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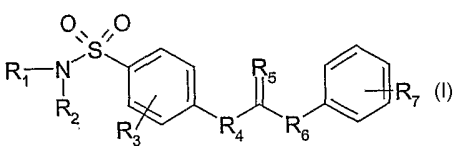
(54) Title: SULFONAMIDO COMPOUNDS THAT ANTAGONISE THE VANILLOID TRPV1 RECEPTOR



(57) Abstract: The invention relates to sulfonamido derivatives of formula (I) wherein R_1-R_7 are as defined in the description. Compounds (I) antagonize the 10 vanilloid receptor and can be used for the preparation of medicaments for the treatment of inflammatory states.

SULFONAMIDO COMPOUNDS THAT ANTAGONIZE THE VANILLOID TRPV1 RECEPTOR

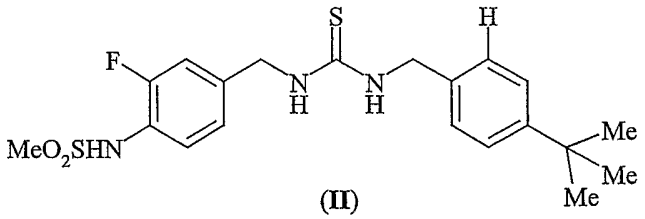
Field of the invention

The present invention relates to antagonists of the vanilloid receptor, in particular to sulfonamido derivatives that antagonize the TRPV1 receptor.

Background of the invention

Recent experimental evidences have demonstrated that expression of the vanilloid TRPV1 receptor (transient receptor potential channel) increases in inflammatory conditions. This led to hypothesize that TRPV1 antagonists could be useful for the treatment of inflammatory processes, for example chronic pain and inflammatory hyperalgesia.

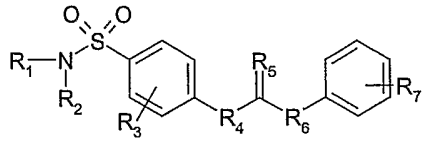
A number of antagonists of the vanilloid receptor are known; some of them derive from capsaicin and are called capsaicinoid antagonists. In particular, Wrigglesworth, R. et al (J. Med. Chem. 1996, 39, 4941-4951) disclosed the thiourea of formula (II):



(II)

Disclosure of the invention

The present invention relates to compounds of general formula (I)



(I)

in which:

R_1 is hydrogen;

R_2 is benzyl or 2-phenylethyl, in which the aromatic ring is optionally substituted with one or more groups selected from halogen, hydroxy and methoxy;

R_3 is hydrogen, halogen or an alkoxy group;

R_4 is a $-(CH_2)_nNH-$ group, in which n ranges from 0 to 3;

R_5 is S or O;

R_6 is $-NHCH_2-$;

R_7 is *t*-butyl or trifluoromethyl.

For the purposes of the present invention, halogen means fluorine, chlorine, bromine or iodine.

Preferred compounds of formula (I) are those wherein R_5 is S and R_6 is a $-NHCH_2-$ group, in particular those wherein R_3 is hydrogen and R_7 is selected from 4-*t*-butyl or 4-trifuoromethyl.

Among them, a first group of preferred compounds is that wherein R_4 n is 0.

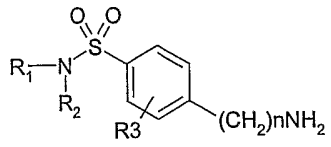
A second group of preferred compounds is that wherein in the group R_4 n is 2; among them, particularly preferred are the compounds in which R_1 is hydrogen and R_2 is benzyl or 2-phenylethyl, optionally substituted as indicated above.

In the compounds of formula (I) in which R_2 is benzyl or 2-phenylethyl wherein the aromatic ring is substituted, those in which R_2 is 2-iodo-4-hydroxy-5-methoxy-benzyl are preferred.

The compounds of formula (I) proved active as inhibitors of the vanilloid TRPV1 receptor and can therefore be used for the preparation of pharmaceutical compositions for the therapy of inflammatory states, for example chronic pain and inflammatory hyperalgesia. These formulations will be prepared with conventional methods and excipients, such as those described in Remington's Pharmaceutical Sciences Handbook, XVII Ed. Mack

Pub., N.Y., U.S.A..

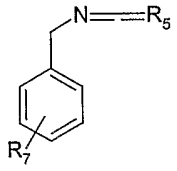
The compounds of formula (I) can be conveniently prepared according to conventional known techniques, for example by reaction of a sulfonamide of formula (III):



(III)

in which R_1, R_2, R_3 and n are as defined above;

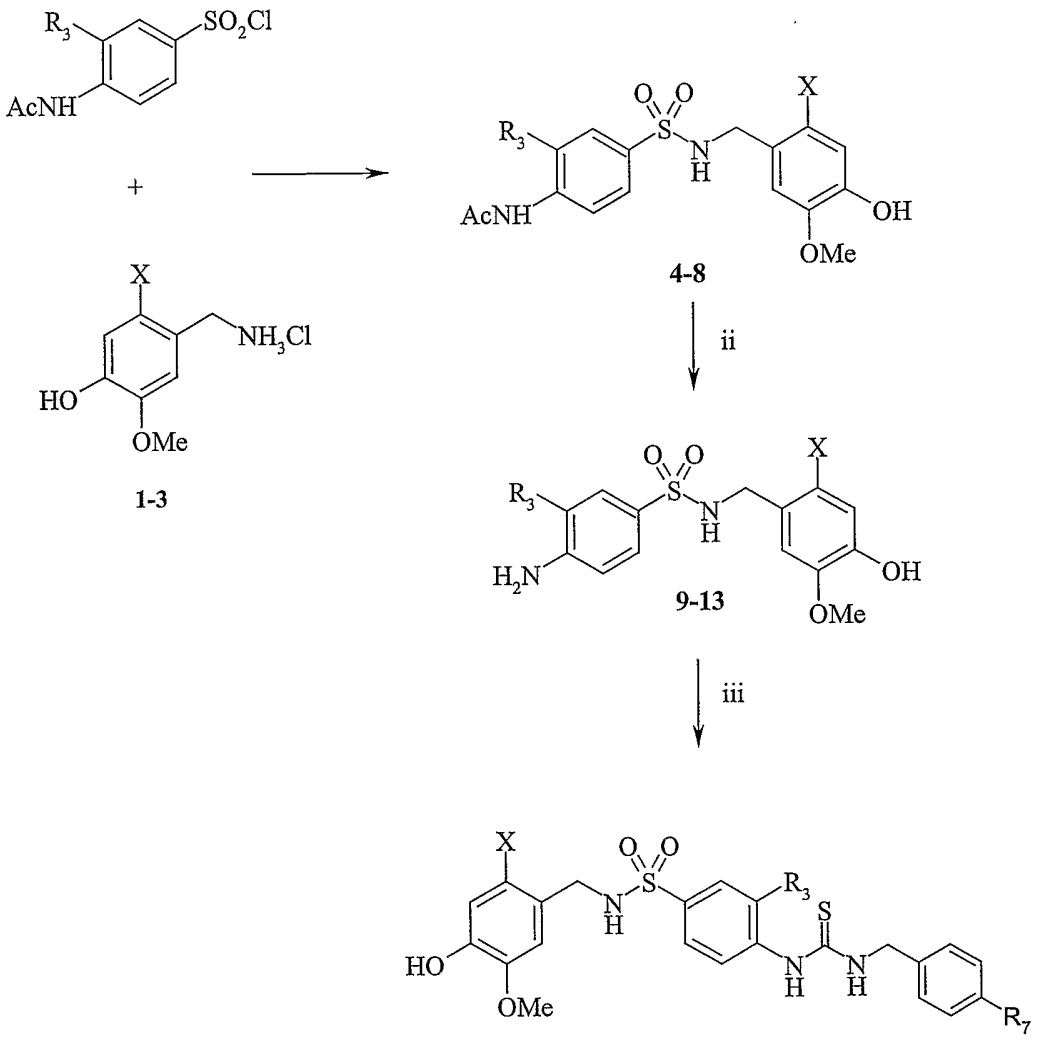
with an isocyanate or an isothiocyanate of formula (IV)



