

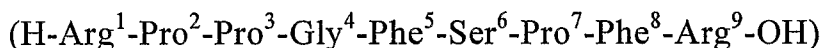
NON-PEPTIDE BRADYKININ ANTAGONISTS AND
PHARMACEUTICAL COMPOSITIONS THEREFROM

FIELD OF THE INVENTION

The present invention relates to non-peptide compounds containing a quaternary ammonium group, having activity as specific antagonists of bradykinin (BK) B2 receptor, pharmaceutical compositions containing them
5 and the use thereof for the treatment of all the conditions in which activation of bradykinin B2 receptors are involved.

PRIOR ART

Bradykinin (BK) belongs to Kinins and forms, together with Kallidin and T-Kinin, the sub-group of Kinins present in mammals. Kinins play an
10 important role as mediators of pain and inflammation, both in the central and peripheral nervous system. Bradykinin is, in particular, a nonapeptide



produced by the body in physiopathological conditions.

Two types of Kinins receptors exist, B1 and B2. The main characteristic
15 of the B1 receptor is that it is more inducible than constitutive. It is expressed in tissues in inflammation or stress conditions. On the other hand, B2 is a constitutive receptor normally present in all tissues and acts a mediator during the inflammatory processes. Bradykinin and Kallidin are released from their protein precursors (known as kininogens), by proteolytic enzymes named
20 kininogenases. Among these, the main role is played by Kallikreins which however, once released by the precursor, can exert their action only for a short time as they are quickly destroyed by a series of circulating enzymes and membranes generically defined as Kininases. One of these Kininases cleaves bradykinin at the C-terminal arginine thus forming a des-Arg-BK which acts
25 as B1 receptor agonist.

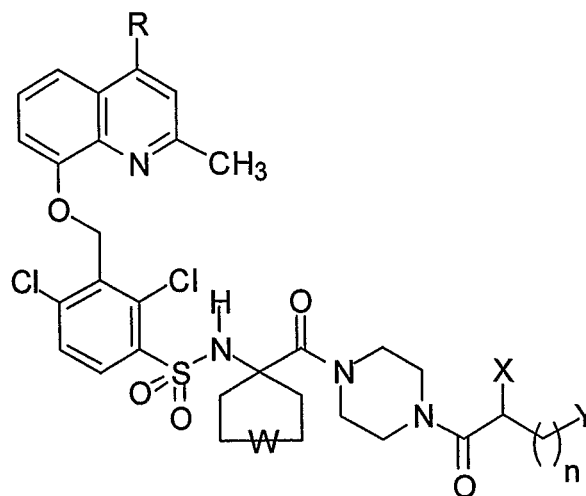
The activation of bradykinin B1 and B2 receptors induces relaxation of
vasal muscles with consequent hypotension, increase in vascular permeability,
contraction of smooth muscles of intestine and respiratory tract, stimulation of
nociceptive neurons, alteration of ionic epithelial secretion, production of
5 nitroxide and release of cytokines by leukocytes and eicosanoids from
different cell types. As a consequence, antagonistic compounds of BK
receptors can be considered a novel class of medicaments supposedly active in
various disorders. Possible therapeutical applications for said antagonists are
inflammatory, allergic and autoimmune disorders, such as asthma and chronic
10 bronchitis (also induced by irritants), allergic, vasomotor and viral rhinitis,
obstructive pulmonary disease (COPD), rheumatoid arthritis, chronic
inflammatory diseases of the bowel (Crohn's disease and ulcerative colitis),
glomerulonephritis, psoriasis, rash, acute and chronic cystitis; degenerative
disorders characterized by fibrosis, such as hepatic cirrhosis, glomerulopathies
15 and pulmonary fibrosis, arteriosclerosis; thanks to their analgesic activity, in
the treatment of both acute and chronic pain, for example in burns, cephealea,
insects bites, chronic pain in cancer patients; in disorders of the cardiovascular
apparatus such as septic, allergic and post-traumatic shocks, and hepatic
cirrhosis by hepatorenal syndrome; as anticancer and antiangiogenetics; in the
20 treatment of hypotension and of alopecia.

Different peptide and non-peptide antagonists of bradykinin B2 receptor
are known in literature. WO03103671 discloses a large family of compounds
with antagonistic activity on bradykinin B2 receptor. The compounds of the
present invention, although being included in the general formula of
25 WO03103671, are not described or characterized in said document.

DETAILED DISCLOSURE

The present invention relates to non-peptide compounds which show
high affinity and antagonistic activity towards B2 receptor, having general

formula (I):



(I)

in which:

- 5 - R is hydrogen or methyl;
- W is a single bond or an oxygen atom;
- $n = 3, 4$;
- X is hydrogen or a $-NR_1R_2$ amino group in which R_1 and R_2 can be independently hydrogen or a group selected from methyl, ethyl, n-propyl, isopropyl;
- 10 - Y is a $-NR_3R_4R_5$ quaternary ammonium group in which R_3, R_4, R_5 can be independently a group selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, n-pentyl; and

the salts thereof with pharmaceutically acceptable acids.

- 15 Preferably, compounds (I) are salified with inorganic or organic acids selected from hydrochloric, hydrobromic, hydroiodic, sulfuric, phosphoric, acetic, trifluoroacetic, propionic, oxalic, malic, maleic, succinic, malonic, aspartic, glutamic acids. Moreover, due to the presence of a chiral center, the invention also comprises the two enantiomers or mixtures thereof, in any
- 20 proportion, including racemic mixtures.

