Pharmacological characterization of RP 62203, a novel 5-hydroxytryptamine 5-HT₂ receptor antagonist

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1 RP 62203 (2-[3-(4-(4-fluorophenyl)-piperazinyl)propyl]naphtho[1,8-cd]isothiazole-1,1-dioxide) is a novel naphthosultam derivative which shows very high affinity for 5-HT₂ receptors in the rat cerebral cortex ($K_i = 50.0$ pM).

2 RP 62203 is relatively selective for this sub-type of 5-hydroxytryptamine (5-HT) receptor, having lower affinity for the 5-HT_{1A} receptor and very low affinity for the 5-HT₃ receptor. RP 62203 displayed low to moderate affinity for α_1 -adrenoceptors, dopamine D₂ receptors and histamine H₁ receptors.

3 *In vivo* binding experiments demonstrated that oral administration of low doses of RP 62203 led to a long-lasting (> 6 h) occupation of cortical 5-HT₂ receptors (ID₅₀ = 0.39 mg kg⁻¹).

4 In cortical slices from the neonatal rat, RP 62203 potently inhibited inositol phosphate formation evoked by 5-HT, with an IC₅₀ of 7.76 nM.

5 The activity of neurones in the raphe and their responses to microiontophoretically applied 5-HT were studied with extracellular recording electrodes in the anaesthetized rat. RP 62203 potently and dose-dependently blocked excitations evoked by 5-HT when administered at doses of 0.5–4.0 mg kg⁻¹, i.p. In contrast, neither 5-HT-evoked depressions nor glutamate-evoked excitations of raphe neuronal firing were blocked by RP 62203 at doses as high as 8.0 mg kg⁻¹, i.p.

6 Head twitches induced by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) could be abolished by low doses of RP 62203 in mice (ED₅₀ = 0.44 mg kg⁻¹, p.o.) and in rats (ED₅₀ = 1.54 p.o.). Similar results were obtained with mescaline and 5-hydroxytryptophan (5-HTP).

7 The potency of RP 62203 was compared with that of three other 5-HT₂ receptor antagonists, ritanserin, ICI 169,369 and ICI 170,809. In all models, RP 62203 showed similar activity to ritanserin, whilst either ICI 169,369 or ICI 170,809 was several fold less active.

8 It is concluded that RP 62203 is a potent and selective antagonist at 5-HT₂ receptors in the rodent central nervous system.

Keywords: 5-Hydroxytryptamine; 5-HT₂ receptor antagonists; RP 62203; binding; inositol phosphate; raphe; neuronal activity; head-twitch

Introduction

5-Hydroxytryptamine (5-HT) is a widely-distributed neurotransmitter in the mammalian central nervous system, where it is thought to play a role in the neural circuits involved in pain perception, sleep, mood, anxiety, appetite and sexual behaviour (see Willis, 1984 for review). Understanding of the physiological role of 5-HT has, however, been hampered by the multiplicity of receptor subtypes and the complexity of their pharmacology (Bradley *et al.*, 1986), discrepancies between binding studies and functional tests (Leysen, 1984), and by species and tissue differences (Hoyer *et al.*, 1986a,b; Hoyer & Middlemiss, 1989; Mylecharane, 1989). The best-characterized of these receptor subtypes is the 5-HT₂ receptor, due to the availability of reasonably selective agonists and antagonists (Shannon *et al.*, 1984; Leysen *et al.*, 1985; Blackburn *et al.*, 1988a).

From electrophysiological studies in the rat, both *in vivo* and *in vitro*, it has been shown that 5-HT excites neurones in the cortex (Davies *et al.*, 1987), the nucleus accumbens (North & Uchimura, 1989), the hippocampus (Andrade & Nicoll, 1987) and the brainstem (Davies *et al.*, 1988) through a 5-HT₂ receptor. The neuronal excitation which follows 5-HT₂ receptor activation results from the closure of an inwardly rectifying K⁺ conductance which induces a slow depolarization. It has been suggested that 5-HT₂ receptors could be linked to protein kinase C through inositol phospholipid hydrolysis

(North & Uchimura, 1989), although in rat facial motoneurones Aghajanian (1990) has reported that this response is mediated by G proteins but not protein kinase C. This 5-HT-evoked firing is readily antagonised by ketanserin, which agrees well with biochemical data from rat brain (Leysen, 1984).

The raphe nuclei of the mammalian brainstem are the origin of the major 5-hydroxytryptaminergic projections throughout the brain and spinal cord and the rate of raphe neuronal firing thus exerts considerable control over many regions in the central nervous system. Compounds which interact with raphe receptors are therefore of significant clinical interest.

In behavioural models of 5-HT-mediated functions, however, the involvement of 5-HT₂ receptors is less clear. Perhaps the best evidence for a behavioural response mediated by 5-HT₂ receptors has been found in the case of 5-HT agonist-induced head-twitches (Green *et al.*, 1983). These can be observed after administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), and the 5-HT₂ receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and mescaline (Corne & Pickering, 1967; Peroutka *et al.*, 1981; Heaton *et al.*, 1988). These responses can be blocked by 5-HT₂ receptor antagonists, such as ritanserin (Goodwin & Green, 1985).

This report describes the properties of a novel 5-HT₂ receptor antagonist belonging to the naphthosultam family, RP 62203 (Figure 1), which shows high affinity and selectivity for 5-HT₂ receptors in rodent brain. The activity of RP 62203 has been compared to those of three reference compounds, ritanserin (Leysen *et al.*, 1985), ICI 169,369 (Blackburn *et al.*,

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Figure 1 Structure of RP 62203 (2-(3-(4-(4-fluorophenyl)-piperazinyl)propyl)naphtho(1,8-c,d)isothiazole-1,1-dioxide).

1988a; 1990) and ICI 170,809 (Blackburn *et al.*, 1988b; Frenken & Kauman, 1989). The properties of RP 62203 have been studied in radioligand binding assays and three functional tests of 5-HT₂ receptor activity, inositol phosphate formation, neuronal firing in the raphe nuclei and 5-HT agonist-induced head-twitches. Preliminary reports of some of these data have been published (Doble *et al.*, 1990; Piot *et al.*, 1990; Dupuy *et al.*, 1991).