(IV)

The synthesis of some compounds of formula (I) is illustrated in Schemes 1-3 and is explained in greater detail in the following examples.

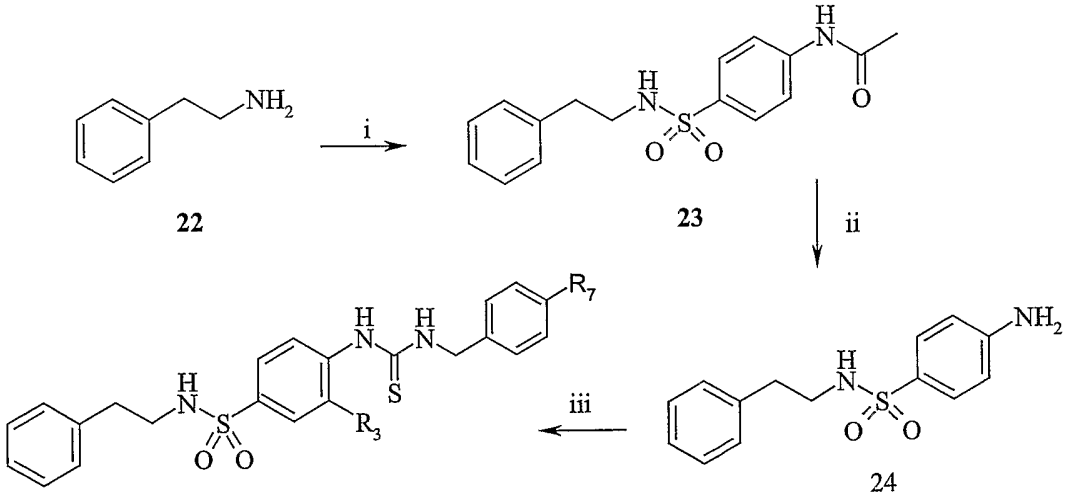
Scheme 1



Reagents: (i) Dioxane, TEA, 80°C; (ii) aq. 20% HCl, dioxane; (iii) 4-*tert*-butylbenzyl isothiocyanate or 4-trifluoromethylbenzyl isothiocyanate, ethanol, rfx.

R_3= H, F, OCH_3; X= I, Cl, Br; R_7= *t*-butyl, trifluoromethyl.

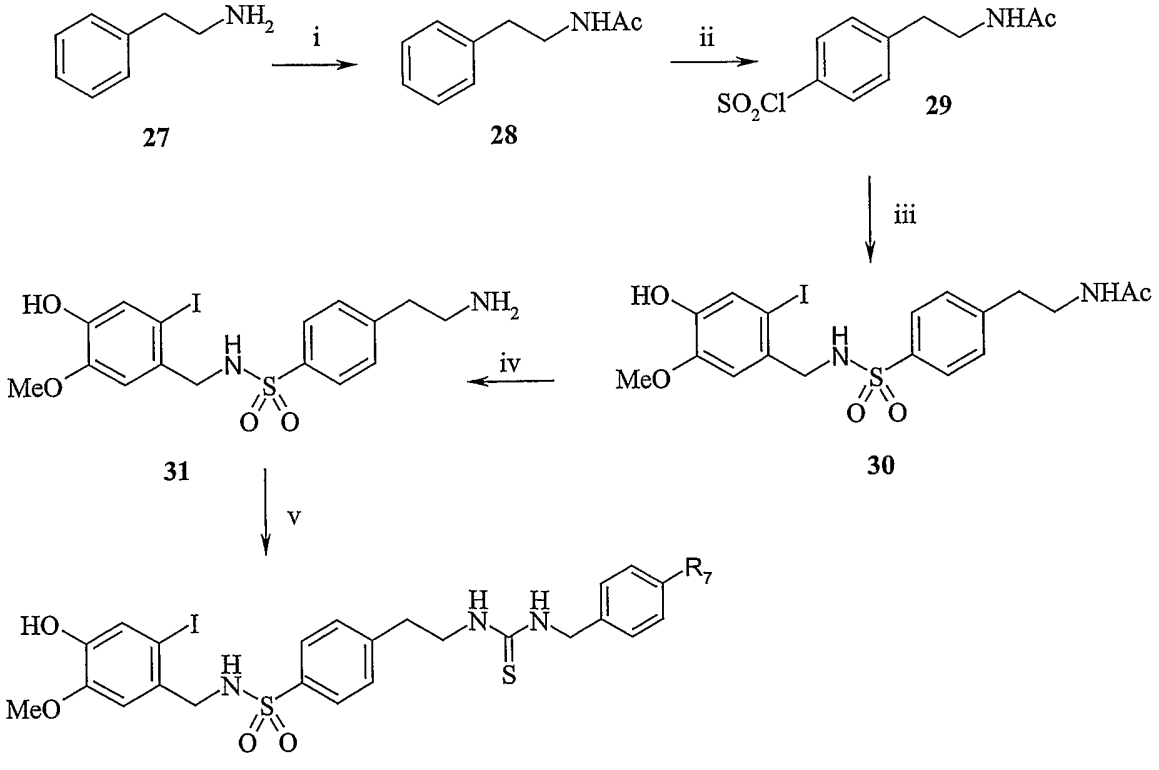
Scheme 2



Reagents: (i) 4-acetamidobenzene sulfonyl chloride, dioxane; (ii) aq. 20% NaOH; (iii) 4-*t*-butylbenzyl isothiocyanate or 4-trifluoromethylbenzyl isothiocyanate.

R_7= *t*-butyl, trifluoromethyl.

Scheme 3



Reagents: (i) Acetic anhydride, (ii) chlorosulfonic acid, 0°C; (iii) 2-iodo(chloro, bromo)-5-methoxy-4-hydroxy benzylamine hydrochloride, dioxane; (iv) aq. 20% NaOH; (v) 4-*tert*-butylbenzyl isothiocyanate or 4-trifluoromethylbenzyl isothiocyanate, ethanol, rfx.

R_7= *t*-butyl, trifluoromethyl.