The compounds of general formula (I) have both *in vivo* and *in vitro*

antagonistic activity towards B2 receptor higher than the more structurally similar analogues as described in WO03103671.

Preferred are the compounds of general formula (I) in which:

$n = 3$;

5 $X = \text{hydrogen or a } -\text{NH}_2 \text{ group};$

$Y = -\text{N}(\text{CH}_3)_3^+$ quaternary ammonium group;

the other substituents being as defined above.

Particularly preferred are the compounds (I) wherein:

R is hydrogen or methyl;

10 W is an oxygen atom;

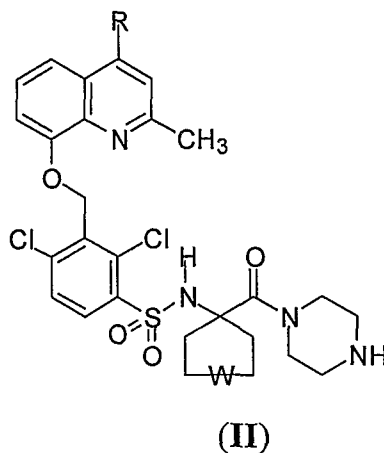
$n = 3$;

X is hydrogen or a group $-\text{NH}_2$;

Y is a $-\text{N}(\text{CH}_3)_3^+$ quaternary ammonium group.

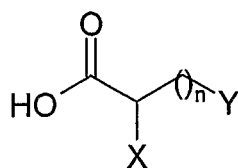
15 The compounds object of the present invention can be prepared according to well known synthetic routes.

Preferably, the compounds of general formula (I) as defined above are prepared by condensation, in the presence of a suitable condensing agent, of the intermediate of general formula (II), obtained as disclosed in WO03103671



20 with the compound of formula (10)

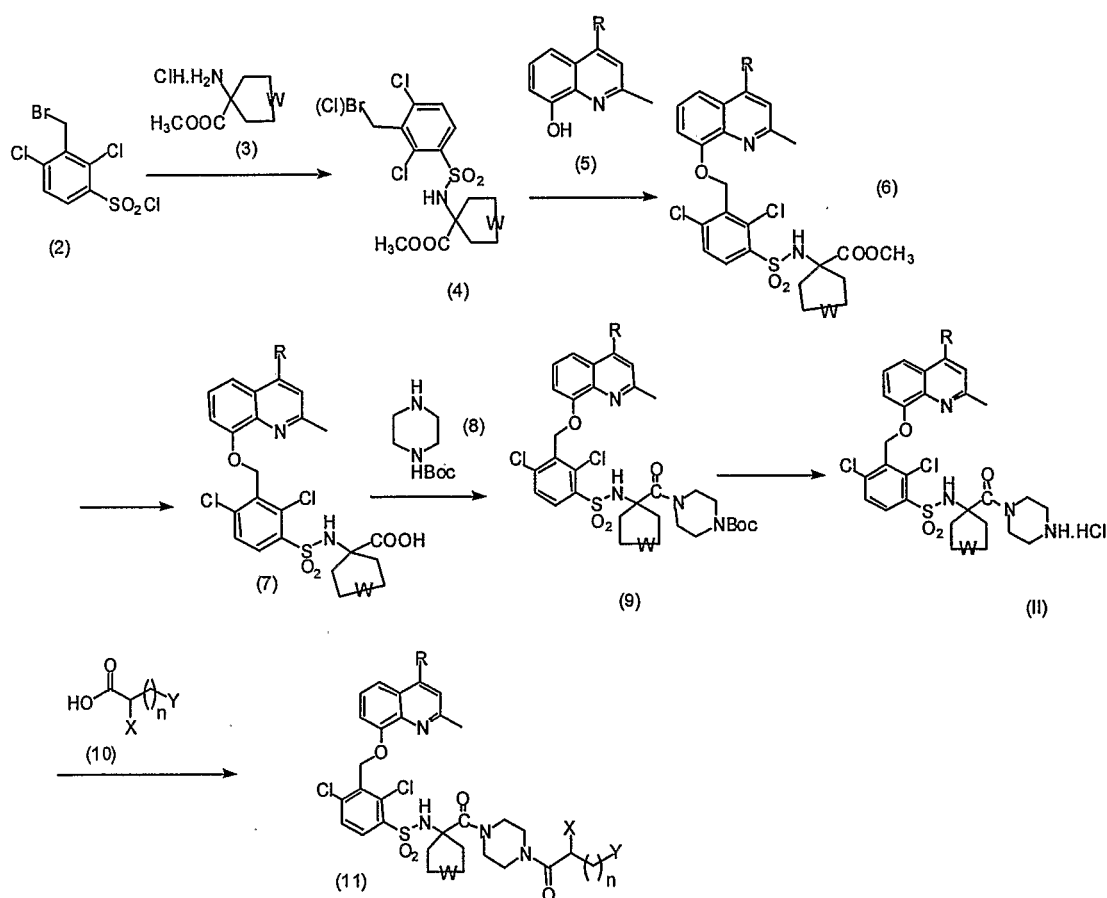
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(10)

or a derivative thereof in which the carboxylic group is suitably activated.

5 The synthetic process is illustrated in Scheme 1



Scheme 1

10 The compound of formula (2) is prepared as described in *J. Med. Chem.* 2001, 44, 1674-1689 by bromination of the corresponding toluene derivative, which is in turn obtained as described in *J. Fluorine Chemistry*, 2000. 101:85-89.

