PHARMACEUTICAL COMPOSITIONS CONTAINING SULPHONIC ACID DERIVATIVES

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ABSTRACT
A naphthalenesulfonic acid or quinolinesulfonic acid of formula (I), wherein A is N or a CR3 formula group, where R8 is H, OH, NR10OR11, independently from one another, where R10 and R11 represent H or C1-C4 alkyl or a group of formula NH—CO—R5, where R5 is C1-C4 alkyl or C6-C10 aryl; R8 and R9 represent H or SO3R3, independently from one another, where R3 is H, ammonium or a cation of an alkali or alkaline-earth metal; R8 is H or OH; and R5, R9 and R7 represent H, an NR10R11 or NH—CO—R12 group, independently from one another; on the condition that (i) at least one of R5 or R9 is SO3R3, and (ii) at least one of R5, R9, R8 or R7 is an NR10R11 or NH—CO—R12 group, or their pharmaceutically acceptable salts; and a pharmaceutically acceptable excipient.
1-NMS Series

FIG. 1A
2-NMS Series

Differential Absorbance

(mM)  (mM)  (mM)

FIG. 1B
Others

Differential Absorbance

C-20

C-21

C-22

C-23

C-24

C-25

(mM)

FIG. 1C
FIG. 3

Vascularization Inhibition by C-9

Vascularization (µm²)

0 0.008 0.08 0.8 8

Inhibitor (mg/kg)
PHARMACEUTICAL COMPOSITIONS CONTAINING SULPHONIC ACID DERIVATIVES

FIELD OF THE INVENTION

This invention refers to pharmaceutical compositions comprising at least one naphthalenesulfonic or quinoline-sulfonic acid derivative and to their therapeutic and/or diagnostic applications.

BACKGROUND OF THE INVENTION

Acid and base fibroblast growth factors (aFGF and bFGF, respectively) are two polypeptides the biochemical and biological properties of which are very similar and are considered paradigmatic for the entire mitogen family (FGFs) they belong to. FGFs typically show a strong affinity for heparin and for the glycoside portion of heparan sulfate, having been shown that the binding to any of these polysulfates is required for the FGFs to recognize their specific tyrosine kinase receptor on the cell surface and to transduce their presence in a cell division signal. FGFs are very important angiogenesis promoters and inappropriate FGF expression could contribute to develop cancers and other types of pathologies. Angiogenesis is a process characterized by the formation of new blood vessels in a tissue or organ taking place in certain normal physiological situations, for example, in wound healing, in fetal and embryonic development and in the formation of the corpus luteum, the endometrial and the placenta. Angiogenesis further constitutes the etiological basis of certain pathological conditions, for example, cancer, diabetic retinopathy, rheumatoid arthritis and the like. Therefore, it is believed that angiogenesis, particularly FGF inhibition activity, may be a form of pharmacological treatment for these diseases, particularly for cancer, and especially for solid tumors. When solid tumors become malignant they induce the formation of dense vascular networks by means of which they receive the supplies required for growth and they eliminate their catabolism products. The collapse of the tumor is caused by preventing the formation of this vascular network due to the lack of nutrients and auto-toxication.


There are numerous antiangiogenic agents for oncology in different stages of clinical development [Krueger et al. (2001) Seminars in Oncology 28, 570-576], a considerable number of which are polypeptides that the organism uses to counteract the effect of the positive angiogenesis regulators [Hagedorn, M. & Bkifalvi, A. (2000) Crit. Rev. Onc. Hemat. 34, 89-110]. However, when said polypeptide s are compared with other compounds having a considerably lower molecular weight, their pharmacological drawbacks are clearly shown. Polysulfonated binaphthyl ureas known as suramin are considered to be potential antiangiogenic and anticancerous agents [Manetti, F. et al. (2000) Curr. Pharm. Des. 6, 1897-1924], at least partially due to their capacity to break the interaction of many growth factors with their membrane receptors, as in the case of FGFs and their tyrosine kinase receptors. Given that it has been shown that heparin breaks aFGF/suramin complexes and counteracts the effect of these polysulfonated ureas, it is thought that suramin acts by means of blocking the FGF heparin binding sites.

Another group of antiangiogenic and anticancerous compounds is formed by the suradistats, a type of synthetic derivatives of sulfonic binaphthlene distyrycine A. These compounds closely interact with FGFs, inhibit the binding of these polypeptides to the cell membrane tyrosine kinase receptors and suppress FGF-induced angiogenesis and neovascularization in vivo.

On the other hand, it has been discovered that 1,3,6-naphthalensulfonic acid (1,3,6-NTS) constitutes a minimum model for aFGF mitogenic activity inhibition by means of suramin and suradistats [Lozano, R. M. et al. (1998) J. Mol. Biol. 281, 899-915]. Said compound (1,3,6-NTS) has been tested, with positive results, both in vitro and in vivo as an aFGF-induced angiogenesis and gloma proliferation inhibitor [Lozano, R. M. et al. (1998) J. Mol. Biol. 281, 899-915; Cuevas, P. et al. (1999) Neurol. Res. 21, 191-194; Cuevas, P. et al. (1999) Neurol. Res. 21, 481-487; Cuevas, P. et al. (1999) Neurosci. Lett. 275, 149-151], suggesting new potential routes for developing new antiangiogenic compounds. The studies by Lozano et al. (mentioned above) also clearly showed that certain naphthalene derivatives containing a reduced number of sulfonate groups per aromatic ring, specifically 1,5-naphthalensulfonic acid (1,5-NTS) and naphthalenesulfonic acid (1-NTS), acted as better aFGF mitogenic activity inhibitors than NTS. However, these compounds showed a clear toxicity at concentrations at which they inhibited aFGF mitogenic activity.

Therefore, it is still necessary to find FGF activity inhibiting compounds and, preferably, compounds having said inhibitory capacity and low cellular toxicity.