1. EXAMPLES

The reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{245} Merck plates) and the products visualized with an iodine or potassium permanganate solution. ^{1}H NMR spectra were recorded in $CDCl_3$, CF_3COOD or DMSO-d_6 with a Varian VXR 200 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hz. IR spectra were recorded on a Pye Unicam SP 300 spectrometer using the KBr Wafer technique. Mass spectra were obtained with a Shimadzu QP5050 DI 50 spectrometer. The expression "Light petroleum ether" refers to petroleum fraction boiling at 40-60°C. Melting points (M.p.) were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatographies were performed using Merck 60-200 mesh silica gel. The synthesized compounds showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were within ±0.4% of the theoretical values for C, H, and N.

1. Preparation of 2-(substituted)-4-hydroxy-5-methoxy-benzylamine hydrochlorides 1-3

1.1. Synthesis of 4-acetyloxy-3-methoxy-*N*-acetyl-benzylamine

Acetic anhydride (1 ml, 10.5 mmol) was added to a solution of 4-hydroxy-3-methoxy-benzylamine hydrochloride (0.5 g, 2.63 mmol) in pyridine (5 ml) and the mixture was stirred at room temperature for 6 hours. The solvent was removed under reduced pressure and the residue was suspended in water (100 ml). The aqueous layer was extracted with ethyl

acetate (3 x 20 ml) and the combined organic phases were anhydrified (Na_2SO_4) and evaporated under reduced pressure to afford the title compound as white solid (0.45 g, yield 75%).

^{1}H-NMR($CDCl_3$) δ 2.01 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.38 (d, 2H, J=6, CH_2), 5.90 (bs, 1H, NH), 6.90 (m, 3H, aromatic).

MS: *m/z* 238.1 (M^+ $C_{12}H_{15}NO_4$).

1.2. Synthesis of 2-iodo-4-acetyloxy-5-methoxy-*N*-acetyl benzyl amine

The diacetyl derivative of example 1.1 and a catalytic amount of trifluoromethane sulfonic acid (5-6 drops) were added to a solution of IPy_2BF_4[1,2] (0.69 g, 6.9 mmol) in CH_2Cl_2 (40 ml). The resulting mixture was stirred at room temperature for 5 hours, then added with 10% aq. sodium thiosulfate until it became completely clear. The aqueous layer was extracted with CH_2Cl_2 (3 x 25 ml) and the organic phases were anhydrified (Na_2SO_4) and evaporated under vacuum. The residue was recrystallized from a mixture of CH_2Cl_2/Et_2O to afford the title compound as pale yellow solid (0.38 g, yield 65%).

^{1}H-NMR($CDCl_3$) δ 2.06 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 4.41 (d, 2H, J=5.6, CH_2), 6.0 (t, 1H, NH), 7.04 (s, 1H, aromatic), 7.44 (s, 1H, aromatic).

Bidimensional NOESY ($CDCl_3$): coupling between the singlet at 7.44 ppm and the singlet at 2.33 ppm confirms that iodine is at the 2-position of the aromatic ring.

MS: *m/z* 364 (M^+ $C_{12}H_{14}INO_4$).

1.3. Synthesis of 2-chloro-4-acetyloxy-5-methoxy-*N*-acetyl benzyl amine

N-chlorosuccinimide (3.15 mmol, 0.42 g) was added to a solution of 4-acetyloxy-3-methoxy-*N*-acetyl-benzylamine of Example 1.1 (0.5 g, 2.1

mmol) in dry DMF (6 ml) and the mixture was stirred for 30' at 0°C and then for 16 hours at room temperature.

When water was added to the reaction (40 ml) the formation of a white precipitate was observed.

The solid was filtered off and washed twice with cold water (2 x 20 ml), then dried over P_2O_5 to afford the title compound as white solid (0.45g, 83% yield).

^1H NMR(DMSO-d_6) δ 1.89 (s, 3H), 2.24 (s, 3H), 3.76 (s, 3H, OCH_3),4.27 (d, 2H, CH_2, J=8), 7.09 (s, 1H, aromatic), 7.25 (s, 1H, aromatic), 8.35 (t, 1H, NH).

Bidimensional NOESY (DMSO-d_6): coupling between the singlet at 2.24 ppm and the singlet at 7.25 ppm confirms that chlorine is at the 2-position of the aromatic ring.

MS: *m/z* 272.1 (M^+ $C_{12}H_{14}ClNO_4$).

1.4. Synthesis of 2-bromo-4-acetyloxy-5-methoxy-*N*-acetyl benzyl amine

N-bromosuccinimide (3.15 mmol, 0.42 g) was added to a solution of 4-acetyloxy-3-methoxy-*N*-acetyl-benzylamine of Example 1.1 (0.5 g, 2.1 mmol) in dry DMF (6 ml) and the mixture was stirred for 30' at 0°C and then for 16 hours at room temperature.

When water was added to the reaction (40 ml) the formation of a white precipitate was observed.

The solid was filtered off and washed twice with cold water (2 x 20 ml), then dried over P_2O_5 to afford the title compound as white solid (0.46 g, 81% yield).

^1H NMR(DMSO-d_6) δ 1.90 (s, 3H), 2.49 (s, 3H), 3.76 (s, 3H, OCH_3),4.26 (d, 2H, CH_2, J=8), 7.093 (s, 1H, aromatic), 7.39 (s, 1H, aromatic), 8.36 (t, 1H, NH).

Bidimensional NOESY (DMSO-d_6): coupling between the singlet at

2.49 ppm and the singlet at 7.39 ppm confirms that bromine is at the 2-position of the aromatic ring.

MS: *m/z* 315.1 (M^+ $C_{12}H_{14}BrNO_4$).

1.5. Synthesis of 2-iodo(chloro,bromo)-4-hydroxy-5-methoxy-benzylamine hydrochloride 1-3

37% hydrochloric acid (0.2 ml) was added to a solution of 2-iodo(chloro, bromo)-4-acetyloxy-5-methoxy-*N*-acetyl-benzylamine (0.1 g, 0.27 mmol) in abs. ethanol (5 ml) and the mixture was refluxed for 12 hours. After cooling, the solvent was evaporated off under reduced pressure and the residue was recrystallized from dry acetone to afford the title compounds as pale yellow solid in quantitative yield.

2. Preparation of N-[(2-substituted-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-3-R_3-4-acetamides 4-8

To a solution of 3-(substituted)-sulfonyl chloride (2.1 mmol) in dioxane (50 mL) was added TEA (2 mol eq) and 2-substituted-4-hydroxy-5-methoxy benzylamine hydrochlorides (2 mol eq). The mixture was heated at reflux for 3 h, the solvent was removed at reduced pressure and water (50 mL) was added to the residue. The solid formed was filtered off, dried and recrystallized from ethanol to give the desired products as white solids.

2.1. N-[(2-iodo-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-4-acetamide 4

Yield 80%; mp.: 123°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,11 (s, 3H), 3,76 (s, 3H), 3,88 (d, 2H, J=8), 6,61 (d, 2H, J=4)), 6,80 (s, 1H), 7,11 (t, 1H), 7,15 (s, 1H), 7,23 (bs, 1H), 7,53 (d, 2H, J=4), 8,80 (bs, 1H).

