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# *In vitro* and *in vivo* profile of SB 206553, a potent 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist with anxiolytic-like properties

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**1** SB 206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) displays a high affinity ($pK_I$ 7.9) for the cloned human 5-$HT_{2C}$ receptor expressed in HEK 293 cells and the 5-$HT_{2B}$ receptor ($pA_2$ 8.9) as measured in the rat stomach fundus preparation. SB 206553 has low affinity for cloned human 5-$HT_{2A}$ receptors expressed in HEK 293 cells ($pK_I$ 5.8) and ($pK_I$ <6) for a wide variety of other neurotransmitter receptors.

**2** SB 206553 appears to be a surmountable antagonist of 5-HT-stimulated phosphoinositide hydrolysis in HEK 293 cells expressing the human 5-$HT_{2C}$ receptor ($pK_B$ 9.0).

**3** The compound potently ($ID_{50}$ 5.5 mg $kg^{-1}$, p.o., 0.27 mg $kg^{-1}$, i.v.) inhibited the hypolocomotor response to *m*-chlorophenylpiperazine (mCPP), a putative model of 5-$HT_{2C}$/5-$HT_{2B}$ receptor function *in vivo*.

**4** At similar doses (2-20 mg $kg^{-1}$, p.o.) SB 206553 increased total interaction scores in a rat social interaction test and increased punished responding in a rat Geller-Seifter conflict test. These effects are consistent with the possession of anxiolytic properties.

**5** SB 206553 also increased suppressed responding in a marmoset conflict model of anxiety at somewhat higher doses (15 and 20 mg $kg^{-1}$, p.o.) but also reduced unsuppressed responding.

**6** These results suggest that SB 206553 is a potent mixed 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist with selectivity over the 5-$HT_{2A}$ and all other sites studied and possesses anxiolytic-like properties.

**Keywords:** 5-$HT_{2C}$ receptors; 5-$HT_{2B}$ receptors; 5-HT; anxiety; *m*-chlorophenylpiperazine

## Introduction

We have previously described the synthesis of SB 200646A, the first of a class of ligands which demonstrate higher affinity for the 5-$HT_{2C}$ and 5-$HT_{2B}$ receptors than the 5-$HT_{2A}$ site (Forbes *et al.*, 1993). This compound was found to act as a surmountable antagonist at the 5-$HT_{2C}$ and 5-$HT_{2B}$ receptors and possessed a number of behavioural actions. In particular, it was observed to antagonize both the hypolocomotor and hypophagic responses to *m*-chlorophenylpiperazine (mCPP) in rats, putative models of central 5-$HT_{2C}$/5-$HT_{2B}$ receptor function (Kennett *et al.*, 1994b). Furthermore, SB 200646A prevented the anxiogenic-like response to mCPP in the rat social interaction test when given at similar doses to those blocking the hypolocomotor and hypophagic responses to the drug (Kennett *et al.*, 1994b), suggesting that this too is 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor mediated. As mCPP is widely observed to induce anxiety and panic-like attacks in both healthy volunteers and patients (see Kennett, 1993, for review) this suggested that SB 200646A might have anxiolytic-like properties should 5-$HT_{2C}$ receptors be stimulated during periods of anxiety. Indeed, SB 200646A, when given alone, was observed to have anxiolytic-like actions in both the rat social interaction (Kennett *et al.*, 1994b) and Geller-Seifter models of anxiety and in a marmoset conflict test (Kennett *et al.*, 1995). The activity of SB 200646A in three anxiety paradigms with different aversive and motivational bases is compelling evidence for an anxiolytic mode of action.

However, although SB 200646A has a unique pharmacological profile, it is not an ideal ligand. The compound has relatively low affinity for both the 5-$HT_{2C}$ ($pK_I$ 6.9) and 5-$HT_{2B}$ ($pA_2$ of 7.2) receptors with a selectivity of only 50 fold over 5-$HT_{2A}$ sites and 80 fold over all other sites tested (Forbes *et al.*, 1993). SB 200646A also had poor oral potency in reversing both the mCPP-induced hypolocomotion and hypophagia models with an $ID_{50}$ of approximately 20 mg $kg^{-1}$, p.o. when given 1 h pretest (Kennett *et al.*, 1994b). The present study reports the properties of a new 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist, SB 206553 (Forbes *et al.*, 1995) with enhanced affinity for both sites together with increased selectivity and *in vivo* potency. The behavioural profile of this compound strengthens the probability that blockade of 5-$HT_{2C}$/5-$HT_{2B}$ receptors causes anxiolysis.

