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Pharmacological profile of SB-357134: A potent, selective, brain penetrant, and orally active 5-HT_6 receptor antagonist

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Abstract

N-(2,5-Dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulfonamide (SB-357134) potently inhibited [^{125}I]SB-258585 and [^{3}H]LSD binding in a HeLa cell line expressing human 5-HT_6 receptors (pK_i = 8.6 and 8.54, respectively). Furthermore, SB-357134 inhibited [^{125}I]SB-258585 binding in human caudate–putamen and in rat and pig striatum membranes (pK_i = 8.82, 8.44, and 8.61, respectively). SB-357134 displayed over 200-fold selectivity for the 5-HT_6 receptor versus 72 other receptors and enzymes. 5-HT-stimulated cyclic AMP (cAMP) accumulation in human 5-HT_6 receptors was competitively antagonised by SB-357134 (pA_2 = 7.63). SB-357134 inhibited ex vivo [^{125}I]SB-258585 binding in the rat with an ED_{50} of 4.9 ± 1.3 mg/kg po, 4 h postdose. In the rat maximal electroshock seizure threshold (MEST) test, SB-357134 produced a potent and dose-dependent increase in seizure threshold, with a minimum effective dose of 0.1 mg/kg po. At 10 mg/kg po, maximum activity occurred between 4 and 6 h postdose. Good exposure was observed with SB-357134 at 10 mg/kg po, reaching maximal blood and brain concentrations of 4.3 ± 0.2 and 1.3 ± 0.06 μM, respectively, 1 h postdose. In addition, SB-357134 (10 mg/kg po) enhanced memory and learning following chronic administration (twice a day for 7 days) in the rat water maze. Overall, these studies demonstrate that SB-357134 is a potent, selective, brain penetrant, and orally active 5-HT_6 receptor antagonist.

Keywords: SB-357134; 5-HT_6 receptor; [^{125}I]SB-258585; [^{3}H]LSD; cAMP; Maximal electroshock seizure threshold (MEST) test; Morris water maze; Memory; Learning

1. Introduction

The discovery of the rat 5-HT_6 receptor (Ruat et al., 1993; Monsma et al., 1993), which is positively coupled to adenylate cyclase, instigated the search for tool compounds to explore the pharmacological role of this receptor in vitro and in vivo. In recent years, a number of potential candidates have been identified, including the first selective 5-HT_6 receptor antagonists, 4-amino-*N*-(2,6-bis-methylamino-pyrimidin-4-yl)-benzenesulfonamide and 4-amino-*N*-(2,6-bis-methylamino-pyridin-4-yl)-benzenesulfonamide (Ro-04-6790 and Ro-63-0563, respectively; Sleight et al., 1998). Similarly, we have recently reported a potent, selective, and orally acting 5-HT_6 receptor antagonist 5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046; Routledge et al., 2000).

These agents have, to date, revealed a potential role of 5-HT_6 receptors in a number of centrally mediated functions, most notably, cognition (Sleight et al., 1998; Bentley et al., 1999; Meneses, 2001; Rogers and Hagan, in press). This role is further supported by earlier evidence associating 5-HT_6 receptor activity with cholinergic transmission

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(Bourson et al., 1995; Sleight et al., 1996). In addition, it has been suggested that 5-HT_6 receptors may also modulate GABA function and excitatory amino acid neurotransmission (Dawson et al., 2000), implicating a potential role for 5-HT_6 receptors in anxiety and related disorders (Hamon et al., 1999). A number of known antipsychotic and antidepressant agents have also been found to possess high affinity for the cloned rat 5-HT_6 receptor, suggesting a role for this receptor in the pathogenesis of schizophrenia and depression (Monsma et al., 1993; Roth et al., 1994; Kohen et al., 1996). These potential functional roles of the 5-HT_6 receptor are supported by the regional distribution of 5-HT_6 receptor mRNA within the central nervous system (CNS), being evident in the nucleus accumbens, striatum, olfactory tubercle, substantia nigra, and hippocampus (Ward et al., 1995).

The implication of several prospective functions for 5-HT_6 receptors in CNS disorders highlights an important need for centrally acting tool compounds to identify the most relevant and likely role(s) for this receptor.

Hence, in the present report, a comprehensive pharmacological profile of the recently described selective and brain penetrant 5-HT_6 receptor antagonist SB-357134 (Bromidge et al., 2001) is presented. In addition, further supporting evidence for the role of 5-HT_6 receptors in cognitive function is discussed.

2. Methods

All experimental work was conducted in compliance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, and was reviewed and approved by the GlaxoSmithKline Procedures Review Panel.

2.1. Radioligand binding studies

Membranes from cells expressing human recombinant 5-HT_6 receptors and rat, pig, and human striatal tissues were prepared as previously described (Hirst et al., 2000a). Radioligand binding studies were carried out in a buffer containing 50 mM Tris–HCl, 10 μM pargyline, 5 mM $MgCl_2$, 5 mM ascorbate, and 0.5 mM EDTA (pH 7.4). In competition binding experiments, 10 concentrations of SB-357134, methiothepin, or 5-HT were tested (concentration range: 0.03 nM–1 μM and 0.3 nM–10 μM), together with 400 μl of membrane suspension (corresponding to approximately 15 μg protein/well for the recombinant cells and 60 μg protein/well for the brain tissue) and 50 μl of [^{125}I]SB-258585 (specific activity, 2000 Ci/mmol). Membranes were incubated with either 0.1 nM [^{125}I]SB-258585 (Hirst et al., 2000a) or 2 nM [^{3}H]LSD (Monsma et al., 1993). Non-specific binding was measured in the presence of 10 μM methiothepin. Following a 45-min incubation at 37 °C, the experiments were terminated by rapid filtration through Whatman GF/B filters, pretreated with 0.3% (v:v) polyethyleneimine (PEI), and washed with 6–9 ml of ice cold buffer. Radioactivity was determined by gamma spectrometry using a Packard Cobra II gamma counter.