The inventors of the present invention have observed that certain sulfonic acid derivatives of formula (I), particularly, certain naphthalenesulfonic acid or quinoline-sulfonic acid derivatives comprising a sulfonic/sulfonate group and optionally a polar group capable of forming hydrogen bonds, such polar group being an amine group for example, located at certain positions in the aromatic ring, are aFGF-induced mitogenic activity inhibitors on fibroblasts in culture as well as angiogenesis inhibitors, and they further inhibit tumor formation in tests with animals without showing signs of toxicity in the tested animals. Therefore, said compounds are potentially useful in treating cancer, especially treating solid tumors. Likewise, said sulfonic acid derivatives are also potentially useful in treating other non-tumor angiogenesis-dependent diseases, for example, rheumatoid arthritis, endometriosis, obesity, arteriosclerosis, restenosis, psoriasis, etc.

Therefore, one aspect of this invention is related to a pharmaceutical composition comprising at least one of said naphthalenesulfonic acid or quinoline-sulfonic acid derivatives, together with a pharmaceutically acceptable excipient.

In another aspect, the present invention is related to the use of said naphthalenesulfonic acid or quinoline-sulfonic acid derivatives in preparing a medicinal product for treating cancer, for treating non-tumor angiogenesis-dependent diseases, for example rheumatoid arthritis, endometriosis, obesity, arteriosclerosis, restenosis, psoriasis, etc.
In other additional aspects, this invention is related to the use of the derivatives of formula (I) in preparing a medicinal product to increase cancer cell sensitivity to chemotherapy or radiotherapy, as well as to their use in preparing a diagnostic kit for diseases or conditions linked to FGFR biological activity.

Some 2-naphthalenesulfonyl acid derivatives are known, for example 5-amino-2-naphthalenesulfonic acid (Aldrich) is used in the dye industry. The sodium salt of said 5-amino-2-naphthalenesulfonic acid is also known [J. Chem. Soc. 3172 (1959); Helv. Chim. Acta 45, 1608 and 1611 (1962)], as well as sodium 5-acetylamino-2-naphthalenesulfonate [J. Med. Chem. 38 (8), 1344-1354 (1995); J. Med. Chem. 40(6), 920-929 (1997)].

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A, 1B and 1C are a set of graphs representing the differential absorbance (y-axis) for the concentration (x-axis) of the assayed compound, and they illustrate the effect of increasing concentrations of the C-1 to C-25 compounds: (A) C-1 to C-7; (B) C-8 to C-19; (C) C-20 to C-25; (see Example 1 and Table 1) on aFGR-induced mitogenesis on fibroblasts in culture in minimum medium supplemented with myo-inositol hexa-sulphate (MHS) both in the absence of aFGR (a) and in the presence of aFGR (b).

FIG. 2 shows illustrative photographs of the antiangiogenic effect of the C-9 compound (see Example 2), which show histological sections representative of sponge implants soaked in PBS (A) and in PBS+aFGR (B and C). The histological sections represented in photograph (C) shows that sponges implanted in mice intraperitoneally treated with 8 mg/kg of the C-9 compound. The arrow in (C) indicates a vessel containing erythrocytes and leukocytes. Note that the exuberant cellular infiltration shown in (B) disappears in the sponge implants soaked in aFGR and intraperitoneally treated with the C-9 compound. The photographs are shown with an original amplification of a magnification of 50 (50x).

FIG. 3 shows a bar graph illustrating vascularization inhibition by the C-9 compound at different concentrations (see Example 2). The inhibitor (C-9) concentration is shown on the x-axis while the measurement of the surface area containing erythrocytes (vascularization) is represented on the y-axis.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the invention is related to a pharmaceutical composition comprising:

(i) at least one compound of formula (I)

(i) at least one compound of formula (I)

wherein:

A is N or a CR₅ group;

R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ represent H, SO₂R⁹, OR¹₀, CO₂R¹₀, NR¹⁰R¹¹, NH—CO—R¹² or C₆H₅ alky, independently from one another;

R² is H, ammonium or a cation of an alkali or alkaline-earth metal;

R¹⁰ and R¹¹ represent H, C₆H₅ alky or C₆H₅C₁₀ ary, independently from one another;

R¹² OH, C₆H₅ alky or C₆H₅C₁₀ ary;

on the condition that:

(a) at least one of R¹ to R⁸ is SO₂R⁹, and

(b) at least one of the remaining R¹ to R⁸ is an OR¹₀, CO₂R¹₀, NR¹⁰R¹¹, NH—CO—R¹² group.

where R⁵, R¹⁰, R¹¹ and R¹² have the previously mentioned meanings;

or one of their pharmaceutically acceptable salts or prodrugs; and

(ii) at least one pharmaceutically acceptable excipient.

The term “C₆H₅ alky” as it is used in this description refers to a radical derived from a saturated, linear or branched hydrocarbon of 1 to 6 carbon atoms, for example methyl, ethyl, isopropyl, etc.

The term “C₆H₅C₁₀ ary” as it is used in this description refers to a radical derived from an aromatic hydrocarbon of 6 to 10 carbon atoms, for example phenyl, naphtyl, etc.

The term “pharmaceutically acceptable salts or prodrugs” includes any salt, ester, amide, solvate, hydrate, polymorphic form, etc. susceptible to be used in pharmaceutical forms and which is capable of directly or indirectly providing a compound of formula (I) in vivo. The nature of the salt is not critical as long as it is pharmaceutically acceptable.

The salts of the compound of formula (I) can be obtained from organic or inorganic acids or bases by conventional methods well known by a person skilled in the art, by reacting the suitable acid or base with the compound of formula (I).