## Methods

### *Cell culture*

Human embryonic kidney cells (HEK 293, American Type Culture Collection CRL 1573) were stably transfected with either the human cloned 5-$HT_{2C}$ or 5-$HT_{2A}$ receptor (Saltzman *et al.*, 1991). HEK 293 cells were maintained in Minimum Essential Medium (MEM) containing Earle's salts, L-glutamine, 10% foetal calf serum (FCS) and geneticin (0.4 mg $ml^{-1}$) in a humidified incubator at 37°C gassed at 5% $CO_2$. The cells were harvested and pelleted by centrifugation at 1,000 *g* for 5 min at room temperature.

### *Binding assays*

The binding of [$^3$H]-mesulergine and [$^3$H]-ketanserin was carried out with conventional radioligand binding techniques.

[$^3$H]-mesulergine binding was carried out essentially according to the method of Westphal & Sanders-Bush (1994). Membranes were prepared by homogenizing cell pellets (Polytron, setting 6, 10 s) in 10 vols of ice-cold Tris buffer (50 mM Tris-HCl, pH 7.4 at 37°C) and centrifugation

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(40,000 *g* for 15 min at 4°C). The resultant pellet was resuspended in 20 volumes of ice-cold Tris buffer and washed a further two times by centrifugation and resuspension with an intermediate 15 min incubation at 37°C to remove endogenous 5-HT. The final pellet was resuspended in Tris buffer (approximately $10^7$ cells per ml) and frozen in 1 ml aliquots at −80°C until required. On the day of the experiment, an aliquot was thawed and resuspended using a polytron homogeniser in 80 ml total volume ice-cold Tris buffer. Binding assays were carried out with cell homogenate (0.4 ml) incubated with [$^3$H]-mesulergine (0.5 nM final) for 40 min at 37°C with test compound (at ten concentrations ranging from 100 mM to 3 nM) and either buffer or mianserin (1000 nM final) to determine total and non-specific binding respectively all in a final volume of 0.5 ml. The reaction was terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester and was followed by five 1 ml washes with ice-cold Tris buffer. The filter mats were pre-soaked in 0.01% polyethyleneimine (PEI) to reduce filter binding. The retained radioactivity was then determined by liquid scintillation spectrometry using Meltilex scintillator (Wallac) and a Wallac Betaplate counter. All reagents were dispensed into deep well microtitre plates using a Tecan RSP 5052 robotic sample processor.

[$^3$H]-ketanserin binding was performed with human cloned 5-$HT_{2A}$ receptor stably expressed into HEK 293 cells. The experimental procedure used was essentially as described above with the following exceptions: filters were not soaked in PEI, cell membrane aliquots were stored at a concentration of approximately $2 \times 10^7$ original cells $ml^{-1}$ and the radioligand used was [$^3$H]-ketanserin (0.5 nM final concentration).

Other binding assays were taken from published methods as described in Forbes *et al.* (1993) with the following exceptions: binding to the cloned human $D_2$ and $D_3$ receptors was carried out according to Bowen *et al.* (1993), to the human cloned $D_4$ receptor according to Van Tol *et al.* (1991), to the cloned human 5-$HT_7$ receptor by the method of To *et al.* (1995), to the cloned human 5-$HT_{1E}$ receptor, by a method based on that described by Hamblin & Metcalf (1991) for 5-$HT_{1D\alpha}$ receptors and to the human cloned and 5-$HT_{1F}$ receptor by the method of Adham *et al.* (1993).

### *Rat stomach fundus*

The rat stomach fundus was set up as detailed in Baxter *et al.* (1994). Briefly, mucosa denuded strips (1.5 × 20 mm) of longitudinal muscle were obtained from the stomach fundus of male Sprague Dawley rats (200–350 g) and suspended under an initial resting tension of 1 g in oxygenated (95% $O_2$/5% $CO_2$) Tyrode solution at 37°C. Experiments were conducted in the presence of indomethacin (3 μM) and after tissues had been exposed to pargyline (100 μM for 15 min). Two concentration effect curves to 5-HT were constructed in each preparation, the first in the absence and the second, 1 h later, in the presence of SB 206553. Time control curves to 5-HT at a 1 h interval were carried out in the same way without adding SB 206553.