Receptor selectivity of SB-357134 versus 5-$HT_{1A,1B,1D,1E,1F,2A,2B,2C,4,7}$, adrenergic alpha$_{1B}$, and $D_{2,3}$ was initially determined according to Hirst et al. (2000a). Subsequently, more detailed analysis was performed against a diverse range of receptors and enzymes by CEREP (Le Bois l'Eveque, 86600 Celle L'Evescault, France, CEREP Task Order 882085).

2.2. In vitro functional studies

Cyclic AMP (cAMP) levels in cells were determined by radioimmunoassay (NEN, SMP004). In brief, cells were washed once with Ca^{2+}-free PBS and centrifuged at $400 \times g$ (1000 rpm) for 5 min at 21 °C. The supernatant was discarded and the pellet resuspended in manufacturers stimulation buffer and approximately 50,000 cells were added to the appropriate wells of the NEN flashplates together with increasing concentration of 5-HT (0.03 nM–0.1 μM) in the presence of vehicle, 0.1, 0.3, 1, or 3 μM SB-357134. Plates were incubated for 15 min at 37 °C, before the addition of the manufacturers detection mixture containing [^{125}I]cAMP tracer (0.16 μCi/ml) to the wells. Plates were covered and left for 12 h prior to counting on a Packard TopCount.

2.3. Ex vivo binding

2.3.1. Animals

Male Sprague–Dawley rats (200–250 g, Charles River, UK) were housed in groups of six at a room temperature of 20–22 °C. Animals were maintained on a 12-h light/dark cycle with lights at 07:00 h. Food (Harlan Maintenance Diet) and water were available ad libitum.

2.3.2. Experimental procedure

Rats received vehicle or SB-357134 (0.1–100 mg/kg po) and at 4 h postdose were sacrificed and the striatum removed. The tissue was homogenised in cold buffer containing 50 mM Tris, 5 mM $MgCl_2$, 5 mM ascorbate, and 0.5 mM EDTA (pH 7.4). Radioligand binding was measured as described in Section 2.1, using 0.1 nM [^{125}I]SB-258585.

2.4. Maximal electroshock seizure threshold (MEST) test

2.4.1. Animals

Male Sprague–Dawley rats (93–165 g, Charles River, UK) were housed in groups of 10 at a room temperature of 20–22 °C. Animals were maintained on a 12-h light/dark cycle with lights on at 07:00 h. Food (Harlan Maintenance Diet) and water were available ad libitum. Drug treatments were evaluated between 14:00 and 18:00 h alongside time-matched, vehicle-treated controls.

2.4.2. Apparatus

The threshold current for electroshock-induced tonic hindlimb extensor seizure was determined using a Hugo Sachs Electronik stimulator (Germany), which delivered an adjustable constant current (1–300 mA) of 0.3-s duration, 50 Hz, sine waveform, via corneal electrodes.

2.4.3. Experimental procedure

Animals were individually assessed for production of a tonic hindlimb extensor seizure following a single electroshock using an 'up and down' method of shock titration, from a typical baseline of 25 mA (see Upton et al., 1997, for details). Data generated from treatment groups of $n = 12$ were used to calculate the seizure threshold ± S.E. values (current producing tonic hindlimb extensor seizure in 50% of animals) according to the method of Kimball et al. (1957). Elevation of seizure threshold is indicative of an anticonvulsant effect. The effect of the selective 5-HT_6 receptor antagonist SB-357134 (0.03–30 mg/kg po, 4 h pretest) on seizure threshold was determined. In addition, the duration of action of this compound was assessed at 10 mg/kg po over a 24-h period. A 1-ml/kg dose volume was used for all treatments, and doses are expressed as free base. Following the conclusion of the duration of action study, whole brain and blood samples were taken from randomly selected animals ($n = 4–5$) at each assessed time point. Samples were assayed for SB-357134 using a method based on protein precipitation with acetonitrile, followed by LC/MS/MS analysis employing positive-ion electrospray ionisation, with a lowest limit of quantification (LLQ) of 0.01 μM.

2.5. Morris water maze

2.5.1. Animals

Male Lister hooded rats (195–280 g at the beginning of the study, Harlan Olac, UK) were housed in groups of four in a controlled environment (temperature = 21.9 ± 1 °C; humidity = 53 ± 2%) and maintained on a 12-h light/dark cycle with lights on at 07:00 h with food and water available ad libitum.

2.5.2. Apparatus

Details for the apparatus used is described by Rogers and Hagan (2001). Briefly, the water maze consisted of a white Perspex pool (200-cm diameter) filled with water made opaque white by the addition of an odourless latex compound (Opacifier E308, Morton Thiokol, UK), with a 15-cm Perspex disk platform anchored below the surface. A video camera connected to an image analyser (HVS Image, Hampton, UK) was positioned directly above the pool.

2.5.3. Experimental procedure

Task retention, in the form of a transfer test, was assessed in rats following a 5-day training period to locate a submerged platform from various start positions around the maze, as described by Rogers and Hagan (2001). On the fifth day of training, rats were also subjected to their first transfer test.

Measurements of latency, path length, number of platform crossings, and quadrant percent time were calculated electronically. Acquisition of the platform position by each rat was quantified by analysis of latency to find the platform, the path length of each trial during the training procedure, and percentage time spent in the platform quadrant during the transfer test. Retention of the task was assessed at 7 days after training, in which each animal received a single 60-s transfer test with the platform removed from the pool, and percent time in the platform quadrant was calculated. Swimming speed was determined by analysis of the path length during the transfer tests, which had a fixed trial duration of 60 s.