The first step concerns the formation of the sulfonamido bond (4)

obtained by condensation of intermediates (2) and (3). This reaction is carried out at room temperature, preferably in acetonitrile/water (2:1), in the presence of sodium hydrogen carbonate (NaHCO_3). Said reaction takes place with exchange of chlorine and bromine at the benzyl position: the resulting products mixture is directly used in the subsequent step. The reaction of the halogen derivatives mixture with a disubstituted hydroxyquinoline (5), in the presence of potassium carbonate (K_2CO_3) and potassium iodide (KI), in acetone under reflux, yields the ester derivative (6).

Compound of formula (5) i.e. 2,4-dimethyl-8-hydroxy quinoline, in which $\text{R}_4=\text{R}_5=\text{CH}_3$, is prepared as disclosed in WO9640639.

The methyl ester of formula (6) is hydrolysed under basic conditions to carboxylic acid (7), which is condensed with Boc-piperazine (8), to yield intermediate (9). Said condensation reaction is carried out according to a known procedure for the peptide synthesis, using hydroxybenzotriazole to activate the carboxylic moiety, a condensing agent such as 1-ethyl-3-(3'-dimethylpropyl) carbodiimide and an amount of tertiary amine, namely diisopropylethylamine, of three equivalents on the basis of the condensing agent. Compound (II) is obtained by cleavage of the Boc group from intermediate (9), by means of a hydrochloric acid solution (4N) in dioxane and isolating the free amine instead of the hydrochloride.

Derivative (11) is obtained by condensation of intermediate (10) with the amino acid (11) according the procedure described for the preparation of (9) from (7). Any Boc group present can be removed from intermediate (11), with a hydrochloric acid solution (4N) in dioxane, thus obtaining the final compound. When case the trialkylammonium group is not present in any commercially available intermediates, it can be synthesized starting from the corresponding amine with known procedures (Rapoport et al, *J. Org. Chem.*, 1977, 42:139-141; Chen et al, *J. Biochem.*, 1978, 56:150-152).

The compounds of the invention are used in the treatment of all those disorders in which the activation of bradykinin receptor has to be blocked or reduced. They are particularly suitable for the treatment of inflammatory, allergic and autoimmune disorders, such as asthma and chronic bronchitis, 5 allergic, vasomotor and viral rhinitis, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, chronic inflammatory diseases of the bowel (Crohn's disease and ulcerative colitis), glomerulonephritis, psoriasis, rash, acute and chronic cystitis, hepatic cirrhosis, glomerulopathies and pulmonary fibrosis, arteriosclerosis, both acute and chronic pain, septic, allergic and post- 10 traumatic shocks, hepatic cirrhosis by hepatorenal syndrome, hypotension, alopecia, or as anticancer and antiangiogenetics.

For use in therapy, the compounds of the invention will be suitably formulated together with pharmaceutically acceptable carriers/excipients. Preferred are pharmaceutical forms suitable for the oral administration, such 15 as tablets, capsules, granules, powders, solutions, suspensions, syrups or the like. These pharmaceutical preparations can be prepared with conventional procedures using ingredients known in technique, such as ligands, disintegrants, lubricants, fillers, stabilizing agents, diluents, dyes, flavours, wetting agents and other excipients known to those skilled in the art. The oral 20 formulations also comprise protracted-release forms, such as enteric-coated tablets or granules. The solid oral compositions can be prepared with conventional mixing, filling or compression methods. The liquid oral preparations can be in the form of, for example, aqueous or oily suspensions or solutions, emulsions, syrups, or can be presented as dry product for 25 reconstitution with water or other suitable carrier before use.

The dosage can range depending on the age and general conditions of the patient, nature and severity of the disease or disorder and route and type of administration. As a rule, in case of oral administration to a human adult

patient, the compounds of the present invention will be generally administered in a total daily dosage ranging from 1 to 1000 mg, preferably from 5 to 300 mg, in a single dose or in subdivided doses.