Methods

Binding assays

The affinity of the different compounds for 5-HT₂ receptors was assessed by use of the [³H]-ketanserin binding assay described by Leysen *et al.* (1982). Male Sprague-Dawley rats (Charles River France) were killed by decapitation and their brains rapidly removed on ice. The cerebral cortices were dissected out and homogenized in 10 volumes of ice-cold Tris-HCl buffer (50 mM; pH 7.6). The homogenate was centrifuged for 10 min at 50,000 *g* at 4°C and the supernatant discarded. The pellet was washed by resuspension in seven volumes of the same buffer and recentrifugation, the final pellet was then resuspended in five volumes of Tris-HCl buffer, distributed in 1 ml aliquots in plastic vials (Nunc) and frozen at -80°C until required. Protein content was measured according to the BCA method (Smith *et al.*, 1985) with bovine serum albumin as the standard.

Aliquots (1 ml) of cortical membranes at a protein concentration of 0.15 mg ml⁻¹ in Tris-HCl buffer were incubated for 15 min at 37°C with the compound of interest or with 10⁻⁶ M methysergide (to define the non-specific binding) in the presence of [³H]-ketanserin (final concentration 0.4 nM). The binding interaction was terminated by filtration across Whatman GF/B glass fibre filters using a Skatron cell harvester, and was followed by two washes with 2.5 ml of ice-cold buffer. The radioactivity retained on the filters was determined by liquid scintillometry in 4.5 ml of Ready-Gel scintillant (Beckman).

The specific binding was defined as that displaceable by 10⁻⁶ M methysergide. The percentage of the specific binding obtained with each concentration of the test compound was calculated, and the IC₅₀ (concentration that inhibits 50% of the specific binding) determined by non-linear regression analysis using an iterative curve-fitting procedure to a simple Langmuir isotherm performed on an IBM-PC computer (McPherson, 1985).

The other binding assays were carried out on frozen crude membrane preparations from rat brain (or guinea-pig brain, in the case of histamine H₁, cholecystokinin, neurotensin and opiate receptors). The protocols were taken from published methods: α₁-adrenoceptors (Greengrass & Bremner, 1979), α₂-adrenoceptors (Rouot & Snyder, 1979), benzodiazepine binding sites (Speth *et al.*, 1978), bradykinin receptors (Manning *et al.*, 1986), CCK_A and CCK_B cholecystokinin receptors (Saito *et al.*, 1981), dopamine D₂ receptors (Urwyler & Coward, 1987), histamine H₁ receptors (Laduron *et al.*,

1982), 5-HT_{1A} receptors (Hall *et al.*, 1985), 5-HT_{1B} receptors (Hoyer *et al.*, 1985), 5-HT₃ receptors (Kilpatrick *et al.*, 1987), 5-HT uptake sites (Hebert *et al.*, 1985), N-methyl-D-aspartate receptors (Vignon *et al.*, 1983), muscarinic receptors (Yamamura & Snyder, 1974), neuropeptide Y receptors (Unden *et al.*, 1984), neurotensin receptors (Schotte *et al.*, 1986), opiate receptors (Kosterlitz *et al.*, 1981), somatostatin receptors (Tran *et al.*, 1985), tachykinin NK₁ receptors (Lee *et al.*, 1983), vasoactive intestinal peptide receptors (Robberecht *et al.*, 1978), calcium channels (Galizzi *et al.*, 1984) and sodium channels (Pauwels *et al.*, 1986).

In vivo binding experiments

In the rat, [³H]-spiperone can be used to determine the occupation *in vitro* of 5-HT₂ receptors in the frontal cortex and dopamine D₂ receptors in the striatum (Leysen *et al.*, 1985). Similarly, in the mouse brain, [³H]-methylspiperone has been proposed as a useful ligand for the study of D₂ and 5-HT₂ receptors *in vivo* (Frost *et al.*, 1987).

Male rats (170–190 g, Charles River France) were used for all experiments. Each animal received an injection of [³H]-spiperone (0.185 MBq in 100 μl, approximately 0.5 μg kg⁻¹) into the left saphenous vein. Animals were pretreated orally or subcutaneously 60 min before administration of [³H]-spiperone, and killed a further 60 min later. The cerebellum, striatum and cerebral cortex were dissected out, and homogenized in 1 ml H₂O (frontal cortex and striatum) or 2.5 ml H₂O (cerebellum). The radioactivity was determined by counting the entire homogenate (frontal cortex and striatum) or a 1 ml aliquot (cerebellum) in 10 ml of HP Beckman scintillant.

Male mice (23 g, Iffa-Credo France) were injected with [³H]-methylspiperone (2.22 or 3.03 MBq ml⁻¹, New England Nuclear) at a dose of 0.5 μg kg⁻¹ into the dorsal tail vein. Animals were pretreated subcutaneously (RP 62203) or intraperitoneally (ritanserin) 30 min before the injection of [³H]-methylspiperone, and killed 60 min later. The cerebellum, hippocampus and cerebral cortex were dissected out, weighed, solubilized in 2 ml Soluene, and the radioactivity estimated by liquid scintillometry in 10 ml Instagel.

The non-specific binding was attributed to the radioactivity accumulated in the cerebellum, and this 'blank' subtracted from the values obtained in the striatum and frontal cortex to yield the 'specific' binding. The doses which inhibited specific [³H]-spiperone binding by 50% *in vivo* (ID₅₀) were determined by linear regression analysis of the log dose-response curves.

Inositol phosphate formation

5-HT-stimulated inositol phosphate formation was measured in cortical slices prepared from neonatal rat brain by a modification of the method of Claustre *et al.* (1988). Neonatal rats of either sex (8–10 days, Sprague-Dawley, Charles River France) were killed by decapitation and their cortices removed. These were cut into slices (350 μm bidimensionally) using a McIlwain tissue chopper and preincubated for 1 h in Krebs-HCO₃ buffer, composition as follows (mM): NaCl 144, KCl 5, MgSO₄ 1.8, NaH₂PO₄ 1.8, CaCl₂ 2, NaHCO₃ 10 and glucose 20.2, equilibrated with O₂/CO₂ (95%/5%) at 37°C before use. The slices were then resuspended in 10 ml of fresh buffer containing [³H]-myo-inositol (4.8 × 10⁻⁷ M; 3.7 × 10⁶ Bq) for a further 60 min, then rinsed and allowed to settle. Aliquots of the slices (50 μl) were distributed into assay tubes containing pargyline (10 μM), LiCl (10⁻² M) and the antagonist to be studied in a total volume of 250 μl. Fifteen minutes later, 5-HT (10⁻⁵ M) was added, and the incubation continued for a further 60 min before isolation of the inositol phosphates. All incubations were performed at 37°C. Quadruplicate determinations were performed for each treatment condition.