Each rat received four consecutive trials on Day 1 of training and six trials on Days 2–4. On Day 5, each rat received six trials followed by their first transfer test. At the beginning of each trial the rat was lowered gently feet first into the water, facing the wall at a start position (north, south, east, and west), which was predetermined randomly. A remote control was used to activate the computer and the rat was allowed to swim for 60 s to find the platform. If the platform was found during this time, the trial was stopped, the recording terminated using the remote control, and the rat left on the platform for 10 s. If the platform was not found during this time, the rat was retrieved quickly from the water and placed on the platform for 10 s.

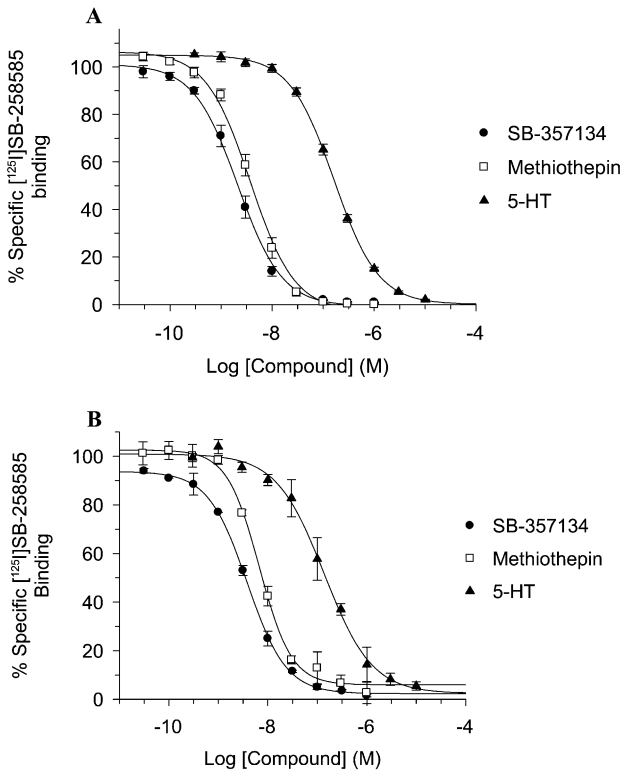

Fig. 1. Competition binding with [^{125}I]SB-258585 at human recombinant 5-HT_6 receptors (A) and rat striatal membranes (B). Data points represent the mean ± S.E. mean of four to six independent experiments. Average pK_i and standard error values are given in Table 2.

Groups of rats ($n=16$) were orally administered with either vehicle or SB-357134 (10 mg/kg, twice a day for 7 days) prior to the start of training. A third group ($n=16$) of animals were not pretreated. All treatments were also administered 4 h prior to each training session, including the third group of animals, which received acute SB-357134 (10 mg/kg po) 4 h before each training session. Seven days after completion of training, rats received the above treatments, 4 h prior to the transfer test. A 2-ml/kg dose volume was used for all treatments and doses are expressed as free base.

3. Materials

SB-357134 (*N*-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulfonamide) was synthesised by GlaxoSmithKline Pharmaceuticals (Harlow, UK) and dissolved in 1% methyl cellulose (Sigma, Poole, UK)) in water for oral administration. [^{125}I]SB-258585 was prepared at GlaxoSmithKline (Synthetic Isotope Chemistry) by reaction of the tributyltin derivative of SB-258585 with chloramine-T and sodium [^{125}I]iodide. [^{3}H]LSD was obtained from Amersham International, UK. Methiothepin mesylate and 5-hydroxytryptamine creatine sulfate (5-HT) were purchased from Sigma. Cell culture reagents were obtained from Life Technologies (Paisley, UK). All other reagents were obtained from Sigma or Merck-BDH (Lutterworth, UK) and were of analytical grade.

4. Data analysis

In concentration–response studies, the concentration of drug inhibiting specific radioligand binding by 50% (IC_{50})

Table 1

A. Recombinant human receptor binding profile of SB-357134

Receptor	Affinity (pK_i)
5-HT_6	8.54 ± 0.03
5-HT_{1A}	< 5.2
5-HT_{1B}	< 5.4
5-HT_{1D}	< 5.4
5-HT_{1E}	< 5.0
5-HT_{1F}	< 5.0
5-HT_{2A}	< 5.1
5-HT_{2B}	< 5.4
5-HT_{2C}	< 5.3
5-HT_4	< 5.3
5-HT_7	< 5.0
Adrenergic alpha$_{1B}$	< 5.1
D_2	< 5.3
D_3	< 5.3

Data taken from Bromidge et al. (2001). 5-HT_6 receptor density was ~7 pmol/mg protein.