The prodrugs can be obtained, for example, from a free hydroxyl group converted into an ester or from an amino group converted into an amide by means of processes well known by a person skilled in the art, by reacting the compounds of formula (I) with a carboxylic acid, an anhydride or an acyl halide in the presence of a base or catalyst.

Included among the compounds of formula (I) are naphthalenesulfonic acid derivatives [compounds of formula (I) in which A is CR₅] and quinolinesulfonic acid derivatives [compounds of formula (I) in which A is nitrogen].

In a particular embodiment, the compound of formula (I) is a naphthalenesulfonic acid derivative belonging to the 2-NMS series (2-naphthalenesulfonic acid derivatives) wherein:

A is CR₅;

R¹ is H or C₆H₅ alky;

R² is SO₂R⁹;

R³ is H, OH or C₆H₅ alky;

R⁴, R⁵, R⁶, R⁷ and R⁸ represent H, OR¹₀, CO₂R¹₀, NR¹⁰R¹¹, NH—CO—R¹² or C₆H₅ alky, independently from one another;

R² is H, ammonium or a cation of an alkali or alkaline-earth metal;

R¹⁰ and R¹¹ represent H, C₆H₅ alky or C₆H₅C₁₀ ary, independently from one another;

R¹² is OH, C₆H₅C₆H₅ or C₆H₅C₁₀ ary;
on the condition that at least one of R², R³, R⁶, R⁷ or R⁸ is OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹², where R¹⁰, R¹¹ and R¹² have the previously mentioned meanings.

Preferred compounds of the 2-NMS series include compounds of formula (I) wherein:

A is CR₄, where R₄ is H or OH;

R² is H;

R⁵ is SO₂R⁶, where R⁶ is H or sodium;

R⁷ is H; and

R⁸, R⁹, R¹⁰ and R¹¹ represent H, OH, OCH₃, COOH, NH₂, NHCOCH₃, NHCOCH₂H₃, NHCH₃, or N(CH₃)₂, independently from one another;

on the condition that at least one of R², R⁶ or R⁸ is OH, OCH₃, COOH, NH₂, NHCOCH₃, NHCOCH₂H₃, NHCH₃ or N(CH₃)₂.

Illustrative examples of compounds of the 2-NMS series include:

sodium 5-amino-2-naphthalenesulfonate,

sodium 5-acetylamino-2-naphthalenesulfonate,

sodium 5-benzoylamino-2-naphthalenesulfonate,

sodium 8-amino-2-naphthalenesulfonate,

sodium 8-acetylamino-2-naphthalenesulfonate, and

di-3-hydroxy-6-amino-2-naphthalenesulfonate.

Within this 2-NMS series, the sodium 5-amino-2-naphthalenesulfonate, sodium 5-acetylamino-2-naphthalenesulfonate and sodium 4-hydroxy-6-amino-2-naphthalenesulfonate compounds are especially preferred, and particularly sodium 5-amino-2-naphthalenesulfonate, given that they are potent aFGF-induced mitogenic activity inhibitors, aFGF-induced angiogenic activity inhibitors, and furthermore they are not toxic for fibroblasts in culture.

In another particular embodiment, the compound of formula (I) is a naphthalenesulfonic acid derivative belonging to the 1-NMS series (1-naphthalenesulfonic acid derivatives) wherein:

A is CR₄,

R² is SO₂R⁶;

R⁵ is H or C₆H₅CH₂alkyl;

R⁷ is H, OH or C₆H₅CH₂alkyl;

R⁸, R⁹, R¹⁰ and R¹¹ represent H, OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹² or C₆H₅CH₂alkyl, independently from one another;

R² is H, ammonium or a cation of an alkali or alkaline-earth metal;

R¹⁰ and R¹¹ represent H, C₆H₅CH₂alkyl or C₆H₅C₁₀aryl, independently from one another;

R¹² is OH, C₆H₅CH₂alkyl or C₆H₅C₁₀aryl;

on the condition that at least one of R², R³, R⁶ or R⁸ is OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹², where R¹⁰, R¹¹ and R¹² have the previously mentioned meanings.

Preferred compounds of the 1-NMS series include compounds of formula (I) wherein:

A is CR₄,

R² is SO₂R⁶, where R⁶ is H or sodium;

R⁷ is H;

R⁸ is H; and

R⁹, R¹⁰, R¹¹ and R¹² represent H, OH, OCH₃, COOH, NH₂, NHCOCH₃, NHCOCH₂H₃, NHCH₃, or N(CH₃)₂, independently from one another;

on the condition that at least one of R², R³, R⁶, R⁷ or R⁸ is OH, OCH₃, COOH, NH₂, NHCOCH₃, NHCOCH₂H₃, NHCH₃ or N(CH₃)₂.
composition is useful for administration and/or application in or on the body of a mammal, preferably a human being.

[0101] The use of the compounds of formula (I) in preparing said pharmaceutical composition is a further aspect of this invention.

[0102] The compounds of formula (I) can be administered to treat cancer, especially solid tumors or, alternatively, non-tumor angiogenesis-dependent diseases, for example rheumatoid arthritis, endometriosis, obesity, arteriosclerosis, restenosis or psoriasis, by any means allowing contact between the compound of formula (I) and the action site thereof on the body of a mammal. The therapeutically effective amount of the compound of formula (I) that must be administered as well as its dosage for treating a pathological condition with said compounds of formula (I) and/or pharmaceutical compositions of the invention will depend on several factors, including among these factors the disease to be treated, age, patient condition, seriousness of the disease, the administration method and frequency, the compound of formula (I) to be used, etc.

[0103] Pharmaceutical compositions containing the compounds of formula (I) provided by this invention may be presented in any administration form that is considered appropriate, for example, a solid or liquid, and they may be administered by any suitable method, for example orally, parenterally, rectally or topically, to which end they will include the pharmaceutically acceptable excipients necessary for the formulation of the desired administration form. A review of different dosage forms for administering medicinal products and the excipients necessary for obtaining them can be found, for example, in the “Tratado de Farmacia Galénica”, C. Faulii Trillo, 1993, Luzín 5, S.A. Ediciones, Madrid.