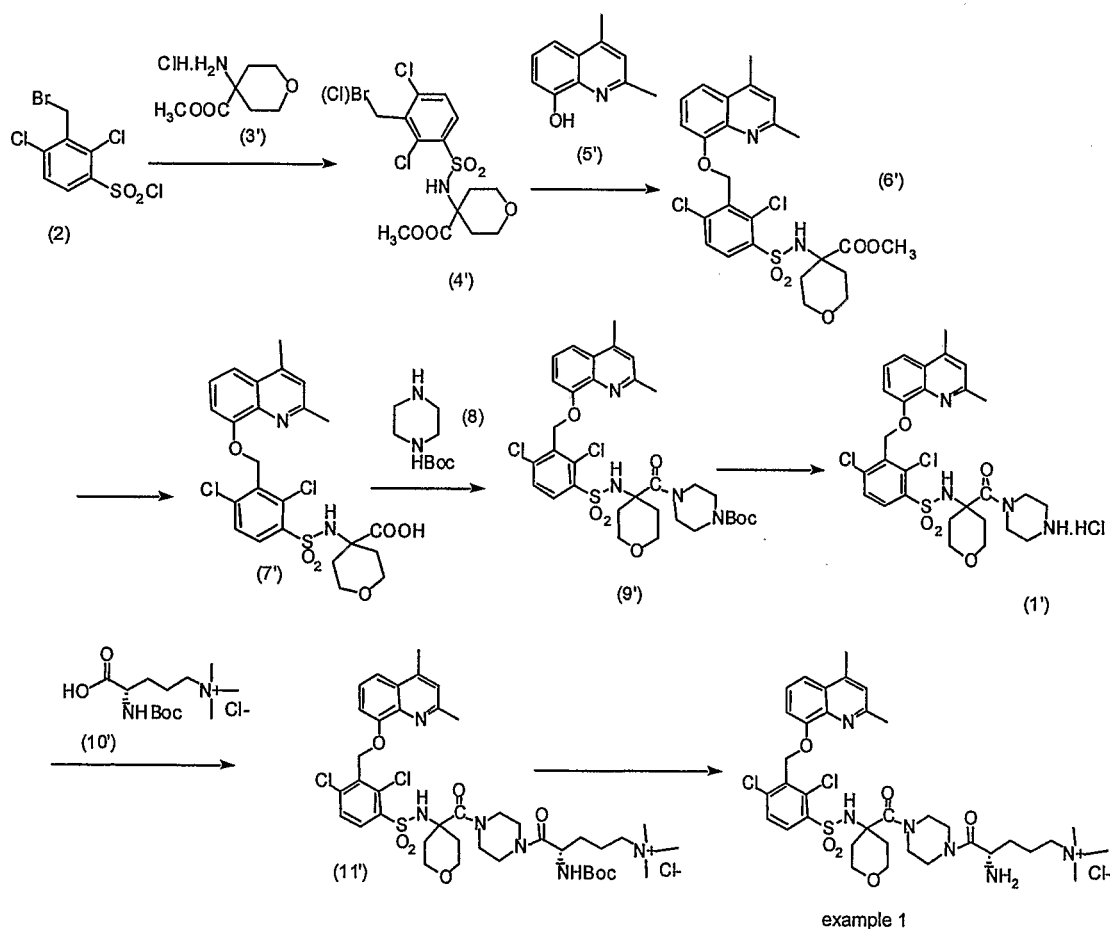
The following examples illustrate the invention in greater detail.

5 EXAMPLE 1

(4-(S)-amino-5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]tetrahydropyran-4-carbonyl}piperazin-1-yl)-5-oxopentyl]trimethyl-ammonium chloride, dihydrochloride

(Compound of general formula I in which R = CH₃, W = -O-, X = NH₂, n = 3, Y = N(CH₃)₃⁺Cl⁻).

The compound is synthesized following the synthetic route illustrated in Scheme 2



Scheme 2

GENERAL METHODS: analytic HPLC: *Flow:* 1 ml/min; *Mobile phase:* A-0.1% trifluoroacetic acid in water, B-0.1% trifluoroacetic acid in acetonitrile; *Column:* Zorbax Eclipse XDB C8, 5 micron, 150 x 4,6 mm.

Intermediate (2) 2,4-Dichloro-3-bromomethyl-benzenesulfonyl chloride

5 10 ml of chlorosulfonic acid are dropwise added with 4.8 ml of 2,6-dichlorotoluene in two hours, under magnetic stirring at room temperature. After completion of the addition, the mixture is heated at 40°C for two hours, thereby obtaining a purple solution, which is cooled and carefully poured into ice-water (0.5 l), stirring vigorously. The separated white solid is filtered,
10 triturated, washed with water, dried over KOH and purified by washing with n-hexane, adding 200 ml of solvent under strong stirring. The mixture is filtered, the solid is discarded and the solvent is evaporated to dryness to obtain 2,4-dichloro-3-methyl-benzenesulfonyl chloride as a crystalline white solid. Yield: 85%.

15 HPLC purity: 86% (30% B, 3%/min, Rt=19.7 min).

¹H-NMR (CDCl₃): δ (ppm) 2.6 (s, 3H), 7.5 (d, 1H), 7.95 (d, 1H);

ESI(+)-MS: m/z 260 [M+H]⁺.

This intermediate is brominated under the following conditions: 20 mmols of 2,4-dichloro-3-methyl-benzenesulfonyl chloride are dissolved in
20 acetonitrile. 2 eq of NBS are added under stirring at room temperature until completed solubilization of NBS. Finally, 0.1 eq of azo-bisisobutyronitrile (AIBN) is added and the mixture is heated at 70°C for approx. 6 hours. The solution is evaporated, the residue is taken up with ethyl acetate, washed with H₂O and 5% NaHCO₃, dried over dry Na₂SO₄ and filtered. The organic phase
25 is evaporated thereby obtaining a viscous, light colored liquid which is taken up into petroleum ether. The residue is filtered, and the solution yields (2') as a light colored crystalline solid.

HPLC purity: 95% (50% B to 5%/min, Rt=18.72).

¹H-NMR (CDCl₃): δ (ppm) 4.85 (s, 2H), 7.58 (d, 1H), 8.08 (d, 1H);
ESI(+)-MS: m/z 338.1 [M+H]⁺.

Intermediate (3') 4-Amino-tetrahydropyran-4-carboxylic acid methyl ester hydrochloride

5 4-Amino-tetrahydropyran-4-carboxylic acid hydrochloride (0.025 mols) is suspended in 13 ml of CH₃OH, cooled to -60°C and dropwise added with SOCl₂ (3 eq) under stirring. After completion of the addition, the mixture is left to warm to room temperature, then gradually heated to ebullition to obtain a clear solution (approx. 2 hours), which is cooled, the residue is filtered and
10 concentrated under vacuum.

Yield 80%. Purity (NMR): 85%.

