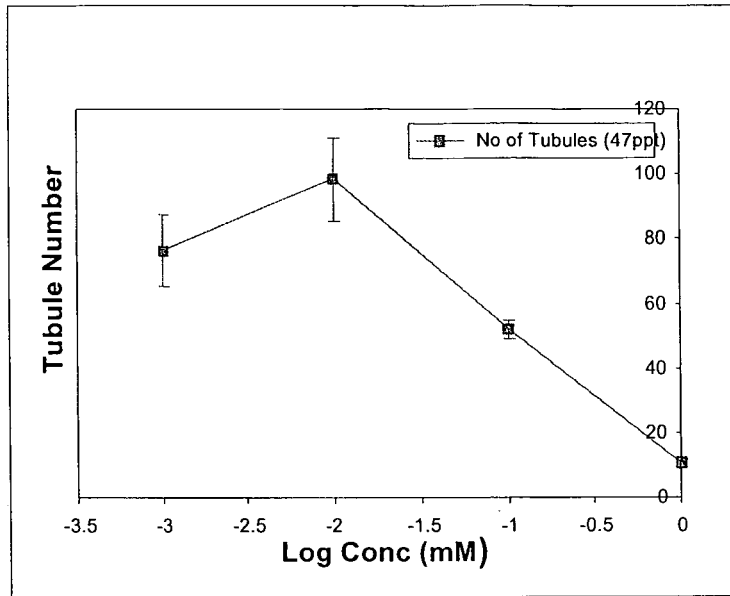


Fig 7C

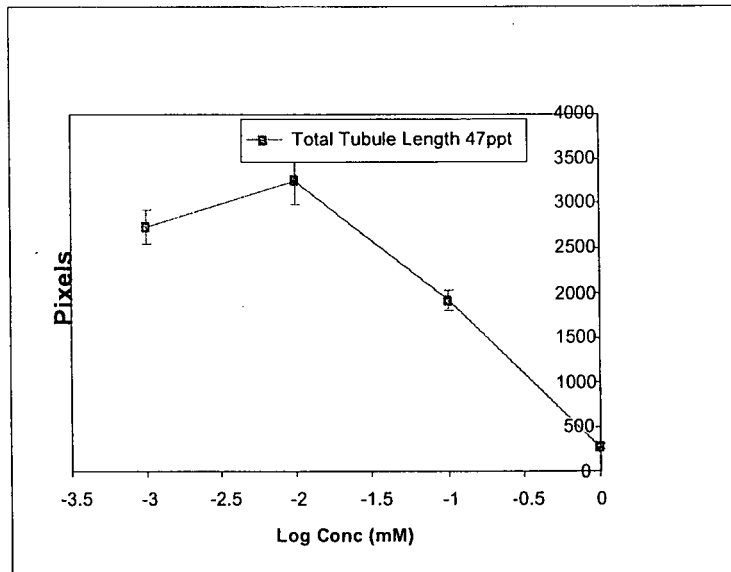


Fig 7D

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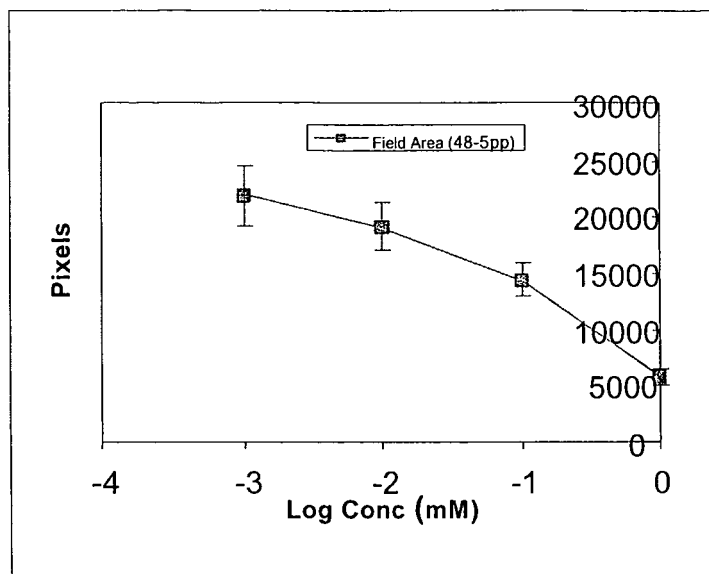