At the end of the incubation period, chloroform/methanol (1:2 v:v; 0.9 ml) was added to lyse the cells and liberate the

inositol phosphates formed. A further 0.3 ml of chloroform and of water were added to separate the organic and aqueous phases. An aliquot (0.75 ml) of the aqueous phase was removed and applied to an anion exchange column (0.25 ml Dowex). The inositol phosphates were eluted by serial elution with increasing concentrations of formate as described by Berridge *et al.* (1982) and modified by Brown *et al.* (1984). Free inositol was eluted with distilled water (10 ml), glycerophosphoinositides with sodium formate (60 mM)/sodium tetraborate (5 mM) (10 ml) and the inositol phosphates with ammonium formate (1.0 M)/formic acid (0.1 M) (1 ml). Preliminary experiments showed that 95% of the radioactivity in the last fraction co-eluted with inositol monophosphate. The radioactivity in the inositol phosphate fraction was determined by liquid scintillometry in 4 ml of Ready-Gel scintillant (Beckman).

Inositol formation stimulated by histamine (10^{-5} M) was studied using a similar protocol in slices of guinea-pig cerebellum, and by noradrenaline (10^{-5} M) in primary cultures of mouse astrocytes using an identical procedure to that described for tachykinins by Torrens *et al.* (1989).

Electrophysiological recordings in the raphe nuclei

All experiments were carried out on 250–350 g male Sprague-Dawley rats (Charles River France). The animals were anaesthetized with chloral hydrate (400 mg kg^{-1} , i.p.) and subsequent doses of anaesthetic were administered when necessary to maintain a stable level of anaesthesia throughout the experiment. An intraperitoneal cannula was inserted and the animals were then placed in a stereotaxic frame and their rectal temperature was maintained at $36\text{--}37^\circ\text{C}$. The skin overlying the skull was incised and reflected and a small hole was drilled in the skull overlying the region of study. The smallest possible piece of dura was removed to allow insertion of the recording electrodes into the brain. For stereotaxic coordinates, the atlas of Paxinos & Watson (1982) was used. The coordinates for the raphe were (mm): dorsal raphe nucleus (DRN) A-P Bregma -7.8 , Lateral O, Vertical $6.0\text{--}7.0$ and median raphe nucleus (MRN) A-P Bregma -7.3 , Lateral O, Vertical $8.0\text{--}9.0$.

Extracellular recordings of action potentials were made with the central barrel (containing 3 M NaCl) of seven barrelled glass microelectrodes with a tip diameter of $5\text{--}7 \mu\text{m}$. The remaining barrels were filled with combinations of the following drugs and solutions: 200 mM NaCl (current balancing), sodium-L-glutamate (0.2 M, pH 8), γ -aminobutyric acid (GABA) (0.2 M, pH 3.5), 5-hydroxytryptamine creatinine sulphate (0.05 M, pH 4). All solutions were freshly prepared immediately before the experiment. One electrode barrel contained pontamine sky blue (PSB, 2% in 0.5 M sodium acetate) for histological verification of the recording site at the end of the experiment.

Extracellular recordings of single unit activity were amplified, filtered and displayed by conventional methods. For microiontophoretic application of drugs, automatic current balancing was routinely used and a small retaining current was applied to each drug barrel to prevent unwanted diffusional leakage. On-line analysis of firing rate was carried out with an IBM microcomputer and interface and recordings were also stored on magnetic tape for further analysis off-line.

Measurement of 5-hydroxytryptamine agonist-induced head-twitches

Rats (male, 180–250 g, Sprague-Dawley, Charles River France or Wistar, Iffa-Credo France) and mice (male or female, 19–25 g, CD₁ COBS, Charles River France) were used. Animals were given free access to food and water and maintained on a 12 h light/dark cycle at an ambient temperature of $22 \pm 2^\circ\text{C}$. To observe head-twitches, the animals were placed in individual perspex chambers without bedding material ($12.5 \times 13.5 \times 11.5 \text{ cm}$ for mice and $18 \times 22 \times 32 \text{ cm}$ for rats).

The head-twitch response consisted of a rapid movement of the head with little or no involvement of the trunk.

5-Hydroxytryptophan-induced head-twitches in mice Mice were injected with carbidopa (6 mg kg^{-1} , s.c.) and 15 min later with DL-5-HTP (180 mg kg^{-1} , i.p.). The presence or absence of head-twitches was recorded during a 2 min period, 15 min after 5-HTP administration (Corne *et al.*, 1963).

DOI-induced head-twitches in mice and rats Animals were injected with DOI (0.63 mg kg^{-1} , i.p.) and head-twitches recorded between the fifth and fifteenth minute after treatment in mice and during the 20 min following treatment in rats. Animals showing less than three head-twitches were considered to be protected (Heaton *et al.*, 1988).

Mescaline-induced head-twitches in mice and rats Mice were injected with mescaline (50 mg kg^{-1} , i.p.) and head-twitches recorded as described above (see also Corne & Pickering, 1967). In rats, head-twitches were counted during the 15 min following mescaline administration (20 mg kg^{-1} , i.v.; Niemegeers, 1983).

In mice, antagonists (6 or 8 mice per dose) were administered 60 (s.c.) or 90 (p.o.) min before the agonist. In rats, antagonists (6 rats per dose) were administered 60 min (s.c. or p.o.) before the agonist. The results are expressed as the ED₅₀, defined as the dose which protected 50% of the animals from head-twitches.

Measurement of amphetamine-induced stereotypies

Antagonists were administered orally or subcutaneously to groups of five rats (male, 160–200 g, Sprague-Dawley, Charles River France) 30 min before treatment with a single dose of amphetamine sulphate (12.5 mg kg^{-1} , i.p.). The rats were placed in individual cages ($14 \times 19 \times 19 \text{ cm}$) and scored for stereotypies 60 and 90 min following amphetamine treatment, with a visual rating scale of 0–3. The results are expressed as the ED₅₀, defined as the dose which produced a 50% decrease in the mean stereotypy score compared to animals treated with amphetamine alone.