### *Phosphatidylinositol hydrolysis assay*

The accumulation of inositol phosphates were assayed following prelabelling of the inositol phospholipids and inducing hydrolysis by agonist in the presence of lithium. HEK 293 cells expressing the human 5-$HT_{2C}$ receptor were suspended in Dulbecco's modification of Eagles' medium (DMEM) containing 10% FCS and were plated out into 24 well tissue culture plates at a density of 250,000 cells per well in 1 ml medium. These were incubated for 6 h after which the medium was aspirated and replaced with 1 ml/well inositol-free DMEM containing 1% dialysed FCS and 1 μCi $ml^{-1}$ [$^3$H]-*myo*-inositol. This was incubated for 48 h after which the medium was aspirated and replaced with FCS-free, inositol-free DMEM and incubated overnight. The medium was then aspirated and replaced with phosphate-buffered saline containing 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 5.5 mM glucose (final incubation volume 0.5 ml per well). This was incubated for 30 min in the presence or absence of antagonist after which 5-HT (50 μl) containing lithium chloride (10 mM final) was added and the incubation continued for a further 20 min. The reaction was terminated by the removal of the medium followed by addition of 0.75 ml cold methanol (−40°C). After at least 10 min the contents of the wells were pipetted into mini scintillation vials and the wells washed with 0.75 ml 2% HCl which was also transferred to the vials. Chloroform (0.75 ml) was then added to the vials and the water soluble $^3$H-labelled inositol phosphates separated by conventional anion exchange chromatography using Dowex AG1-X8 (Sigma). Radioactivity was determined by liquid scintillation counting.

### *Animals*

Male Sprague Dawley (CD) rats (220–250 g) were housed in groups of six under a 12 h light/dark cycle (lights on 07 h 00 min) with free access to food (CRMX, special Diet Services) and water.

### *mCPP-induced hypolocomotion*

Rats were placed in a room adjacent to the experimental room on the day of the procedure. They were dosed either orally 1 h or i.v. 20 min before the locomotion test with SB 206553 or vehicle, and injected i.p. 20 min before the test with mCPP or saline in groups of four. Rats were returned to their home cages after dosing. At 0 h they were each placed in automated locomotor activity cages (57 × 16.6 × 25.3 cm) made of black perspex with a clear perspex lid and sawdust covered floor, under red light for 10 min. During this time, locomotion was recorded by means of alternately breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level.

### *Social interaction*

Rats were housed singly in a room adjacent to the testing room on day 1. On day 5 they were dosed orally 1 h before the test with SB 206553 or vehicle in treatment and weight (±5 g) matched pairs unfamiliar to each other and returned to their home cages. Rats were then placed in a white perspex test box (54 × 37 × 26 cm) for 15 min under bright white light (150 lux) in an adjacent darkened room containing a fan to generate white noise. Active social interaction (sniffing, following, grooming, biting, boxing and crawling over or under) was scored by a 'blind' observer by remote video monitoring and a computerised score pad. At the end of each test, the box was thoroughly wiped with moistened tissue paper.

### *Geller-Seifter test*

Forty male Sprague-Dawley CFY rats (Interfauna 400–600 g) were housed in pairs under a 12 h light/dark cycle (lights on 07 h 00 min) and fed a restricted diet to maintain their body weight to 80% of a free-feeding animal. The rats were part of a colony and were trained initially in a typical operant box (Campden Instruments Ltd) to associate pressing of a lever with a food pellet reward. As training progressed, the rats were introduced to a multiple schedule of reinforcement, i.e. five 3 min variable interval components [10–50 (mean 30) s, VI30] alternating with five 3 min fixed ratio (one reinforcement every five lever presses; FR5) components. The FR component was signalled to the rat by a light above the lever and in this component reinforcement was contingent with a footshock of pulse width 15 ms at intervals of 200 ms for 1 s. The magnitude of footshock was individually titrated for each rat up to a maximum of 0.75 mA, to give a lever pressing rate of between two and seven reinforcements during each of the five, 3 min punished responding periods. Fully trained rats also had a high level of responding in the VI phases (typically 180 presses in

3 min) to detect non-specific effects such as sedation or stimulant properties. Before use, all rats used had met specific performance criteria (see Kennett *et al.*, 1995) and had shown a significant positive response to a reference anxiolytic drug (e.g. chlordiazepoxide). A period of at least seven days was left between subsequent tests. No rat received two consecutive doses of the same drug or type of drug and no rat received more than five treatments.

