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(54) Title: CARBAZOLE DERIVATIVES AND THEIR USES AS HEPARANASE INHIBITORS

(57) Abstract: Carbazole derivatives having at the 9-position a 3-(substituted)amino-2-hydroxypropyl group and fluorene derivatives having at the 9-position a =N-NH-R4 group, wherein R4 is a (substituted) carboxamido, (substituted) thiocarboxamido or (substituted) hydrazido group, are provided as heparanase inhibitors suitable for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

CARBAZOLE DERIVATIVES AND THEIR USES AS HEPARANASE INHIBITORS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to heparanase inhibitors, particularly to certain carbazole and fluorene derivatives, and to their use in the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as cancer, inflammatory disorders and autoimmune diseases.

Heparan sulfate proteoglycans (HSPGs) are ubiquitous macromolecules associated with the cell surface and with the extracellular matrix (ECM) of various tissues. They consist of a protein core to which several linear heparan sulfate (HS) chains are covalently attached. Studies on the involvement of ECM molecules in cell attachment, growth and differentiation revealed a central role of HSPGs in embryonic morphogenesis, angiogenesis, neurite outgrowth, tissue repair, and metastasis. HSPGs are also prominent components of blood vessels. In capillaries they are found mainly in the subendothelial basement membrane, where they support proliferating and migrating endothelial cells and stabilize the structure of the capillary wall.

Several cellular enzymes such as collagenase IV, plasminogen activator, cathepsin B, and elastase are thought to be involved in the degradation of basement membrane. Another enzyme of this type is heparanase, an endo-β-D-glucuronidase that cleaves HS at specific intrachain sites (Nakajima et al., 1984). Heparanase released from cells removes HS molecules from the basement membrane resulting in increase of basement membrane permeability. Heparanase also facilitates proteolytic degradation of the core structural components such as type IV collagen in collaboration with gelatinases. Thus, blood-borne cells accomplish penetration through the basement membrane. In fact, HS catabolism is observed in wound repair, inflammation, and in diabetes.

Expression of heparanase was found to correlate with the metastatic potential of mouse lymphoma (Vlodavsky et al., 1983), fibrosarcoma and melanoma cells (Nakajima et al., 1988). Similar correlation was observed in human breast, colon, bladder, prostate, and liver carcinomas (Vlodavsky et al., 1999). Moreover, elevated levels of heparanase

were detected in sera of metastatic tumor bearing animals (Nakajima et al., 1988) and of cancer patients, in urine of highly metastatic patients (Vlodavsky et al., 1997), and in tumor biopsies (Vlodavsky et al., 1988). Treatment of experimental animals with heparanase substrates or inhibitors (e.g., non-anticoagulant species of low molecular weight heparin and polysulfated saccharides) considerably reduced the incidence of lung metastases induced by B16-F10 melanoma, pancreatic adenocarcinoma, Lewis lung carcinoma, and mammary adenocarcinoma cells (Vlodavsky et al., 1994; Nakajima et al., 1988; Parish et al., 1987; Lapierre et al., 1996), indicating that heparanase inhibitors may inhibit tumor cell invasion and metastasis.

Heparanase is involved also in primary tumor angiogenesis. Most primary solid tumors (1-2 mm diameter) obtain their oxygen and nutrient supply through a passive diffusion from pre-existing blood vessels, however the increase in their mass beyond this size requires angiogenesis. Heparin-binding polypeptides such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are highly mitogenic for vascular endothelial cells, and are among the most potent inducers of angiogenesis. bFGF has been extracted from the subendothelial ECM produced in vitro, and from basement membranes of cornea, suggesting that ECM may serve as a reservoir for bFGF. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues and blood vessels. bFGF binds to HSPG in the ECM and can be released in an active form by HS-degrading enzymes. Heparanase expressed by platelets, mast cells, neutrophils, and lymphoma cells was found to be involved in the release of active bFGF from ECM and basement membranes, suggesting that heparanase activity may not only function in cell migration and invasion, but may also elicit an indirect neovascular response (Elkin et al., 2001).

Heparanase catalytic activity correlates with the ability of activated cells of the immune system to leave the circulation and elicit both inflammatory and autoimmune responses. Interaction of platelets, granulocytes, T and B lymphocytes, macrophages, and mast cells with the subendothelial ECM is associated with degradation of HS by heparanase (Vlodavsky et al., 1992). The enzyme is released from intracellular compartments (e.g., lysosomes, specific granules) in response to various activation signals (e.g., thrombin, calcium ionophore, immune complexes, antigens, mitogens), suggesting its regulated involvement in inflammatory sites and in autoimmune diseases.

Indeed, treatment of experimental animals with heparanase substrates (e.g., non-anticoagulant species of low molecular weight heparin) markedly reduced the incidence of experimental autoimmune encephalomyelitis (EAE), adjuvant arthritis and graft rejection, indicating that heparanase inhibitors may inhibit autoimmune and inflammatory diseases (Lider et al., 1989).

Heparanase inhibitors have been proposed for treatment of human metastasis, for example, derivatives of siastatin B (Nishimura et al., 1994; Kawase et al., 1995), a pyran derivative isolated from the fungal strain *Acremonium* sp. MT70646 (PCT/KR00/01493), suramin, a polysulfonated naphthylurea (Nakajima et al., 1991), sulfated oligosaccharides, e.g., sulfated maltotetraose and maltohexaose (Parish et al., 1999), and sulfated polysaccharides (Parish et al., 1987; Lapierre et al., 1996).

U.S. Patent No. 5,968,822 discloses a polynucleotide encoding a polypeptide having heparanase catalytic activity and host cells, particularly insect cells, expressing said polypeptide. The recombinant polypeptide having heparanase activity is said to be useful for potential treatment of several diseases and disorders such as wound healing, angiogenesis, restenosis, inflammation and neurodegenerative diseases as well as for development of new drugs that inhibit tumor cell metastasis, inflammation and autoimmunity. International Patent Publication No. WO 99/57244 of the present applicants discloses bacterial, yeast and animal cells and methods for over expressing recombinant heparanase in cellular systems. U.S. Patent No. 6,190,875, assigned to the present applicants, discloses methods of screening agents inhibiting heparanase catalytic activity and hence potentially inhibiting tumor metastasis, autoimmune and inflammatory diseases which comprises interacting a native or recombinant heparanase enzyme with a heparin substrate in the presence or absence of an agent and determining the inhibitory effect of said agent on the catalytic activity of said heparanase enzyme towards said heparin substrate. Both U.S. 5,968,822 and U.S. 6,190,875 and further WO 99/57244 are herein incorporated by reference in their entirety as if fully disclosed herein.

European Patent Application No. EP 1094063 discloses certain piperazine derivatives of carbazoles, particularly 9-(piperazinylalkyl)carbazoles, for use in the treatment of disorders associated with the modulation of the Bax function and/or the Bax activation.

None of the above-mentioned publications discloses or suggests the heparanase inhibitors of the present invention.

SUMMARY OF THE INVENTION

The present invention provides, in one aspect, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one heparanase inhibitor selected from a carbazole or fluorene derivative of the general Formula I hereinafter or a pharmaceutically acceptable salt thereof.

The pharmaceutical composition of the invention is particularly useful for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as, but not being limited to, cancer, inflammatory disorders and autoimmune diseases.

In another aspect, the present invention relates to the use of a carbazole or fluorene derivative of the general Formula I for the manufacture of a pharmaceutical composition. In one embodiment, said compositions are for treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as, but not being limited to, cancer, inflammatory disorders and autoimmune diseases.

In a further aspect, the present invention provides certain novel carbazole derivatives of the general Formula I.

In still another aspect, the present invention relates to a method for treatment of a patient suffering from a disease or disorder caused by or associated with heparanase catalytic activity such as cancer, an inflammatory disorder or an autoimmune disease, which comprises administering to said patient an effective amount of a carbazole or fluorene derivative of the general Formula I.

BRIEF DESCRIPTION OF THE FIGURES

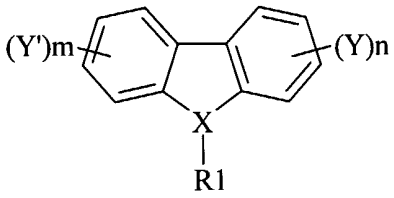
Fig. 1 is a graph showing the total number of cells extracted from three polyvinyl alcohol (PVA) sponges implanted in BALB/c mice untreated (control) or treated with **Compound 3** (at doses of 1 mg/ml/mouse and 0.2 mg/ml/mouse).

Fig. 2 is a graph showing the myeloperoxidase (MPO) activity in a suspension of cells squeezed from the PVA sponges implanted in BALB/c mice untreated (control) or treated with **Compound 3** (at doses of 1 mg/ml/mouse and 0.2 mg/ml/mouse).

Fig. 3 is a graph showing changes in TNF-α concentration in the supernatant extracted from the PVA sponges implanted in BALB/c mice untreated (Control 2) or treated with **Compound 3** (at doses of 1 mg/ml/mouse and 0.2 mg/ml/mouse). Control 1 – TNF-α level in a normal mouse (not implanted with the PVA sponges, without inflammation).

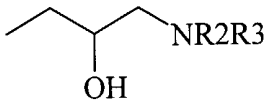
DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, pharmaceutical compositions are provided, particularly for treatment of diseases and disorders caused by or associated with heparanase catalytic activity, said compositions comprising a pharmaceutically acceptable carrier and at least one heparanase inhibitor which is a carbazole or fluorene compound of the general Formula I:



wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the formula:



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

m and n, the same or different, are integers from 0 to 4;

and pharmaceutically acceptable salts thereof.

As used herein the term "C1-C6 alkyl" typically refers to a straight or branched alkyl radical having 1-6 carbon atoms and includes for example methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-heptyl, 2,2-dimethylpropyl, n-hexyl and the like. The term "C2-C6 alkenyl" refers to straight or branched hydrocarbon radicals having 2-6 carbon atoms and one double bond, preferably a terminal double bond, and includes for example vinyl, prop-2-en-1-yl, but-3-en-1-yl, pent-4-en-1-yl, and hex-5-en-1-yl.

The term "C1-C6 alkoxy" refers to the group C1-C6 alkyl-O-, wherein C1-C6 alkyl is as defined above. Examples of alkoxy are methoxy, ethoxy, hexoxy and the like. The term "C1-C6 alkylthio" refers to the group C1-C6 alkyl-S-, wherein C1-C6 alkyl is as defined above. Examples of alkylthio are methylthio, ethylthio, butylthio and the like.

The term "C6-C14 aryl" refers to an aromatic carbocyclic group having 6 to 14 carbon atoms consisting of a single ring or multiple condensed rings such as phenyl, naphthyl, and phenanthryl optionally substituted by C1-C6 alkyl. The term "heteroaryl" in radicals R, R', R2, and R3 refers to a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S. Particular examples are pyridyl, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, quinolinyl, thiazolyl, pyrazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, isobenzofuryl, indolyl, imidazo[1,2-a]pyridyl, benzimidazolyl, benzthiazolyl and benzoxazolyl. The term "heteroaryl" in radical R5 refers to a radical derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S, such as, but not limited to, quinazolinyl, quinolinyl, benzofuryl, isobenzofuryl, indolyl, imidazo[1,2-a]pyridyl, benzimidazolyl, benzthiazolyl and benzoxazolyl, that may be substituted as defined above. In one preferred embodiment, R is quinazolinyl substituted by –NRR', wherein R is H and R' is dimethoxyphenyl.

The term "halogen" refers to fluoro, chloro, bromo or iodo.

The group –NR2R3 may be $-NH_2$, when R2 and R3 are both hydrogen, or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl or heteroaryl and R3 is –CONRR', -CSNRR', or –CONHNRR', as defined above, or R2 and R3 together with the nitrogen atom to which they are attached form a saturated 5-7 membered heterocyclic ring, preferably a 6-membered ring, optionally containing at least one further heteroatom selected from nitrogen, oxygen and/or sulfur. Such rings may be substituted, for example

with one or two C1-C6 alkyl groups, preferably at the N atom. Examples of such rings include, without being limited to, pyrrolidino, piperidino, morpholino, thiomorpholino, piperazino, N-C1-C6 alkylpiperazino, e.g. N-methylpiperazino and the like.

Also contemplated by the present invention are pharmaceutically acceptable salts of the compounds of formula I, both salts formed by any carboxy or sulfo groups present in the molecule and a base as well as acid addition and/or base salts.

Pharmaceutically acceptable salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge S. M., et al., "Pharmaceutical Salts," (1977) J. of Pharmaceutical Science, 66:1-19). The salts can also be pharmaceutically acceptable quaternary salts such as a quaternary salt of the formula – NRR'R" + Z' wherein R, R' and R" each is independently hydrogen, alkyl or benzyl and Z is a counterion, including chloride, bromide, iodide, O-alkyl, toluenesulfonate, methylsulfonate, sulfonate, phosphate, or carboxylate.

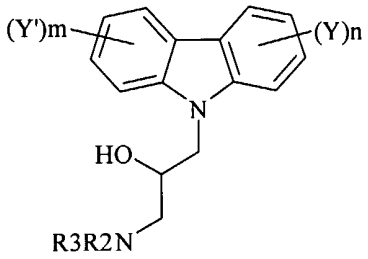
Pharmaceutically acceptable acid addition salts of the compounds include salts derived from inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorous, and the like, as well as salts derived from organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are salts of amino acids such as arginate and the like and gluconate or galacturonate (see, for example, Berge S. M., et al., "Pharmaceutical Salts," (1977) J. of Pharmaceutical Science, 66:1-19).

The acid addition salts of said basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the

conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

In a preferred embodiment of the present invention, the pharmaceutical composition comprises a compound of the general Formula I wherein X is N and R1 is a 3-amino-2-hydroxy-propyl radical, as exemplified by a compound of the formula Ia:



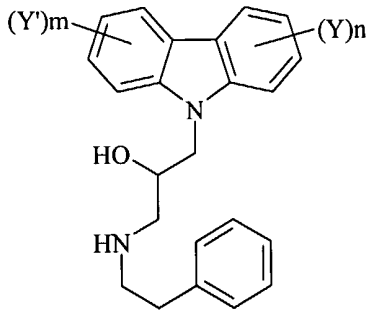
(Ia)

wherein Y, Y', R2, R3, m and n are as defined above.

In one preferred embodiment, in the compound of formula Ia, R2 is H and R3 is selected from C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic

heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as described above.