2.2. N-[(2-chloro-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-4-acetamide 5

Yield 68%; mp.: 132°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 2.08 (s, 3H), 3,37 (s, 3H), 4.04 (d, 2H, J=8), 6,72 (s, 1H)), 6,83 (s, 1H), 7,72 (s, 4H), 7,92

(t, 1H), 9.47 (bs, 1H), 10.30 (s, 1H).

2.3. N-[(2-bromo-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-4-acetamide 6

Yield 72%; mp.: 129°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 2.07 (s, 3H), 3,69 (s, 3H), 3.99 (d, 2H, J=8), 6,76 (s, 1H)), 6,87 (s, 1H), 6.95 (t, 1H), 7,66 (s, 4H), 8.44 (bs, 1H), 9.71 (bs, 1H).

2.4. N-[(2-iodo-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-3-fluoro-4-acetamide 7

Yield 96%; mp.: 211°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3.57 (s, 3H), 3.79 (s, 3H), 3.98 (d, 2H, J=7.8), 7.06 (s, 1H), 7.21 (s, 1H), 7.51 (m, 1H), 7.82 (m, 2H), 8.35 (bs, 2H), 10.08 (bs, 1H).

2.5. N-[(2-iodo-4-hydroxy-5-methoxy)-benzyl]aminosulfonyl benzene-3-methoxy-4-acetamide 8

Yield 81%; mp.: 208°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3.42 (s, 3H), 3.77 (s, 3H), 3.81 (s, 3H), 4.01 (d, 2H, J=7.8), 7.16 (s, 1H), 7.23 (s, 1H), 7.60 (m, 1H), 7.80 (m, 2H), 8.44 (bs, 2H), 9.98 (bs, 1H).

3. General procedure for the hydrolysis of the N-acetyl functionalities of example 2

To a solution of the acetyl derivatives of examples 2 (1 g) in dioxane (15 mL) was added aq. 20% HCl (20 mL) and the mixture was heated at reflux for 1 h. The solvent was removed at reduced pressure and water was added to the residue. The solution obtained was neutralized with aq. 20% NaOH and the solid formed was filtered off and washed with cold water (30 mL). The precipitate was dried and recrystallized from abs. ethanol to afford the free amino compounds as solids in a quantitative yield.

3.1. N-(2-iodo-4-hydroxy-5-methoxybenzyl)-4-aminobenzene sulfonamide 9

Pale yellow solid, mp.: 111°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,74 (s, 3H), 3,90

(d, 2H), 5,10 (bs, 2H), 6,61 (d, 2H, J=4), 6,83 (s, 1H), 7,00 (t, 1H), 7,16 (s, 1H), 7,50 (d, 2H, J=5), 8,80 (s, 1H).

3.2. N-(2-chloro-4-hydroxy-5-methoxybenzyl)-4-aminobenzene sulfonamide 10

Pale yellow solid, mp.: 111°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,69 (s, 3H), 3,87 (d, 2H), 5,93 (bs, 2H), 6,56 (d, 2H, J=4), 6,73 (s, 1H), 6.85 (t, 1H), 7,41 (d, 2H), 7,56 (t, 1H), 9.22 (s, 1H).

3.3. N-(2-bromo-4-hydroxy-5-methoxybenzyl)-4-aminobenzene sulfonamide 11

Pale yellow solid, mp.: 115°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,69 (s, 3H), 3,90 (d, 2H), 5,41 (bs, 2H), 6,63 (d, 2H, J=6), 6,87 (s, 1H), 6.90 (s, 1H), 7.46 (d, 2H, J=7), 7.64 (bt, 1H), 8.90 (bs, 1H).

3.4. N-(2-iodo-4-hydroxy-5-methoxybenzyl)-3-fluoro-4-aminobenzene sulfonamide 12

Pale yellow solid, mp.: 113°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,72 (s, 3H), 3,88 (d, 2H), 5,88 (bs, 2H), 6,63 (d, 1H, J=4), 6,91 (s, 1H), 6.95 (s, 1H), 7.81 (m, 2H), 7.69 (t, 1H), 9.05 (bs, 1H).

3.5. N-(2-iodo-4-hydroxy-5-methoxybenzyl)-3-methoxy-4-aminobenzene sulfonamide 13

Yellow solid, mp.: 120°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,44 (s, 3H), 3.77 (s, 3H), 3,94 (d, 2H), 6.01 (bs, 2H), 6,72 (d, 1H, J=4), 6,92 (s, 1H), 7.01 (s, 1H), 7.83 (m, 2H), 7.72 (t, 1H), 9.02 (bs, 1H).

4. General Procedure for the synthesis of *N*-(4-R_7-benzyl)-*N'*-[4-(2-substituted)-4-hydroxy-5-methoxybenzyl)-3-R_3-aminosulfonyl]phenyl thiourea derivatives 14-21

To a solution of compounds 9-13 (0.23 mmol) in abs. ethanol (10 mL) was added 4-*t*-butylbenzyl isothiocyanate or 4-trifluoromethylbenzyl isothiocyanate (1.2 mol eq, Wrigglesworth, R.et al. *J. Med. Chem.* 1996, *39*,

4942-4951) and the mixture was refluxed for 16 h. The solvent was removed at reduced pressure and the residue was purified by flash chromatography (EtOAc:etere petrolio 1:1) to give derivatives 14-21 as solids.

4.1. *N*-(4-*t*-butyl-benzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 14

Pale yellow solid, yield 42%, m.p.: 95°C; ^{1}H-NMR ($CDCl_3$) δ: 1,31 (s, 9H), 3,79 (s, 3H), 4,14 (d, 2H, J=4), 4,85 (d, 2H, J=4,2), 5,11 (t, 1H), 5,52 (bs, 1H), 6,75 (s, 1H), 7,05 (s, 1H), 7,27 (m, 4H), 7,41 (d, 2H), 7,74 (d, 2H), 7,86 (bs, 1H), 8,36 (bs, 1H).

MS: *m/z* 640,6 (M^+ $C_{26}H_{30}IN_3O_4S_2$).

IR (KBr) cm^{-1}: 1548 (C=S).

Anal. C, H, N, ($C_{26}H_{30}IN_3O_4S_2$): calculated C, 48,83; H, 4,73; N, 6,57. Found C, 48,80; H, 4,69; N, 6,55.

4.2. *N*-(4-triflouromethyl-benzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 15

Pale yellow solid, yield 38%, m.p.: 102°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3,69 (s, 3H), 3,88 (d, 2H, J=4), 4,76 (d, 2H, J=4), 6,51 (t, 1H), 6,69 (s, 1H), 7,05 (s, 1H), 7,36 (d, 2H), 7,44 (d, 2H), 7,55 (d, 2H), 7,63 (m, 3H), 7,81 (t, 1H), 9,37 (bs, 1H).

MS: *m/z* 651,4 (M^+ $C_{23}H_{21}F_3IN_3O_4S_2$).