### *Marmoset conflict test*

Ten marmosets, male and female, (bred in house, 400–500 g) on a normal diet, were trained to lever press for a banana milkshake reinforcement in a black perspex operant box, lit by a single house light. The box contained a stainless steel reinforcement well lit by an orange light set 1.5 cm above in the wall. The rim of the well was surrounded by air holes. The reinforcement was fed via a valve up into the well. On shut off (after 1.5 s), the milk shake drained away from the well. Animals were initially trained on a continuous reinforcement schedule (1 press per reinforcement) until they achieved an average of 79 reinforcements over eight 2 min periods. The rate of reinforcement was then reduced (using a fixed ratio schedule) until responding reached a maximum for each individual marmoset. The conditions in four of the alternate 2 min intervals were then changed to associate a light cue with air blasts given through the holes surrounding the well of the reward dispenser. These were increased until each marmoset's response rate was reduced to 30% of its unsuppressed response rate. When marmosets had shown consistent rates of responding on consecutive control days and a positive response to 5 mg $kg^{-1}$, p.o. diazepam, they were used to test the effect of novel compounds. Each unpunished and punished period was separated by a 30 s time out period during which lever pressing was unrewarded and the light cue was off. Thus the total duration of each test session was 20 min. During this study marmosets received an average 3 successive treatments at monthly intervals. For further details see Kennett *et al.* (1995).

### *Materials*

[$^3$H]-mesulergine (70–85 Ci $mmol^{-1}$) and [$^3$H]-*myo*-inositol (with PT6-271 stabilzer) were purchased from Amersham International (Little Chalfont, Bucks) and [$^3$H]-ketanserin (60–90 Ci $mmol^{-1}$) was purchased from DuPont N.E.N. (Stevenage). Tris, polyethyleneimine indomethacin, pargyline hydrochloride, 5-HT hydrochloride and other chemicals were purchased from Sigma (Poole, Dorset). Mianserin, HCl was purchased from RBI (Natick, MA, U.S.A.). Cell culture reagents were purchased from Gibco BRL (Paisley, Scotland). SB 206553 (5-methyl-1-(3-pyridylcarbamomyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) was synthesized in the Department of Medicinal Chemistry, SmithKline Beecham. For *in vitro* studies it was initially dissolved in dimethylsulphoxide and 50 $\mu$l 4 M tartaric acid to give a $10^{-2}$ M solution prior to dilution. For *in vivo* studies, SB 206553 was given orally as a suspension after grinding (with a mortar and pestle) into a 1% methyl cellulose solution in 0.9% NaCl containing a drop of BRIJ 35 (Sigma) or i.v. as a 1 ml bolus in 10% polyethylene glycol by volume and 8% hydroxypropyl-$\beta$-cyclodextrin by weight. Chlordiazepoxide and diazepam (all synthesized by SmithKline Beecham Pharmaceuticals) were treated similarly and injection volumes of 2 ml $kg^{-1}$ (or 1 ml total volume for marmosets and i.v. studies) were used. In the social interaction test, the vehicle also contained 10 mg $ml^{-1}$ $BaSO_4$ (Sigma) and 10 $\mu$l egg yellow food dye (Supercook, Leeds) to obscure the slightly creamy colour of drug containing suspensions. Drug and appropriate vehicle suspensions were then independently coded prior to experiments to establish blind conditions. Oral dosing took place 1 h before testing, i.v. dosing 20 min before testing. mCPP was dissolved in 0.9% NaCl and given a 1 ml $kg^{-1}$ volume i.p. 20 min before testing.