[0104] Said compounds of formula (I) can be used in preparing a medicinal product for treating cancer, especially solid tumors [Rosti G. et al. (2002) *Crit. Rev. Oncol. Hematol.* 41, 129-240], for example breast cancer, prostate cancer, etc. Alternatively, the compounds of formula (I) can be used in preparing a medicinal product for treating other angiogenesis-dependent diseases, for example skin diseases, rheumatoid arthritis, endometriosis, obesity, arteriosclerosis, restenosis, psoriasis, etc.

[0105] Other active agents, for example anticancerous agents, can be used together with a compound of formula (I). The different active agents can be administered simultaneously in a single formulation or in several, or sequentially.

[0106] The use of the derivatives of formula (I) in preparing a medicinal product for increasing cancer cell sensitivity to chemotherapy or radiotherapy as well as their use in preparing a diagnostic kit for diseases or conditions linked to FGF biological activity are other further aspects of the present invention.

[0107] The following examples illustrate the invention and must not be considered as limiting on the scope thereof.

**EXAMPLE 1**

aFGF-Induced Mitogenic Activity Inhibition

[0108] The works of Lozano et al. [Lozano, R. M. et al. (1998) *J. Mol. Biol.* 281, 899-915] clearly showed that certain naphthalenesulfonic compounds, such as 1,3,6-naphthalenetrisulfonic acid (1,3,6-NTS), 1,5-naphthalenedisulfonic acid (1,5-NDS) and 1-naphthalenesulfonic acid (1-NMS) are aFGF-induced mitogenic activity inhibitors. 1-NMS was a more potent inhibitor than 1,3,6-NTS and 1,5-NDS. However, 1-NMS and 1,5-NDS are toxic at concentrations at which they inhibit aFGF-induced mitogenic activity.

[0109] Now, for the purpose of finding other arylsulfonic compounds with better potential pharmacological profiles, an iterative test-error process has been followed by means of which certain functional groups with a different steric volume or load were placed in different positions of different aromatic compounds, testing their aFGF-induced mitogenesis inhibitory activity on fibroblasts in culture in the presence of an aFGF activator. The materials used as starting materials in the search for said arylsulfonic compounds that inhibit aFGF-induced mitogenic activity were 1-naphthalenesulfonic acid (1-NMS), which gave rise to the series called 1-NMS, and 2-naphthalenesulfonic acid (2-NMS), which gave rise to the series called 2-NMS. Like 1-NMS, 2-NMS inhibits aFGF-induced mitogenic activity (although it does so at lower concentrations) and is toxic for quiescent fibroblasts within the concentration range in which it inhibits aFGF-induced mitogenic activity. Other arylsulfonic compounds were also tested (see Table 1).

1.1 Tested Compounds

[0110] Table 1 lists all the tested compounds, while their preparation is described in Example 4.

<table>
<thead>
<tr>
<th>Code</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-NMS Series</td>
</tr>
<tr>
<td>C-1</td>
<td>1-naphthalenesulfonic acid</td>
</tr>
<tr>
<td>C-2</td>
<td>sodium 2-acetylamino-1-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-3</td>
<td>sodium 4-amino-1-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-4</td>
<td>sodium 4-acetylamino-1-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-5</td>
<td>sodium 4-benzoylamino-1-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-6</td>
<td>sodium 5-dimethylaminoo-1-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-7</td>
<td>sodium 4-amino-5-hydroxy-1-naphthalenesulfonate</td>
</tr>
<tr>
<td></td>
<td>2-NMS Series</td>
</tr>
<tr>
<td>C-8</td>
<td>2-naphthalenesulfonic acid</td>
</tr>
<tr>
<td>C-9</td>
<td>sodium 5-amino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-10</td>
<td>sodium 5-acetylamino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-11</td>
<td>sodium 5-benzoylamino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-12</td>
<td>sodium 2-(((6-oxo-1,2-naphthyl)(amino)carbonyl)benzoic acid</td>
</tr>
<tr>
<td>C-13</td>
<td>sodium 5-(((6-oxo-1,2-naphthyl)(amino)carbonyl)amino)2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-14</td>
<td>sodium 8-amino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-15</td>
<td>sodium 8-acetylamino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-16</td>
<td>sodium 8-benzoylamino-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-17</td>
<td>sodium 6-amino-4-hydroxy-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-18</td>
<td>sodium 7-amino-4-hydroxy-2-naphthalenesulfonate</td>
</tr>
<tr>
<td>C-19</td>
<td>sodium 7-acetylamino-4-hydroxy-2-naphthalenesulfonate</td>
</tr>
<tr>
<td></td>
<td>Others</td>
</tr>
<tr>
<td>C-20</td>
<td>sodium 4-aminobenzensulfonate</td>
</tr>
<tr>
<td>C-21</td>
<td>sodium 4-(aminomethyl)benzensulfonate</td>
</tr>
<tr>
<td>C-22</td>
<td>sodium 4-(aminomethyl)benzenesulfonate</td>
</tr>
<tr>
<td>C-23</td>
<td>sodium 6-quinolinesulfonate</td>
</tr>
<tr>
<td>C-24</td>
<td>sodium 8-quinolinesulfonate</td>
</tr>
<tr>
<td>C-25</td>
<td>2-[(dimethylamino)methyl]-1H-indole-5-sulfonic acid</td>
</tr>
</tbody>
</table>
zano, R. M. et al. (1998) J. Mol. Biol. 281, 899-915], briefly consisting in placing increasing concentrations of the compound to be tested in contact with fibroblasts in culture, for 60 hours, in minimum medium supplemented with myo-inositol hexafluoride (MIES) (20 μg/mL) in the absence and in the presence of aFGF (0.32 ng/mL) [the aFGF of 139 amino acid residues is not the concentration of the compound tested]. The absence of floating cells or other cell rupture signals in said mitogenic tests also indicates that many of the tested compounds are not toxic for fibroblasts in the absence or in the presence of aFGF. On the other hand, since aFGF-induced mitogenesis inhibition by means of the use of the tested compounds may be compensated by raising the heparin concentrations (an aFGF mitogenic activity activator) [data not shown], it can be asserted that said compounds are aFGF-induced mitogenic activity inhibitors which act by blocking the binding between the protein and its activator.