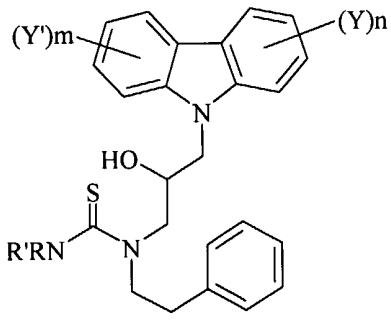
In the formula Ia, R3 is preferably C1-C6 alkyl, most preferably ethyl, substituted by C6-C14 aryl, preferably phenyl, as identified in the formula Ib below:



(Ib)

In the compound of formula Ib, wherein Y and Y' are both Br or Cl, and m and n are both 1, there are obtained the compounds herein identified as **Compounds 1** and **2,** respectively, in the Appendix A just before the Claims. **Compounds 1** and **2** have been described in the literature (CAS Nos. 301160-13-4 and 301160-12-3, respectively), but no biological activity has been disclosed for them.

In another preferred embodiment, in the compound of formula Ia, R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', -SO_3H, or -SO_2NRR', and R3 is -CONRR', –CSNRR', or -CONHNRR'. Preferably, R2 is ethyl substituted by C6-C14 aryl, preferably phenyl, and R3 is –CSNRR', as depicted in formula Ic:

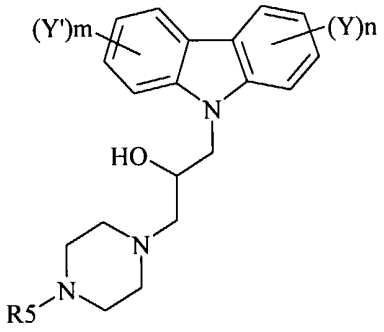


(Ic)

wherein R, R', Y, Y', m and n are as described hereinabove.

In a preferred embodiment, in the compound of formula Ic, R is H and R' is phenyl substituted at the para position by $-SO_3H$, Y and Y' are Br and m and n are 1, as exemplified by the novel compound herein identified as **Compound 3** in the Appendix A.

In yet another embodiment of the present invention, the pharmaceutical composition comprises a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated 6-membered heterocyclic ring containing at least one further heteroatom selected from N, O, and/or S, preferably piperazino substituted at the further N atom by R5, as depicted in formula Id:



(Id)

wherein R5, Y, Y', m and n are as described hereinabove.

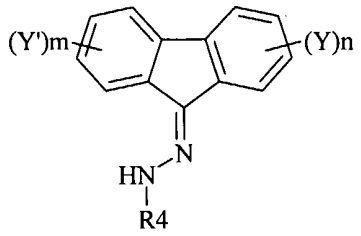
In one preferred embodiment of the present invention, the pharmaceutical composition comprises a compound of the formula Id, wherein R5 is heteroaryl derived from a bicyclic ring containing two N atoms, preferably quinazolinyl substituted by -NRR', wherein R is hydrogen and R' is C6-C14 aryl substituted by at least one radical selected from halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, or C1-C6 alkylthio.

In the compound of formula Id, when R5 is quinazolin-2-yl substituted at the 4-position by –NRR', R is H and R' is 3,4-dimethoxyphenyl, there is obtained the novel compound herein identified as **Compound 4** in the Appendix A.

In yet another embodiment of the present invention, the pharmaceutical composition comprises a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio, preferably a 3-carbazolyl-2-hydroxy-

propyl group as exemplified by the compound herein identified as **Compound 5** in Appendix A. This compound is described in the literature [CAS No. 300392-69-2] but no biological activity has been disclosed for the compound.

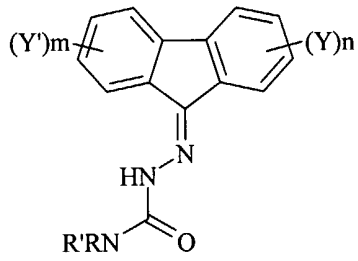
In a further preferred embodiment of the present invention, the pharmaceutical composition comprises a fluorine compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as described hereinabove.

In one embodiment, in the compound of formula Ie, R4 is –CONRR', as depicted in formula If:



(If)

In the formula If, when Y and Y' are -SO_2NRR', R is H and R' is C1-C6 alkyl substituted by –OH, preferably 2-hydroxyethyl, and R and R' in the group –NRR' are both H, the compound is herein identified as **Compound 6** in the Appendix A. This compound is described in the literature [CAS No. 321941-87-1] but no biological activity has been disclosed for the compound.

The **Compounds 1, 2, 4** and **5** may be prepared from unsubstituted or the appropriately substituted carbazole as depicted in **Scheme 1**. Thus, the carbazole is reacted with epichlorohydrin under basic conditions, and the resulting 9-oxiranylmethyl-9H-carbazole is reacted with a suitable primary or secondary amine, the oxiranyl ring is therefore opened and the desired β-amino alcohol is formed.

Compound 3 was prepared in one step from **Compound 2** as shown in **Scheme 2** by stirring a mixture of **Compound 2** and *p*-sulfophenylisothiocyanate.

Compound 4 was prepared in one step by reacting 3,6-dibromo-9-oxiranylmethyl-9H-carbazole with 2-piperazino-4-(3,4-dimethoxyphenyl)amino-quinazoline, as shown in **Scheme 3**.

Although the procedures given are used specifically for the synthesis of the carbazole derivatives of this invention, the methods apply widely to analogous compounds of Formula I, given appropriate consideration to protection and deprotection of reactive functional groups by methods standard to the art of Organic Chemistry. For example, in order to prevent unwanted side reactions, hydroxy groups generally need to be converted to ethers or esters during chemical reactions at other sites in the molecule. The hydroxy protecting group is readily removed to provide the free hydroxy group. Amino groups and carboxylic acid groups are similarly derivatized to protect then against unwanted side reactions. Typical protecting groups, and methods for attaching and cleaving them, are described fully by Greene and Wuts in Protective Groups in Organic Synthesis, John Wiley and Sons, New-York (2nd Ed, 1991) and McOmie, Protective Groups in Organic Chemistry, Plenum Press, New-York, 1973.

The inhibitory effect of the compounds of the present invention on heparanase activity can be evaluated by several methods carried out in vitro, ex vivo, or in vivo.

Some of the in vitro assays used according to the present invention were described in US 6,190,875. In these assays, heparanase is incubated with a heparanase substrate in the presence and in the absence of a compound of the present invention, and the inhibitory effect of the compound on the catalytic activity of the heparanase on its substrate is evaluated.

The heparanase may be natural mammalian heparanase, such as human heparanase purified as described in U.S. Patent 5,362,641 or, preferably, recombinant mammalian, e.g. human or mouse recombinant heparanase as described in US 5,968,822, US 6,190,875, and WO 99/57244, in purified or non-purified form. A source of non-purified recombinant heparanase is, for example, an extract of cells in which mammalian heparanase cDNA is expressed.

The heparanase substrate may be a natural heparan sulfate substrate, or an alternative substrate of the enzyme as described in U.S. 6,190,875, for example, heparin

(e.g. heparin immobilized on a gel such as Sepharose), heparin fragments (e.g. several species of low molecular weight heparin), modified non-anticoagulant species of heparin, other sulfated polysaccharides (e.g. pentosan polysulfate), soluble HSPG or ECM.

Evaluation of the inhibitory effect can be carried out, for example, as described in US 6,190,875, by a size separation assay adapted for detection of degradation products of the heparanase substrate. Examples of such assays include gel electrophoresis and column chromatography.

Qualitative and quantitative evaluation of the catalytic activity of heparanase on its substrate and the inhibitory effect of a candidate inhibitor can be effected, for example, by colorimetric assays. Any colorimetric assay based on any color producing reaction is envisaged by the invention, be it a simple color reaction, which is readily detectable, or a fluorimetric or a luminiscent (e.g., chemiluminiscent) reaction, which are readily detectable by fluorescence detecting techniques. Examples of such suitable colorimetric assays include, but are not limited to, the dimethylmethylene blue (DMB), tetrazolium blue and carbazole assays. Qualitative colorimetric assays include the dimethylmethylene blue (DMB) assay, which yields color shift in the presence of polyanionic compounds such as sulfated glycosaminoglycans having different sizes that are released from the substrate (soluble or immobilized), and the carbazole assay, which detects uronic acid derivatives present in complete hydrolyzates of products released from an immobilized substrate, both assays being applicable for crude extracts of heparanase and for the purified enzyme as well.

In a preferred embodiment, a quantitative evaluation is desired and the preferred in vitro assays are those which are adapted for detection of reducing moieties associated with degradation products of the heparanase substrate, preferably a reducing sugar assay. An example of a quantitative colorimetric assay is the tetrazolium blue assay which allows colorimetric detection of reducing moieties released from the substrate, e.g. heparan sulfate, which may be present either in soluble or immobilized form.

Another possibility, although less preferred, consists in evaluating the catalytic activity of heparanase on the substrate by radioactive techniques, in which case the substrate used is radiolabeled, either in vitro or metabolically.

The ex vivo assays for evaluating the inhibitory effect of the compounds on heparanase activity include angiogenic sprout formation and transmigration assays. The

angiogenic sprout formation assay is carried out in the rat aorta model (Nicosia et al., 1997; Nicosia and Ottinetti, 1990), whereby rat aorta rings are embedded in a basement membrane-like matrix composed of ECM-derived proteins such as laminin and collagen type IV, and HSPG, thus constituting a relevant heparanase substrate. The rings then develop angiogenic sprouts and angiogenesis can be quantitated. The compounds to be tested are added to the embedded aortic rings and their effect on angiogenic sprout formation is then evaluated.

In the ex vivo transwell migration assay, immune cell migration is evaluated, optionally in the presence of a chemoattractant factor such as stromal cell-derived factor 1 (SDF-1), a process which mimics in vivo extravasation of immune cells from the vasculature to sites of inflammation. In this assay, immune cells such as lymphocytes are let to migrate from the upper to the lower chamber through a transwell filter coated with a basement membrane-like matrix composed of ECM-derived proteins. The migration rate of the cells through the filter is then evaluated by counting the number of cells migrated through the filter (e.g. using a FACSort) compared to the number of cells added on top of the upper chamber. Over expression of heparanase in the immune cells results in an increase in the transmigration rate of the cells while addition of a heparanase inhibitor reduces the transmigration rate of the cells.

The inhibitory effect of the compounds on heparanase activity may be also assayed in vivo, for example, using the primary tumor growth or metastasis animal models or the sponge inflammation assay.

In the primary tumor animal model, animals are injected subcutaneously (s.c.) with tumor cells and treated with the heparanase inhibitors. Tumor growth is measured when animals in untreated control group start to die. For example, primary tumors may be generated with B16-F1 melanoma cells or with a highly metastatic subclone thereof injected s.c. into the flanks of mice. The mice are treated with heparanase inhibitors injected intraperitoneally (i.p.) twice a day starting 4 days after cell injection and are sacrificed and the tumor measured about 3 weeks after cell injection.

In the metastasis animal model, animals are injected intravenously (i.v.) with tumor cells and treated with the heparanase inhibitors. The number of lung metastasis is counted when animals in untreated control group start to die or about 3 weeks after cell injection. For example, metastasis may be generated with B16-F1 melanoma cells or with

a highly metastatic subclone thereof injected i.v. to mice. The mice are treated with heparanase inhibitors injected i.p. at certain times following cell injection, and are then sacrificed and the number of lung metastasis is counted.

In the sponge inflammation assay, polyvinyl alcohol (PVA) sponges are implanted under the mouse skin and the mouse is kept untreated or is treated with a test inhibitor agent. One day later, the mouse is sacrificed, the sponges are taken out, squeezed into a tube and the number of cells in each sample is determined. After centrifugation, the myeloperoxidase (MPO) content may be determined in a suspension of the cell pellets, and the TNF-α content in the supernatant of the sample. This assay mimics the inflammatory reaction resulting from the presence of a foreign body in the organism.

The heparanase inhibitors of the present invention can be used for the treatment of diseases and disorders caused by or associated with heparanase catalytic activity such as, but not limited to, cancer, inflammatory disorders and autoimmune diseases.

Thus, in one embodiment of the present invention, the compounds can be used for inhibition of angiogenesis, and are thus useful for the treatment of diseases and disorders associated with angiogenesis or neovascularization such as, but not limited to, tumor angiogenesis, ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration, reperfusion of gastric ulcer, and also for contraception or for inducing abortion at early stages of pregnancy.

In another embodiment of the invention, the compounds of the general formula I are useful for treatment or inhibition of a malignant cell proliferative disease or disorder.

According to this embodiment and due to the angiogenesis inhibitory activity of the compounds, they can be used for the treatment or inhibition of non-solid cancers, e.g hematopoietic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma, as well as for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and

malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

It is to be understood that the compounds of the general formula I are useful for treating or inhibiting tumors at all stages, namely tumor formation, primary tumors, tumor progression or tumor metastasis.

The compounds of general formula I are also useful for inhibiting or treating other cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma, and for inhibition or treatment of other diseases or disorders such as polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, and arteriovenous malformation.

In a further embodiment, the compounds of general formula I are useful for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial such as, but not limited to, treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders, or of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

In another preferred embodiment, the compounds of general formula I are useful for treatment of or amelioration of an autoimmune disease such as, but not limited to, Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis,

vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease and autism.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be acceptable in the sense that it is compatible with the other ingredients of the composition and it is not deleterious to the recipient thereof.

The term "carrier" refers to a diluent, adjuvant, excipient, or any other suitable vehicle. Such pharmaceutical carriers can be sterile liquids such as water and oils.

The pharmaceutical composition can be administered systemically, for example by parenteral, *e.g.* intravenous , intraperitoneal or intramuscular injection. In another example, the pharmaceutical composition can be introduced to a site by any suitable route including intravenous, subcutaneous, transcutaneous, topical, intramuscular, intraarticular, subconjunctival, or mucosal, *e.g.* oral, intranasal, or intraocular.

In one specific embodiment, the pharmaceutical composition is administered to the area in need of treatment. This may be achieved by, for example, local infusion during surgery, topical application, direct injection into the inflammed joint, directly onto the eye, etc.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or in solid form as tablets, capsules and the like. For administration by inhalation, the compositions are conveniently delivered in the form of drops or aerosol sprays. For administration by injection, the formulations may be presented in unit dosage form, *e.g.* in ampoules or in multidose containers with an added preservative.

The compositions of the invention can also be delivered in a vesicle, in particular in liposomes. In another embodiment, the compositions can be delivered in a controlled release system.

The amount of the therapeutic or pharmaceutical composition of the invention which is effective in the treatment of a particular disease, condition or disorder will depend on the nature of the disease, condition or disorder and can be determined by standard clinical techniques. In general, the dosage ranges from about 0.01 mg/kg to

about 50-100 mg/kg. In addition, in vitro assays as well in vivo experiments may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. For example, in order to obtain an effective mg/kg dose for humans based on data generated from mice or rat studies, the effective mg/kg dosage in mice or rats is divided by twelve or six, respectively.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

For convenience and better understanding, the section of the Examples is divided into two subsections: (I) the Chemical Section describing the synthesis of the carbazole and fluorene compounds, and (II) the Biological Section describing the biological activity of the compounds.