B. CEREP-determined selectivity profile of SB-357134

Assay	Calculated pIC_{50}	Assay	Calculated pIC_{50}	Assay	Calculated pIC_{50}	Assay	Calculated pIC_{50}	Assay	Calculated pIC_{50}	Assay	Calculated pIC_{50}
A1 (h)	< 5	BZD#	5.61	NMDA	< 5	kappa (h)	5.48	PDE II (h)	< 5	MC3 (h)	< 5
A2a (h)	< 5	CGRP (h)	< 5	H1#	< 5	mu (h)	< 5	PDE III (h)	< 5	MC4 (h)	< 5
Alpha 1A	< 5	CB1 (h)#	5.52	H2	< 5	5-HT_3 (h)	5.31	PDE IV (h)	< 5	NPY (nonselective)	< 5
Alpha 2A	< 5	CB2 (h)$	< 5	I2#	< 5	estrogen (h)	< 5	PDE V (h)	5.28	NPY1 (h)	5.09
Alpha 2B	< 5	D1 (h)	< 5	LTB4 (h)	< 5	progesterone (h)	< 5	elastase (h)	< 5	NPY2 (h)	< 5
Alpha 2C	5.34	ETA (h)	< 5	M1 (h)	< 5	testosterone	< 5	ATPase (Na^+/K^+)	< 5	NT (nonselective)	< 5
Beta 1 (h)	< 5	ETB (h)	< 5	M2 (h)	< 5	Ca^{2+} channel (L, DHP site)	< 5	protein kinase C	< 5	NT1 (h) (NTS1)	< 5
Beta 2 (h)	< 5	GABAA	5.73	NK3 (h)	< 5	Na^+ channel (Site 1)	5.37	EGF-tyrosine kinase	< 5	PACAP-null (h) (PAC1)	< 5
Beta 3	5.13	AMPA	< 5	N#	< 5	Na^+ channel (Site 2)	5.50	CCKA (h) (CCK1)	< 5	PACAP-sv-1 (h) (PAC1)	< 5
AT2 (h)	< 5	kainate	< 5	delta (h)	5.42	PDE I	< 5	GABAB	< 5		

h: human, #central, $peripheral, PDE: phosphodiesterase. Data obtained from CEREP Task Order 882085. IC_{50} values were calculated from single percentage binding data as described in Section 4. A: adenosine; AT: angiotensin; BDZ: benzodiazepine; CGRP: calcitonin gene-related peptide: CB: cannabinoid; D: dopamine; ET: endothelin; PDE: phosphodiesterase; CCK: cholecystokinin; H: histamine; I: imidazoline; LT: leukotriene; M: muscarinic; NK: neurokinin; N: nicotinic; MC: melanocortin; NP: neuropeptide; NT: neurotensin.

Table 2
Affinity of SB-357134 at rat, pig, and human 5-HT_6 receptors

Competing compound	Human recombinant 5-HT_6 receptors	Rat striatal membranes	Pig striatal membranes	Human caudate membranes
SB-357134	8.99 ± 0.02	8.44 ± 0.05	8.61 ± 0.10	8.82 ± 0.02
Methiothepin	8.72 ± 0.14	8.28 ± 0.11	7.94 ± 0.09	8.26 ± 0.03
5-HT	6.79 ± 0.02	6.96 ± 0.07	7.07 ± 0.06	6.96 ± 0.09

Data represent mean ± S.E. mean pK_i values for $n = 4-6$ independent experiments.

was determined by iterative curve fitting (Bowen and Jerman, 1995). pK_i values (the negative $\log_{10}$ of the molar K_i) for receptor binding were then calculated from the IC_{50} values as described by Cheng and Prusoff (1973) using the K_d values determined in the saturation binding studies.

Single percentage binding data derived by CEREP was also used to estimate IC_{50} values, according to the formula below. This approximation was dependent on a single binding site and the binding mechanism being competitive.

From standard mass action equations, it can be shown that the ratio of bound ligand in the presence of the inhibitor to the bound ligand in the absence of the inhibitor (B_r) is given by

$$B_r = \frac{K_d + F}{K_d I / K_i + K_d + F} \quad (1)$$

where K_d and K_i are the equilibrium constants for the radioligand and inhibitor, respectively, and F and I are the unbound concentrations of the radioligand and inhibitor. In practice, I equals the total inhibitor concentration as the free is rarely known and for all but the highest affinity inhibitors this assumption is acceptable.

From this, Eq. (2) can be derived (see Cheng and Prusoff, 1973),

$$K_d + F = \frac{IC_{50} K_d}{K_i} \quad (2)$$

where IC_{50} is the concentration of inhibitor that gives 50% inhibition.

Substituting Eq. (2) into Eq. (1) and rearranging gives Eq. (3)

$$IC_{50} = \frac{B_r I}{(1 - B_r)} \quad (3)$$

or using percentage binding (Eq. (4))

$$IC_{50} = \frac{\%\ \text{binding} \times \text{inhibitor concentration}}{(100 - \%\ \text{binding})}. \quad (4)$$

Drug concentration–response curves from cAMP accumulation assays were fitted to a four-parameter logistic equation using GRAFIT (Erithacus Software), constraining the E_{max} of each curve to 100%. The pA_2 for antagonism was determined by Schild analysis of the data where, for a reversible competitive antagonist, provided the slope is not significantly different from unity, the pA_2 = pK_B. The pK_B is the $-\log$ of the antagonist equilibrium dissociation constant, i.e., $-\log$(antagonist concentration/[concentration ratio − 1]), where the concentration ratio is the ratio of the agonist EC_{50} in the absence and presence of the antagonist.

5. Statistical analysis

In the MEST studies, significant differences between drug- and vehicle-treated animals on seizure threshold were determined according to the method of Litchfield and Wilcoxon (1949).

In the Morris water maze study acquisition and retention, data were analysed by repeated measures ANOVA with post hoc testing using Scheffe multiple comparisons and one-way ANOVA followed by Dunnett's t test, respectively. All statistical analyses were carried out using Statistica for Windows (StatSoft, Tulsa, USA, Release 5.1, 97 edition).