### *Data analysis and statistics*

Data from receptor binding and phosphoinositol hydrolysis functional studies were analysed by the four parameter-logistic function (DeLean *et al.*, 1978) to determine the $IC_{50}$ (concentration of test compound that inhibits specific binding or maximal response to 5-HT by 50%) or $EC_{50}$ (concentration producing 50% of maximal response). The $IC_{50}$ was then corrected to inhibitory affinity constant ($K_I$) according to Cheng & Prusoff (1973) and expressed as the negative $\log_{10} K_I$ ($pK_I$). $K_B$ was defined as concentration of antagonist divided by [dose-ratio ($EC_{50}$ for 5-HT observed in the presence of antagonist/that obtained in its absence) − 1]. The significance of altered basal levels of phosphoinositide hydrolysis was determined by Student's *t* test. Results are expressed as the mean ± standard error of the mean (s.e.mean) or in the rat stomach fundus experiments, standard deviation (s.d.) from a number ($n$) of separate experiments. The $pA_2$ of SB 206553 versus 5-HT in the rat stomach fundus was calculated by Schild regression analysis, plotting $\log_{10}$ molar antagonist concentration against $-\log_{10}$ of the concentration ratios (CR − 1) determined in individual experiments as detailed in Baxter *et al.* (1994). The effect of SB 206553 on mCPP-induced hypolocomotion was determined by 1-way ANOVA and Newman-Keuls test. The dose producing 50% disinhibition of mCPP ($ID_{50}$) was also estimated by the four parameter-logistic function. Social interaction test data were subjected to 1-way ANOVA and Dunnett's test while Geller-Seifter and marmoset conflict test data were analysed by 2-way ANOVA (treatment x subjects) of the number of lever presses on the 2 consecutive days before the test day (2 scores per subject), and on the test day itself (1 score per subject). Both control day scores were included in 1 treatment group for the purposes of this analysis and these were compared with the relevant test day scores for each subject. All data are cited as the mean ± s.e.mean unless otherwise indicated.

## Results

### In vitro *studies*

*Receptor binding* SB 206553 potently inhibited the binding of [$^3$H]-mesulergine to the cloned human 5-$HT_{2C}$ receptor with a $pK_I$ of 7.92 ± 0.03 (7) and Hill coefficient of 1.11 ± 0.03 (Figure 1). In contrast, SB 206553 was a weak inhibitor of [$^3$H]-ketanserin binding to the cloned human 5-$HT_{2A}$ receptor with a $pK_I$ of 5.78 ± 0.03 (7) and a slope factor of 0.98 ± 0.06 (Figure 2).

With respect to other 5-HT and monoamine receptors (except the 5-$HT_{2B}$ receptor reported below), SB 206553 showed little activity (Table 1). SB 206553 was also inactive at the benzodiazepine and *t*-butylbicyclophosphoro[$^{35}$S]thionate (TBPS) binding sites of the $\gamma$-aminobutyric acid $(GABA)_A$ receptor (Table 1).

*Rat stomach fundus assay* SB 206553 (0.1–10 $\mu$M) caused a concentration-dependent rightward displacement of contractile concentration effect curves to 5-HT, with no depression

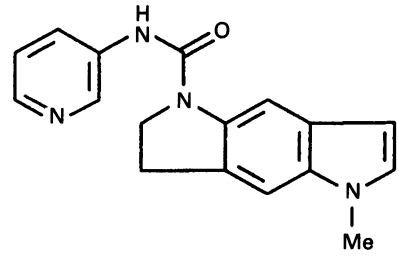


**Figure 1** Structure of SB 206553.

of the maximum response. A $pA_2$ value of $8.89 \pm 0.07$ (4) (mean ± s.d.) associated with a slope of $0.80 \pm 0.01$ (mean ± s.d.) was determined by Schild regression analysis of data from 4 separate experiments each testing three different concentrations of SB 206553 (Figure 3). Response curves to 5-HT generated at 1 h intervals as time controls in the absence of SB 206553 produced mean (± s.e.mean) $pEC_{50}$ values of $8.32 \pm 0.01$ and $8.27 \pm 0.04$ respectively ($n=4$) and were not significantly different by Student's $t$ test.