¹H-NMR (DMSO-d₆): δ (ppm) 1.91-2.04 (m, 4H), 3.78 (s, 3H), 3.60-3.85 (m, 4H), 9.00 (s, 3H). ESI(+)-MS: m/z 160.1 [M+H]⁺.

Intermediate (4') 4-(3-Bromomethyl-2,4-dichloro-benzenesulfonylamino)-tetrahydropyran-4-carboxylic acid methyl ester

15 The intermediate (3') (1.1 eq) is dissolved in water together with 4 equivalents of K₂CO₃. This solution is added with a solution of 1 equivalent (10 mmols) of intermediate (2) in acetonitrile and stirred at room temperature until a precipitate forms (4 hours). The solvent is evaporated off and the
20 residue is dissolved in ethyl acetate and 0.1M HCl (1/1). The organic phase is separated and dried over Na₂SO₄. The solvent is evaporated off, the resulting solid is washed with cyclohexane, thereby obtaining a white solid in which chloro/bromo derivatives are present in 10/1 ratio. Yield: 60%.

HPLC purity: 88% (20% B at 3%/min; Rt=14.11 (Br) and 14.47 (Cl)).

25 ¹H-NMR (CDCl₃): δ (ppm) 1.81-1.99 (2H, m), 2.07-2.25 (2H, m), 3.49-3.71 (7H, m), 4.81 (1.5H, s, [Br]), 4.94 (0.3H, s, [Cl]), 5.30 (1H, brs), 7.47-7.53 (1H, m), [7.49 (d, J 8.5Hz, X = Br), 7.51 (d, J 8.5Hz, X = Cl)], 7.91-7.98 (1H, m), [7.94 (d, J 8.5Hz, X = Br), 7.96 (d, J 8.5Hz, X = Cl)].

Intermediate (6') 4-[2,4-Dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]tetrahydropyran-4-carboxylic acid methyl ester

Quinoline (5') (0.48 mmols) and LiOH (2.5 eq) are mixed at room temperature under nitrogen in methyl ethyl ketone (MEK). The mixture is kept
5 under stirring and under nitrogen for 90 min. Intermediate (4) is dissolved in MEK/dry DMF (2/1) (42 ml, 12 ml/mmol), and the solution containing the quinoline is dropwise added to the reaction mixture, under stirring. Stirring is kept for 16 hours. The reaction mixture is concentrated under vacuum and the residue dissolved in ethyl acetate (50 ml, 100 ml/mmol). The organic phase is
10 washed (3x50 ml) with a buffer solution pH=4.2, dried over Na₂SO₄, filtered and concentrated under vacuum to obtain a yellow oil. Yield: 33%. HPLC purity: 77% (20% B, 3%/min; Rt=9.54).

¹H-NMR (DMSO-d₆): δ (ppm) 1.80-1.95 (m, 4H), 2.56 (s, 3H), 2.64 (s, 3H), 3.32-3.40 (m, 2H), 3.42-3.55 (m, 2H), 3.60 (s, 3H), 5.57 (s, 2H),
15 7.30 (s, 1H), 7.39 (d, 1H), 7.50 (dd, 1H), 7.67 (d, 1H), 7.78 (d, 1H), 8.02 (d, 1H), 8.77 (bs, 1H); ESI(+)MS: m/z 553.1 [M+H]⁺.

Intermediate (7') 4-[2,4-Dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]tetrahydropyran-4-carboxylic acid

Intermediate of formula (6') is dissolved in THF and the solution is
20 added with 10 eq of 1M LiOH in water. The mixture is stirred for 4 hours at 40°C, then the solvent is evaporated off. The residue is dissolved in water and 0.1M HCl is added to pH=4. The aqueous phase is extracted with dichloromethane and the organic phase is dried over Na₂SO₄. Solvent is evaporated off and to obtain a yellow solid residue. Yield: 90%. HPLC purity:
25 99% (20%B, 3%/min; Rt=7.72).

¹H-NMR (DMSO-d₆): δ (ppm) 1.75-1.90 (m, 4H), 2.56 (s, 3H), 2.64 (s, 3H), 3.10-3.35 (m, 2H), 3.38-3.50 (m, 2H), 5.58 (s, 2H), 7.30 (s, 1H),

7.37 (d, 1H), 7.46 (t, 1H), 7.67 (d, 1H), 7.75 (d, 1H), 8.03 (d, 1H), 8.64 (bs, 1H). ESI(+)MS: m/z 539.1 [M+H]⁺.

Intermediate (9') 4-tert-butoxycarbonyl-((4-(2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)benzenesulfonylamino)-tetrahydropyran-4-carbonyl)-piperazin-1-yl)

(7') (1.3 mmols) and HOBt (1.1 eq) are suspended in 50 ml of dry DMF in a 100 ml round-bottom flask under nitrogen. The mixture is cooled to +4°C and added with EDCI. HCl (1.1 eq) under stirring. Stirring at +4°C is continued for an hour, then DIPEA (2 eq) and Boc-piperazine (1 eq) are added and the mixture is left to warm to room temperature, under stirring. After 12 h the solvent is evaporated off, the residue is dissolved in 40 ml of DCM and the organic phase is washed with brine (20 ml) and dried over Na₂SO₄. The solvent is evaporated off to obtain an oil which is purified on a Varian Mega Bond (flash master system) 70 g column (ethyl acetate, R_f=0.50), thereby obtaining a yellow solid.

Yield: 96%. HPLC purity: 98% (20% B, 3% B/min, R_t=11.14).