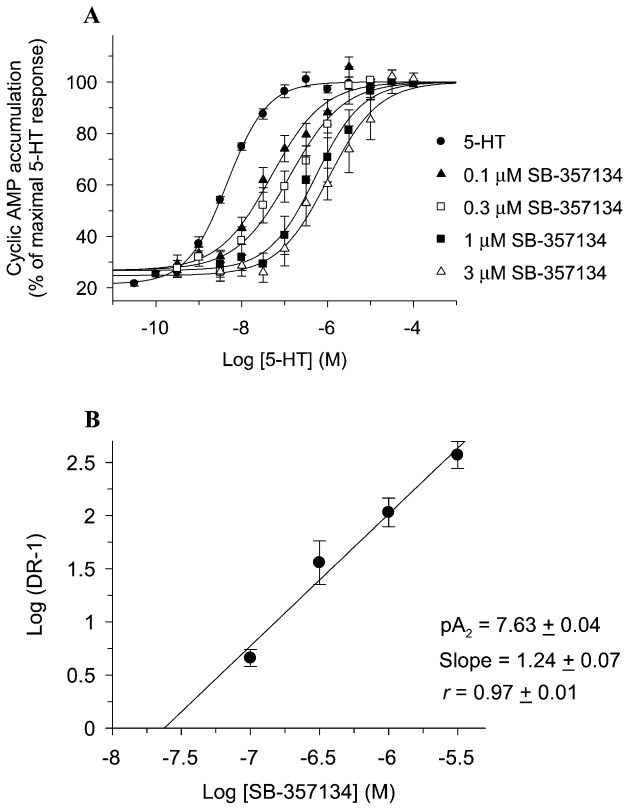

Fig. 2. Effect of SB-357134 (0.1–3 μM) on 5-HT-stimulated cAMP accumulation in HeLa cells expressing human recombinant 5-HT_6 receptors (A). Data points represent the mean ± S.E.M. of four independent experiments. Results are expressed as % of the maximal 5-HT response in each assay. Linear regression analysis of these data is expressed as a Schild plot (B).

6. Results

6.1. Radioligand binding studies

SB-357134 displayed high affinity at recombinant human 5-HT_6 receptors. In competition binding experiments with [^{3}H]LSD and [^{125}I]SB-258585, pK_i values were 8.54 ± 0.03, and 8.99 ± 0.02, respectively (Fig. 1A and Tables 1 and 2). In addition, SB-357134 inhibited [^{125}I]SB-258585 binding at native 5-HT_6 receptors with pK_i values of 8.44 ± 0.05, 8.61 ± 0.1, and 8.82 ± 0.02 for rat and pig striatum and human caudate, respectively (Fig. 1B and Table 2). SB-357134 had higher affinity than 5-HT and comparable affinity to methiothepin at both the recombinant human and native 5-HT_6 receptors (Table 2). Furthermore, SB-357134 displayed over 200-fold selectivity for the 5-HT_6 receptor versus 72 other receptors and enzymes (Table 1A and B).

Table 3
Estimated 'free brain concentrations' of SB-357134

Dose of SB-357134 (mg/kg po, 4 h pretest)	% Specific binding	Estimated 'free brain concentration' (nM)
0.1	86.6	0.4 ± 0.03
1	76.2	1.04 ± 0.11
10	26.6	9.68 ± 1.31
50	7.9	45.7 ± 4.28
100	6.9	55.3 ± 3.81

Data represent mean ± S.E.M. values from $n = 3–6$ animals per dose group calculated from the inhibition curve of SB-357134 in Fig. 3B.

6.2. In vitro functional studies

In studies with the human cloned 5-HT_6 receptor expressed in a HeLa cell line, 5-HT mediated concentration-dependent increases in cAMP levels with a pEC_{50} of 8.29 ± 0.06 (Fig. 2A). SB-357134 (0.1, 0.3, 1, and 3 μM) produced a concentration-dependent rightward shift of the 5-HT concentration effect curve, which was surmountable at high 5-HT concentrations, consistent with competitive antagonism. Linear regression analysis of the Schild plot (Fig. 2B) revealed a correlation coefficient of 0.97 ± 0.01, a slope of 1.24 ± 0.07, and a pA_2 of 7.63 ± 0.04.

6.3. Ex vivo binding

Fig. 3A shows the dose-dependent inhibition of specific [^{125}I]SB-258585 binding in rats, which had received vehicle or 0.1–100 mg/kg po of SB-357134 4 h prior to sacrifice. Specific binding (10,715 ± 954 cpm/mg protein) comprised 80% of the total signal. Iterative curve fitting of these data