*PI hydrolysis studies* Basal inositol phosphate release was significantly reduced ($P<0.005$, Figure 4) in the presence of SB 206553 (100 nM). Incubation of HEK 293 cells expressing the cloned human 5-$HT_{2C}$ receptor with 5-HT resulted in an increase in inositol phosphates release with a $pEC_{50}$ of $8.5 \pm 0.1$ (9) and a % stimulation over basal of $349 \pm 21$ (9). SB 206553 (100 nM) shifted the concentration-effect curve to 5-HT to the right in a parallel fashion (Figure 4) with a $pK_B$ of $9.00 \pm 0.06$ (3). SB 206553 at 100 nM had no effect on the maximal response seen with 5-HT. SB 206553 had no agonist-like effects on its own.

## In vivo *studies*

*mCPP-induced hypolocomotion* mCPP (7 mg $kg^{-1}$, i.p. 20 min pretest) significantly reduced locomotion [$F(1,91)=22.2$, $P<0.01$]. SB 206553 5–40 mg $kg^{-1}$, p.o. 1 h pretest had no effect on locomotion when given alone, but significantly reversed the effect of mCPP (mCPP × SB 206553; $F(4,91)=7.45$, $P<0.01$). The effect of mCPP was fully antagonized by SB 206553 and there was no loss of efficacy over the

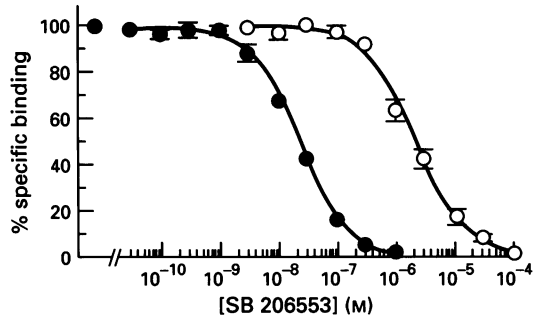


**Figure 2** Inhibition of [$^3$H]-mesulergine to the human cloned 5-$HT_{2C}$ (●) and of [$^3$H]-ketanserin to cloned human 5-$HT_{2A}$ (○) receptors by SB 206553. Data are shown as % specific binding and represent the mean ± s.e.mean from 4 separate determinations.

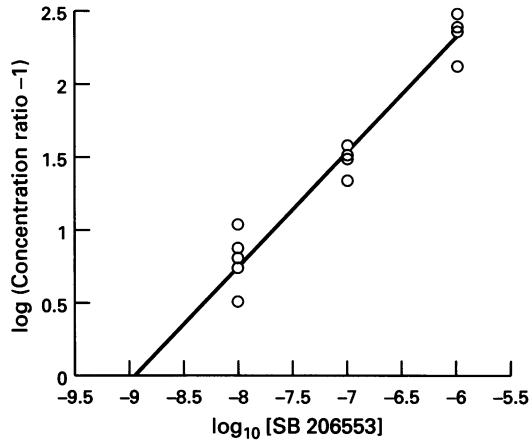


**Figure 3** Schild regression analysis of the effect of SB 206553 at three concentrations on 5-HT-induced contractions of the rat stomach fundus preparation. Each point represents the result of an individual determination. $pA_2=8.89 \pm 0.07$; slope $=0.80 \pm 0.01$.

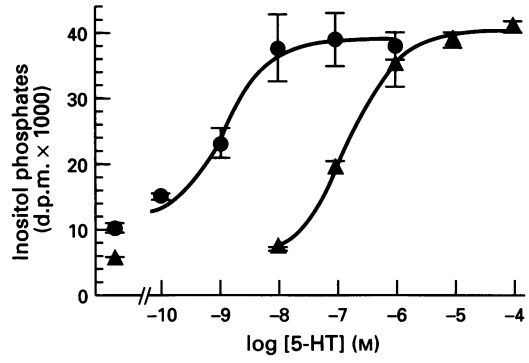


**Figure 4** Antagonism by SB 206553 (100 nM, ▲) of the 5-HT-stimulated inositol phosphate production in HEK 293 cells expressing the human 5-$HT_{2C}$ receptor; (●) control. Data are from a single experiment with standard errors from triplicate determinations to show experimental variability. The experiment was repeated a total of three times with similar results.