Fig 8A

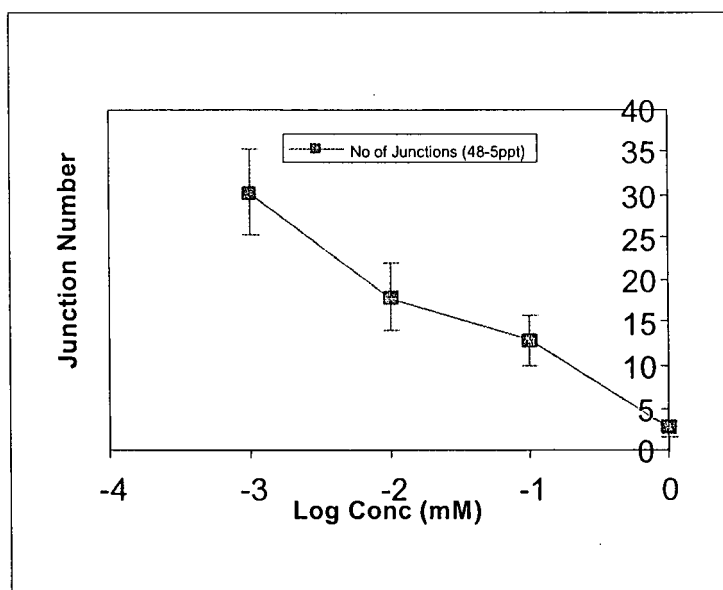


Fig 8B

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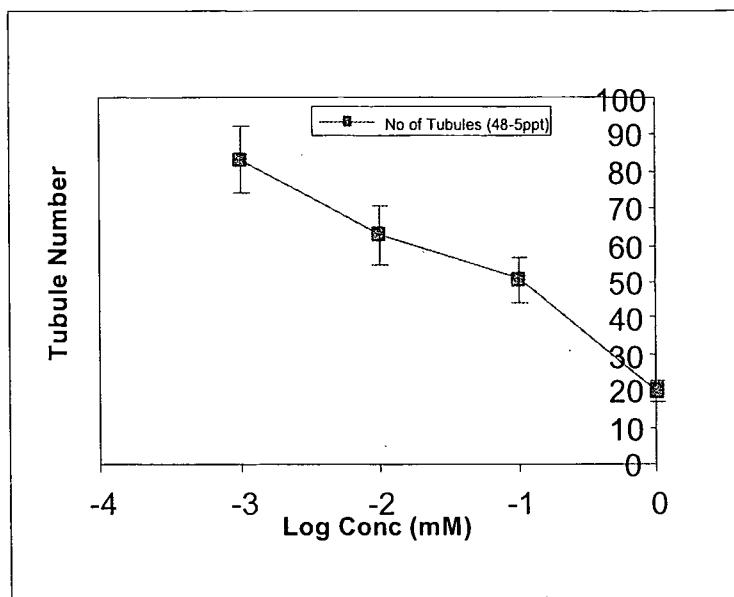