IR (KBr) cm^{-1}: 1550 (C=S).

Anal. C, H, N, F ($C_{23}H_{21}F_3IN_3O_4S_2$): calculated C, 42,40; H, 3,25; N, 6,45; F, 8,75. Found C, 42,35; H, 3,22; N, 6,43; F, 8,76.

4.3. *N*-(4-*t*-butyl-benzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)-3-fluoro-aminosulfonyl]phenyl thiourea 16

White solid, 41% yield, m.p.: 105°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 1.29 (s, 9H), 3.62 (s, 3H), 3.83 (d, 2H, J=4), 4.13 (d, 2H, J=4.1), 6.44 (bt, 1H), 6.83 (s, 1H), 7.25 (m, 3H), 7.39 (m, 5H), 7.6 (bs, 1H), 7.80 (bs, 1H), 8.21 (bs, 1H).

MS: *m/z* 658.5 (M^+ $C_{26}H_{29}FIN_3O_4S$).

Anal. C, H, N, F ($C_{26}H_{29}FIN_3O_4S$): calculated C, 47.49; H, 4.45; N, 6.39. Found C, 47.41; H, 4.44; N, 6.36.

4.4. *N*-(4-*t*-butylbenzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)-3-methoxy-aminosulfonyl]phenyl thiourea 17

Pale yellow solid, 31% yield, m.p.: 103°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 1.27 (s, 9H), 3.82 (s, 3H), 3.84 (s, 3H), 4.07 (d, 2H), 4.81 (m, 2H), 6.41 (bs, 1H), 6.85 (m, 2H), 7.16 (s, 1H), 7.26 (m, 6H), 7.52 (m, 2H), 8.05 (bs, 1H).

MS: *m/z* 670.4 (M^+ $C_{27}H_{32}IN_3O_5S_2$).

Anal. C, H, N, F ($C_{27}H_{32}IN_3O_5S_2$): calculated C, 48.43; H, 4.82; N, 6.28. Found C, 48.40; H, 4.84; N, 6.25.

4.5. *N*-(4-*t*-butyl-benzyl)-*N'*-[4-(2-chloro-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 18

Pale yellow solid, 48% yield, m.p.: 124°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 1.27 (s, 9H), 3.67 (s, 3H), 3.96 (m, 2H), 4.18 (d, 2H, J=6), 6.31 (t, 1H), 6.72 (s, 1H), 6.86 (s, 1H), 7.18 (d, 2H), 7.31 (m, 4H), 7.65 (s, 2H), 8.40 (t, 1H), 9.46 (s, 1H), 9.85 (bs, 1H).

MS: *m/z* 549.4 (M^+ $C_{26}H_{30}ClN_3O_4S_2$).

Anal. C, H, N, F ($C_{26}H_{30}ClN_3O_4S_2$): calculated C, 56.97; H, 5.52; N, 7.67. Found C, 56.92; H, 5.50; N, 7.62.

4.6. *N*-(4-trifluoromethyl-benzyl)-*N'*-[4-(2-chloro-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 19

Yellow solid, 45% yield, m.p.: 122°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3.69 (s, 3H), 3.95 (d, 2H, J=6), 4.27 (d, 2H, J=6.1), 6.60 (t, 1H), 6.74 (s, 1H), 6.92 (m, 3H), 7.48 (d, 2H), 7.68 (m, 4H), 7.85 (t, 1H), 8.22 (bs, 1H), 9.41 (bs, 1H).

MS: *m/z* 561.4 (M^+ $C_{23}H_{21}ClF_3N_3O_4S_2$).

Anal. C, H, N, F ($C_{23}H_{21}ClF_3N_3O_4S_2$): calculated C, 49.33; H, 3.78; N, 7.50; F, 10.18. Found C, 49.30; H, 3.74; N, 7.45; F, 1011.

4.7. *N*-(4-*t*-butyl-benzyl)-*N'*-[4-(2-bromo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 20

Pale yellow solid, 51% yield, m.p.: 118°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 1.29 (s, 9H), 3.58 (s, 3H), 3.81 (m, 2H), 4.19 (d, 2H, J=6), 6.22 (t, 1H), 6.81 (s, 1H), 6.91 (s, 1H), 7.23 (d, 2H), 7.40 (m, 4H), 7.69 (s, 2H), 8.43 (t, 1H), 9.49 (s, 1H), 10.01 (bs, 1H).

MS: *m/z* 598.3 (M^+ $C_{26}H_{30}BrN_3O_4S_2$).

Anal. C, H, N, F ($C_{26}H_{30}BrN_3O_4S_2$): calculated C, 52.70; H, 5.10; N, 7.09. Found C, 52.66; H, 5.08; N, 7.12.

4.8. *N*-(4-trifluoromethyl-benzyl)-*N'*-[4-(2-bromo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl]phenyl thiourea 21

Pale yellow solid, 49% yield, m.p.: 120°C; ^{1}H-NMR ($DMSO_{d6}$) δ: 3.62 (s, 3H), 4.01 (d, 2H, J=6), 4.35 (d, 2H, J=6.1), 6.63 (t, 1H), 6.81 (s, 1H), 6.90 (m, 3H), 7.55 (d, 2H), 7.72 (m, 4H), 8.01 (t, 1H), 8.42 (bs, 1H), 9.81 (bs, 1H).

MS: *m/z* 605.3 (M^+ $C_{23}H_{21}BrF_3N_3O_4S_2$).

Anal. C, H, N, F ($C_{23}H_{21}BrF_3N_3O_4S_2$): calculated C, 45.70; H, 3.50; N, 6.95; F, 9.43. Found C, 45.63; H, 3.52; N, 6.92; F, 9.46.

5. Synthesis of *N*-(2-phenylethyl)-4-acetamidobenzene sulfonamide 23

A solution of sulfonyl chloride (0.5 g) in anhydrous dioxane (30 ml) is added with 2-phenylethyl-amine (1.6 eq, 0.43 ml) and the misture is refluxed for about 1 hour. The solvent is removed under reduced pressure and the residue is taken up with water (35 ml). The resulting solid is filtered under reduced pressure, dried and crystallised from ethanol to give compound 23 a white solid. (0,61 g, 84% yield).

M.p.: 98°C.

^{1}H-NMR ($DMSOd_6$) δ: 2.88 (s, 3H), 3,01 (t, 2H, J=8), 3.22 (q, 2H, J=8) 6.25 (t, 1H), 6.42 (d, 2H, J=6), 6.80 (m, 5H), 6,91 (bs, 1H), 7.01 (d, 2H, J=6).

6. Synthesis of N-(2-phenylethyl)-4-aminobenzene sulfonamide 24

To a solution of **23** (0.6 g, 1.7 mmol) in dioxane (8 ml) 20% NaOH is added (13 ml) and the mixture is refluxed for 1.5 hours. The solvent is concentrated under reduced pressure and the aqueous phase is added with 20% NaOH to pH=7. The resulting solid is filtered under reduced pressure, washed with water and dried to give compound **24** as white solid (0.43 g, 83% yield).

M.p.: 114°C.