I CHEMICAL SECTION

The **Compounds 1-6,** which formulas are presented in Appendix A hereinafter, are identified in the Examples by their numbers in bold.

Materials

All reagents were purchased from Sigma-Aldrich Israel, Ltd., (Rehovot, Israel) and were used without further purification unless stated otherwise.

Compounds 1, **2**, **5**, and **6** were purchased from ChemDiv, Chemical Diversity (San Diego, CA, USA). **Compound 4** was purchased from Tripos Inc. (St. Louis, MO, USA).

Example 1. General approach for the synthesis of Carbazole Compounds 1, 2, 4, and 5

The carbazole compounds may be readily synthesized from the appropriate carbazole derivative, as shown in **Scheme 1**. The carbazole is reacted with epichlorohydrin in the present of sodium hydride, thus obtaining 9-oxiranylmethyl-9H-carbazole. The oxiranyl ring opening is achieved in the presence of a primary or secondary amine, which produces a secondary or tertiary β-amino alcohol. Such reactive primary or secondary amine may be, for example, phenethylamine, 3-phenylallyl amine, and N-substituted piperazines.

Compounds 1 and **2** are prepared by reacting 3-phenethylamine with dihalo 9-oxiranylmethyl-9H-carbazole. **Compounds 4** and **5** are prepared by reacting the 9-oxiranylmethyl-9H-carbazole with the appropriate N-substituted piperazine.

Example 2. Synthesis of 1-[3-(3,6-Dibromocarbazol-9-yl)-2-hydroxypropyl]-1-phenethyl -3-*p*-sulfonyl thiourea (Compound 3)

Compound 3 was prepared from **Compound 1** as shown in **Scheme 2**, as follows:

Compound 1 (500 mg, 1 mmol) was dissolved in dimethylformamide (20 mL) at room temperature before *p*-sulfophenyl isothiocyanate sodium salt monohydrate (500 mg, 2 eq.) was added in one portion. The reaction mixture was stirred for 48 hours after which time the solvent was evaporated under reduced pressure and the product was separated on a column using silica gel RP-18 (gradient methanol: water starting from ratio 1:1 to 3:1). Evaporation of the chromatographic solvent mixture gave a cloudy white solution. The solution was dried under reduced pressure for 12 hours, thus affording **Compound 3** as white needles in a total weight of 530 mg (73 % yield).

^{1}H (DMSO-d_6): δ= 8.46 (d, 1H), 8.31 (s, 1H), 7.67 (d, 2H), 7.61 (dd, 2H), 7.52 (d, 2H), 7.28 (t, 2H), 7.23 (s, 2H), 7.2 (d, 2H), 4.42 (m, 2H), 4.08 (q, 1H), 3.32 (m, 2H), 3.17 (d, 2H), 2.94 (m, 2H).

Example 3. Synthesis of 1-(3,6-dibromocarbazol-9-yl)-3-{4-[4-(3,4-dimethoxyphenyl amino) quinazolin-2-yl]-piperazine-1-yl} propan-2-ol (Compound 4)

Compound 4 was prepared as described in Example 1 from 9-oxiranylmethyl-9H-carbazole and 2-piperazino-4-(3,4-dimethoxyphenylamino)-quinazoline, as shown in **Scheme 3**.

II BIOLOGICAL SECTION

Materials

Heparin Sepharose CL-6B was purchased from Pharmacia (Amersham Pharmacia Biotech) Uppsala, Sweden ; 1,9-Dimethylmethylene blue (DMB), tetrazolium blue and heparan sulfate were purchased from Sigma-Aldrich (Rehovot, Israel); MCDB 131 medium was purchased from Clonetics (San Diego, CA, USA); DMEM and fetal calf serum were purchased from Gibco BRL (InVitrogen Corporation, CA, USA) ; glutamine and gentamicin were purchased from Biological Industries (Bet Haemek, Israel). Matrigel was kindly provided by Dr. H. Kleinmann, NIDR, NIH, Bethesda, MD, USA.

Methods

(a) In vitro Dimethylmethylene blue (DMB) assay for heparanase activity

Heparin Sepharose CL-6B beads were added up to the top of the wells of a multiscreen column loader (Millipore). A 96-well multiscreen plate containing 0.65 μm hydrophilic, low protein binding, Durapore membrane (Millipore) was placed, upside down, on top of the multiscreen column loader. The column loader and the multiscreen plate were held together, turned over, and the beads were uniformly transferred from the column loader to the multiscreen plate. Double-distilled water (DDW) was then added to the beads, which were allowed to swell for one minute, and then washed (three times) with DDW under vacuum. Heparin concentration was estimated to be 20 μM/well.

Human recombinant heparanase of at least 50% purity was obtained by expression in the CHO cells S1-11 subclone (generated as described for CHO clones S1PPT-4 and S1PPT-8 in WO 99/57244). Active human recombinant heparanase, purified from the CHO cell extracts by ion exchange chromatography (as described for the CHO 2TT1-8

subclone in WO 99/57244), was added (5 ng/well) to a reaction mixture containing 20 mM phosphate citrate buffer, pH 5.4, 1 mM $CaCl_2$, 1 mM NaCl, and 1 mM dithiothreitol (DTT; total volume of 100 µl). After 3-hour incubation at 37° C in a incubator on a vortex shaker, the heparanase reaction products were filtered under vacuum and collected into a 96-well polystyrene flat bottom plate (Greiner Cat. No. 655101). To each well, phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; 75 µl/well) and DMB (32 mg of DMB were dissolved in 5 ml ethanol, diluted to 1 liter with formate buffer containing 4 g sodium formate and 4 ml formic acid; 125 µl /well) were added. Color was developed after 5 minutes, and the absorbance of the samples was determined using a spectrophotometer (CECIL CE2040) at 530 nm. The absorbance correlated to heparanase activity. As a control, heparanase was added to the heparin Sepharose swollen beads in the multiscreen plate and the heparanase reaction products were filtered immediately thereafter and the absorbance of these control samples was subtracted from all other samples.

Alternatively, instead of the partially purified human recombinant heparanase enzyme as above, crude extracts of CHO cells S1-11 subclone expressing human recombinant or crude extracts of CHO cells mhG9 clone expressing mouse recombinant heparanase (generated with the mouse heparanase cDNA as described for CHO clones expressing human recombinant heparanase in WO 99/57244) were used. The cell extracts were centrifuged and resuspended in 20 mM phosphate citrate buffer, pH 5.4 containing 50 mM NaCl. The cells were lysed by three cycles of freezing and thawing. The cell lysates were centrifuged (10000xg for 5 min), supernatants were collected and then assayed for heparanase activity using the DMB assay.

In order to examine whether a test compound exhibits an inhibitory effect on the heparanase activity, each compound was dissolved in dimethylsulfoxide (DMSO) and added, at a concentration range of 1-30 µM, to the heparin Sepharose swollen beads in the 96-multiscreen plate. The partially purified human recombinant heparanase or the crude cell extracts expressing either human or mouse recombinant heparanase was added for a 3-hour incubation and the reaction continued as described above. Color was developed and the absorbance was measured as described above. The IC_{50} value (the concentration at which the heparanase activity was inhibited by 50%) for each compound was evaluated.

(b) In vitro tetrazolium blue assay for heparanase activity

Human recombinant heparanase of at least 50% purity (obtained by expression in the CHO cells S1-11 subclone as described in (a) above) was added (4 ng) to each well of a 96-well microplate and incubated in a reaction mixture containing 20 mM phosphate citrate buffer, pH 5.4, 1 mM $CaCl_2$, 1 mM NaCl, and 4 μM heparan sulfate (final volume of 100 μl). After 3 hours of incubation at 37° C in an incubator on a vortex shaker, the reaction was stopped by the addition of tetrazolium blue reagent (0.11% tetrazolium blue in 0.1 M NaOH; 100 μl/well). Color was developed by incubation of the plates at 60°C for 2 hours. For each assay, a control reaction, which did not contain the substrate (heparan sulfate), was included. Color intensity was quantitatively determined in a microplate reader (Dynatech) at 580 nm. Heparanase activity was calculated as the difference between the O.D of the sample containing the substrate, and the O.D. of the sample not containing the substrate. The background O.D. produced by the substrate was also subtracted from all the samples. The absorbance correlated to heparanase activity. The IC_{50} value (the concentration at which the heparanase activity was inhibited by 50%) for each compound was evaluated.

(c) Ex vivo angiogenic sprout formation assay for heparanase activity

As described in the Background section, previous studies have demonstrated the involvement of heparanase in angiogenesis. In order to test whether the heparanase inhibitors of the present invention can inhibit angiogenesis, the rat aorta model of angiogenesis as previously described (Nicosia et al., 1997; Nicosia and Ottinetti, 1990) was used with some modifications. In this model, the rat aortic endothelium exposed to a three-dimensional matrix of collagen or other ECM-derived proteins, switches to a microvascular phenotype, generating branching networks of microvessels. Angiogenesis is triggered by the injury caused by the dissection procedure and does not require stimulation by exogenous growth factors. Therefore, the rat aorta model can be used to investigate the endogenous mechanisms by which blood vessels regulate angiogenesis during wound healing.

Briefly, thoracic aortas were excised from 2- to 3-month-old Fischer 344 male rats, rinsed in serum-free MCDB 131 growth medium containing 50 μg/ml gentamicin,

cleaned of periadventitial fibroadipose tissue, and cross-sectioned at ~1 mm intervals. Freshly cut aortic rings were rinsed in serum-free MCDB 131 medium and each ring was embedded in Matrigel (a basement membrane-like matrix composed of ECM-derived proteins such as laminin and collagen type IV and others, and HSPG, thus constituting a relevant heparanase substrate). Matrigel cultures were transferred to 18-mm wells of 4-well plates (Nunc) and grown at 35.5°C in 0.5 ml of serum-free MCDB131 medium that was changed 3 times a week. Angiogenesis was quantitated by counting the number of neovessels according to published criteria (Nicosia and Ottinetti, 1990). In order to examine the inhibitory effect, a test compound was added to the Matrigel aortic ring cultures and its effect on reduction of the number of new microvessels was determined in comparison with untreated cultures.

(d) <u>In vivo mouse melanoma primary tumor growth assay for heparanase activity</u>

Instead of using a primary tumor cell line, primary tumor was generated in C57BL mice by cells herein designated FOR cells, which were generated as follows: B16-F1 mouse melanoma cells (ATCC No. 6326) were grown in DMEM containing 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin. A subclone of the B16-F1 cell line, F1-J, produced large amounts of melanin and exhibited a highly metastasis potential. These highly metastatic F1-J cells were injected to syngeneic mice (100,000 cells, s.c.). Cells from metastases that were formed were cultured in different conditions. A clone, F1-LG, designated herein FOR, was selected by its high heparanase expression and activity using the reverse transcriptase-polymerase chain reaction (RT-PCR) and the radiolabeled ECM degradation analyses, respectively, as previously described (Vlodavsky et al., 1999; U.S. 6,190,875).

FOR cells were grown in DMEM containing 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin until they reached confluence (typically 4-5 days) and then splitted (1:5). This splitting yielded subconfluent and growing cells at day 7, the day of cell injection, at which the cells were trypsinized, washed with PBS and counted to yield a cell suspension of 10^6 cells/ml in PBS. Male C57BL mice (~20 gram each; at least 10 mice/group) were injected s.c. on the flank with a suspension of the FOR cells (100 μl/mouse). Four days later, a test compound dissolved in DMSO was injected (100 μl) i.p to the mice, twice a day (morning and evening). Each compound was injected at

concentration of 1 mg/mouse/day). Control mice were injected i.p. with DMSO only (100 µl). Mice were observed daily, and usually three weeks after cell injection, mice were sacrificed, the tumors were harvested and weighted.

(e) In vivo mouse melanoma metastasis assay for heparanase activity

FOR cells were cultured as described in (d) above. After trypsinization, the cells were washed with PBS and counted to yield a cell suspension of $1.5x10^6$ cells/ml in PBS. Male C57BL mice (~20 gram each; at least 10 mice/group) were injected i.v. with a suspension of the FOR cells (100 µl/mouse). A test compound dissolved in DMSO was injected (100 µl) i.p to the mice 4 and 8 hours after cell injection. The compound was injected at concentration of 0.5 mg/mouse/day). Control mice were injected i.p. with DMSO only. Mice were observed daily, and three weeks after cell injecion, mice were sacrificed, the lungs were fixed in Bouen's solution and scored for the number of metastatic nodules as previously described (Vlodavsky et al., 1994).

(f) In vivo sponge inflammation assay

The sponge inflammation assay mimics the inflammatory reaction resulting from the presence of a foreign body in the organism. It was carried out by placing 3 polyvinyl alcohol (PVA) sponges (10 x 0.4 mm) under the skin of BALB/c male mice. The compound to be tested was then injected to the mouse in the following order: (i) immediately following sponge implantation, (ii) 4 hours after sponge implantation, and (iii) 8 hours after sponge implantation. The total volume of all three injections is 1 ml. The starting concentration of the tested compound was 1mg/ml/mice. At the next morning, the sponges were taken out and all three were squeezed into an Eppendorf tube (the total volume collected was about 0.6 ml). The number of cells was counted with a hemacytometer. Then the tubes were centrifuged, the soup was removed and saved. The cell pellets were washed once with PBS and the myeloperoxidase (MPO) content and activity were determined. TNF-α concentrations were determined in the supernatant (in pg/ml) with mouse TNF-α ELISA kit according to the manufacturer's instructions (Bender Medsystems, Vienna, Austria).

MPO is an oxidoreductase that catalyzes the reaction of hydrogen peroxide and halide ions to produce cytotoxic acids (such as hypochlorous acid) and other

intermediates; these play a role in oxygen-dependent killing of microorganisms and tumor cells. MPO is a green hemoprotein found in the azurophil granules of neutrophils and its quantification serves as an index of neutrophils infiltration.

The cells in the cell pellets obtained from the squeezed sponges were suspended in ice cold 0.5% hexadecyltrimethylammonium bromide (HTAB) in 50 mM potassium phosphate buffer, pH 6.0, frozen at -80^0C and then warmed at 60^0C for 24 minutes. MPO was measured spectrophotometrically: 10 µl of the cell homogenate were mixed with 380 µl of a 50 mM phosphate buffer, at pH 6.0 and containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% H_2O_2. The reaction was quenched with 10 µl H_2SO_4 (final concentration 3.3M) and then the change in absorbance at 460 nm was measured by an ELISA reader (MRX, Dinatec).