Fig 8C

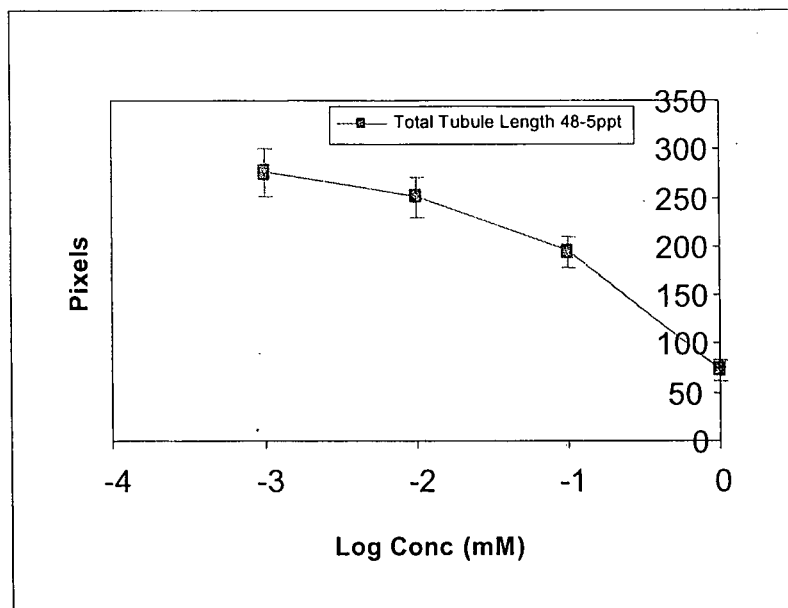


Fig 8D

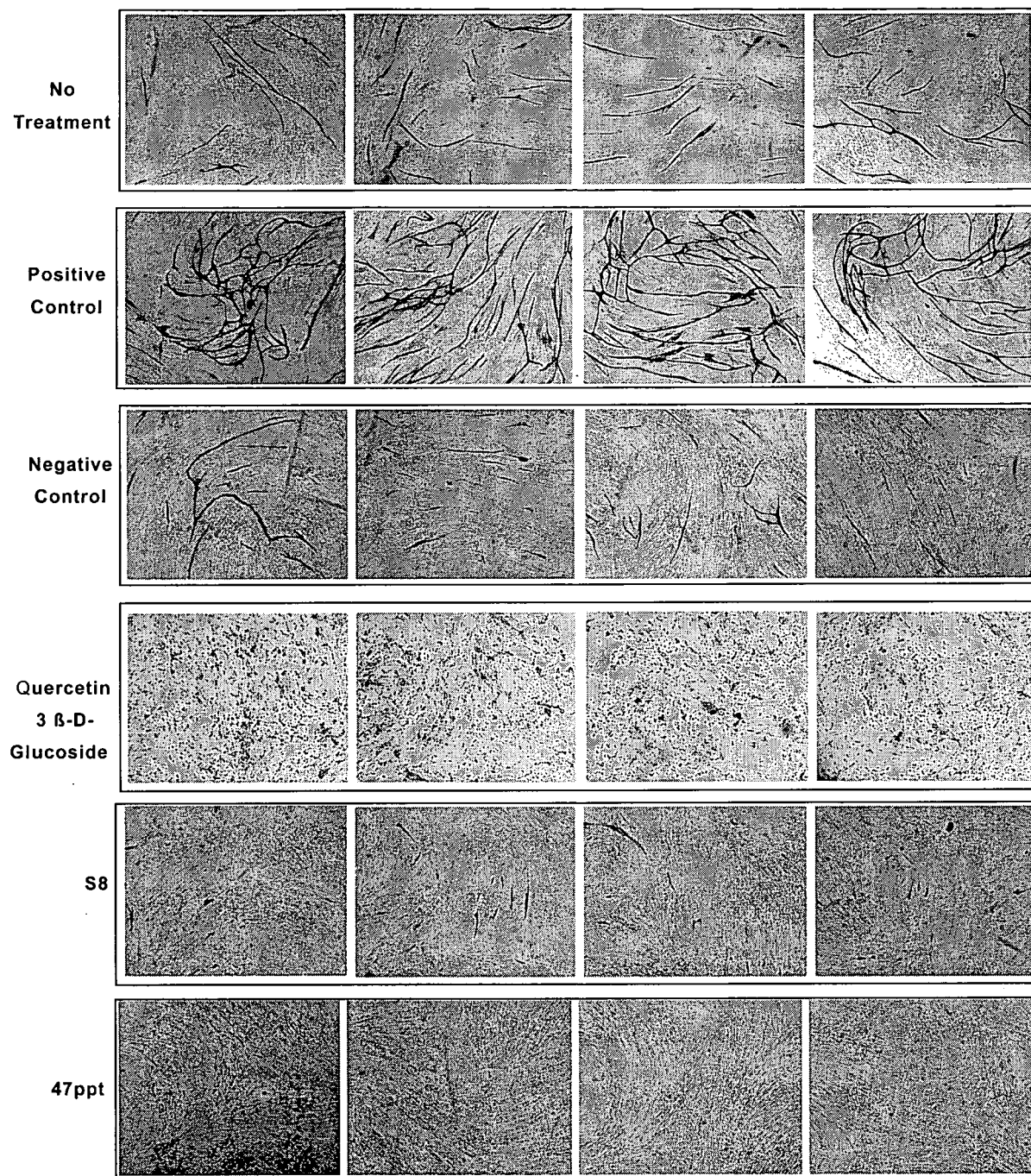


Fig. 9