1H-NMR (DMSOd6) δ: 2.2,75 (t, 2H, J=8), 3.3,20 (q, 2H, J=8), 4.4,18 (bs, 2H), 6.6,68 (d, 2H, J=6), 7.7,09 (d, 2H, J=5), 7.7,24 (m, 4H), 7.7,59 (d, 2H, J=6).

7. General procedure for the synthesis of N-(4-R_7-benzyl)-N'-[4-(2-phenylethyl-amino)sulfonyl] phenyl thioureas 25, 26

A solution of compound **24** (150 mg) in absolute ethanol (12 ml) is added with *t*-butyl-benzyl isothiocianate ((Wrigglesworth, R. et al. J. Med. Chem. 1996, 39, 4942-4951) or 4-trifluoromethyl benzyl isothiocianate (1.2 eq) and the solution is refluxed for about 16 hours. The solvent is evaporated under reduced pressure and the residue is purified by chromatography (AcOEt:petroleum ether 1:1) to give compounds **25, 26** as solids.

7.1 *N*-(4-*t*-butyl-benzyl)-N'-[4-(2-phenylethyl-amino)-sulfonyl] phenyl thiourea 25

White solid, 40% yield, m.p.: 97°C.

^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H), 2.77 (t, 2H, J=8), 3.22 (q, 2H, J=8), 4.61 (bs, 2H), 4.82 (d, 2H), 6.20 (bs, 1H), 7.08 (d, 2H), 7.25 (m, 5H), 7.37 (d, 2H), 7.77 (d, 2H), 7.82 (bs, 1H), 8.40 (bs, 1H).

MS: m/z 481.1,1 (M^+ $C_{26}H_{31}N_3O_2S_2$).

Anal. C, H, N, S ($C_{26}H_{31}N_3O_2S_2$): calculated C, 64.83; H, 6. 49; N,

8.72; S, 13.31. Found C, 64. 80; H, 6.42; N, 8.69; S, 13.29.

7.2 *N*-(4-trifluromethyl-benzyl)-N'-[4-(2-phenyletyl-amino) sulfosulfonyl]phenyl thiourea 26

White solid, 70% yield, m.p.: 102°C.

^{1}H-NMR ($CDCl_3$) δ: 2.77 (t, 2H, J=6), 3.21 (q, 2H, J=6), 4.51 (t, 1H), 4.90 (d, 2H), 6.63 (d, 2H), 7.09 (d, 2H), 7.20 (m, 5H), 7.35 (d, 2H), 7.58 (d, 2H), 7.92 (bs, 1H), 8.32 (bs, 1H).

MS: m/z 494.4,3 (M^+ $C_{23}H_{22}F_3N_3O_2S_2$).

Anal. C, H, N, F ($C_{23}H_{22}F_3N_3O_2S2$): calculated C, 55. 97; H, 4.49; N, 8. 51; F, 1.1,55. Found C, 55.94; H, 4.47; N, 8.48; F, 11. 52.

8. Synthesis of N-acetyl-2-phenylethylamine 28

A commercially available solution of 2-phenylethylamine (2 ml, 15.8 mmol) in pyridine (5 ml) is added with acetic anhydride (2 eq., 3 ml) and the solution is stirred at room temperature for 12 hours. The solvent is evaporated off under reduced pressure and the residue is taken up with water (40 ml) and the aqueous phase is extracted with EtOAc (4x25 ml). The organic extracts are pooled, anhydrified over Na2SO4 and evaporated under reduced pressare. The residue is crystallised from petroleum ether to give compound **28** as white solid (2.56 g, quantitative yield).

^{1}H-NMR ($CDCl_3$) δ: 1.93 (s, 3H), 2.81 (t, 2H, J=8), 3.51 (t, 2H, J=8), 5.61 (bs, 1H), 7.18 (m, 5H).

9. Synthesis of 4-acetamidoethylbenzene sulfonyl chloride 29

Compound **28** (0.5 g, 3 mmol) is added with HSO_3Cl (1.1 ml) drop by drop, keeping the temperature at 0°C. At the end of the addition the reaction mixture is heated to a 100° for about 1 hour, then cooled and poured over crushed ice. The resulting precipitate is filtered and washed with water to give compound **29** as white solid (0.51 g, yield 65%).

^{1}H-NMR ($DMSO_{d6}$) δ: 1.94 (s, 3H), 2.79 (t, 2H), 3.40 (m, 2H), 7.19

(d, 2H, J=4), 7.72 (d, 2H, J=4), 8.08 (bs, 1H).

10. Synthesis of *N*-[(2-iodo-4-hydroxy-5-methoxy)-benzyl]amino sulfonyl-benzene-*N*-ethyl-4-acetamide 30

A solution of **29** (250 mg, 0.9 mmol) in anhydrous dioxane (20 ml) is added with 2-iodo-4-hydroxy-5-methoxy benzylamine hydrochloride (1.6 eq, 480 mg) and TEA (1.8 eq, 0.24 ml) and the mixture is refluxed for about 1 hour. At the end of the addition the solvent is evaporated under reduced pressure and the residue is taken up with water (40 ml). The resulting precipitate is filtered with suction, washed with water and dried to give compound **30** as pale yellow solid (380 mg, yield 84%). The crude compound is used for the following deprotection reaction.

11. Synthesis of *N*-[(2-iodo-4-hydroxy-5-methoxy)-benzyl]-4-(2-ethylamino)benzene sulfonamide 31

A solution of compound **30** (110 mg, 0.22 mmol) in dioxane (5 ml) is added with 20% NaOH (6 ml) and the mixture is heated to 80°C for about 3 hours. At the end of the addition the solvent is removed under reduced pressure and the pH of the residue is adjusted to 7 with 20% HCl. The resulting precipitate is filtered under reduced pressure, dried and e crystallised from ethyl ether to give compound **31** as pale yellow solid (90 mg, 88% yield).

M.p.: 291°C.

^{1}H-NMR ($DMSOd_6$) δ: 2.48 (s, 3H), 2.67 (m, 2H), 3.52 (t, 2H), 3.67 (bs, 3H), 3.87 (s, 2H), 6.78 (s, 1H), 7.11 (s, 1H), 7.36 (d, 2H, J=8), 7.66 (d, 2H, J=8).

12. General procedure for the synthesis of *N*-(4-R_7-benzyl)-*N*'-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)aminosolfonyl-2-phenylethyl] thiourea derivatives 32 and 33

A solution of compound **31** (100 mg, 0.2 mmol) in absolute ethanol

(12 ml) is added with 4-*t*-butyl-benzyl isothiocianate (Wrigglesworth, R. et al. J. Med. Chem. 1996, 39, 4942-4951) or 4-trifluoromethyl benzyl isothiocianate (1.2 eq.) and the solution is refluxed for about 16 hours. Thereafter, the solvent is evaporated under reduced pressare and the residue is purified by chromatography (EtOAc: petroleum ether 1:1) to give compounds **32** and **33** as solids.

12.1. *N*-(4-*t*-butyl-benzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl-2-phenylethyl] thiourea 32

White solid, 41% yield, m.p.: 195°C.