1.3.3 Results

0112 The obtained results are shown in Fig. 1, which summarizes the results of the aFGF-induced mitogenesis inhibition tests on fibroblasts in culture for all the tested compounds (o), together with the toxicity analyses for said compounds for quiescent fibroblasts ( ).

0113 Briefly, said results clearly show that virtually all the tested compounds progressively inhibit aFGF-induced mitogenesis at concentrations exceeding 1 nM (see the decrease in the differential absorbance units (o) as the concentration of the test compound increases). The absence of floating cells or other cell rupture signals in said mitogenic tests also indicates that many of the tested compounds are not toxic for fibroblasts in the absence or in the presence of aFGF. On the other hand, since aFGF-induced mitogenesis inhibition by means of the use of the tested compounds may be compensated by raising the heparin concentrations (an aFGF mitogenic activity activator) [data not shown], it can be asserted that said compounds are aFGF-induced mitogenic activity inhibitors which act by blocking the binding between the protein and its activator.

0114 More specifically, with regard to the 1-NMS series, it can be observed that the C-1, C-2, C-4, C-6 and C-7 compounds are aFGF-induced mitogenic activity inhibitors even though they show a certain toxic effect on quiescent fibroblasts within the concentration range in which they inhibit aFGF mitogenic activity. Compound C-7 is the one that shows the lowest IC₅₀ value, though the opposite effect is observed at concentrations exceeding 0.2 nM (that is, the proliferation of quiescent fibroblasts cultured in the absence of aFGF significantly increases). Compounds C-3 and C-5 show a low aFGF-induced mitogenesis inhibitory activity within the tested concentration range although they are not toxic for the quiescent fibroblasts within said concentration range.

0115 With respect to the 2-NMS series, it is observed that compound C-8 inhibits aFGF-induced mitogenic activity at lower concentrations than compound C-1; however, it is also toxic for quiescent fibroblasts within the concentration range in which it inhibits aFGF-induced mitogenic activity. Compounds C-12 and C-13 are also effective aFGF-induced mitogenesis activity inhibitors, although they have a high mitogenesis inducer activity even in the absence of aFGF. However, this last effect does not occur in compound C-9 which had the second best IC₅₀ value, nor was the toxic effect described for compounds C-1 and C-8 on the quiescent fibroblasts observed, at least in the concentration range in which it inhibits aFGF-induced mitogenic activity. Two other compounds with an inhibitory activity that is slightly less than that of C-9 have been found, specifically compounds C-10 and C-17 which, like C-9, were innocuous for the quiescent fibroblasts. Compounds C-15 and C-16 are aFGF-induced mitogenic activity inhibitors and are innocuous for quiescent fibroblasts within the tested concentration range. Compounds C-11, C-14 and C-18 are also aFGF-induced mitogenic activity inhibitors, although compound C-14 shows a certain toxic effect on quiescent fibroblasts within the tested concentration range. Compound C-19 shows a certain tumorigenic effect on quiescent fibroblasts. The inhibitory activity IC₅₀ of compound C-9 is 265 μM, exceeding that of 1,3-bis-NTS by more than two orders of magnitude [Lozano, R. M. et al. (1998) J. Mol. Biol. 281, 899-915] and was chosen to evaluate its use as an angiogenesis inhibitor in vivo by means of a conventional angiogenesis test in mice (Example 2) and as an inhibitor of tumor formation in animals (Example 3).

0116 Compounds C-20, C-22 and C-24 showed little aFGF-induced mitogenesis inhibitory activity at the tested concentrations, although they were innocuous for quiescent fibroblasts. Compound C-23 is a potent aFGF-induced mitogenic activity inhibitor although it shows a toxic effect on quiescent fibroblasts within the concentration range in which it inhibits aFGF-induced mitogenic activity. Compounds C-21 and C-25 show a certain tumorigenic effect on quiescent fibroblasts.

0117 The obtained results allow establishing a series of stereochemical guidelines that improve the potential pharmacological applicability of arylsulfonic derivatives, particularly naphthalenesulfonic acid derivatives, in angiogenesis, specifically:

0118 a) the sulfonate group must preferably be located in position 2 of naphthalene since the 2-NMS series derivatives are generally more potent aFGF-induced mitogenic activity inhibitors than the 1-NMS series derivatives;

0119 b) among the 2-NMS series derivatives, those containing an amino group in position 5 or 6 of the naphthalene ring are better aFGF-induced mitogenic activity inhibitors (see C-9 and C-17 vs. C-14 and C-18, respectively); and

0120 c) the size of the functional group present in position 5 seems to have certain relevance since the substitution of an amino group by a small amide does not substantially alter the inhibitory activity (see C-9 vs. C-10); however, a significant reduction is observed when a bulky amide is introduced (see C-9 vs. C-11 and C-13).

0121 Although the naphthalene ring seems to constitute the nucleus of the most suitable inhibitor, it is to be expected that quinolines with sulfonic groups in position 2 and amine groups in position 5 or 6 can also be used.

0122 Compound C-7 constitutes an exception to the previously mentioned rules since it is the compound that showed the best aFGF-induced mitogenic activity inhibitory activity, although it is toxic for quiescent fibroblasts.