Measurement of tetrabenazine-induced ptosis

Groups of six mice (male or female, 16–26 g, CD₁ COBS, Charles River France) were treated orally with antagonists 1 h before administration of tetrabenazine (50 mg kg^{-1} , p.o.). The appearance of the palpebral fissure was evaluated 30 and 60 min after tetrabenazine treatment; a visual rating scale of 0 (fully closed) to 2 (fully open) was used. The results are expressed as the ED₅₀, defined as the dose which produced a 50% decrease in the mean ptosis score compared to animals treated with tetrabenazine alone.

Protection against noradrenaline-induced lethality

Antagonists were administered subcutaneously to groups of eight mice (male or female, 10–26 g, CD₁ COBS, Charles River France) 1 h before intravenous injection of noradrenaline (0.5 mg kg^{-1}). The number of mice surviving was counted after 15 min and 24 h. The results are expressed as the ED₅₀, defined as the dose which protected 50% of the animals against noradrenaline-induced lethality.

Drugs

RP 62203 (2-[3-(4-(4-fluorophenyl)-piperazinyl)propyl]-naphto[1,8-cd]isothiazole-1,1-dioxide), mescaline, amphetamine sulphate, prazosine, tetrabenazine and mepyramine were synthesized by the Chemistry Department at Rhône-Poulenc Rorer, CRVA, Vitry-sur-Seine, France.

ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride) and ICI 170,809 (2-(2-dimethylamino-2-

Table 1 Affinity of RP 62203 and other 5-HT₂ antagonists for 5-HT₂ receptors in rat frontal cortex

Antagonist	IC ₅₀ (nM)
RP 62203	0.12 ± 0.03
Ritanserin	0.24 ± 0.03
Spiperone	0.27 ± 0.10
Ketanserin	0.76 ± 0.14
ICI 170,809	0.77 ± 0.20
Pipamperone	1.13 ± 0.13
Methysergide	2.00 ± 0.32
Carpipramine	3.00 ± 0.20
Mianserin	3.67 ± 0.45
ICI 169,369	61.3 ± 17.1

The concentrations at which the compounds displaced 50% of the specific binding (IC₅₀) were obtained from seven-point displacement curves using [³H]-ketanserin as the radiolabel. Data are expressed as the mean ± s.e.mean of three to four independent determinations of the IC₅₀ values.

methylpropylthio)-3-phenylquinoline hydrochloride) were kindly supplied by Dr G.R. Birchall, I.C.I. Pharmaceuticals, Macclesfield.

Radioligands were purchased from Amersham, unless otherwise stated. Other compounds used were obtained from the following commercial sources: ritanserin, Janssen Life Sciences Products; noradrenaline bitartrate, sodium-L-glutamate, γ-aminobutyric acid (GABA), 5-hydroxytryptamine creatinine sulphate and 5-hydroxytryptophan, Sigma; 8-OH-DPAT (8-hydroxy-N,N-dipropyl-2-aminotetralin) and DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), Research Biochemicals Incorporated.

Results

In vitro binding studies

RP 62203 potently inhibited the binding of [³H]-ketanserin to 5-HT₂ receptors on rat frontocortical membranes with an IC₅₀ = 1.2 × 10⁻¹⁰ M (Figure 2). Of all the compounds tested in this binding assay, RP 62203 is as yet the most potent detected (Table 1), being twice as active as ritanserin (IC₅₀ = 2.4 × 10⁻¹⁰ M vs [³H]-ketanserin) and six times more active than ICI 170,809 (IC₅₀ = 7.7 × 10⁻¹⁰ M). ICI 169,369 was considerably less potent (IC₅₀ = 6.1 × 10⁻⁸ M), in agreement with published data (Blackburn *et al.*, 1988a).

RP 62203 was less active at 5-HT_{1A} binding sites in the rat hippocampus, labelled with [³H]-8-OH-DPAT (IC₅₀ = 7.4 × 10⁻⁸ M) and displayed very low affinity (IC₅₀ > 10⁻⁶ M) for

Figure 2 Displacement by RP 62203 (●), ritanserin (▲) and ICI 170,809 (■) of [³H]-ketanserin binding to 5-HT₂ receptors in rat cortical membranes. Each point represents the mean of three independent determinations; s.e.mean shown by vertical bars. The curves represent the best-fit solutions to Langmuir isotherms generated by computer-assisted non-linear regression analysis using the IC₅₀ values in Table 1. The Hill coefficients calculated by regression analysis of the corresponding Hill transformations were 0.93 (RP 62203), 1.10 (ritanserin) and 0.91 (ICI 170,809).

5-HT_{1B} receptors in rat cortex labelled with [¹²⁵I]-iodocyanopindolol and for 5-HT₃ receptors in rat entorhinal cortex labelled with [³H]-GR 65630 (Table 2). Neither ritanserin nor ICI 170,809 was active (IC₅₀ > 10⁻⁶ M) in these binding assays.

With respect to other monoamine receptors, RP 62203 displaced the binding of [³H]-mepyramine to histamine H₁ receptors in the guinea-pig cerebellum with an affinity of 1.31 × 10⁻⁸ M. The affinities of ritanserin and ICI 170,809 in this assay were 2.24 × 10⁻⁸ M and 6.78 × 10⁻⁷ M respectively (Table 2). At dopamine D₂ receptors in the rat striatum labelled with [³H]-spiperone, RP 62203 (IC₅₀ = 3.94 × 10⁻⁷ M) and ICI 170,809 (IC₅₀ = 7.79 × 10⁻⁷ M) displayed only very low affinity, whilst ritanserin (IC₅₀ = 3.5 × 10⁻⁸ M) was much more active (Table 2). RP 62203 was most active in binding to α₁-adrenoceptors in the rat cortex labelled with [³H]-prazosin (IC₅₀ = 4.33 × 10⁻⁹ M). The affinities of ritanserin and ICI 170,809 at this binding site were 1.33 × 10⁻⁸ M and 9.23 × 10⁻⁸ M respectively (Table 2).