^{1}H-NMR ($CDCl_3$) δ: 1.31 (s, 9H), 2.85 (t, 2H), 4.71 (m, 2H), 3.79 (s, 3H), 4.14 (d, 2H, J=6), 4.49 (bs, 2H), 5.18 (t, 1H), 5.74 (bs, 1H), 5.91 (bs, 1H), 6.18 (bs, 1H), 6.70 (s, 1H), 7.2 (m, 5H), 7.37 (d, 2H), 7.66 (d, 2H).

MS: m/z 668,5 (M^+ $C_{28}H_{34}IN_3O_4S_2$).

Anal. C, H, N ($C_{28}H_{34}IN_3O_4S_2$): calculated C, 50,37; H, 5,13; N, 6,29. Found C, 50,19; H, 5,11; N, 6,21.

12.2. *N*-(4-trifluoromethyl-benzyl)-*N'*-[4-(2-iodo-4-hydroxy-5-methoxy-benzyl)aminosulfonyl-2-phenylethyl] thiourea 33

Pale yellow solid, 52% yield, m.p.: 186°C.

^{1}H-NMR ($CDCl_3$) δ: 2.93 (t, 2H), 3.75 (m, 2H), 3.79 (s, 3H), 4.14 (d, 2H, J=8), 4.76 (d, 2H), 5.20 (t, 1H), 5.81 (bs, 1H); 5.92 (bs, 1H), 6.20 (t, 1H), 6.70 (s, 1H), 7.11 (s, 1H), 7.16 (d, 2H, J=6), 7.42 (d, 2H), 7.63 (m, 4H).

MS: m/z 680,3 (M+ $C_{25}H_{25}F_3IN_3O_4S_2$).

Anal. C, H, N ($C_{25}H_{25}F_3IN_3O_4S_2$): calculated C, 44,19; H, 3,71; N, 6,18. Found C, 44,15; H, 3,52; N, 5,99.

2. Pharmacology

Materials and Methods

Animals and Tissues

Newborn and adult Sprague-Dawley rats (~250 g) were used (Harlam,

Italy). All experiments complied with the national guidelines and were approved by the regional ethics committee.

Radioligand binding assays

Male Sprague-Dawley rats with body weight between 250 to 350 g at the time for testing were used. For binding assays rats were sacrificed by decapitation under anesthesia and spinal cord was removed and disrupted using a Polytron tissue homogenizer in ice cold buffer containing 5 mM KCl, 5.8 mM NaCl, 0.75 mM $CaCl_2$, 2 mM $MgCl_2$, 320 mM sucrose, 10 mM Hepes, pH 8.6 (Szallasi and Blunberg, 1992; 1993). Tissue homogenized was centrifuged at 1000 xg for 10 min at 4°C and the surnatant was centrifuged again at 35000 xg for 30 min at 4°C (Beckman Avanti J25). The pellet was resuspended in the same buffer as described above and used in binding experiments. In saturation experiments, 150 μg protein/sample from membrane suspensions were incubated with ([^{3}H]-Resiniferatoxin, Perkin Elmer, Boston, MA) [^{3}H]-RTX (0.003-3 nM) in the assay buffer containing 0.25 mg/ml fatty acid-free bovine serum albumin at 37°C for 60 min. In competition experiments, the membranes were incubated at 37°C for 60 min with [^{3}H]RTX (0.4 nM) and increasing concentrations of examined compounds in the range from 0.1 nM to 3 μM. Non specific binding was defined in the presence of 1 μM RTX. After incubation the reaction mixture was cooled at 0°C and incubated with bovine α1-acid glycoprotein (200 μg per tube) for 15 min to reduce non-specific RTX binding. Membrane-bound RTX was separated from the free through the centrifugation of the samples at 18500 xg for 15 min. The tip of the microcentrifuge tube containing the pellet was cut off and the radioactivity was determined by scintillation counting (Packard 2500 TR). The protein concentration was determined according to a Bio-Rad method with bovine serum albumin as a standard reference (Bradford, 1976). Saturation and competition studies were analyzed with the program Ligand (Munson and

Rodbard, 1980).

Ca^{2+} fluorescence measurements in cultured rat trigeminal ganglia neurons

Newborn rats (2 days old) were terminally anaesthetized and decapitated. The trigeminal ganglia were removed and rapidly placed in cold phosphate buffered solution (PBS) before being transferred to collagenase/dispase (1 mg/ml dissolved in Ca^{2+}-Mg^{2+}-free PBS) for 35 min at 37°C (Rigoni et al, 2003). After the enzymatic treatment ganglia were rinsed three times with Ca^{2+}-Mg^{2+}-free PBS and then placed in 2 ml of cold DMEM supplemented with 10% foetal bovine serum (FBS, heat inactivated), 2 mM L-glutamine, 100 u/ml penicillin and 100 mg/ml streptomycin. The ganglia were then dissociated into single cells by several passages through a series of syringe needles (23G down to 25G). Finally, the complex of medium and ganglia cells were sieved through a 40 mm filter to remove debris and topped up with 8 ml of DMEM medium and centrifuged (200 x g for 5 min). The final cell pellet was re-suspended in DMEM medium (supplemented with 100 ng/ml mouse Nerve Growth Factor (mouse-NGF-7S) and cytosine-b-D-arabino-furanoside free base (ARA-C) 2.5 mM). Cells were plated on poly-L-lysine (8.3 mM) and laminin (5 mM) coated 25 mm glass cover slips and kept for 2 to 5 days at 37°C in a humidified incubator gassed with 5% CO_2 and air. Plated neurons were loaded with Fura-2-AM-ester (3 μM) in Ca^{2+} buffer solution of the following composition (mM): $CaCl_2$ 1.4, KCl 5.4, $MgSO_4$ 0.4, NaCl 135, D-glucose 5, HEPES 10 with BSA 0.1%, at pH 7.4, for 40 min at 37°C, washed twice with the Ca^{2+} buffer solution and transferred to a chamber on the stage of Nikon eclipse TE300 microscope. The dye was excited at 340 and 380 nm to indicate relative $[Ca^{2+}]_i$ changes by the F_{340}/F_{380} ratio recorded with a dynamic image analysis system (Laboratory Automation 2.0, RCS, Florence, Italy). Capsaicin (0.1 μM) and ionomycin (5 μM) were added to the chamber.

A calibration curve using a buffer containing Fura-2-AM-ester and determinant concentrations of free Ca^{2+} (Kudo et al., 1986) was used to convert the data obtained from F_{340}/F_{380} ratio to $[Ca^{2+}]_i$ (nM).

The effects of all compounds were tested against capsaicin-induced calcium mobilisation. Antagonistic compounds were incubated for 10 minutes prior to the capsaicin challenge. The inhibitory effect of the TRPV1 antagonist, capsazepine, was also tested.

Wiping test in rats

The irritant effect (induction of wiping movements) of capsaicin was assessed by applying capsaicin 3 μg/eye (10 μl) on the rat conjunctiva and the number of wiping movements was recorded during the 60 sec period that followed the application. In other set of experiments, rats were treated intraperitoneally with diverse doses of 14 and capsaicin-induced wiping was studied.