**Table 1** Receptor profile of SB 206553

| *Receptor* | *Tissue source* | *Non-specific ligand* | *Radioligand* | *SB 206553 ($pK_I$)* |
|---|---|---|---|---|
| 5-$HT_{2C}$ | Human clone | Mianserin (1 μM) | [$^3$H]-mesulergine | 7.92 |
| 5-$HT_{2A}$ | Human clone | Mianserin (1 μM) | [$^3$H]-ketanserin | 5.78 |
| 5-$HT_{2B}$ | Rat stomach fundus | | | 8.89* |
| 5-$HT_{1A}$ | Rat hippocampus | Spiperone (10 μM) | [$^3$H]-8-OH-DPAT | < 5 |
| 5-$HT_{1D}$ | Guinea-pig cortex | 5-HT (10 μM) | [$^3$H]-5-HT | < 6 |
| 5-$HT_{1E}$ | Human clone | 5-HT (10 μM) | [$^3$H]-5-HT | < 5.3 |
| 5-$HT_{1F}$ | Human clone | 5-HT (10 μM) | [$^3$H]-5-HT | < 5.3 |
| 5-$HT_3$ | Rat entorhinal cortex | ICS 205–930 (1 μM) | BRL 43694 | < 5 |
| 5-$HT_4$ | Rat oesophagus | | | 5.3* |
| 5-$HT_7$ | Human clone | 5-HT (10 μM) | [$^3$H]-5-CT | < 6 |
| Dopamine $D_2$ | Human clone | Iodo-sulpride (10 μM) | [$^{125}$H]-Iodo-sulpride | < 5 |
| Dopamine $D_3$ | Human clone | Iodo-sulpride (10 μM) | [$^{125}$H]-Iodo-sulpride | < 5 |
| Dopamine $D_4$ | Human clone | YM 09151-2 (10 μM) | [$^3$H]-YM 09151-1 | < 5 |
| Adrenoceptor $\alpha_1$ | Rat cortex | Phentolamine (10 μM) | [$^3$H]-prazosin | < 5 |
| Histamine $H_1$ | Guinea-pig cortex | Promethazine (10 μM) | [$^3$H]-mepyramine | < 5 |
| Adenosine $A_1$ | Rat cortex | 2-chloro-adenosine (100 μM) | [$^3$H]-PIA | < 4.3 |
| TBPS | Rat cortex | Picrotoxin (100 μM) | [$^{35}$S]-TBPS | < 4.3 |
| Benzodiazepine | Rat cortex | Diazepam (10 μM) | [$^3$H]-flunitrazepam | < 4.3 |

*$pA_2$ values from isolated tissue functional assays.

dose-range studied (Table 2). Analysis of the data of four parameter logistic function generated an $ID_{50}$ figure (±confidence limits) of 5.5±1.7 mg $kg^{-1}$. SB 206553 also completely blocked mCPP-induced hypolocomotion after i.v. administration; ($F(6,31)=11.3$, $P<0.01$) with an estimated $ID_{50}$ of 0.27±0.49 mg $kg^{-1}$. The vehicle used for these studies did not affect basal activity compared with saline + saline-treated rats (Table 3).

*Social interaction* SB 206553 significantly increased time spent in active social interaction ($F(6,79)=12.6$, $P<0.01$) at doses between 2 and 20 mg $kg^{-1}$, p.o. given 1 h before testing. The magnitude of the effect was fully equal to that of the benzodiazepine positive control chlordiazepoxide (5 mg $kg^{-1}$) used in this study. SB 206553 also increased locomotor activity in the test ($F(6,79)=6.9$, $P<0.01$) at the highest doses tested (10 mg $kg^{-1}$ + 26%, 20 mg $kg^{-1}$ + 31%), as did chlordiazepoxide (+45%) (Figure 5).

*Geller-Seifter test* The mean(±s.e.mean) number of lever presses on the two days preceding challenge with SB 206553 or chlordiazepoxide was 939.1±43.6 during unpunished, as opposed to 23.2±0.7 during punished trials. SB 206553 significantly increased lever pressing under punished conditions at 2 mg $kg^{-1}$ and above, as did the benzodiazepine anxiolytic, chlordiazepoxide. The maximal effect of chlordiazepoxide was, however somewhat greater (+336% at 5 mg kg, p.o. as opposed to +257% for SB 206553 at 10 mg $kg^{-1}$, p.o.). At one dose (5 mg $kg^{-1}$, p.o.) SB 206553 also caused a small increase in unpunished responding while chlordiazepoxide had a more substantial effect at both 2.5 and 5 mg $kg^{-1}$ (Table 4).