No displacement by RP 62203 of [³H]-p-aminoclonidine binding to α₂-adrenoceptors or of [³H]-dihydroalprenolol to β-adrenoceptors was observed in the rat cortex at a concentration of 10⁻⁶ M. The binding of [³H]-quinuclidinyl benzoate ([³H]-QNB) to muscarinic acetylcholine receptors in the rat cortex was not displaced by RP 62203 at a concentration of

Table 2 Receptor binding profile of RP 62203

Receptor	Tissue source	Non-specific (M)	RP 62203	IC ₅₀ (M) ritanserin	ICI 170,809
5-HT ₂	Rat frontal cortex	Methysergide (10 ⁻⁶)	1.2 ± 0.3 × 10 ⁻¹⁰	2.4 ± 0.3 × 10 ⁻¹⁰	7.7 ± 2.0 × 10 ⁻¹⁰
5-HT _{1A}	Rat hippocampus	5-HT (10 ⁻⁵)	7.5 ± 2.6 × 10 ⁻⁸	55.5% at 10 ⁻⁶ *	NA 10 ⁻⁶
5-HT _{1B}	Rat cortex	5-HT (10 ⁻⁵)	22.5% at 10 ⁻⁶ *	32.7% at 10 ⁻⁶ *	ND
5-HT ₃	Rat entorhinal cortex	Ondansetron (10 ⁻⁶)	6.6 ± 2.0 × 10 ⁻⁶	2.5 ± 1.1 × 10 ⁻⁶	ND
α ₁ -Adrenoceptor	Rat cortex	Phentolamine (10 ⁻⁶)	4.3 ± 0.7 × 10 ⁻⁹	1.3 ± 0.1 × 10 ⁻⁸	9.2 ± 0.5 × 10 ⁻⁸
α ₂ -Adrenoceptor	Rat cortex	Phentolamine (10 ⁻⁶)	NA 10 ⁻⁶	8.6 ± 0.2 × 10 ⁻⁸	ND
Dopamine D ₂	Rat striatum	Sulpiride (10 ⁻⁵)	3.9 ± 0.6 × 10 ⁻⁷	3.5 ± 0.4 × 10 ⁻⁸	7.8 ± 0.8 × 10 ⁻⁷
Histamine H ₁	Guinea-pig cerebellum	Promethazine (10 ⁻⁵)	5.0 ± 0.6 × 10 ⁻⁸	4.2 ± 0.2 × 10 ⁻⁹	4.8 ± 0.2 × 10 ⁻⁷
Muscarinic	Rat cortex	Atropine (10 ⁻⁶)	NA 10 ⁻⁶	NA 10 ⁻⁶	1.1 ± 0.4 × 10 ⁻⁷

Data are expressed as the mean ± s.e.mean of three to four independent determinations of the IC₅₀ values. NA: no displacement observed at the highest concentration tested (10⁻⁶ M). ND: not determined. * Percentage displacement seen at the indicated concentration. In addition, RP 62203 (10⁻⁶ M) did not displace radioligand binding to the following sites: benzodiazepine binding site ([³H]-flunitrazepam), bradykinin receptors ([³H]-bradykinin), CCK_A and CCK_B cholecystokinin receptors ([³H]-pCCK₈), 5-HT uptake sites ([³H]-paroxetine), N-methyl-D-aspartate receptors ([³H]-TCP), neuropeptide Y receptors ([³H]-neuropeptide Y), neurotensin receptors ([³H]-neurotensin), opiate receptors ([³H]-ethylketocyclazocine), somatostatin receptors ([¹²⁵I]-SRIF), tachykinin NK₁ receptors ([³H]-substance P), vasoactive intestinal peptide receptors ([¹²⁵I]-VIP), calcium channels ([³H]-desmethoxyverapamil) and sodium channels ([³H]-batrachotoxin).

Table 3 Occupation of 5-HT₂ receptors *in vivo*

Antagonist	Species	p.o.	ID ₅₀ (mg kg ⁻¹)	
			s.c.	i.p.
RP 62203	Rat	0.39 ± 0.10	0.07 ± 0.04	ND
	Mouse	ND	0.06 ± 0.02	ND
Ritanserlin	Rat	3.28 ± 0.90	0.58 ± 0.18	ND
	Mouse	ND	ND	0.10 ± 0.04

The ability of RP 62203 and ritanserlin to inhibit the *in vivo* binding of [³H]-spiperone (rats) or [³H]-methylsipiperone (mice) was evaluated as described in the Methods. ID₅₀ values (with standard errors) were determined from five-point dose-response curves (0.02–20 mg kg⁻¹) obtained on groups of five animals per dose. ND: not determined.

Figure 3 Antagonism by RP 62203 (●), ritanserlin (▲) and ICI 170,809 (■) of 5-hydroxytryptamine (5-HT)-stimulated inositol phosphate (IP) formation in slices of neonatal rat cortex. The data are presented as a percentage of the maximal 5-HT-evoked response (214 ± 9% of basal IP formation) and represent the mean of three (ICI 170,809) or four (RP 62203 and ritanserlin) independent determinations; s.e.mean shown by vertical bars.

10⁻⁶ M. RP 62203 was inactive in radioligand binding assays for a number of other neurotransmitter receptors. The affinity of ICI 169,369 for receptors other than the 5-HT₂ receptor was not assessed, since this information is already published (Blackburn *et al.*, 1988a).

In vivo binding studies

In rats, RP 62203 was a potent inhibitor of specific [³H]-spiperone binding in the frontal cortex (Table 3). When administered orally, the ID₅₀ value for RP 62203 was 0.39 mg kg⁻¹, compared to 3.28 mg kg⁻¹ for ritanserlin. The activity of RP 62203 was also observed after subcutaneous administration (ID₅₀ = 0.07 mg kg⁻¹). It is noteworthy that in neither case did RP 62203 modify the binding of [³H]-spiperone to D₂ receptors in the striatum: binding in animals injected with RP 62203 (20 mg kg⁻¹) corresponded to 93.5% (p.o.) and 96.7% (s.c.) of the binding seen in vehicle-injected controls. Occupation of 5-HT₂ receptors by RP 62203 was long-lasting, substantial inhibition of [³H]-spiperone binding still being observed 6 h following treatment (ID₅₀ = 0.78 mg kg⁻¹, p.o. and 0.33 mg kg⁻¹, s.c.). Similar findings were obtained in mice, where RP 62203 and ritanserlin also potently inhibited specific [³H]-methylsipiperone binding in the frontal cortex, yielding ID₅₀ values of 0.06 mg kg⁻¹, s.c. and 0.102 mg kg⁻¹, i.p. respectively. Again, RP 62203 did not modify the binding of [³H]-methylsipiperone to D₂ dopamine receptors in the striatum (binding in animals injected with 2.5 mg kg⁻¹ s.c. corresponded to 103% of that seen in vehicle-injected controls).