Drugs and solubility

Drugs and reagents were obtained from the indicated companies: capsaicin, ionomycin, laminin, poly-L-lysine and capsazepine (Sigma, Italy); mouse NGF-7S and collagenase/dispase (Roche Diagnostics, Italy); Dulbecco's Modified Eagle's medium (DMEM), foetal bovine serum (FBS) heat inactivated, L-glutamine (200 mM), penicillin/streptomycin (10,000 IU/ml ± 10,000 UG/ml), Ca^{2+}-Mg^{2+}-free phosphate buffered solution (PBS) (Gibco, Italy); Fura-2-AM- ester (Societa' Italiana Chimici, Italy). The stock concentrations of capsaicin (10 mM), were prepared in 100% ethanol. Mother solutions of all the PharmEste compounds (100 mM), Fura-2-AM-ester (100 mM) and ionomycin (100 mM) were prepared in DMSO. The appropriate dilutions were then made in Krebs buffer solution.

Results

General overview

Compounds **14**, **19** and **20** exhibited the ability to bind and activate the

TRPV1 receptor.

Binding assay

Competition binding experiments of [^{3}H]-RTX showed that 3 compounds had a great affinity versus the TRPV1 receptor expressed in rat spinal cord (table 1). In particular **14** revealed affinity values less than 100 nM. The order of potency of these compounds was: **14>20>19**.

Ca^{2+} fluorescence

Capsaicin (0.1 μM) caused an increase in $[Ca^{2+}]_i$ in the vast majority (87%) of rat trigeminal neuronal cells, that therefore were identified as TRPV1 expressing neurons. For IC_{50} values of all the compounds please referrer to table 1. Data are expressed as Mean and 95% fiducial limits.

Table 1: Affinities (K_i, nM) and potencies (IC_{50}, nM) values of TRPV1 antagonists

Compound code	K_i (nM) (Fiducial limits)	IC_{50} (nM) (Fiducial limits)
Capsazepine	**NT**	**2168** (1528-3080)
14	**90** (73-110)	**60** (43-85)
20	**493** (340-716)	**212** (90-590)
19	**756** (515-1109)	**270** (127-574)

Affinity (K_i) and potency (IC_{50}) values were obtained by using [^{3}H]-RTX competition binding assays and intracellular calcium assay in cultured rat trigeminal neurons. NT: not tested.

Wiping test in rats

Intraperitoneal compound **14**, 60 minutes prior to the capsaicin challenge, caused a dose dependent reduction of the capsaicin-induced wiping behaviour in rats (the dose of 1 mg/kg produced a 24% of inhibition).

Conclusions

In *in vitro* and *in vivo* studies, **14** was able to inhibit TRPV1-dependent

responses with an affinity that was significantly greater than that of the classic TRPV1 receptor antagonist, capsazepine. Furthermore, the compounds **19** and **20** did demonstrate high affinity for the TRPV1 receptor in vitro. All the compounds mentioned above may be an important tool for future studies in pain and neurogenic inflammatory models.

References

Bradford MM, *Anal Biochem* (1976) 72: 248-254.

Kudo Y. *et al.,* Japanese. Journal of Pharmacology (1986) 41, 345-151.

Munson PJ *et al., Anal Biochem* (1980) 107: 220-239.

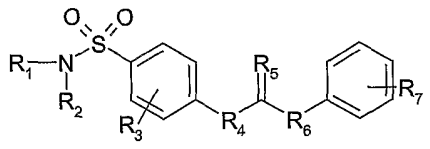
Rigoni M. *et al.,* British Journal of Pharmacology (2003) 138, 977-985.

Szallasi A. and Blunberg PM, ed. P.M. Conn (Academic Press, Orlando, FL) (1992) vol. 8,.p. 368.

Szallasi A. and Blunberg PM, *Naunyn Schmiedeberg's Arch Pharmacol* (1993) 347: 84-91.

CLAIMS

1. Compounds of formula (**I**):



(I)

wherein:

R_1 is hydrogen;

R_2 is benzyl or 2-phenylethyl, wherein the aromatic ring is optionally substituted with one or more groups selected from halogen, hydroxy and methoxy;

R_3 is hydrogen, halogen or an alkoxy group;

R_4 is a $-(CH_2)_nNH-$ group, wherein n ranges from 0 to 3;

R_5 is S or O;

R_6 is a $-NHCH_2-$;

R_7 is *t*-butyl or trifluoromethyl.

2. Compounds according to claim 1 wherein R_5 represents S and R_6 represents a $-NHCH_2-$ group.

3. Compounds according to claim 2 wherein R_3 is hydrogen, R_7 is selected from 4-*t*-butyl or 4-trifuoromethyl.

4. Compounds according to claim 3 wherein, in the R_4 group, n is 0.

5. Compounds according to claim 3 wherein, in the R_4 group, n is 2.

6. Compounds according to claim 5 wherein R_1 is hydrogen and R_2 is benzyl or 2-phenylethyl, wherein the aromatic ring is optionally substituted with one or more groups selected from halogen, hydroxy and methoxy.

7. Compounds according to any one of claims 1 to 6 wherein R_1 is hydrogen and R_2 2-is iodo-4-hydroxy-5-methoxy-benzyl.

8. Compounds of formula (**I**) as defined in any one of claims 1 to 7 for use as medicament.

9. Use of compounds of formula (**I**) as defined in any one of claims 1 to 7 for the preparation of pharmaceutical compositions for the therapy of inflammatory states.

10. The use according to claim 9 wherein the inflammatory state is chronic pain or inflammatory hyperalgesia.

11. Pharmaceutical compositions containing a compounds of formula (**I**) as defined in any one of claims 1 to 7 in admixture with suitable excipients and/or vehicles.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP2005/011206

A. CLASSIFICATION OF SUBJECT MATTER
C07C335/12 C07C335/16 C07C311/21 C07C311/37 A61K31/17
A61K31/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/16318 A (PACIFIC CORPORATION; SUH, YOUNG, GER; OH, UH, TAEK; KIM, HEE, DOO; LEE) 28 February 2002 (2002-02-28) page 60, Example 12; claims	1-11
A	SHAH, K. J. ET AL: "Benzylthioureas. III" J. INDIAN CHEM. SOC. , 36, 507-8, 1959, XP009059692 page 507, Table 1; page 508, Table II	1-11
A	EP 0 693 386 A (NIPPON PAPER INDUSTRIES CO., LTD) 24 January 1996 (1996-01-24) page 7, compound A-37; page 11, compound B-16	1-11

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]/EP2005/011206

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0216318 A	28-02-2002	AU 8022901 A	04-03-2002
		CA 2417507 A1	28-02-2002
		CN 1418191 A	14-05-2003
		EP 1303483 A1	23-04-2003
		JP 2004506713 T	04-03-2004
		KR 2002039226 A	25-05-2002
		NZ 523882 A	26-11-2004
EP 0693386 A	24-01-1996	CA 2154323 A1	22-01-1996
		DE 69500152 D1	20-03-1997
		DE 69500152 T2	18-09-1997
		US 5665675 A	09-09-1997