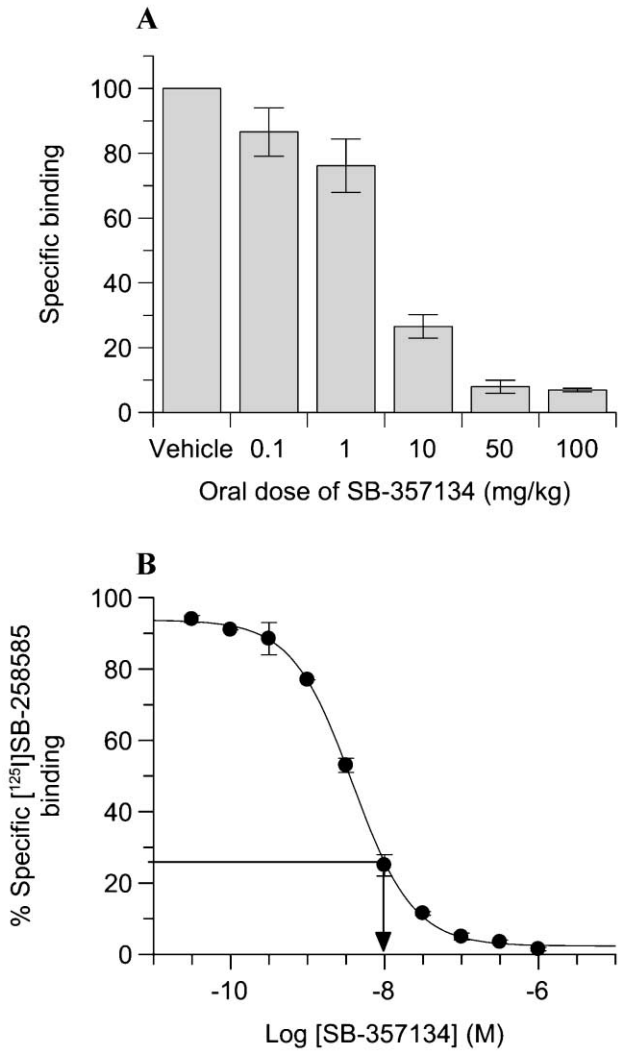

Fig. 3. (A) Inhibition of ex vivo [^{125}I]SB-258585 binding by systemically administered SB-357134 (0.1–100 mg/kg, 4 h pretest). Specific binding (10,715 ± 954 cpm/mg protein) comprised 80% of the total signal. (B) In vitro competition binding experiment on control striata used to estimate 'free brain concentration' of SB-357134 in ex vivo binding experiments shown in (A). Data points represent the mean ± S.E.M. from three to six rats.

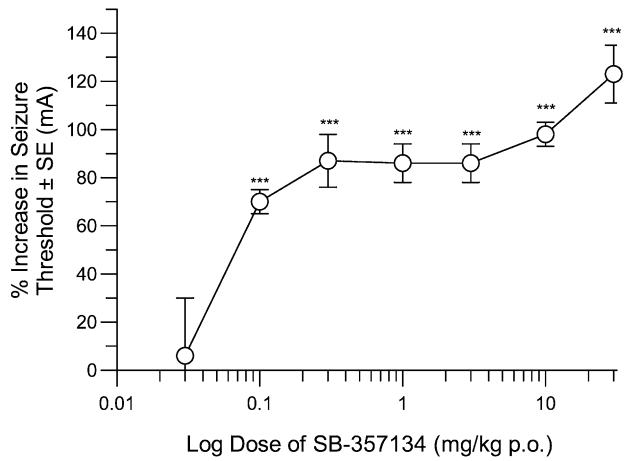

Fig. 4. Effect of SB-357134 on seizure threshold in the rat MEST test. SB-357134 was administered at 0.03–30 mg/kg po, 4 h pretest. Data represent percentage increases from control CC_{50} ± S.E. values for $n = 12$ animals per dose. *** $P < .001$ compared to zero dose control group according to Litchfield and Wilcoxon (1949).

Table 4
Time course profile of SB-357134 in the rat MEST test

Time postdose (h)	Seizure threshold (mA)
0	21.7 ± 1.7
0.5	23.3 ± 1.7
1	26.7 ± 1.7 *
2	30.8 ± 1.0* * *
3	26.7 ± 1.7 *
4	39.2 ± 1.0* * *
6	40.5 ± 3.7* * *
9	33.8 ± 1.1* * *
12	20.5 ± 2.4
15	35.0 ± 1.3* * *
18	37.5 ± 2.3* * *
21	31.3 ± 1.1* * *
24	29.5 ± 3.7

SB-3573134 was administered at 10 mg/kg po. Data represent calculated CC_{50} ± S.E. values for groups of 12 animals per time point. Zero dose represents vehicle-treated control group.

* $P < .05$, compared to corresponding vehicle control group according to Litchfield and Wilcoxon (1949).

*** $P < .001$, compared to corresponding vehicle control group according to Litchfield and Wilcoxon (1949).

gave an estimated ED_{50} of 4.9 ± 1.3 mg/kg. In addition, the 'free brain' concentration of SB-357134, i.e., compound available to interact with and compete for [^{125}I]SB-258585 binding, was estimated by extrapolation of the competition binding curves generated from in vitro experiments using striata from vehicle-treated rats ($n = 3$) (Fig. 3B). For example, 10 mg/kg SB-357134 inhibited 73.4% of the specific [^{125}I]SB-258585 binding, corresponding to a 'free brain concentration' of approximately 10 nM (Table 3).

6.4. MEST test

Following oral administration, SB-357134 produced a potent and dose-related anticonvulsant effect in the rat MEST model, with a minimum significantly effective dose of 0.1 mg/kg. Seizure threshold was increased by up to 123 ± 12% at the highest dose tested of 30 mg/kg (Fig. 4). At 10 mg/kg po, SB-357134 exhibited a rapid onset of action, significantly increasing seizure threshold from a control value of 21.7 ± 1.7 to 26.7 ± 1.7 mA at 1 h postdose, as shown in Table 4. Peak activity for SB-357134 was observed within 4–6 h postdose. With the exception of an unexplained loss of activity at 12 h, SB-357134 had a long duration of action of 21 h in this model (Table 4). In this duration of action study, SB-357134 displayed good exposure in both blood and brain, reaching C_{max} values of 4.3 ± 0.2 and 1.3 ± 0.06 μM, respectively, at 1 h postdose (Fig. 5).

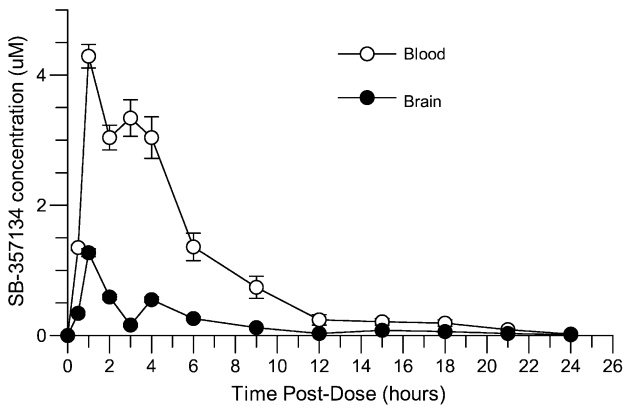

Fig. 5. Blood and brain concentrations of SB-357134 in rats following oral administration at a single dose of 10 mg/kg at various intervals over a 24-h period. Data represent mean ± S.E.M. of micromolar concentrations for groups of four to five animals per time point assessed, with a lowest limit of detection of 0.01 μM.