Effects on 5-hydroxytryptamine-stimulated inositol phosphate formation

Incubation of neonatal rat cortical slices with 5-HT (10⁻⁵ M) led to a two fold increase in the accumulation of inositol phosphate. This effect was also seen with the selective 5-HT₂ receptor agonist, DOI, but not with the 5-HT_{1A} receptor agonist 8-OH-DPAT (data not shown). RP 62203 potently antagonized the stimulatory effect of 5-HT, with an IC₅₀ of 7.76 ± 2.09 × 10⁻⁹ M. A similar activity

was observed for ritanserlin (IC₅₀ = 7.58 ± 1.32 × 10⁻⁹ M), whilst ICI 170,809 was much less active (IC₅₀ = 5.89 ± 1.55 × 10⁻⁷ M) (Figure 3). RP 62203 (10⁻⁶ M) also antagonised inositol phosphate formation stimulated by 10⁻⁴ M DOI (66 ± 3% inhibition, n = 3). RP 62203 seemed to be a selective antagonist of the 5-HT₂ receptor-mediated response (Table 4), since it had little effect on either basal or carbachol-stimulated inositol phosphate (IP) accumulation. In the guinea-pig cerebellum, IP formation stimulated by histamine (10⁻⁴ M) was only weakly inhibited by RP 62203 (35% inhibition at 10⁻⁶ M). High concentrations (IC₅₀ = 1.48 ± 0.13 × 10⁻⁶ M) of RP 62203 were needed to antagonize noradrenaline-stimulated IP formation in primary cultures of mouse astrocytes, compared to the reference α₁-receptor antagonist, prazosin (IC₅₀ = 6.20 ± 1.33 × 10⁻⁸ M).

Electrophysiological recordings in the raphe nuclei

Spontaneously active neurones in the raphe nuclei typically fired short duration action potentials at frequencies of 5–20 Hz. Cells giving reproducible responses to micro-iontophoretically applied 5-HT (50–100 nA for 30–60 s) were used for the antagonist studies. Changes in activity following drug administration were expressed as a percentage of the control firing rate of the cell. Histological examination of the location of the PSB dye spot confirmed correct electrode placement in all studies. The effects of RP 62203 were studied on 29 cells affected by 5-HT. RP 62203 was administered intraperitoneally in physiological saline after dissolving the compound in 1% Tween vehicle. Injection time was 1 ml min⁻¹ and in a volume of not more than 1 ml kg⁻¹ at pH 6. In 3 control injections, no

Table 4 Effect of RP 62203 on monoamine-stimulated inositol phosphate formation

Agonist		Tissue	IC ₅₀ (M)
None		Rat cortex	9.5 ± 2.9%*
5-Hydroxytryptamine	10 ⁻⁵ M	Rat cortex	7.8 ± 2.1 × 10 ⁻⁸
Carbachol	10 ⁻⁴ M	Rat cortex	24.4 ± 11.1%*
Histamine	10 ⁻⁴ M	Guinea-pig cerebellum	39.1 ± 16.1%*
Noradrenaline	10 ⁻⁵ M	Mouse astrocytes	1.5 ± 0.2 × 10 ⁻⁶

The indicated tissues and agonists were used as described in the Methods. Data are expressed as the mean ± s.e.mean of four independent determinations.
* Percentage displacement seen at a concentration of 10⁻⁶ M.

Figure 4 Antagonism of 5-HT₂ receptor-mediated responses in raphe neurones. Excitatory responses of a median raphe neurone to microiontophoretically applied 5-hydroxytryptamine (5-HT, 60 nA, 40 s) and glutamate (Glu; 40 nA, 30 s). RP 62203 was administered as a cumulative dose of 1.0 mg kg⁻¹, i.p. at the times indicated by the horizontal bars. The diminution of the neuronal responses to 5-HT is clear, whereas the responses to the control agonist, glutamate, were not antagonized by RP 62203.

effects of vehicle alone were seen on the rate of firing of recorded neurones.

In all studies, RP 62203 depressed and eventually blocked excitatory responses to microiontophoretically applied 5-HT in a dose-dependent manner at doses of 0.1–4.0 mg kg⁻¹, i.p. ($n = 21$, e.g. Figure 4). At a dose of 0.5 mg kg⁻¹, i.p., RP 62203 reduced 5-HT responses by $57.5 \pm 6.6\%$, and at 1.0 mg kg⁻¹, i.p., the responses were blocked completely. The effects of RP 62203 were long lasting and recovery from the effects of RP 62203 was not observed within a period up to 2 h after administration of the compound. In 5 studies, glutamate (20–40 nA, 10–20 s) was cycled with 5-HT as the control agonist to examine the specificity of the antagonist. RP 62203 was administered at doses of 0.5–2.0 mg kg⁻¹, i.p. The excitatory responses to 5-HT were potently depressed and eventually blocked at these doses, whereas responses to glutamate remained. In 5 studies, glutamate was cycled alone on cells that responded to 5-HT. RP 62203 at higher doses of 6.0 mg kg⁻¹, i.p. did not affect the responses to glutamate.

Eight neurones were studied in which 5-HT depressed their activity. This effect has been correlated with the activation of a 5-HT₁-like receptor. RP 62203 had no effect at this receptor when injected at doses from 2.0 to 8.0 mg kg⁻¹, i.p. (e.g. Figure 5). In 3 of the studies, 5-HT and GABA (10–20 nA for 5–10 s) were cycled together. Inhibitory responses to these two transmitters were not blocked by RP 62203 at doses as high as 8.0 mg kg⁻¹, i.p. However, it was observed that at this high dose, RP 62203 decreased the firing rate of the recorded neurone.

Ritanserin ($n = 5$) and ICI 169,369 ($n = 4$) also depressed 5-HT-evoked excitations. Ritanserin had similar activity to RP 62203, depressing responses to 5-HT by $71.8 \pm 6\%$ at 0.5 mg kg⁻¹, i.p. whereas ICI 169,369 was less potent, depressing 5-HT-evoked firing by $41.3 \pm 6\%$ at 2.0 mg kg⁻¹, i.p. (Figure 6). With neither antagonist was a recovery from the effects seen.

In some studies in which microiontophoretically applied glutamate was cycled with 5-HT as the control agonist, an increase in the amplitude of the glutamate response accompanied the antagonism of the excitatory response to 5-HT. An illustration of this effect is shown in Figure 4.