Example II (1). In vitro inhibition of heparanase activity by compounds of the invention.

The inhibition of heparanase activity by the compounds of the present invention was first detected in two colorimetric in vitro assays, i.e., the DMB assay and the tetrazolium blue assay as described in Methods (a) and (b) above. The human recombinant heparanase (designated h-hepa) expressed in CHO cells S1-11 subclone was used herein either in its partially purified form (50% purity) or in crude cell extracts, and the mouse recombinant heparanase (designated m-hepa) expressed in the CHO cells mhG9 clone was used herein in crude cell extracts only.

The results of the IC_{50} values of the different compounds are shown in Table 1. All the tested compounds were found to inhibit heparanase activity at micromolar concentrations. However, **Compound 3** was shown to be potent (IC_{50} values in the range of 2.2 to 12 µM compared to IC_{50} values in the range of 6 to 36 µM for the other compounds).

Table 1. IC_{50} values of the tested compounds for inhibition of heparanase as detected by the in vitro DMB and tetrazolium assays.

Compound	DMB (h-hepa) IC_{50}[μM]	Tetrazolium (h-hepa) IC_{50}[μM]	DMB of cell Extract (h-hepa) IC_{50}[μM]	DMB of cell Extract (m-hepa) IC_{50}[μM]
1	16.1		6	13
2	25			
3	12	2.2	9	5
4	8.4		14	36
5	21.9			
6	18.7		20	31

Example II (2). Inhibition of angiogenesis by Compound 3

The angiogenesis inhibitory effect of **Compound 3** was assayed using the angiogenic sprout formation assay described in Method (c) above. **Compound 3** showed inhibitory concentration of 120 μM.

Example II (3). Inhibition of mouse melanoma primary tumor growth and of metastasis by Compound 3

Since **Compound 3** was shown in Example II (2) above to inhibit angiogenic sprout formation, and since tumor progression is angiogenic-dependent, the effect of **Compound 3** on melanoma primary tumor growth and on melanoma metastasis was assayed as described in Methods (d) and (e) above. The results are summarized in Tables 2 and 3 below.

Table 2. Effect of Compound 3 on mouse melanoma primary tumor growth

Dose [mg/mouse/day]	Control	1.0
Tumor weight (gr)		
	0.75	0.7
	0.8	0.16
	1.1	0.32
	1.07	0.33
	0.86	0.24
	1.02	0.26
	0.34	0.13
	0.11	0.25
	1.79	
	0.31	
	0.16	
Median	0.8	0.255
Range	0.16 - 1.79	0.13 - 0.70

Table 3. Effect of Compound 3 on mouse melanoma metastasis

Dose [mg /mouse/day]		
Number of metastasis	Control	0.5
	5	100
	17	0
	6	0
	0	110
	25	5
	100	0
	60	0
	110	0
	120	
Median	25	0
Range	0-100	0-110

As shown in Table 2, untreated control mice developed primary tumors with an average weight of 0.8 g. Treatment with **Compound 3** (1.0 mg/mouse/day) significantly reduced the tumor size to 0.255 g, namely by a factor of 3. The effect of **Compound 3**

was further tested in melanoma metastasis as described in Method (e) above. The results, summarized in Table 3, show that the average number of metastatic nodules in the lungs of control (untreated) mice was 25, while treatment with **Compound 3** at a daily dosage of 0.5 mg/mouse/day completely abolished the lung metastatic nodules.

Example II (4). Measurement of myeloperoxidase (MPO) activity.

Sponge inflammation assay was carried out with the heparanase inhibitor **Compound 3** as described in Method (f) above at concentrations of 1 mg/ml/mouse and 0.2 mg/ml/mouse. The results are shown in Figs. 1-3 (10 mice in each group).

As shown in Fig. 1, administration of 1 mg/ml/mouse of **Compound 3** resulted in about 25% decrease in the total number of cells extracted from the sponges (in comparison to control, untreated mice).

Fig. 2 shows that there was a decrease in the MPO activity in the animal treated with 1 mg/ml of **Compound 3**

The results of Figs. 1 and 2 indicate that fewer neutrophils were recruited to the sponge, indicating that treatment with **Compound 3** resulted in a weaker inflammatory reaction. Most importantly, as shown in Fig. 3, the heparanase inhibitor **Compound 3,** at concentrations of 1 mg/ml/mouse and 0.2 mg/ml/mouse, dramatically reduced the amount of the pro-inflammatory TNF-α in the supernatant extracted from sponge samples in a manner that correlates well with the total number of cells extracted from the sponges (Fig. 1). Thus, by both cellular and molecular criteria, the heparanase inhibitor **Compound 3** was shown to reduce the inflammatory response.

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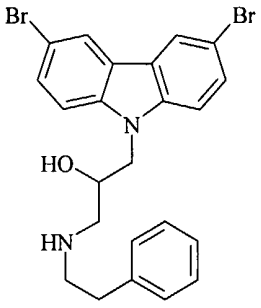
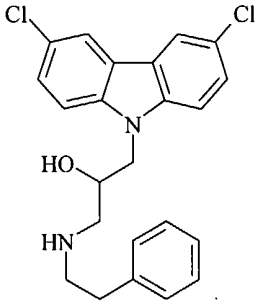
Appendix A- Compounds 1-6

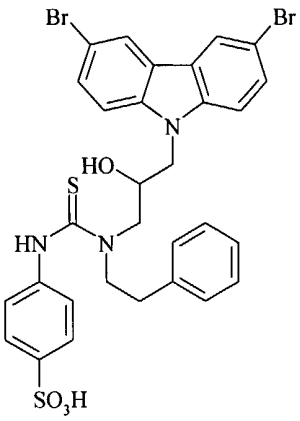
Compound 1



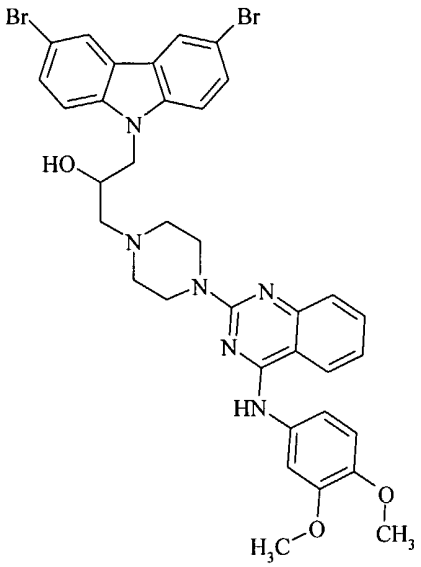
Compound 2



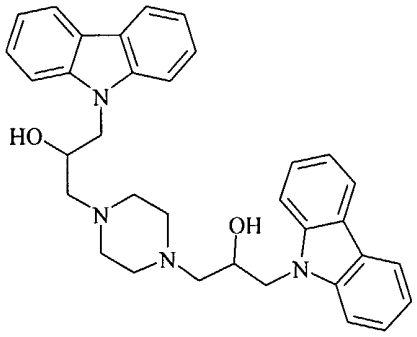
Compound 3



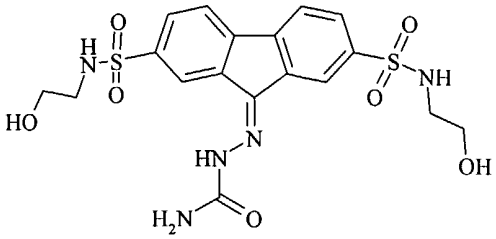
Compound 4



Compound 5

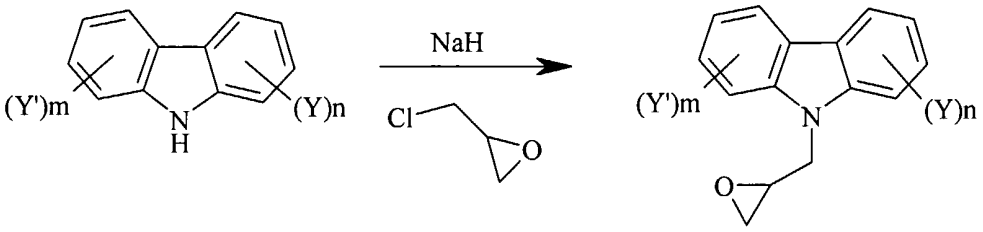


Compound 6

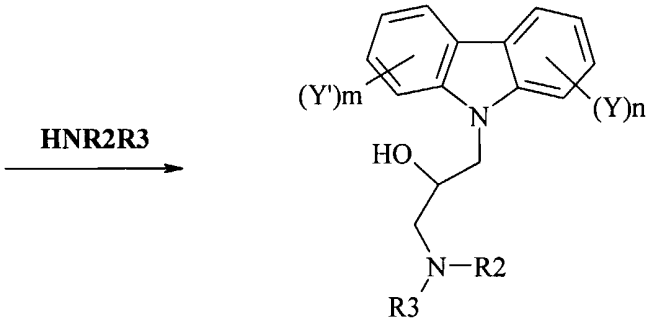


Appendix B-SCHEMES

SCHEME 1

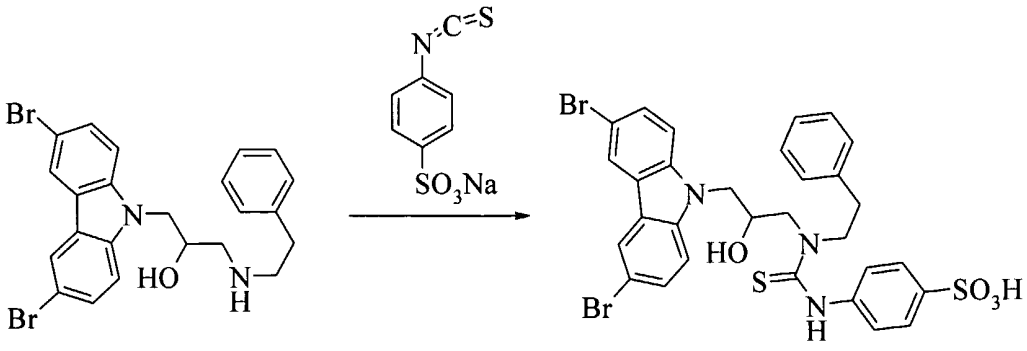


9-oxiranylmethyl-9H-carbazole



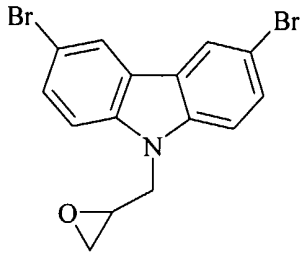
5

SCHEME 2



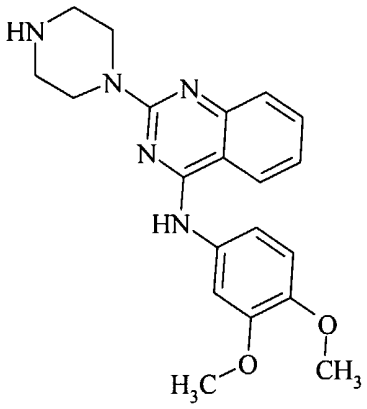
Compound 1 **Compound 3**

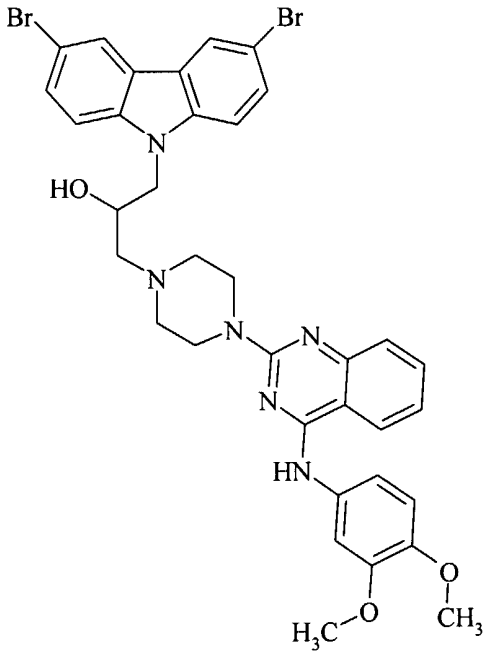
SCHEME 3



3,6-Dibromo-9-oxiranylmethyl-9H-carbazole

+

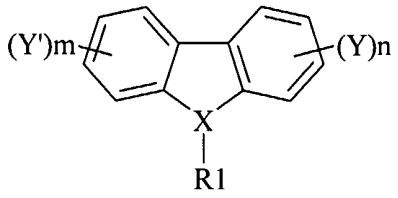




Compound 4

CLAIMS

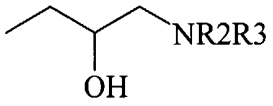
1. A pharmaceutical composition for treatment of diseases and disorders caused by or associated with heparanase catalytic activity, said composition comprising a pharmaceutically acceptable carrier and a heparanase inhibitor which is a carbazole or fluorene compound of the Formula I:



(I)

wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the formula:



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, -NH_2, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, -NH_2, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, -NH_2, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, -NH_2, -SO_3H, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, -NH_2, -SO_3H, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

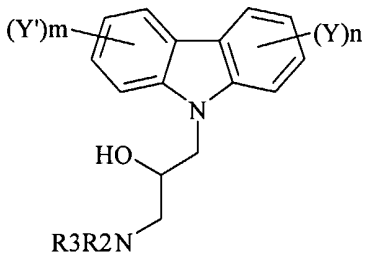
any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', -SO_3H, or -SO_2NRR';

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', -SO_3H, or -SO_2NRR';

m and n, the same or different, are integers from 0 to 4;

and pharmaceutically acceptable salts thereof.

2. A pharmaceutical composition according to Claim 1 comprising a compound of the formula Ia:

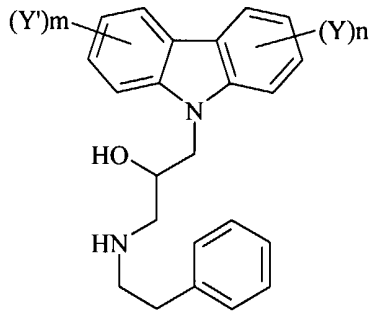


(Ia)

wherein R2, R3, Y, Y', m and n are as defined in Claim 1.

3. A pharmaceutical composition according to Claim 2 comprising a compound of formula Ia, wherein R2 is hydrogen and R3 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as defined in Claim 1.