EXAMPLE 2

Angiogenesis Inhibition Test in Mice

0123 A standard angiogenesis test was performed with mice in order to evaluate the C-9 compound as an angiogenesis inhibitor.

0124 Pathogen-free C57/B16 mice (Charles River, Spain) weighing 22-24 g were used. The animals were kept in plastic cages under controlled temperature and humidity conditions; they had water and food ad libitum and a schedule of 12 hours of daylight and darkness was maintained. The guidelines on animal welfare of the NIH and the European Union were followed meticulously.

0125 10 mm long sterile gelatin sponge cubes (Curaspon Dental, Clnieded Holding, Zwanenburg, Holland) were subcutaneously implanted in the backs of the mice after inducing intrapertoneal anesthesia [Cuevas, P. et al. (1999) Neur.
Res. 21, 191-194]. The animals were distributed into 2
groups: Group A (n=10) formed by animals in which
sponges loaded with 200 μL of phosphate buffered saline
(PBS) containing 29 μg/mL of heparin were implanted; and
Group B (n=40) formed by animals in which sponges soaked
with the same solution as that of Group A but containing 10
μg/mL of αFGF were implanted. The skin was sutured after
implanting the sponge in the subcutaneous bursa. The mice in
Group B were randomly divided into four groups (n=10) that
received 0.008, 0.08, 0.8 and 8 mg/kg of compound C-9,
respectively, by means of intraperitoneal injection of 200 μL
of PBS 24 hours after surgical treatment [Pesenti, E. et al.
Neurol. Res. 21, 191-194]. All procedures were performed
under sterile conditions.

[0126] In order to evaluate angiogenesis, the mice were
again anesthetized as previously described, and the sponges
were surgically extracted, which were treated to perform
histological studies by means of conventional methods [Cuevas,
P. et al. (1999) Neurol. Res. 21, 191-194] 7 days after the
implants. Neovascularization was quantified by means of a
morphometric computer program connected to a microscope.
The growth of new vessels inside the sponges was evaluated
by means of the measurement of the surface area containing
erythrocytes. Neovascularization was analyzed in 4 prede
termined visual fields of 5 different sections at a magnification
of 10. The statistical analyses were performed using Student’s
t-Test.

[0127] Figs. 2 and 3 illustrate the neovascularization inhibi
tion induced by means of αFGF in gelatin sponges
implanted subcutaneously in mice treated with C-9. The calcu
lation of the number of new blood vessels per unit of area
with different doses of the C-9 compound clearly showed that
neovascularization inhibition was already assessable in mice
that received 0.008 and 0.08 mg/kg and was virtually com
plete with doses exceeding 0.8 mg/kg. This data clearly
shows that C-9 is a considerably more effective neovascu
larization inhibitor than suraminases and 1,3,6-NTS since in these
last 2 cases, it seems that concentrations of about 200 mg/kg
are required to reach substantial neovascularization inhibi
results were obtained when αFGF was substituted with bFGF
(data not shown). The highest tested C-9 concentrations did
not cause any toxic death or apparent alterations in the ani
mals, nor did it cause weight changes.

EXAMPLE 3

Angiogenesis and Tumor Growth Inhibition in Ani
mals with Implanted Tumors

[0128] 3.1 Test with Albino Rabbıts

[0129] Albino New Zeland rabbits (3±0.5 kg, male
and female in equal numbers) were used and were
implanted with 5 μL of a glioma C6 cell suspension
[ATCC CCL-107] sub-epithelially injected into the
corner with a 10 μL Hamilton syringe using a surgi
cal microscope. The injection is located at
2 mm from the limbal margin of the cornea. At the same
time, an ophthalmic minipump was subcutaneously implanted
in the neck region for the purpose of assuring continuous infu
sion of the compound to be tested (C-9) or of a saline solution
(control). A catheter guided the solution to the sclera at a
constant rate of 0.2 mg/h. It is thus calculated that a con
centration of about 0.003 mg/mL of the compound to be tested or
saline solution is maintained in the cornea. Treatment was
maintained for 14 days. The corneas were eliminated at the
end of each experiment and at fixed intervals after each treat
ment and processed for histological examination.

[0130] A very positive apoptosis index was observed (apo
ptosis increases with the number of treatment days) and a very
reduced corneal neovascularization area (in comparison with
the untreated animals). The following could be confirmed by
means of microscopic observation: a lower number of glioma
C6 cells, a higher number of apoptotic bodies, the presence of
broken blood vessels and the presence of apoptotic endothel
ial nuclei in treated animals.

3.2 Tests with Rats

[0131] A test similar to the one described in Example 3.1 was
performed with rats, implanting the C6 glioma cells in
the crural area of the brain and the osmotic micropump in
the back of the rats. A catheter led the treatment solution that
contained the C-9 compound or the saline solution to the area
of the glioma implant. Coronal sections of the brain of the rats
thus treated were obtained and compared with coronal sec
tions of the brain of untreated rats. The analysis was done by
means of NMR spectroscopy imaging.

[0132] In this case, it could be seen that the tumor mass
significantly decreased. Intratumor vascularization inhibi
tion was also confirmed (by a factor of six). The “apoptotic index”
was also obtained, which clearly showed that the C-9 com
pound was a potent apoptosis inhibitor in experimental gliomas.

EXAMPLE 4

Preparation of the Tested Compounds

[0133] Unless otherwise indicated, the tested compounds
were obtained from commercially available sources and used
with no further purification. The structure and purity of the
prepared compounds was confirmed by means of NMR. The
solvents were dried and purified using conventional methods.
Particularly compounds C-1 and C-3 and the acid precur
sors of compounds C-6, C-7, C-9, C-14, C-17, C-18, C-20, C-21
and C-22 are commercial products. The remaining tested
compounds were obtained by means of conventional meth
ods, as briefly described below.