¹H-NMR (CDCl₃): δ (ppm) 1.45 (s, 9H); 1.55-1.80 (m, 2H), 2.05-2.20 (m, 4H), 2.56 (s, 3H), 2.64 (s, 3H), 3.38-3.90 (m, 10H), 5.58 (s, 2H), 7.10 (s, 1H), 7.30 (s, 1H), 7.37 (d, 1H), 7.46 (t, 1H), 7.67 (d, 1H), 7.75 (d, 1H), 8.03 (d, 1H), 8.64 (bs, 1H). ESI(+)MS: m/z 707.2 [M+H]⁺.

Intermediate (1') (4-(2,4-Dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino)-tetrahydropyran-4-carbonyl)-piperazin-1-yl

0.62 mmols of (9') are added with 10 ml of HCl/dioxane 4M and the mixture is kept under stirring for 3 hours. The solvent is evaporated off and the residue is freeze-dried, to obtain hydrochloride (1') as yellow solid. Yield: 98%. HPLC purity: 92% (20% B, 3%/min; R_t=5.34).

¹H-NMR (D₂O): δ (ppm) 1.55-2.10 (m, 7H), 2.90-3.10 (m, 9H), 3.20-3.55 (m, 9H), 6.0 (s, 2H), 7.60-8.10 (m, 8H), 8.95 (d, 1H).

ESI(+)-MS: m/z 609.1 $[M+H]^+$.

Intermediate (10') (4-tert-butoxycarbonylamino-4-carboxy-butyl)-
trimethylammonium

10 mmols of Boc-Orn-OH are suspended in methanol (20 ml) and the
5 suspension is added with 44 mmols of isourea. The flask is plugged and kept
under stirring at room temperature for 2 days. The resulting solution is
monitored by TLC (eluent: $CHCl_3/CH_3OH/NH_4OH$ 40/54/6; Boc-Orn-OH
 $R_f=0.29$; (10') R_f : 0.11, detection $KMnO_4$).

Methanol is evaporated off under vacuum and the residue is digested in
10 150 ml of water and filtered. The round-bottom flask and the solid are washed
with water (2x50 ml) and all the washing aqueous fractions are combined,
then concentrated under vacuum (40 ml). The resulting solid (4.068 g) is
suspended in water (40 ml), filtered (to remove any traces of urea) and
purified by FCC on reversed phase LiChroprep RP-18 (40-63 micron). The
15 column (19 x 7 cm) is eluted with 3% CH_3CN in water and the fractions
(approx. 100 ml) are analyzed by TLC. The fractions containing the pure
product (500 ml) are combined, concentrated under vacuum to remove
 CH_3CN , freeze-dried, and finally evaporated from 150 ml of absolute ethanol,
to give 442 mg of a white, highly hygroscopic solid. Yield: 16%.

20 1H -NMR ($DMSO-d_6$): δ (ppm) 1.38 (s, 9H) 1.58-1.75 (m, 4H), 3.03 (s,
9H), 3.29 (m, 2H), 3.45 (m, 1H), 6.49 (d, d, 1H); ESI(+)-MS: m/z 275.2
 $[M+H]^+$.

Intermediate (11') (4-(S)-tert-Butoxycarbonylamino-5-(4-{4-[2,4-dichloro-3-
(2,4-dimethyl-quinolin-8-yloxymethyl)benzenesulfonylamino]tetrahydro-
25 pyran-4-carbonyl}piperazin-1-yl)-5-oxo-pentyl]trimethyl-ammonium chloride

Intermediate (10'), 1.2 mmols, is dissolved in DMF and the solution is
added with dicyclohexylcarbodiimide (1.2 eq) and HOBt (1.2 eq). The mixture
is kept under stirring for 30 min, then added with diisopropylaminomethyl-

polystyrene (1.5 eq) and intermediate (1') (1 eq). The mixture is left under stirring for 24 hours. The resin is filtered, the solvent is evaporated off and the residue is dissolved in water and ethyl acetate. The aqueous phase is separated and freeze-dried. The crude product is purified by preparative HPLC (column
 5 Vydac 218TP, C18, 250x50 mm, flow 60 ml/min, gradient 10% to 70% CH₃CN/0.1% TFA in 120 min, detector UV at 240 nm, collection 55 to 75 min) thereby affording intermediate (11') which is freeze-dried as a white solid. Yield: 46%. HPLC purity: 98% (20% B, 3%/min; Rt=7.68).

¹H NMR (DMSO-d₆) δ: 1.4 (s, 9H), 1.8-1.45 (m, 6H), 1.95-1.85 (m,
 10 2H), 2.81 (m, 6H), 3.08 (s, 9H), 3.70-3.18 (m, 7H), 4.01-3.56 (5H, m), 4.57-4.45 (m, 1H), 5.59 (s, 2H), 7.25 (d, 1H), 7.90-7.43 (m, 4H), 8.02 (d, 1H), 8.85 (s, 1H). ESI(+)MS: m/z 863.2 [M+H]⁺.

(4-(S)-Amino-5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]tetrahydropyran-4-carbonyl}piperazin-1-yl)-5-oxo-
 15 pentyl]trimethyl-ammonium chloride, dihydrochloride

0.45 mmols of (11') are added with 10 ml of HCl/dioxane 4M. The mixture is kept under stirring for 6 hours, the solvent is evaporated off and the residue is freeze-dried, thereby obtaining the final compound as a white solid. Yield: 87%. HPLC purity: 98% (20% B, 3%/min; Rt=5.14).