6.5. Water maze

All rats acquired the task well, finding the platform in less than 10 s by the end of the 5-day training period. Acute administration of SB-357134 (10 mg/kg po) produced no

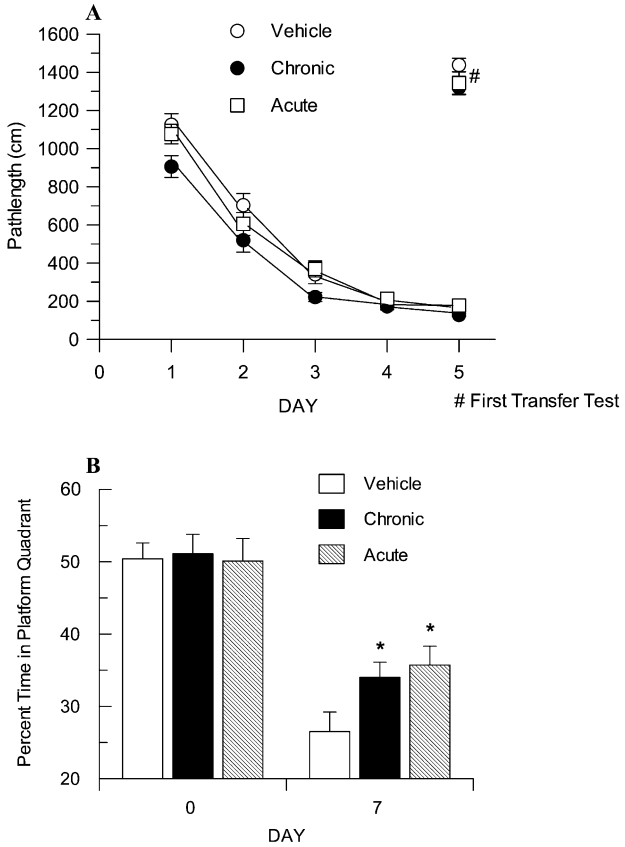

Fig. 6. Effect of acute and chronic SB-357134 (10 mg/kg po) in the water maze in rats. (A) Task acquisition as latency (s) to find the platform (blocked across day) for each of five training days, including path length during the first transfer test. (B) Task retention as percentage time spent in the platform quadrant during transfer tests carried out immediately (Day 0) and 7 days after training. Acute administration was given 4 h prior to each training session. Chronic administration was given twice a day for 7 days prior to the first training session, in addition to 4 h prior to each training session. Data represent mean ± S.E.M. values for $n = 16$ animals per group. * $P < .05$ compared to vehicle-treated control group according to Dunnett's t test following significant ANOVA on Day 7 [$F(2,43) = 3.90$, $P < .05$] after training.

significant effects on latency to finding the platform. However, post hoc statistical analysis following significant repeated measures ANOVA [$F(2,43) = 6.76$, $P < .01$] revealed that chronic administration of SB-357134 (10 mg/kg po, twice a day for 7 days) significantly shortened the path length compared to both vehicle ($P < .05$) and acute SB-357134 ($P < .03$) treatment (Fig. 6A). Furthermore, there was no significant treatment effect on swim speed during the first transfer test, according to Dunnett's t test [$F(2,43) = 1.94$, $P > .5$] (Fig. 6A).

During the first transfer test, SB-357134 had no effect on percent time spent in the platform quadrant (Fig. 6B). However, during the 7-day retention test, both acute and chronic SB-357134 significantly increased the percentage time spent in the platform quadrant (Fig. 6B).

7. Discussion

This report describes the pharmacological profile of SB-357134, a potent, selective, brain penetrant, and orally active 5-HT_6 receptor antagonist. [^{3}H]LSD (Monsma et al., 1993) and [^{125}I]SB-258585 (Hirst et al., 2000a) have both been utilised as radioligands for the 5-HT_6 receptor and were used in the present study to determine the affinity of SB-357134 at recombinant 5-HT_6 receptors. In addition, the highly selective ligand [^{125}I]SB-258585 was employed to determine the affinity of SB-357134 at this receptor in native tissue. SB-357134 was found to exhibit a similar high potency at human recombinant ($pK_i = 8.99$) and native rat, pig, and human 5-HT_6 receptors (pK_i values of 8.44, 8.61, and 8.82, respectively).

In vitro functional studies with SB-357134 produced a lower pA_2 (7.63) than binding affinity for the 5-HT_6 receptor. This reduced potency was not attributed to intrinsic activity at the 5-HT_6 receptor, as SB-357134 had no effect on basal cAMP levels, i.e., in the absence of 5-HT (data not shown). Furthermore, the lower pA_2 value appeared not to be due to the system used to measure the functional antagonism, as a similar series of experiments measuring adenylyl cyclase activation, instead of cAMP accumulation, gave a pA_2 value of 7.67 ± 0.23 (data not shown). Although the reason for this discrepancy is unclear, interpretation of the data obtained shows that SB-357134 is a potent 5-HT_6 receptor antagonist.