[0134] Generally, all the reactions were performed in dry
flasks fitted with a glass stopper or with a rubber septum under
positive argon pressure, unless otherwise indicated. The liq
uids sensitive to air and to humidity and the solutions were
transferred by syringe or stainless steel cannula. The flash
chromatography column was prepared using silica gel of
230-400 mesh diameter. Thin layer chromatography was car
ried out in Kieselgel 60 F254 (Merck). The detection was first
carried out by means of UV (245 mm) and then, after treat
ment with a 20% aqueous sulfuric acid solution (200 mL), in
acetic acid (800 mL). Anhydrous MgSO4 or Na2SO4 were
used to dry the organic solutions. The elimination of the
solvents was carried out under vacuum conditions with a
rotary evaporator.

Sodium Salts

[0135] The sodium salts were prepared by adding the
required amount of 0.1 N sodium hydroxide to a suspension
of the corresponding sulfonic acid derivative in water. Evapo-
ration of the solvents provided the sodium salts.

Acetylamide Derivatives (C-4, C-10, C-15, C-19)

[0136] Acetylamide derivatives were prepared by heating a
suspension of the corresponding sodium salts (C-3, C-9, C-14
and C-18) in acetic anhydride for 4 hours at 90°C. The
solvent was evaporated, producing the acetylamide deriva-
tives.

Benzoylamide Derivatives (C-5, C-11, C-16)

[0137] A slight excess of benzoyl chloride (1.2 equivalents)
was added to a solution of the corresponding sulfonic acid in
pyridine. The solvent was evaporated after 30 minutes and the
residue was chromatographed on silica gel column (1:9
MeOH:CH2Cl2 v:v). The purified product was transformed into
its corresponding sodium salts.

Compound C-2

[0138] Concentrated H2SO4 (0.32 ml) was added to 2-methyl-
naphthalene (2.8 mmoles). The mixture was heated in an
oil bath at 80°C for 3 hours. The solution was cooled, poured
over ice and basified by means of adding 1N NaOH. The
product was separated under cold conditions and isolated by
filtration.

Compound C-8

[0139] Ion exchange resin (Amberlite) (H+form) was
added to a solution of sodium 2-naphthalenesulfonate in
methanol. The mixture was stirred overnight and filtered
and the solvent was evaporated. The residue was lyophilized
yielding 2-naphthalenesulfonic acid.

Compound C-12

[0140] An excess of phthalic anhydride (1.3 equivalents)
was added to a solution of C-9 (0.6 mmoles) in methanol.
The mixture was stirred overnight and evaporated. The residue
was chromatographed on silica gel column (3:7 MeOH:CH2Cl2
v:v) to produce the required product.

Compound C-13

[0141] Adipoyl chloride (0.5 equivalents) was added to a
suspension of C-9 (0.1 mmoles) in pyridine. The stirring was
continued for 48 hours and the solvent was evaporated. The
residue was digested with a small volume of methanol and
the solid that was separated was collected and characterized.

Compound C-23

[0142] Concentrated sulfuric acid (61 mL) is added in por-
tions over a mixture of sulfamic acid (75 g, 390 mmoles),
glycerol (103 mL, 1,41 mmoles) and nitrobenzene (20 mL, 195
mmoles), then the reaction mixture is carefully heated to
about 80°C. The exothermic process is then triggered, sponta-
naneously evolving up to 120-140°C. Then the external heat-
ing is stopped and once the reaction is stabilized (after about
30 minutes), it is heated again externally up to 140-145°C for
4 hours. After this time has elapsed, the reaction mixture is
cooled and poured over 500 ml of a water-ice mixture, keep-
ing it in the refrigerator at 4°C for 4 days. Once this time has
elapsed, the little solid generated is eliminated and the filtra-
tion waters are diluted 6 times their own volume in water and
treated with BaCO3, again filtering the solid residue gener-
ated. The filtration waters are treated with 20% NaOH up to a
pH of 11. The solvent of an aliquot of these alkali waters is
eliminated under vacuum conditions and is purified by means
of chromatography column on silica gel (Merck, Kieselgel H
F254, 7:3 AcOEt/MEOH), isolating an analytically pure
sample of the sodium salt.

Compound C-24

[0143] Quinoline (9 mL, 76.2 mmoles) was added drop-
wise over a flask containing H2SO4 SO3 (20% SO3, 30 mL).
The temperature is maintained under 50°C during the entire
process. Once the addition has ended, the mixture is heated at
140°C for 2 hours. The cooled raw product is then poured
over a water-ice mixture, isolating 8-sulfoquinoline as a white
solid after abundant washing with water. Once dried under
vacuum conditions, 8.25 g of acid (>50%) are quantified. This
acid was used to prepare the corresponding sodium salt as
previously indicated.

Compound C-25

[0144] 6.45 mmoles of gramine were dissolved in 10 mL of
concentrated H2SO4 and stirred for 5 minutes at 0°C. The
solution was poured over ice and basified by means of adding
1N NaOH. This aqueous solution was extracted with dichlo-
rromethane, neutralized by means of adding 1N H2SO4 and
evaporated. The residue was digested with hot ethanol and the
solids separated by filtration. The ethanol solution was evapo-
rated and the residue was subjected to column chromatog-
raphy (ethanol) in order to produce the desired compound.