All three compounds were found to depress action potential amplitude by approximately 5% of control amplitudes in at least some studies. Since the amplitude was carefully monitored throughout counting, on no occasion did the decrease in

Figure 5 The lack of effect of RP 62203 at neuronal 5-HT₁-like receptors. Microiontophoretically applied 5-hydroxytryptamine (5-HT, 65 nA, 50 s) evoked depressions of dorsal raphe neuronal firing which were not blocked by a cumulative dose of RP 62203 (6.0 mg kg⁻¹, i.p.) administered at the times indicated by the horizontal bars.

spike amplitude introduce an error into the recording of the correct frequency of firing.

Arterial blood pressure was not measured during these studies. Administration of RP 62203 at the doses studied had no visible effect on respiration and resulted in no change of the recorded temperature of the animal. These observations were supported by data obtained from experiments carried out in rats in which administration of RP 62203 at doses of 30 mg kg⁻¹, p.o. did not have any effect on arterial blood pressure (S. Mondot, personal communication).

Effects on 5-hydroxytryptamine agonist-induced head-twitches

The effects of RP 62203 on 5-HT agonist-induced head-twitches are presented in Table 5. RP 62203 effectively abolished 5-HTP-induced head-twitches in the mouse after oral and subcutaneous administration ($ED_{50} = 1.27$ mg kg⁻¹ and 0.17 mg kg⁻¹ respectively). The values obtained for ritanserin were 0.17 mg kg⁻¹, p.o. and 0.15 mg kg⁻¹, s.c.

Figure 6 The effect of (a) ritanserin (1.0 mg kg⁻¹, i.p.) and (b) ICI 169,369 (2.0 mg kg⁻¹, i.p.) on excitatory responses to microiontophoretically applied 5-hydroxytryptamine (5-HT). Antagonists were administered after three control applications of 5-HT.

Table 5 Antagonism by RP 62203, ritanserin and ICI 170,809 of head-twitches in rats and mice

Antagonist	Route	ED ₅₀ (mg kg ⁻¹)				
		5-HTP mice	Mescaline mice	rats	DOI mice	rats
RP 62203	p.o.	1.27 (0.55–2.95)	0.43 (0.08–2.28)	0.99 (0.63–1.55)	0.44 (0.24–0.78)	1.54 (0.85–2.78)
	s.c.	0.17 (0.09–0.34)	0.16 (0.08–0.34)	0.65 (0.38–1.12)	0.04 (0.03–0.07)	1.82 (0.90–3.65)
Ritanserin	p.o.	0.17 (0.08–0.28)	0.09 (0.04–0.23)	2.55 (1.22–5.33)	0.08 (0.05–0.13)	2.59 (1.52–4.40)
	s.c.	0.15 (0.08–0.28)	0.29 (0.08–1.07)	0.38 (0.24–0.62)	0.05 (0.04–0.08)	1.54 (0.76–3.12)
ICI 170,809	p.o.	ND	ND	> 20	1.78 (1.27–2.40)	> 20

The data represent the ED₅₀ values (5–95% confidence limits) calculated from four-point dose-response curves, obtained in groups of six to eight animals per dose. ND: not determined.
5-HTP: 5-hydroxytryptophan; DOI: 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

In the case of mescaline-induced head-twitches, protection was observed with RP 62203 in both mice (ED₅₀ = 0.43 mg kg⁻¹, p.o. and 0.17 mg kg⁻¹, s.c.) and rats (ED₅₀ = 0.99 mg kg⁻¹, p.o. and 0.65 mg kg⁻¹, s.c.). The duration of this effect was examined in rats: no decrease in protection was observed after 6 h, and effects could still be seen 16 h after administration of RP 62203 (Table 6).

When head-twitches were evoked by the more selective 5-HT₂ receptor agonist DOI, these were blocked by RP 62203 in both mice (ED₅₀ = 0.44 mg kg⁻¹, p.o. and 0.04 mg kg⁻¹, s.c.) and rats (ED₅₀ = 0.05 mg kg⁻¹, p.o. and 1.82 mg kg⁻¹, s.c.) at doses similar to those for ritanserin, whereas ICI 170,809 (ED₅₀ = 1.78 mg kg⁻¹, p.o. in mice) was less active (Figure 7). This latter compound was considerably less potent in rats.

Effects on dopamine- and noradrenaline-mediated responses

RP 62203 did not affect amphetamine-induced stereotypies in rats up to the highest dose tested (93.4% of controls at 40 mg kg⁻¹, p.o.), suggesting that this compound does not interact with dopamine receptor function *in vivo*. Furthermore, in two tests of adrenergic function in mice, tetrabenazine-induced ptosis (83.4% of controls at 40 mg kg⁻¹, p.o.) and noradrenaline-induced lethality (ED₅₀ > 40 mg kg⁻¹, s.c.), RP 62203 was inactive.

Discussion

These studies reveal the extremely potent 5-HT₂ receptor antagonist properties of RP 62203 both *in vitro* and *in vivo*. In

radioligand binding studies, RP 62203 showed the highest affinity for 5-HT₂ receptors labelled with [³H]-ketanserin of all compounds tested (K_i = 50 pM). Similarly, *in vivo* binding studies demonstrated occupation of central 5-HT₂ receptors following administration of low doses of RP 62203 (<1.0 mg kg⁻¹, p.o.), suggesting good penetration of RP 62203 into the brain following oral administration. Moreover, receptor occupation could still be observed 6 h after treatment.

In functional tests of 5-HT₂ receptor activity, RP 62203 showed potent 5-HT receptor antagonist properties. For example, antagonism of 5-HT-stimulated inositol phosphate formation was observed in the nanomolar range (IC₅₀ = 7.76 nM). RP 62203 was equiactive with ritanserin, but considerably more potent than ICI 170,809.