Due to the high affinity of 5-HT_6 receptors for [^{125}I]SB-258585 compared to their affinity for 5-HT, [^{125}I]SB-258585 has been used for ex vivo binding studies in crude striatal homogenates (Hirst et al., 2000b), enabling determination of receptor occupancy by peripherally administered SB-357134 (although direct assessment of 5-HT_6 receptor occupancy in vivo was not possible, primarily due to limited blood–brain barrier penetration of [^{125}I]SB-258585). Preliminary experiments that compared binding in the crude homogenate with binding in a pellet generated by a single centrifugation step ($20{,}000 \times g$) revealed no differences, suggesting that the SB-357134 was tightly bound to the receptors, rendering any dilution effect in the homogenate as negligible (Hirst et al., 2000b). These data show that following oral administration, SB-357134 occupied native 5-HT_6 receptors with an estimated ED_{50} of 4.9 ± 1.3 mg/kg.

In addition to an excellent in vitro profile, SB-357134 exhibited a favourable pharmacokinetic profile (Bromidge et al., 2001) with excellent oral bioavailability ($65 \pm 11\%$), good CNS penetration (19%), and low clearance (14 ml/min/kg).

In vivo, SB-357134 produced a potent and dose-related anticonvulsant effect against electroshock-elicited seizures. The level of activity achieved compared favourably to the response observed previously in this model with the 5-HT_6 receptor antagonists Ro-04-6790, SB-258510, and SB-271046 (Routledge et al., 2000). In addition, with the exception of an unexplained loss of activity at 12 h, which was not concurrent with reduced blood or brain exposure at this time point, the anticonvulsant effect of SB-357134 was long lasting. There is no known reason for the loss in activity observed at 12 h, although an active metabolite may be a possible cause for the second phase of activity between 15 and 21 h. However, reevaluation would be required to determine whether or not this is a real effect and not just a spurious one. Assessment of the correlation between anticonvulsant activity and exposure of SB-357134 revealed a more indirect relationship than that observed with SB-271046, taking on the form of a more common counter-clockwise hysteresis, indicative of an indirect pharmacological response (Derendorf and Meibohm, 1999). This is characterised by an evident delay between the onset of maximal effect and the observed maximum concentration of SB-357134 in the blood. Although the reason for delayed activity of SB-357134 is as yet unknown, a possible cause could be due to the distribution time required for the compound to reach the site of anticonvulsant action. Another possibility could be that active metabolites of SB-357134 are responsible for the effects on seizure threshold, rather that the parent compound itself. Nonetheless, a growing number of structurally diverse 5-HT_6 receptor antagonists have now been shown to elicit an increase in seizure threshold in the rat MEST model (Routledge et al., 2000), revealing a common pharmacodynamic response for such agents. At the present time, attribution of this anticonvulsant effect, at least in part, to 5-HT_6 receptor blockade, remains speculative. Therefore, further investigation is required to provide definitive evidence for the involvement of 5-HT_6 receptors in seizure propagation. To aid such investigations, the advent of selective 5-HT_6 receptor agonists (Glennon et al., 2000) may provide a possible means to determining whether proconvulsant activity is evoked upon activation of the 5-HT_6 receptor. If seizure activity is modulated by 5-HT_6 receptor function, the effects observed to date provide a more robust behavioural marker than previously identified behaviours, such as yawning and stretching (Bourson et al., 1995). These behaviours were not observed in our studies with SB-357134.

Several studies have implicated a modulatory function for 5-HT_6 receptors in cholinergic neurotransmission (Reavill and Rogers, 2001), alluding to a role of these receptors in memory and learning. A recent report has shown that the 5-HT_6 receptor antagonist Ro-04-6790 improved learning consolidation in rats under normal and dysfunctional memory conditions (Meneses, 2001). Indeed, the selective 5-HT_6 receptor antagonists SB-271046 and SB-357134 have also recently been shown to improve some aspects of cognitive performance in the rat water maze (Rogers and Hagan, in press), following acute administration at 10 mg/kg po. In the present study, we have confirmed the acute effect of SB-357134 on the retention of a spatial memory task in the rat. Furthermore, there was no evidence of tolerance to this effect following chronic administration. In fact, this study has also detected a previously unobserved improvement in learning, which was evident in the absence of any effects on general neurological function, as shown by a lack of effect on swim speed. The cognitive enhancement properties of SB-357134 were elicited at a pharmacodynamically active dose, which, from the ex vivo binding data above, produced $\sim$70% occupancy of the 5-HT_6 receptor. These data support the growing body of evidence for a modulatory role for 5-HT_6 receptors in memory and learning. This includes a recent report by Woolley et al. (2001) in which administration of either 5-HT_6 specific antisense oligonucleotide or administration of the 5-HT_6 receptor antagonist Ro-04-6790 were found to enhance cognitive function in the rat Morris water maze. Although a clear role for 5-HT_6 receptors in memory and learning is emerging, other neurotransmitter systems have been shown to be associated with 5-HT_6 receptor function, such as excitatory amino acid function (Dawson et al., 2000), which may be linked with the procognitive effects of 5-HT_6 receptor antagonists. Overall, this study has demonstrated that SB-357134 is a potent, selective, and brain penetrant 5-HT_6 receptor antagonist. Current interest in the 5-HT_6 receptor has led rise to the synthesis of a number of potentially useful tool compounds (Isaac et al., 2000; Lee et al., 2000; Glennon et al., 2000; Tsai et al., 2000), although in vivo activity for these agents is yet to be determined. Here we have shown in vivo activity with SB-357134, both pharmacodynamically and in a learning model. Furthermore, activity was observed in the absence of any overt behaviour, such as sedation or motor incoordination (data not shown). Hence, SB-357134 represents a suitable pharmacological probe to elucidate the role of the 5-HT_6 receptor in vivo.

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