What is claimed is:

1. A method for preventing or treating a disease or condi-
tion in which FGF biological activity is involved compris-
ing the step of administering to a subject a pharmaceutical
composition comprising a naphthalenesulfonic acid derivative of formula (I)

$$\text{(I)}$$

wherein:
A is a CR8 group;
R1, R2, R3, R4, R5, R6, R7 and R8 represent H, SO3R, OR10,
CO2R10, NR11R11, NH—CO—R12, or C1—C10 alkyl,
indipendently from one another;
R2 is H, ammonium or a cation of an alkali or alkaline-earth
metal;
R12 and R11 represent H, C1—C10 alkyl or C6—C10 aryl,
independently from one another;
R13 is OH, C1—C3 alkyl or C3—C10 aryl;
on the condition that:
(a) at least one of R2 to R8 is SO3R8, and
(b) at least one of the remaining R1 to R8 is an OR10,
CO2R10, NR10R10, R10R12, NH—CO—R12 group,
or one of their pharmaceutically acceptable salts or pro-
drugs; and at least one pharmaceutically acceptable
excipient, thereby preventing or treating a disease or condition in which FGFR biological activity is involved.

2. The method according to claim 1, wherein the disease or condition is an angiogenesis-dependent disease chosen from rheumatoid arthritis, endometriosis, obesity, atherosclerosis, restenosis or psoriasis.

3. The method according to claim 1, wherein the disease or condition is any type of cancer.

4. The method according to claim 1, wherein:
   A is CR²⁸; R² is H or C₁₋₅ alkyl;
   R is SO₂R⁹;
   R³ is H, OH or C₁₋₅ alkyl;
   R⁴, R⁵, R⁶, R⁷ and R⁸ represent H, OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹² or C₁₋₅ alkyl, independently from one another;
   R⁹ is H, ammonium or a cation of an alkali or alkaline-earth metal;
   R¹⁰ and R¹¹ represent H, C₁₋₅ alkyl or C₆₋₁₀ aryl, independently from one another;
   R¹² is OH, C₁₋₅ alkyl or C₆₋₁₀ aryl;
   on the condition that at least one of R⁴, R⁵, R⁶, R⁷ or R⁸ is OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹².

5. The method according to claim 1, wherein:
   A is CR²⁸, where R² is H or OH;
   R is H; and
   R⁴, R⁵, R⁶ and R⁷ represent H, OH, OCH₃, COOH, NH₂, HCOCH₃, HCONH₂, NHCH₃ or N(CH₃)₂, independently from one another;
   on the condition that at least one of R⁴, R⁵, R⁶ or R⁷ is OH, OCH₃, COOH, NH₂, HCOCH₃, HCONH₂, NHCH₃ or N(CH₃)₂.

6. The method according to claim 1, wherein the compound of formula (I) is selected from the group consisting of sodium 5-amino-2-naphthalenesulfonate, sodium 5-acetylaminom-2-naphthalenesulfonate, sodium 5-benzoylaminom-2-naphthalenesulfonate, sodium 5-acetylaminom-2-naphthalenesulfonate, sodium 5-benzoylaminom-2-naphthalenesulfonate, sodium 8-amino-2-naphthalenesulfonate, 8-acetylamino-2-naphthalenesulfonic acid, sodium 4-hydroxy-6-amino-2-naphthalenesulfonate and mixtures thereof.

7. The method according to claim 1, wherein:
   A is CR²⁸;
   R is SO₂R⁹;
   R² is H or C₁₋₅ alkyl;
   R³ is H, OH or C₁₋₅ alkyl;
   R⁴, R⁵, R⁶, R⁷ and R⁸ represent H, OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹² or C₁₋₅ alkyl, independently from one another;
   R is H, ammonium or a cation of an alkali or alkaline-earth metal;
   R¹⁰ and R¹¹ represent H, C₁₋₅ alkyl or C₆₋₁₀ aryl, independently from one another;
   R¹² is OH, C₁₋₅ alkyl or C₆₋₁₀ aryl;
   on the condition that at least one of R⁴, R⁵, R⁶, R⁷ or R⁸ is OR¹⁰, CO₂R¹⁰, NR¹⁰R¹¹, NH—CO—R¹².

8. The method according to claim 7, wherein:
   A is CR²⁸;
   R is SO₂R⁹, where R² is H or sodium;
   R³ is H;
   R⁴ is H; and
   R⁴, R⁵, R⁶, R⁷ and R⁸ represent H, OH, OCH₃, COOH, NH₂, HCOCH₃, HCONH₂, NHCH₃ or N(CH₃)₂, independently from one another;
   on the condition that at least one of R⁴, R⁵, R⁶ or R⁷ is OH, OCH₃, COOH, NH₂, HCOCH₃, HCONH₂, NHCH₃ or N(CH₃)₂.

9. The method according to claim 7, wherein the compound of formula (I) is selected from the group consisting of sodium 4-amino-1-naphthalenesulfonate, sodium 4-acetylamino-1-naphthalenesulfonate, sodium 4-benzoylamino-1-naphthalenesulfonate, sodium 5-dimethylaminom-1-naphthalenesulfonate, sodium 4-amino-3-hydroxy-1-naphthalenesulfonate and mixtures thereof.

10. The method according to claim 5, wherein the compound of formula (I) is selected from the group consisting of sodium 5-amino-2-naphthalenesulfonate, sodium 5-acetylaminom-2-naphthalenesulfonate, sodium 5-benzoylaminom-2-naphthalenesulfonate, sodium 5-acetylaminom-2-naphthalenesulfonate, sodium 5-benzoylaminom-2-naphthalenesulfonate, sodium 8-amino-2-naphthalenesulfonate, 8-acetylamino-2-naphthalenesulfonic acid, sodium 4-hydroxy-6-amino-2-naphthalenesulfonate and mixtures thereof.

11. The method according to claim 8, wherein the compound of formula (I) is selected from the group consisting of sodium 4-amino-1-naphthalenesulfonate, sodium 4-acetylamino-1-naphthalenesulfonate, sodium 4-benzoylamino-1-naphthalenesulfonate, sodium 5-dimethylaminom-1-naphthalenesulfonate, sodium 4-amino-3-hydroxy-1-naphthalenesulfonate and mixtures thereof.