4. A pharmaceutical composition according to Claim 3 comprising a compound of the formula Ib:

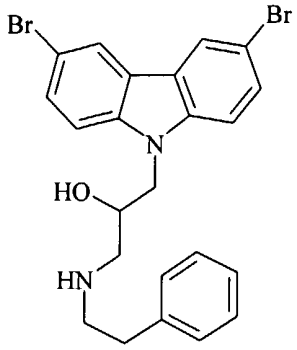


(Ib)

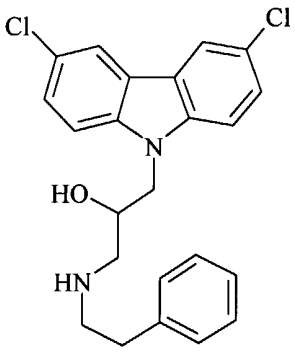
wherein Y, Y', m and n are as defined in Claim 1.

5. A pharmaceutical composition according to Claim 4 comprising a compound of the formula Ib, wherein Y and Y' are halogen and m and n are 1.

6. A pharmaceutical composition according to Claim 5 comprising the compound herein designated **Compound 1** of the formula:

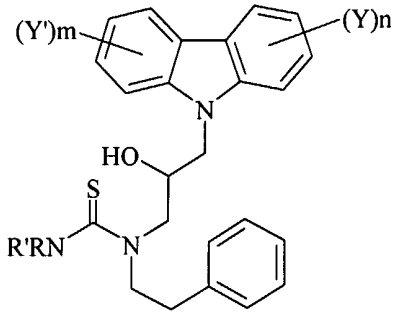


7. A pharmaceutical composition according to Claim 5 comprising the compound herein designated **Compound 2** of the formula:



8. A pharmaceutical composition according to Claim 2 comprising a compound of the formula Ia, wherein R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and R3 is -CONRR', -CSNRR', or -CONHNRR'; and wherein R, R', Y, Y', m, n and heteroaryl are as defined in Claim 1.

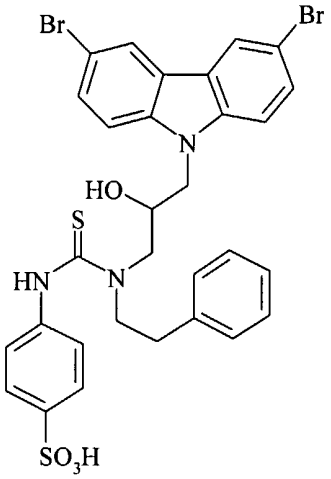
9. A pharmaceutical composition according to Claim 8 comprising a compound of the formula Ic:



(Ic)

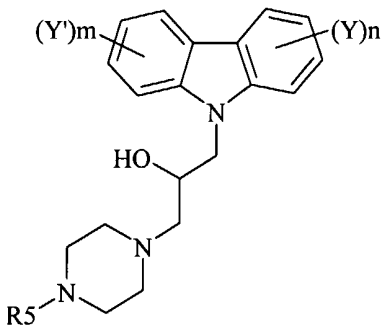
wherein R, R', Y, Y', m and n are as defined in Claim 1.

10. A pharmaceutical composition according to Claim 9 comprising the compound herein designated **Compound 3** of the formula:



11. A pharmaceutical composition according to Claim 2 comprising a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated 6-membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5, wherein R5, Y, Y', m and n are as defined in Claim 1.

12. A pharmaceutical composition according to Claim 11 comprising a compound of the formula Id:

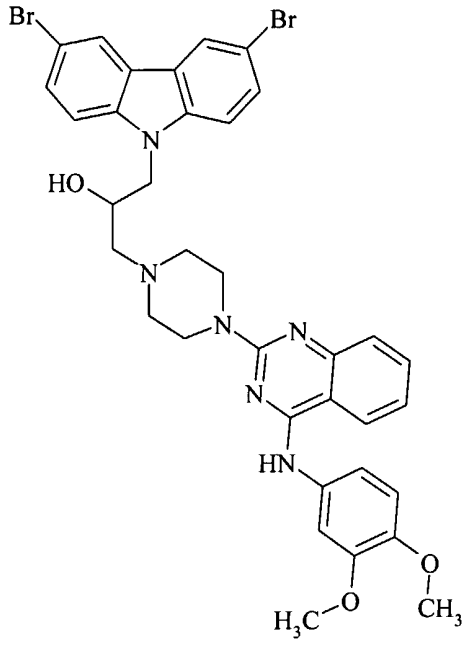


(Id)

wherein R5, Y, Y', m and n are as defined in Claim 1.

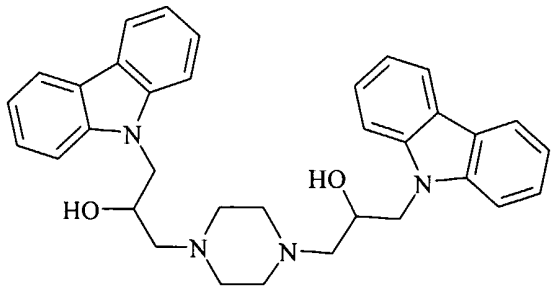
13. A pharmaceutical composition according to Claim 12 comprising a compound of the formula Id, wherein R5 is a heteroaryl derived from a bicyclic heteroaromatic ring containing two N atoms, preferably quinazolinyl, and being preferably substituted by -NRR', wherein R is hydrogen and R' is C6-C14 aryl optionally substituted by at least one radical selected from halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, or C1-C6 alkylthio, preferably a phenyl radical substituted by two methoxy groups.

14. A pharmaceutical composition according to Claim 13 comprising the compound herein designated **Compound 4** of the formula:

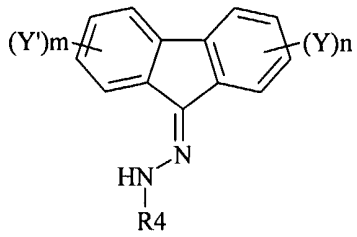


15. A pharmaceutical composition according to Claim 12, comprising a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, -NH_2, C1-C6 alkoxy or C1-C6 alkylthio; wherein Y, Y', m and n are as defined in Claim 1.

16. A pharmaceutical composition according to Claim 15 comprising the compound herein designated **Compound 5** of the formula:



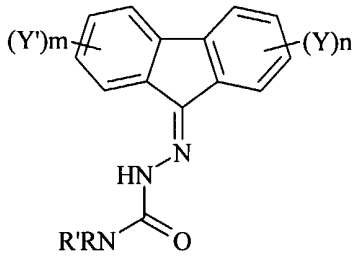
17. A pharmaceutical composition according to Claim 1 comprising a compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as defined in Claim 1.

18. A pharmaceutical composition according to Claim 17 comprising a compound of formula If:

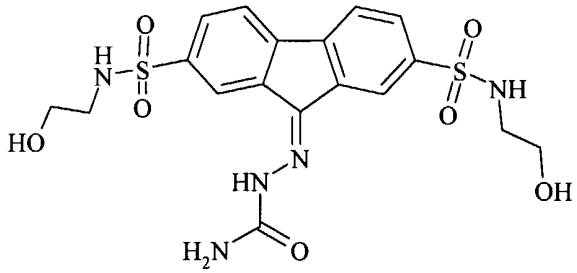


(If)

wherein Y, Y', R, R', m and n are as defined in Claim 1.

19. A pharmaceutical composition according to Claim 18 comprising a compound of the formula If, wherein Y and Y' are $-SO_2NRR'$, wherein R and R' are as defined in Claim 1.

20. A pharmaceutical composition according to Claim 19 comprising the compound herein designated **Compound 6** of the formula:



21. A pharmaceutical composition according to any one of claims 1 to 20 for inhibition of angiogenesis.

22. A pharmaceutical composition according to any one of claims 1 to 20 for treatment or inhibition of a malignant cell proliferative disease or disorder.

23. The pharmaceutical composition according to claim 21 or 22 for the treatment or inhibition of non-solid cancers, e.g hematopoietic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

24. The pharmaceutical composition according to claim 21 or 22 for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

25. The pharmaceutical composition according to claim 23 or 24 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

26. A pharmaceutical composition according to any one of claims 1 to 20 for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

27. The pharmaceutical composition according to any one of claims 1 to 20 for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

28. The pharmaceutical composition according to any one of claims 1 to 20 for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

29. The pharmaceutical composition according to any one of claims 1 to 20, for contraception or for inducing abortion at early stages of pregnancy.

30. The pharmaceutical composition according to any one of claims 1 to 20, for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

31. The pharmaceutical composition according to claim 30, for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

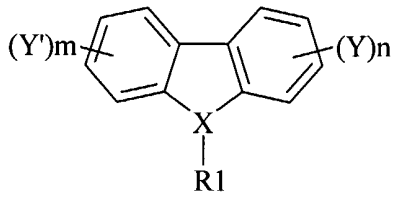
32. The pharmaceutical composition according to claim 30, for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

33. The pharmaceutical composition according to any one of claims 1 to 20, for treatment of or amelioration of an autoimmune disease.

34. The pharmaceutical composition according to claim 33, wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent

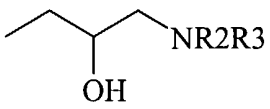
diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

35. Use of a heparanase inhibitor which is a carbazole or fluorene compound of the Formula I:



wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the (I)



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

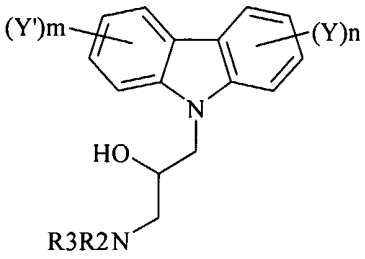
any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

m and n, the same or different, are integers from 0 to 4;

or of a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for treatment of a disease or a disorder caused by or associated with heparanase catalytic activity.

36. Use according to Claim 35 of a compound of the formula Ia:

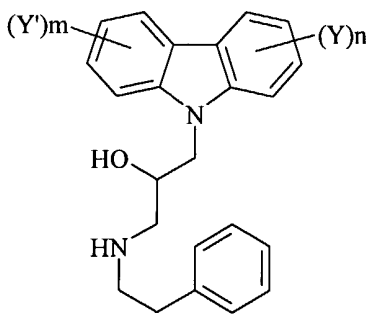


(Ia)

wherein R2, R3, Y, Y', m and n are as defined in Claim 35.

37. Use according to Claim 36 of compound of formula Ia, wherein R2 is hydrogen and R3 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as defined in Claim 35.

38. Use according to Claim 37 of compound of the formula Ib:

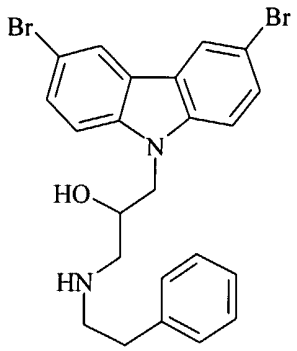


(Ib)

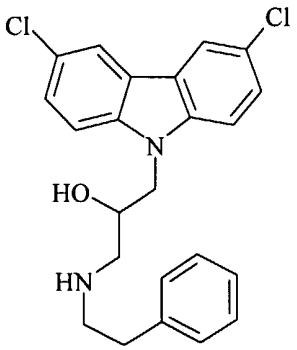
wherein Y, Y', m and n are as defined in Claim 35.

39. Use according to Claim 38 of a compound of the formula Ib, wherein Y and Y' are halogen and m and n are 1.

40. Use according to Claim 39 of the compound herein designated **Compound 1** of the formula:

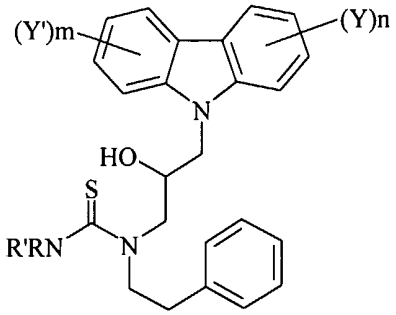


41. Use according to Claim 39 of the compound herein designated **Compound 2** of the formula:



42. Use according to Claim 36 of a compound of the formula Ia, wherein R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and R3 is -CONRR', –CSNRR', or -CONHNRR'; and wherein R, R', Y, Y', m, n and heteroaryl are as defined in Claim 35.

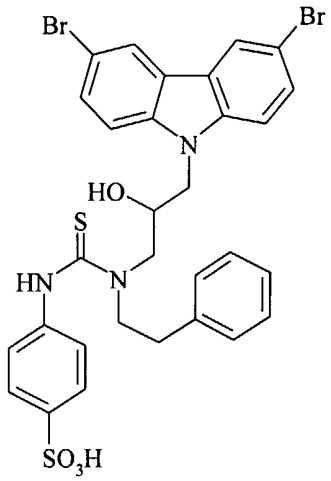
43. Use according to Claim 42 of a compound of the formula Ic:



(Ic)

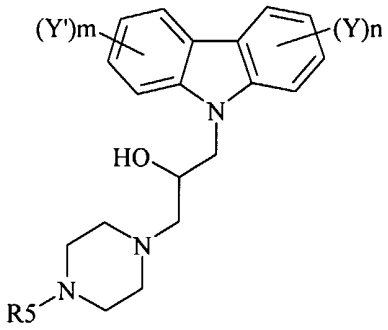
wherein R, R', Y, Y', m and n are as defined in Claim 35.

44. Use according to Claim 43 of the compound herein designated **Compound 3** of the formula:



45. Use according to Claim 36 of a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5, wherein R5, Y, Y', m and n are as defined in Claim 35.

46. Use according to Claim 45 of a compound of the formula Id:

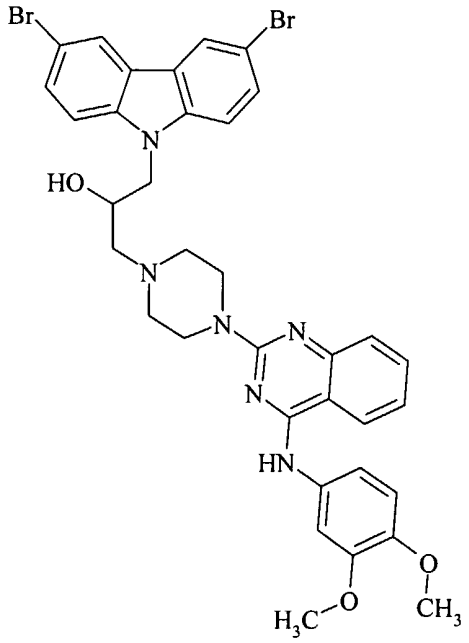


(Id)

wherein R5, Y, Y', m and n are as defined in Claim 35.

47. Use according to Claim 46 of a compound of the formula Id, wherein R5 is heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S and Y, Y', m and n are as defined in Claim 35.