Similarly, 5-HT₂ receptor antagonist activity could be demonstrated *in vivo*: in the raphe nuclei, the electrophysiological data clearly illustrate that RP 62203 can block central effects of 5-HT that are mediated by 5-HT₂ receptors. The absence of activity at other receptors mediating neuronal excitation, such as the excitatory amino acid receptors studied in these experiments, confirms the ability of the compound to discriminate between different excitatory receptors on the same neurone. This demonstrates that RP 62203 does not block 5-HT-evoked firing by a general depressant effect on neuronal activity. This observation is supported by other results obtained in the cortex, where similar doses of RP 62203 can block postsynaptic 5-HT₂ receptors, but not dopamine receptors (Godbout *et al.*, 1992). The two reference compounds, ritanserin and ICI 160,369 also blocked 5-HT₂ receptors in the raphe. As seen with RP 62203, a slight decrease

Table 6 Antagonism by RP 62203 and ritanserin of mescaline-evoked head-twitches in rats: duration of action

Antagonist	Time (h)	ED ₅₀ (mg kg ⁻¹)	
		p.o.	s.c.
RP 62203	1	0.99 (0.63–1.55)	0.65 (0.38–1.12)
	6	2.38 (1.08–5.25)	0.45 (0.22–0.89)
	16	14.2 (10.7–18.8)	5.91 (8.06–11.4)
Ritanserin	1	2.55 (1.22–5.33)	0.38 (0.24–0.62)
	6	1.54 (0.74–3.20)	0.55 (0.34–0.93)
	16	18.4 (9.17–37.0)	> 5

The data represent the ED₅₀ values (5–95% confidence limits) calculated from four-point dose-response curves, obtained on groups of six rats per dose.

Figure 7 Antagonism by RP 62203 (hatched columns), ritanserin (open columns) and ICI 170,809 (filled columns) of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-evoked head-twitches in mice. The data are presented as a percentage of the number of head-twitches observed in control animals (6.29 ± 0.44) and represent the values (mean with s.e.mean shown by vertical bars) obtained in groups of eight mice.

(<5%) in action potential amplitude was seen in some cells after administration of the antagonists; this was not, however, seen in all cases and was not correlated with the dose administered. A possible explanation of this effect could be a local anaesthetic action of the compounds, although this cannot be concluded with certainty. In addition, with high doses of RP 62203 a variable degree of depression of the spontaneous activity of recorded neurones was seen in both regions of study. Since this effect was seen in a heterogeneous population of cells and the pharmacological basis of the effect was not explored, the mechanism underlying this effect cannot at present be proposed. It will be interesting to see if this effect is retained *in vitro*.

Electrophysiological evidence for ritanserin potency has already been published (Uego *et al.*, 1989) and the doses used by these authors (0.5–2.0 mg kg⁻¹, i.v.) agree well with our findings. The effects of ICI 169,369 on neuronal firing are however, reported here for the first time. This compound also displayed 5-HT₂ receptor antagonist activity in the raphé, although at higher doses. The relative potencies of the three compounds were ritanserin > RP 62203 > ICI 169,369.

A behavioural consequence of central 5-HT₂ receptor activation is the head-twitch syndrome induced by 5-HT₂ receptor agonists (Goodwin & Green, 1985). In such tests, RP 62203 antagonized head-twitches induced by 5-HTP, mescaline and DOI in both rats and mice at doses comparable to those active in the *in vivo* binding studies. Again, RP 62203 showed comparable activity to ritanserin in rats, whilst being somewhat less potent following oral administration in mice; ICI 170,809 was once again less active. The effects of RP 62203 are long-lasting, no loss of activity being observed 6 h after administration. These results are in agreement with the data obtained in *in vivo* binding studies.

A major drawback with currently available 5-HT₂ receptor antagonists is their lack of selectivity for this receptor sub-type (Fozard, 1987; Hoyer, 1988; Canton *et al.*, 1990). In this respect, RP 62203 is relatively selective for 5-HT₂ receptors compared to other 5-HT receptor sub-types. In binding studies, its affinity for 5-HT_{1A} receptors, although not negligible (IC₅₀ = 75 nM), is more than five hundred times lower than that at 5-HT₂ receptors. This selectivity was also confirmed in electrophysiological experiments *in vivo*. In the dorsal raphé nuclei, where 5-HT depresses neuronal activity by activation of a 5-HT_{1A} receptor (Lum & Piercey, 1988), RP 62203 did

not block 5-HT-evoked depressions at doses as high as 8.0 mg kg⁻¹, i.p.

The activity of RP 62203 at 5-HT_{1C} receptors in the choroid plexus has been evaluated by [³H]-mesulergine autoradiography (IC₅₀ = 35 nM, compared to 0.1 nM for cortical 5-HT₂ receptors in the same slices); these data will be published elsewhere. It should be noted that neither ritanserin nor ICI 169,369 (Blackburn *et al.*, 1990) discriminate between 5-HT₂ and 5-HT_{1C} receptors. Like other known 5-HT₂ antagonists (Hoyer *et al.*, 1985), RP 62203 had very low (IC₅₀ > 10⁻⁶ M) affinity for 5-HT_{1B} receptors in rat cerebral cortex.

Binding studies revealed that RP 62203 displayed significant activity at α_1 -adrenoceptors (IC₅₀ = 4.3 nM); this selectivity ratio of 40 compared to 5-HT₂ receptors is of the same order as that for ritanserin. Antagonism of noradrenaline-stimulated IP formation was seen at concentrations 150 times higher than those needed to antagonize 5-HT-stimulated IP formation. However, the consequences of this affinity *in vivo* seem to be minimal, since neither noradrenaline-induced lethality nor tetrabenazine-induced ptosis were affected. Furthermore, autoradiographic studies performed *ex vivo* have failed to demonstrate occupation of α_1 -adrenoceptors at the highest dose tested (10 mg kg⁻¹, i.p.) (Malgouris *et al.*, 1990). Overall, the selectivity profile of RP 62203 in binding studies was superior to that of ritanserin, but this compound was less selective than ICI 170,809 (Blackburn *et al.*, 1988b) and ICI 169,369 (Blackburn *et al.*, 1988a).

Since very little is known about the role of particular 5-hydroxytryptaminergic pathways in pathological states, nor about the receptor sub-types activated at individual synapses, the high *in vivo* potency and selectivity of RP 62203 would make it an ideal tool with which to explore such mechanisms.

In the clinic, selective 5-HT₂ receptor antagonists have been proposed to have therapeutic potential in the treatment of dysthymic disorders; indeed, ritanserin has been studied in certain forms of anxiety and depression, as well as in sleep disorders (Idzikowsky *et al.*, 1986; Paiva *et al.*, 1988), and, in experimental animals, has been shown to modify sleep cycles (Dugovic & Wauquier, 1987) and to be active in certain models of anxiety (Critchley & Handley, 1987). The superior selectivity of RP 62203 compared to ritanserin makes it a promising candidate for the treatment of these disorders.

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