¹H NMR (DMSO-d₆) δ: 1.95-1.60 (m, 8H), 2.81 (m, 6H), 3.08 (s, 9H),
 20 3.70-3.18 (m, 12H), 4.57-4.45 (m, 1H), 5.59 (s, 2H), 7.90-7.60 (m, 4H), 8.02 (d, 1H), 8.5 (s, 3H), 8.85 (s, 1H).

ESI(+)MS: m/z 763.1 [M+H]⁺.

EXAMPLE 2

25 (4-(S)-Amino-5-(4-(4-(2,4-dichloro-3-(2-methyl-quinolin-8-yloxymethyl)-benzenesulfonylamino)tetrahydropyran-4-carbonyl)-piperazin-1-yl)-5-oxo-pentyl)-trimethylammonium chloride, hydrochloride

¹H NMR (DMSO-d₆) δ: 8.90 (1H, s), 8.47-8.34 (4H, m), 8.02 (1H, d), 7.81 (1H, d), 7.73-7.37 (4H, m), 5.62 (2H, s), 4.57-4.45 (1H, m), 4.01-3.56 (5H, m), 3.43-3.18 (7H, m), 3.06 (9H, s), 2.78-2.61 (4H, m), 2.89 (1H, s), 1.97-1.60 (9H, m). HPLC: t_R = 9.26 min. MS: [M]⁺ 749.

5 EXAMPLE 3

[5-(4-{4-[2,4-Dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzene-sulfonylamino]tetrahydropyran-4-carbonyl}piperazin-1-yl)-5-oxo-pentyl]-trimethyl-ammonium trifluoroacetate.

¹H-NMR (DMSO-d₆): δ (ppm) 1.53 (s, 2H, m); 1.69 (m, 4H); 1.90 (m, 2 H); 2.45 (t, 2 H); 2.78 (m, 6 H); 3.04 (9 H, s); 3.23 - 3.57 (7 H, m); 5.68 (2H, s); 7.38-8.18 (5 H, m); 8.04 (1H, d, J = 8.42 Hz); 8.82 (1 H, s). HPLC: t_R = 5.65 min. MS: [M]⁺ 748.

EXAMPLE 4

[4-(S)-Amino-5-(4-{1-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]cyclopentanecarbonyl}piperazin-1-yl)-5-oxo-pentyl]-trimethyl-ammonium chloride, dihydrochloride.

¹H NMR (DMSO-d₆) δ: 8.90 (1H, s), 8.48 (3H, s), 8.02 (1H, d), 7.95-7.63 (3H, m), 5.59 (2H, s), 4.57-4.45 (1H, m), 3.97-3.24 (10H, m), 3.08 (9H, s), 2.95-2.61 (5H, m), 1.97-1.72 (8H, m), 1.42 (4H, s); HPLC: t_R = 5.88 min. MS: [M]⁺ 747.2.

Biological Activity

The evaluation of the B2 receptor affinity of the compounds of the present invention was carried out with studies of binding to the human B2 receptor expressed in CHO cells, following the procedure described by Bellucci et al, *Br. J. Pharmacol.* **2003**, 140:500-506; the binding values are reported expressed as pK_i.

Antagonistic activity (expressed as pA₂) was evaluated as the inhibition of the bradykinin-induced production of inositols in CHO cells transfected

with B2 human receptor, according to the procedure described in Bellucci et al, *Br. J. Pharmacol.* **2003**, 140:500-506.

The *in vivo* activity of the compounds of the present invention was evaluated as effectiveness in inhibiting BK-induced bronchospasm in the guinea pig (Tramontana et al., *J. Pharmacol. Exp. Therap.*, 296:1051-1057, 2001), measuring the it dose (it=intratracheal administration) (in nmols/kg) which inhibited by 80% bronchial constriction for at least 210 min.

Example	W	R	X	Y	n	pKi	pA2	it Dose
WO03103671 ex 55	bond	H	NH ₂	NHC(=NH)NH ₂	3	8.7	8.4	300
WO03103671 ex 63	bond	CH ₃	NH ₂	NH ₂	4	9.1	8.9	300
WO03103671 ex 57	bond	H	NH ₂	N(CH ₃) ₂	4	8.8	8.3	300
WO03103671 ex 59	bond	CH ₃	NH ₂	N(CH ₃) ₂	4	8.8	9.0	300
WO03103671 ex 44	bond	CH ₃	NH ₂	NHC(=NEt)NHEt	3	10.1	9.0	300
WO03103671 ex 88	bond	CH ₃	N(CH ₃) ₃	N(CH ₃) ₃	4	9.7	8.2	-
Example 1	O	CH ₃	NH ₂	N(CH ₃) ₃	3	10.3	10.3	30
Example 2	O	H	NH ₂	N(CH ₃) ₃	3	10.2	9.7	100
Example 3	O	CH ₃	H	N(CH ₃) ₃	3	10.1	9.5	100
Example 4	bond	CH ₃	NH ₂	N(CH ₃) ₃	3	10.1	9.4	100

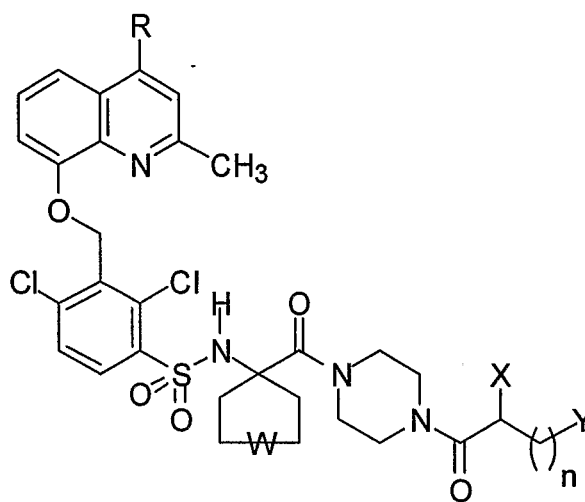
The preferred compounds of the present invention were compared with those more structurally similar disclosed in WO03103671. It has surprisingly been found that the compounds of the invention have *in vivo* and *in vitro* activities higher than the structurally related analogues of WO03103671. Both
5 the antagonistic activity test on cells transfected with the human receptor and the *in vivo* test are highly predictive of the expected dose for therapeutical applications in humans.