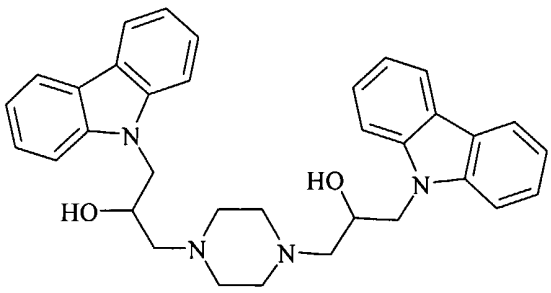
48. Use according to Claim 47 of the compound herein designated **Compound 4** of the formula:



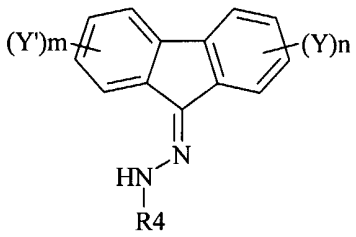
49. Use according to Claim 46 of a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group

selected from halogen, -OH, -SH, -NH_2, C1-C6 alkoxy or C1-C6 alkylthio, and Y, Y', m and n are as defined in Claim 35.

50. Use according to Claim 47 of the compound herein designated **Compound 5** of the formula:



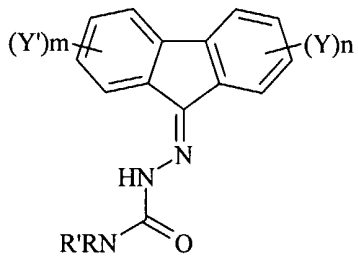
51. Use according to Claim 35 of a compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as defined in Claim 35.

52. Use according to Claim 51 of a compound of formula If:

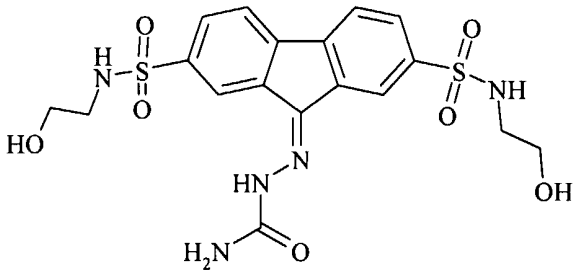


(If)

wherein Y, Y', R, R', m and n are as defined in Claim 35.

53. Use according to Claim 52 of a compound of the formula If, wherein Y and Y' are $-SO_2NRR'$, wherein R and R' are as defined in Claim 35.

54. Use according to Claim 53 of the compound herein designated **Compound 6** of the formula:



55. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibition of angiogenesis.

56. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment or inhibition of a malignant cell proliferative disease or disorder.

57. Use according to claim 55 or 56 for the preparation of a pharmaceutical composition for the treatment or inhibition of non-solid cancers, e.g hematopoietic malignancies such as all types of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

58. Use according to claim 55 or 56 for the preparation of a pharmaceutical composition for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma,

bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

59. Use according to claim 57 or 58 for the preparation of a pharmaceutical composition for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

60. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

61. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

62. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

63. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for contraception or for inducing abortion at early stages of pregnancy.

64. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of or amelioration of inflammatory symptoms

in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

65. Use according to claim 64, wherein said pharmaceutical composition is for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

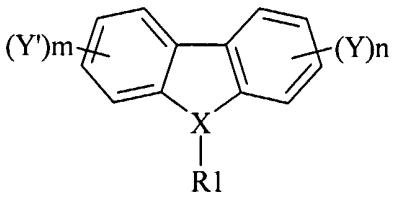
66. Use according to claim 64, wherein said pharmaceutical composition is for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

67. Use according to any one of claims 35 to 54 for the preparation of a pharmaceutical composition for treatment of or amelioration of an autoimmune disease.

68. Use according to claim 67 wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia gravis, plexus disorders e.g. acute brachial neuritis,, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

69. A method for treatment of a patient suffering from a disease or disorder caused by or associated with heparanase catalytic activity, which comprises administering to said patient an effective amount of a heparanase inhibitor or a pharmaceutically acceptable

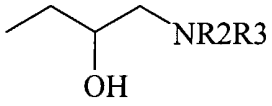
salt thereof, wherein said heparanase inhibitor is a carbazole or fluorene compound of the Formula I:



(I)

wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the formula:



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

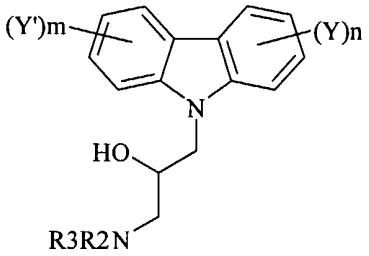
"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and

m and n, the same or different, are integers from 0 to 4.

70. A method according to Claim 69, wherein said heparanase inhibitor is a compound of the formula Ia:



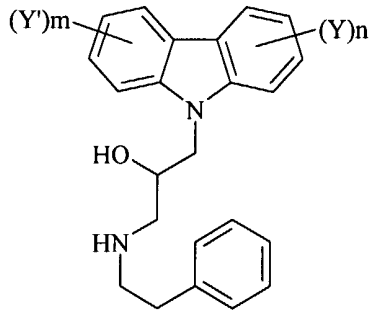
(Ia)

wherein R2, R3, Y, Y', m and n are as defined in Claim 69.

71. A method according to Claim 70, wherein the heparanase inhibitor is a compound of formula Ia, wherein R2 is hydrogen and R3 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at

least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as defined in Claim 69.

72. A method according to Claim 71, wherein the heparanase inhibitor is a compound of the formula Ib:

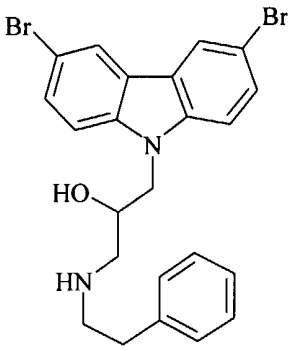


(Ib)

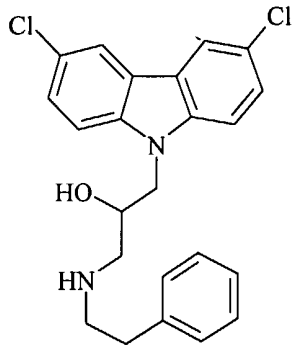
wherein Y, Y', m and n are as defined in Claim 69.

73. A method according to Claim 72, wherein the heparanase inhibitor is a compound of the formula Ib, wherein Y and Y' are halogen and m and n are 1.

74. A method according to Claim 73, wherein the heparanase inhibitor is the compound herein designated **Compound 1** of the formula:

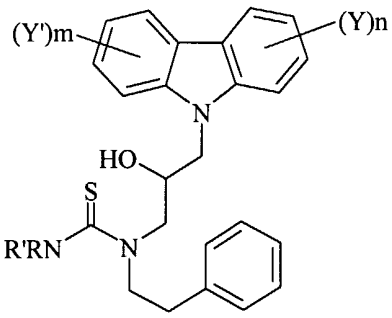


75. A method according to Claim 73, wherein the heparanase inhibitor is the compound herein designated **Compound 2** of the formula:



76. A method according to Claim 70, wherein the heparanase inhibitor is a compound of the formula Ia, wherein R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and R3 is -CONRR', –CSNRR', or -CONHNRR'; and wherein R, R', Y, Y', m, n and heteroaryl are as defined in Claim 69.

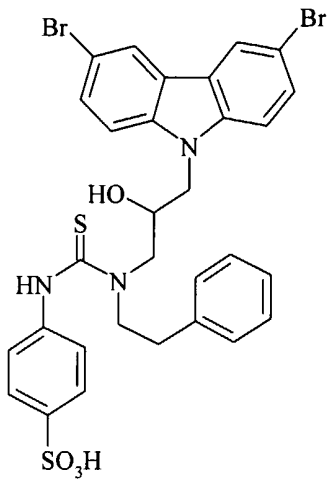
77. A method according to Claim 76, wherein the heparanase inhibitor is a compound of the formula Ic:



(Ic)

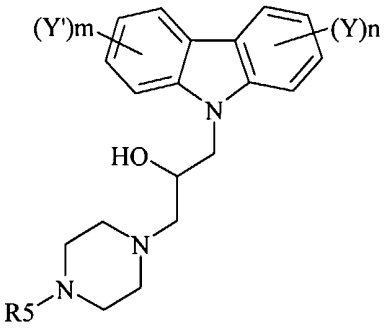
wherein R, R', Y, Y', m and n are as defined in Claim 69.

78. A method according to Claim 77, wherein the heparanase inhibitor is the compound herein designated **Compound 3** of the formula



79. A method according to Claim 70, wherein the heparanase inhibitor is a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5, wherein R5, Y, Y', m and n are as defined in Claim 69.

80. A method according to Claim 79, wherein the heparanase inhibitor is a compound of the formula Id:



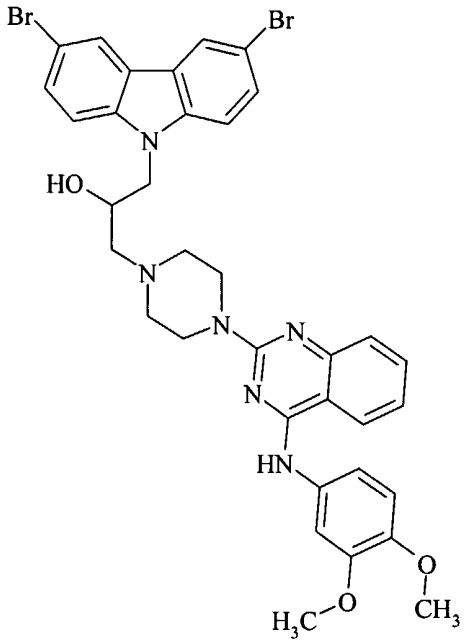
(Id)

wherein R5, Y, Y', m and n are as defined in Claim 69.

81. A method according to Claim 80, wherein the heparanase inhibitor is a compound of the formula Id, wherein R5 is a heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S, and being preferably substituted by NRR', wherein R is H and R' is C6-c14 aryl optionally substituted by at

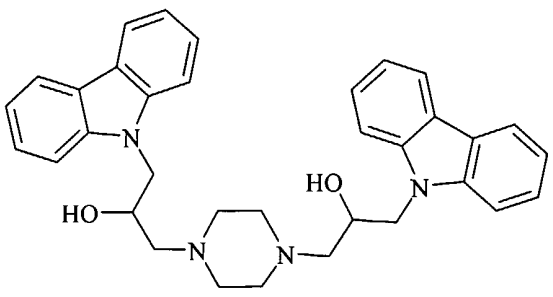
least one radical selected from halogen, -OH, -SH, -NH2, -SO_3H, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy or C1-C6 alkylthio.

82. A method according to Claim 81 wherein said heparanase inhibitor is the compound herein designated **Compound 4** of the formula:

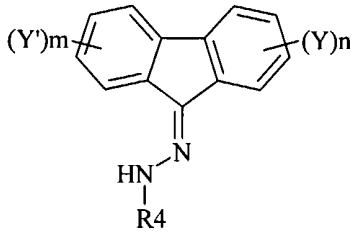


83. A method according to Claim 80, wherein the heparanase inhibitor is a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, -NH_2, C1-C6 alkoxy or C1-C6 alkylthio, and Y, Y', m and n are as defined in Claim 69.

84. A method according to Claim 83 wherein said heparanase inhibitor is the compound herein designated **Compound 5** of the formula:



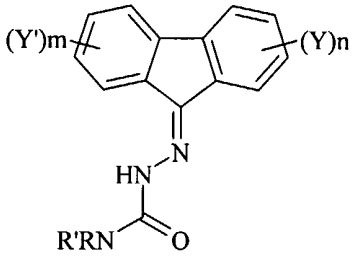
85. A method according to Claim 69, wherein the heparanase inhibitor is a compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as defined in Claim 69.

86. A method according to Claim 85, wherein the heparanase inhibitor is a compound of formula If:

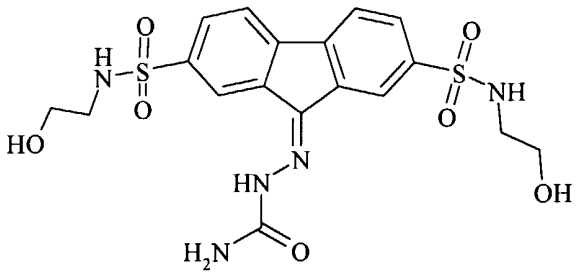


(If)

wherein Y, Y', R, R', m and n are as defined in Claim 69.

87. A method according to Claim 86, wherein the heparanase inhibitor is a compound of the formula If, wherein Y and Y' are $-SO_2NRR'$, wherein R and R' are as defined in Claim 69.

88. A method according to Claim 87 wherein said heparanase inhibitor is the compound herein designated **Compound 6** of the formula:



89. A method according to any one of claims 69 to 88 for inhibition of angiogenesis.

90. A method according to any one of claims 69 to 88 for treatment or inhibition of a malignant cell proliferative disease or disorder.

91. A method according to claim 89 or 90 for the treatment or inhibition of a non-solid cancer, e.g a hematopoietic malignancy such as any type of leukemia, e.g. acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), mast cell leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphomas, Burkitt's lymphoma and multiple myeloma.

92. A method according to claim 89 or 90 for the treatment or inhibition of solid tumors such as tumors in lip and oral cavity, pharynx, larynx, paranasal sinuses, major salivary glands, thyroid gland, esophagus, stomach, small intestine, colon, colorectum, anal canal, liver, gallbladder, extrahepatic bile ducts, ampulla of vater, exocrine pancreas, lung, pleural mesothelioma, bone, soft tissue sarcoma, carcinoma and malignant melanoma of the skin, breast, vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis, ureter, urinary bladder, urethra, carcinoma of the eyelid, carcinoma of the conjunctiva, malignant melanoma of the conjunctiva, malignant melanoma of the uvea, retinoblastoma, carcinoma of the lacrimal gland, sarcoma of the orbit, brain, spinal cord, vascular system, hemangiosarcoma and Kaposi's sarcoma.

93. A method according to claim 91 or 92 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

94. A method according to any one of claims 69 to 88 for treatment of ophthalmologic disorders such as diabetic retinopathy and macular degeneration, particularly age-related macular degeneration.

95. A method according to any one of claims 69 to 88 for inhibiting or treating cell proliferative diseases or disorders such as psoriasis, hypertrophic scars, acne and sclerosis/scleroderma.

96. A method according to any one of claims 69 to 88 for inhibiting or treatment of a disease or disorder selected from polyps, multiple exostosis, hereditary exostosis, retrolental fibroplasia, hemangioma, reperfusion of gastric ulcer and arteriovenous malformation.

97. A method according to any one of claims 69 to 88 for contraception or for inducing abortion at early stages of pregnancy.

98. A method according to any one of claims 69 to 88 for treatment of or amelioration of inflammatory symptoms in any disease, condition or disorder where immune and/or inflammation suppression is beneficial.

99. A method according to claim 98, for treatment of or amelioration of inflammatory symptoms in the joints, musculoskeletal and connective tissue disorders.

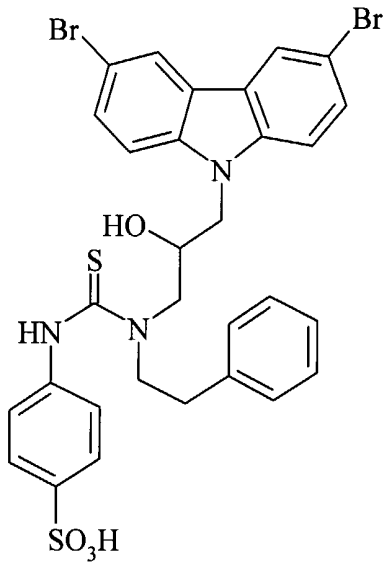
100. A method according to claim 98, for treatment of or amelioration of inflammatory symptoms associated with hypersensitivity, allergic reactions, asthma, atherosclerosis, otitis and other otorhinolaryngological diseases, dermatitis and other skin diseases, posterior and anterior uveitis, conjunctivitis, optic neuritis, scleritis and other immune and/or inflammatory ophthalmic diseases.

101. A method according to any one of claims 69 to 88 for treatment of or amelioration of an autoimmune disease.

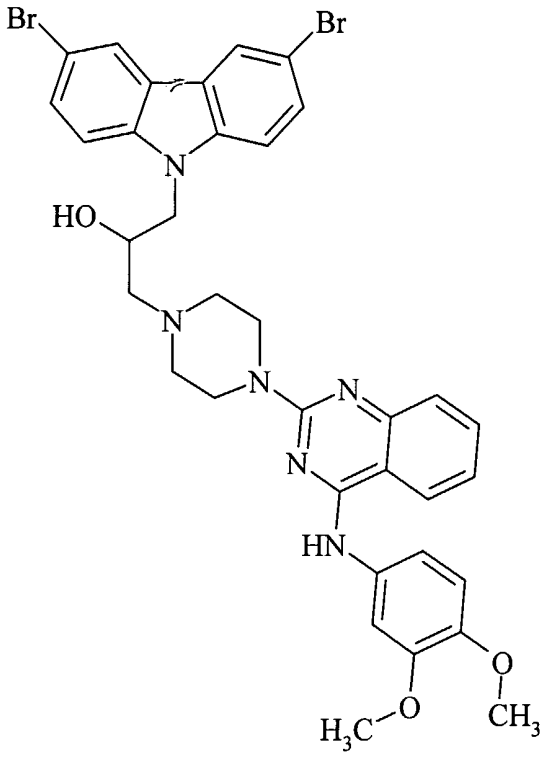
102. A method according to claim 101 wherein said autoimmune disease is Eaton-Lambert syndrome, Goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome, autoimmune hemolytic anemia (AIHA), hepatitis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), multiple sclerosis (MS), myasthenia

gravis, plexus disorders e.g. acute brachial neuritis, polyglandular deficiency syndrome, primary biliary cirrhosis, rheumatoid arthritis, scleroderma, thrombocytopenia, thyroiditis e.g. Hashimoto's disease, Sjögren's syndrome, allergic purpura, psoriasis, mixed connective tissue disease, polymyositis, dermatomyositis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, pemphigus, bullous pemphigoid, dermatitis herpetiformis, Crohn's disease or autism.

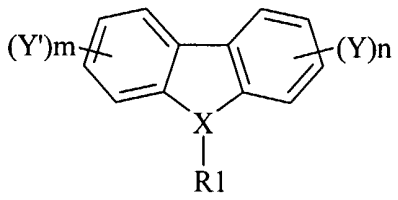
103. A carbazole compound herein designated **Compound 3** of the formula:



104. A carbazole compound herein designated **Compound 4** of the formula:



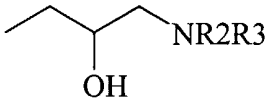
105. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a carbazole or fluorene compound of the Formula I:



(I)

wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the formula:



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

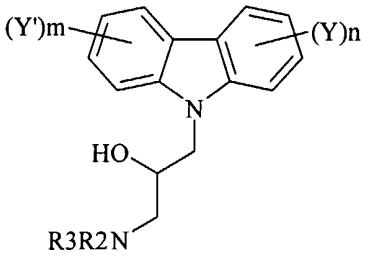
any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

m and n, the same or different, are integers from 0 to 4;

and pharmaceutically acceptable salts thereof.

106. A pharmaceutical composition according to Claim 105 comprising a compound of the formula Ia:

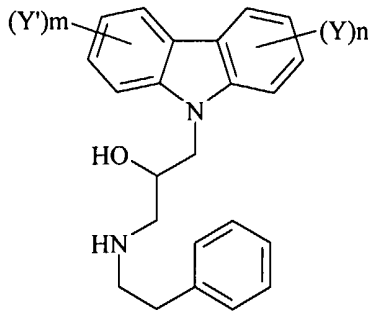


(Ia)

wherein R2, R3, Y, Y', m and n are as defined in Claim 105.

107. A pharmaceutical composition according to Claim 106 comprising a compound of formula Ia, wherein R2 is hydrogen and R3 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as defined in Claim 105.

108. A pharmaceutical composition according to Claim 107 comprising a compound of the formula Ib:

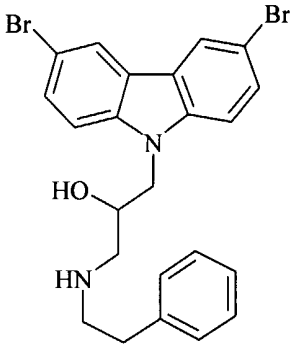


(Ib)

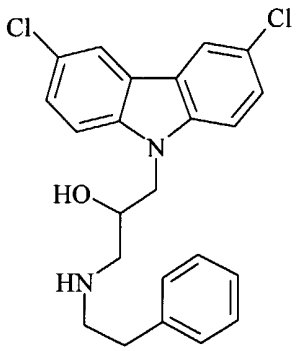
wherein Y, Y', m and n are as defined in Claim 105.

109. A pharmaceutical composition according to Claim 108 comprising a compound of the formula Ib, wherein Y and Y' are halogen and m and n are 105.

110. A pharmaceutical composition according to Claim 109 comprising the compound herein designated **Compound 1** of the formula:

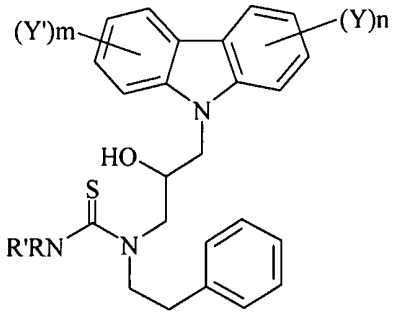


111. A pharmaceutical composition according to Claim 109 comprising the compound herein designated **Compound 2** of the formula:



112. A pharmaceutical composition according to Claim 106 comprising a compound of the formula Ia, wherein R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and R3 is -CONRR', –CSNRR', or -CONHNRR'; and wherein R, R', Y, Y', m, n and heteroaryl are as defined in Claim 105.

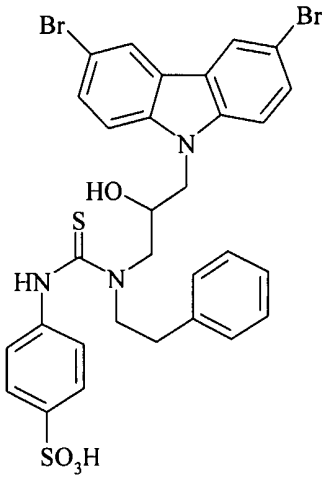
113. A pharmaceutical composition according to Claim 112 comprising a compound of the formula Ic:



(Ic)

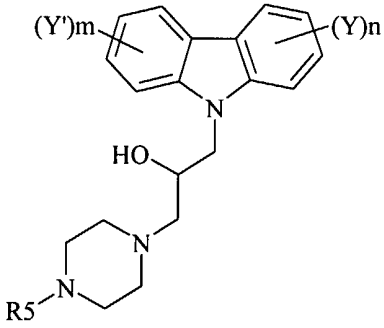
wherein R, R', Y, Y', m and n are as defined in Claim 105.

114. A pharmaceutical composition according to Claim 113 comprising the compound herein designated **Compound 3** of the formula:



115. A pharmaceutical composition according to Claim 106 comprising a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated 6-membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5, wherein R5, Y, Y', m and n are as defined in Claim 105.

116. A pharmaceutical composition according to Claim 115 comprising a compound of the formula Id:

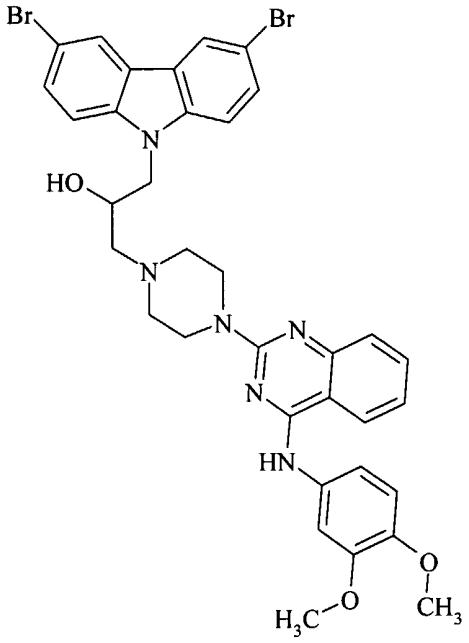


(Id)

wherein R5, Y, Y', m and n are as defined in Claim 105.

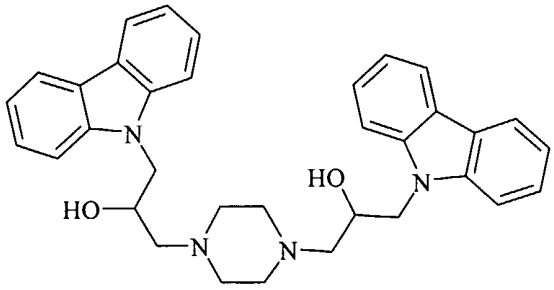
117. A pharmaceutical composition according to Claim 116 comprising a compound of the formula Id, wherein R5 is a heteroaryl derived from a bicyclic heteroaromatic ring containing two N atoms, preferably quinazolinyl, and being preferably substituted by -NRR', wherein R is hydrogen and R' is C6-C14 aryl optionally substituted by at least one radical selected from halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, or C1-C6 alkylthio, preferably a phenyl radical substituted by two methoxy groups.

118. A pharmaceutical composition according to Claim 117 comprising the compound herein designated **Compound 4** of the formula:

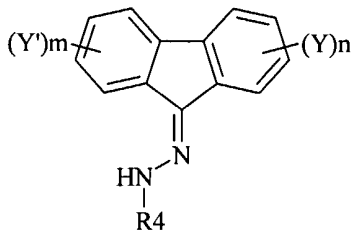


119. A pharmaceutical composition according to Claim 116, comprising a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, -NH_2, C1-C6 alkoxy or C1-C6 alkylthio; wherein Y, Y', m and n are as defined in Claim 105.

120. A pharmaceutical composition according to Claim 119 comprising the compound herein designated **Compound 5** of the formula:



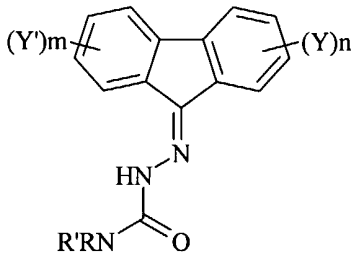
121. A pharmaceutical composition according to Claim 105 comprising a compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as defined in Claim 105.

122. A pharmaceutical composition according to Claim 121 comprising a compound of formula If:

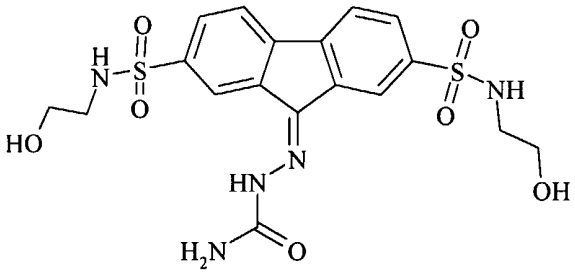


(If)

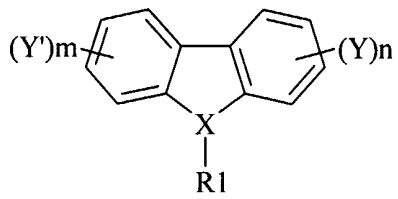
wherein Y, Y', R, R', m and n are as defined in Claim 105.

123. A pharmaceutical composition according to Claim 122 comprising a compound of the formula If, wherein Y and Y' are $-SO_2NRR'$, wherein R and R' are as defined in Claim 105.

124. A pharmaceutical composition according to Claim 123 comprising the compound herein designated **Compound 6** of the formula:



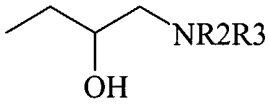
125. Use of a carbazole or fluorene compound of the Formula I:



(I)

wherein

either X is N and R1 is a 3-amino-2-hydroxy-propyl radical of the formula:



or X is C and R1 is a radical of the formula:

=N-NH-R4

and

Y and Y' each independently represents hydrogen, halogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, nitro, -OR, -SR, -CONRR', -NRCONRR', -NRR', $-SO_3H$, or $-SO_2NRR'$;

R2 and R3 each independently represents hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl;

or R2 is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl and R3 is -CONRR', –CSNRR', or -CONHNRR';

or R2 and R3 together with the N atom to which they are attached form a saturated 5-7 membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5;

R4 is –CONRR', -CSNRR' or –CONHNRR';

R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; or heteroaryl derived from a bicyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

R and R' each independently represents (i) hydrogen; (ii) C1-C6 alkyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, and/or heteroaryl; (iii) C2-C6 alkenyl optionally substituted by halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl and/or heteroaryl; (iv) C6-C14 aryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio; or (v) heteroaryl optionally substituted by halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, and/or C1-C6 alkylthio;

"heteroaryl" in radicals R, R', R2, and R3 is a radical derived from a mono- or poly- cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S;

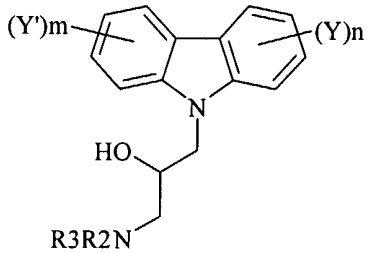
any "C1-C6 alkyl" or "C2-C6 alkenyl" in radicals R2 and R3 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

any "C6-C14 aryl" and "heteroaryl" in radicals R2, R3 and R5 may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$;

m and n, the same or different, are integers from 0 to 4;

or of a pharmaceutically acceptable salt thereof, for the manufacture of a pharmaceutical composition.