ABBREVIATIONS

it = intratracheal administration; iv = intravenous administration; eq = equivalent;
10 DCM = dichloromethane; MeOH = methanol; THF = tetrahydrofuran; DMSO = dimethylsulfoxide; DMF = dimethylformamide; AcOEt = ethyl acetate; AcOH = acetic acid; TFA = trifluoroacetic acid; NBS = N_α-bromosuccinimide; bpo = benzoyl peroxide; Boc = tert-butoxycarbonyl; HOBt = 1-hydroxy-benzotriazole; EDC = 1-ethyl-3-(3'-dimethylpropyl)carbodiimide; DIPEA =
15 diisopropylethylamine; HPLC = high pressure liquid chromatography; TLC = thin-layer chromatography; NMR = nuclear magnetic resonance; ESI = electron spray ionization; MS = mass spectrometry; FCC = Flash Column Chromatography; Rt = retention time.

CLAIMS

1. Compounds of general formula (I)



(I)

in which

- R is hydrogen or methyl;
- W is a single bond or an oxygen atom;
- n = 3, 4;
- X is hydrogen or a -NR₁R₂ amino group in which R₁ and R₂ can be independently hydrogen or a group selected from methyl, ethyl, n-propyl, isopropyl;
- Y is a -NR₃R₄R₅ quaternary ammonium group in which R₃, R₄, R₅ can be independently a group selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, n-pentyl; and

the pharmaceutically acceptable salts, enantiomers and enantiomeric mixtures thereof.

2. Salts of the compounds of general formula (I) with inorganic or organic acids selected from hydrochloric, hydrobromic, hydroiodic, sulfuric,

phosphoric, acetic, trifluoroacetic, propionic, oxalic, malic, maleic, succinic, malonic, aspartic, glutamic acids.

3. Compounds as claimed in claim 1 or 2, wherein:

W is a single bond;

5 n = 3;

X is selected from hydrogen or a -NH₂ group;

Y is a -N(CH₃)₃⁺ quaternary ammonium group;

the other substituents being as defined in claim 1.

4. Compounds as claimed in claim 1 or 2, in which:

10 R is selected from hydrogen or methyl;

W is an oxygen atom;

n = 3;

X is selected from hydrogen or a -NH₂ group;

Y is a -N(CH₃)₃⁺ quaternary ammonium group;

15 the other substituents being as defined in claim 1.

5. The following compound as claimed in claim 3:

[4-(S)-amino-5-(4-{1-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)benzenesulfonylamino]-cyclopentanecarbonyl}piperazin-1-yl)-5-oxo-pentyl]trimethyl-ammonium chloride, dihydrochloride

20 6. The following compounds as claimed in claim 4:

- (4-(S)-amino-5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxy-methyl)benzenesulfonylamino]tetrahydropyran-4-carbonyl}-piperazin-1-yl)-5-oxo-pentyl]trimethyl-ammonium chloride, dihydrochloride;

- (4-(S)-amino-5-(4-(4-(2,4-dichloro-3-(2-methyl-quinolin-8-yloxy-methyl)-benzenesulfonylamino)-tetrahydropyran-4-carbonyl)-piperazin-1-yl)-5-oxo-pentyl)-trimethyl-ammonium chloride, hydrochloride;

25

- [5-(4-{4-[2,4-dichloro-3-(2,4-dimethyl-quinolin-8-yloxymethyl)-benzenesulfonylamino]tetrahydropyran-4-carbonyl}piperazin-1-yl)-5-oxo-pentyl]trimethyl-ammonium trifluoroacetate.

7. Pharmaceutical compositions containing as the active ingredient a
5 compound as claimed in claims 1 to 6 together with pharmaceutically acceptable excipients.

8. The use of a compound as claimed in claims 1 to 6 for the preparation of pharmaceutical compositions for the treatment of all the conditions in which activation of bradykinin B2 receptors is involved.

10 9. The use of a compound as claimed in claim 8 for the preparation of pharmaceutical compositions for the treatment of inflammatory, allergic and autoimmune conditions.

10. The use of a compound as claimed in claim 8 for the preparation of pharmaceutical compositions for the treatment of asthma and chronic
15 bronchitis, allergic, vasomotor and viral rhinitis, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, chronic inflammatory diseases of the bowel (Crohn's disease and ulcerative colitis), glomerulonephritis, psoriasis, rash, acute and chronic cystitis, hepatic cirrhosis, glomerulopathies and pulmonary fibrosis, arteriosclerosis, both
20 acute and chronic pain, septic, allergic and post-traumatic shocks, hepatorenal syndrome by hepatic cirrhosis, hypotension, alopecia, cancer and antiangiogenic diseases.