126. Use according to Claim 125 of a compound of the formula Ia:



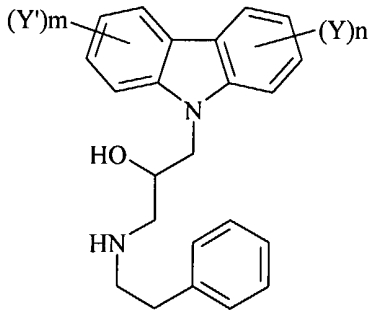
(Ia)

wherein R2, R3, Y, Y', m and n are as defined in Claim 125.

127. Use according to Claim 126 of a compound of formula Ia, wherein R2 is hydrogen and R3 is C1-C6 alkyl, C2-C6 alkenyl, C6-C14 aryl, or heteroaryl, wherein

said C1-C6 alkyl and C2-C6 alkenyl may be substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and wherein said C6-C14 aryl and heteroaryl may be substituted by halogen, -OH, -SH, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, C1-C6 alkylthio, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$; and wherein "heteroaryl" is a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three heteroatoms selected from N, O and/or S; and R, R', Y, Y', m and n are as defined in Claim 125.

128. Use according to Claim 127 of a compound of the formula Ib:

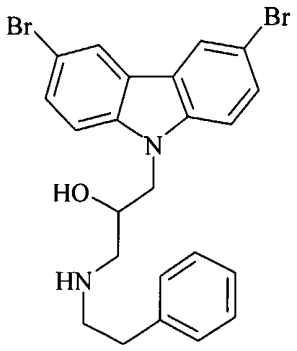


(Ib)

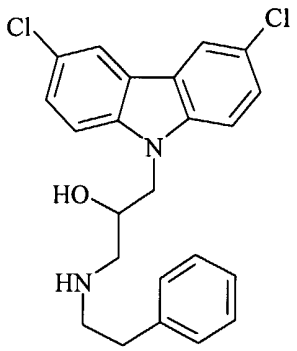
wherein Y, Y', m and n are as defined in Claim 125.

129. Use according to Claim 128 of a compound of the formula Ib, wherein Y and Y' are halogen and m and n are 125.

130. Use according to Claim 129 of the compound herein designated **Compound 1** of the formula:

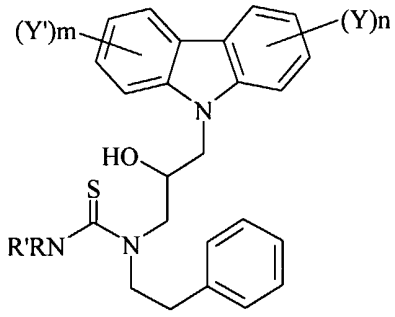


131. Use according to Claim 130 of the compound herein designated **Compound 2** of the formula:



132. Use according to Claim 126 of a compound of the formula Ia, wherein R2 is C1-C6 alkyl optionally substituted by at least one radical selected from halogen, -OH, -SH, C1-C6 alkoxy, C1-C6 alkylthio, C6-C14 aryl, heteroaryl, -NRR', -COOR, -CONRR', $-SO_3H$, or $-SO_2NRR'$, and R3 is -CONRR', –CSNRR', or -CONHNRR'; and wherein R, R', Y, Y', m, n and heteroaryl are as defined in Claim 125.

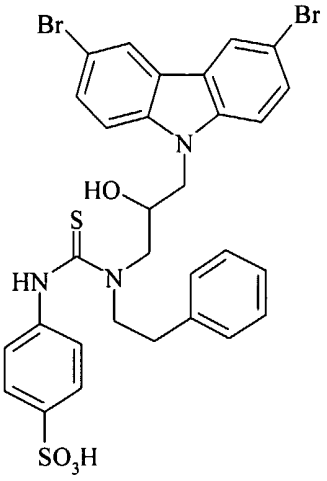
133. Use according to Claim 132 of a compound of the formula Ic:



(Ic)

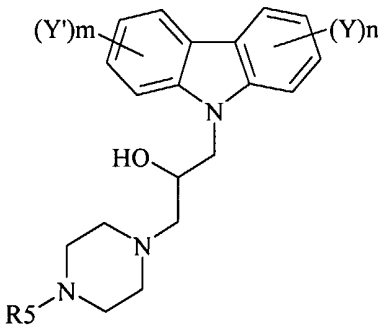
wherein R, R', Y, Y', m and n are as defined in Claim 125.

134. Use according to Claim 133 of the compound herein designated **Compound 3** of the formula:



135. Use according to Claim 126 of a compound of the formula Ia, wherein R2 and R3 together with the N atom to which they are attached form a saturated 6-membered heterocyclic ring optionally containing at least one further heteroatom selected from N, O, and/or S, said at least one further N atom being optionally substituted by R5, wherein R5, Y, Y', m and n are as defined in Claim 125.

136. Use according to Claim 135 comprising a compound of the formula Id:



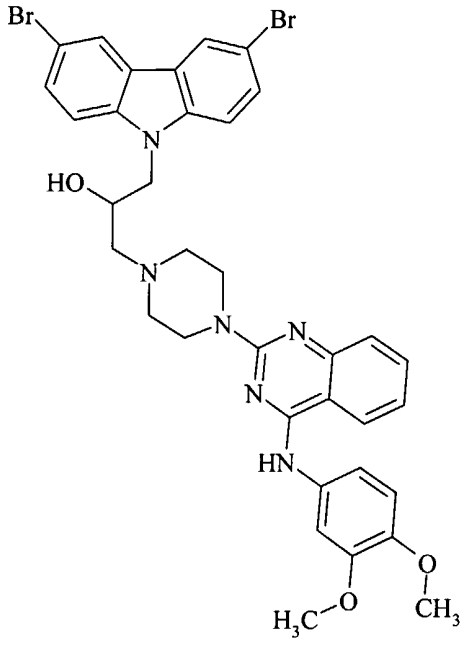
(Id)

wherein R5, Y, Y', m and n are as defined in Claim 125.

137. Use according to Claim 136 of a compound of the formula Id, wherein R5 is a heteroaryl derived from a bicyclic heteroaromatic ring containing two N atoms, preferably quinazolinyl, and being preferably substituted by -NRR', wherein R is hydrogen and R' is C6-C14 aryl optionally substituted by at least one radical selected

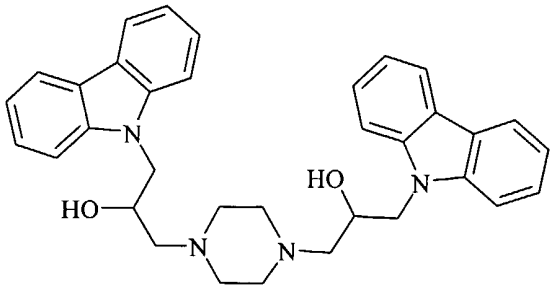
from halogen, -OH, -SH, $-NH_2$, $-SO_3H$, C1-C6 alkyl, C2-C6 alkenyl, C1-C6 alkoxy, or C1-C6 alkylthio, preferably a phenyl radical substituted by two methoxy groups.

138. A pharmaceutical composition according to Claim 137 of the compound herein designated **Compound 4** of the formula:

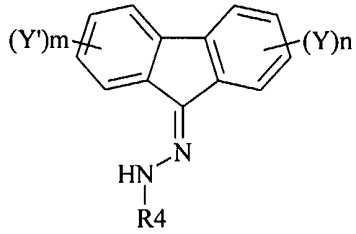


139. Use according to Claim 136 of a compound of formula Id, wherein R5 is C1-C6 alkyl substituted by carbazolyl at the terminal carbon atom and by a further group selected from halogen, -OH, -SH, $-NH_2$, C1-C6 alkoxy or C1-C6 alkylthio; wherein Y, Y', m and n are as defined in Claim 125.

140. Use according to Claim 139 of the compound herein designated **Compound 5** of the formula:



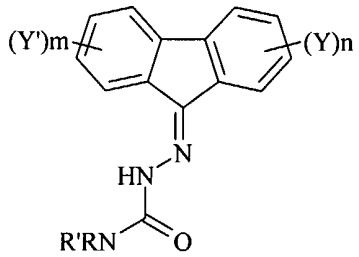
141. Use according to Claim 125 of a compound of formula Ie:



(Ie)

wherein R4, Y, Y', m and n are as defined in Claim 125.

142. Use according to Claim 141 of a compound of formula If:

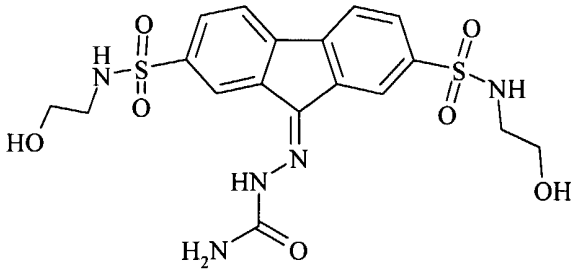


(If)

wherein Y, Y', R, R', m and n are as defined in Claim 125.

143. Use according to Claim 142 of a compound of the formula If, wherein Y and Y' are $-SO_2NRR'$, wherein R and R' are as defined in Claim 125.

144. Use according to Claim 143 of the compound herein designated **Compound 6** of the formula:



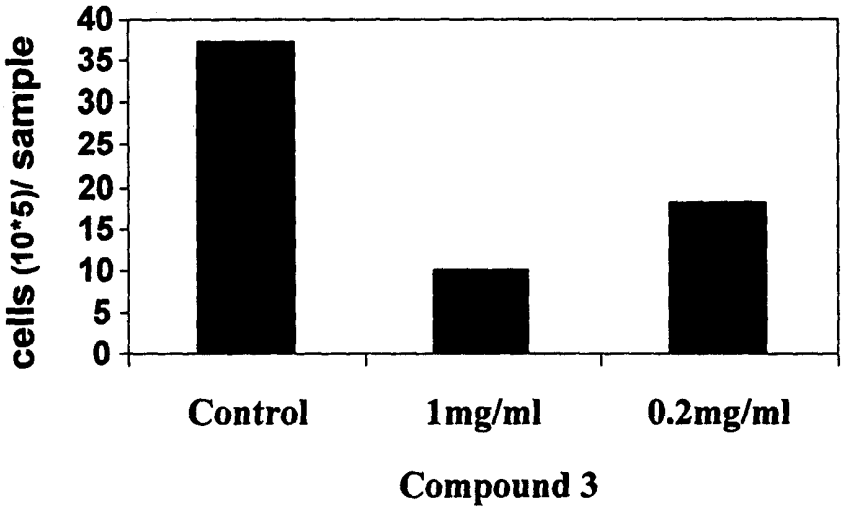

cells (10*5)/ sample
40
35
30
25
20
15
10
5
0
Control
1mg/ml
0.2mg/ml
Compound 3

Fig. 1

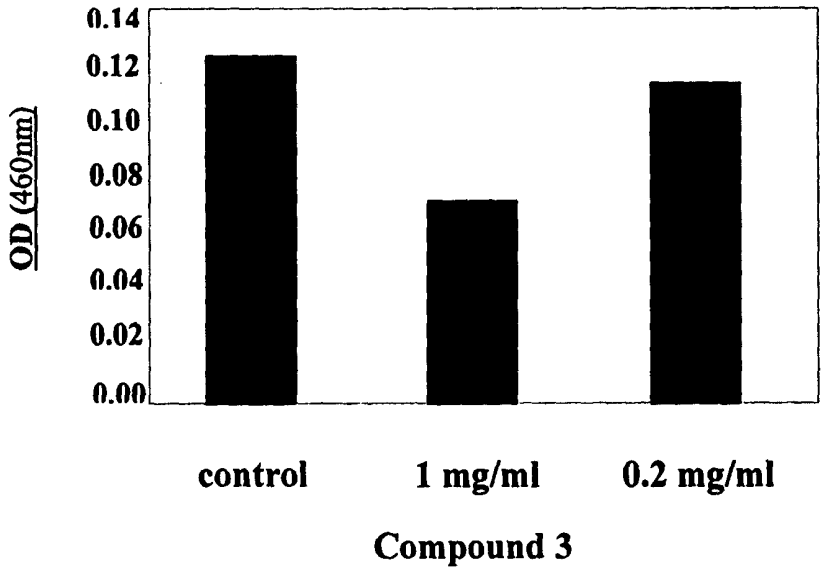

OD (460nm)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
control
1 mg/ml
0.2 mg/ml
Compound 3

Fig. 2

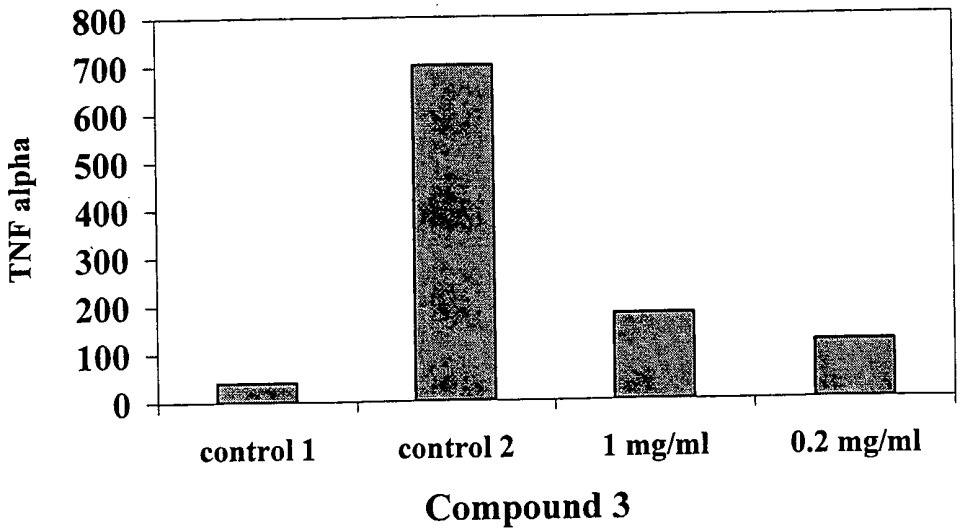

800
700
600
500
400
300
200
100
0
TNF alpha
control 1
control 2
1 mg/ml
0.2 mg/ml
Compound 3

Fig. 3