*Marmoset conflict test* The mean(±s.e.mean) number of lever presses on the two days preceding challenge with SB 206553 or diazepam was 149.3±18.1 during unpunished and 9.6±1.4 during punished trials respectively. SB 206553 increased punished responding at both 15 and 20 mg $kg^{-1}$, p.o. given 1 h before testing, as did the benzodiazepine anxiolytic diazepam at 2 mg $kg^{-1}$, p.o. However, while diazepam increased responding during unpunished trials, SB 206553 caused a marked reduction after either 15 and 20 mg $kg^{-1}$, p.o. (Table 5).

## Discussion

SB 206553 exhibited high affinity for both the 5-$HT_{2C}$ ($pK_I$ 7.9) and the 5-$HT_{2B}$ ($pA_2$ 8.9) receptors, but had 100 fold or greater selectivity over all other sites tested. This contrasts with the lower affinity for both sites of the only other reported selective 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist, SB 200646A ($pK_I$ 5-$HT_{2C}$ = 6.9, $pA_2$ = 7.2 respectively, Forbes *et al.*, 1993). In the PI hydrolysis model of 5-$HT_{2C}$ receptor function, SB 206553 acted as a surmountable antagonist producing a parallel shift in the dose-response curve to 5-HT ($pK_B$ 9.0) with no loss of 5-HT efficacy. Interestingly, SB 206553 (100 nM) lowered the basal level of inositol phosphate production in the absence of agonist in the present study. Thus SB 206553 may possess inverse agonist properties as has been suggested for other ligands at the 5-$HT_{2C}$ receptor (Westphal & Sanders-Bush, 1994; Barker *et al.*, 1994). However, this could also be caused by the presence of low levels of residual 5-HT in the cell incubation medium despite the use of dialysed serum. The apparent higher blocking potency of SB 206553 in the phosphoinositide hydrolysis model of 5-$HT_{2C}$ function compared with its binding affinity may also be consistent with inverse agonism but could equally be accounted for by some other complexity of the interaction of SB 206553 with the 5-$HT_{2C}$ receptor in a heterologous expression system. Further studies to elucidate whether SB 206553 acts as an inverse agonist at 5-$HT_{2C}$ receptors are currently underway.

**Table 2** Effect of oral SB 206553 on mCPP-induced hypolocomotion

| *Pretreatment* (p.o. 1 h before test) | *Treatment* (i.p. 20 min before test) | *Transits/10 min* (mean ± s.e.mean) |
|---|---|---|
| Vehicle | Saline | 21.3±1.0 |
| SB 206553 5 mg $kg^{-1}$ | Saline | 18.4±2.0 |
| SB 206553 10 mg $kg^{-1}$ | Saline | 21.4±2.0 |
| SB 206553 20 mg $kg^{-1}$ | Saline | 24.2±2.8 |
| SB 206553 40 mg $kg^{-1}$ | Saline | 23.9±1.9 |
| Vehicle | mCPP 7 mg $kg^{-1}$ | 1.4±0.3** |
| SB 206553 5 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 7.6±2.2** |
| SB 206553 10 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 21.2±3.6†† |
| SB 206553 20 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 25.0±4.3†† |
| SB 206553 40 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 21.4±3.3†† |

All groups contained 5–16 rats. Significantly different from relevant saline-treated group: **$P<0.01$; from vehicle + mCPP treated group: ††$P<0.01$ by Newman-Keuls test and 2-way ANOVA.

**Table 3** Effect of i.v. administration of SB 206553 on mCPP-induced hypolocomotion

| *Pretreatment* (i.v. 20 min before test) | *Treatment* (i.p. 20 min before test) | *Transist/10 min* (mean ± s.e.mean) |
|---|---|---|
| Saline | Saline | 19.8±2.7 |
| Vehicle | Saline | 15.8±1.7 |
| Vehicle | mCPP 7 mg $kg^{-1}$ | 3.2±1.8** |
| SB 206553 0.03 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 4.7±2.8 |
| SB 206553 0.1 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 2.6±1.4 |
| SB 206553 0.3 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 11.8±2.0† |
| SB 206553 1.0 mg $kg^{-1}$ | mCPP 7 mg $kg^{-1}$ | 19.2±2.3†† |

All groups contain 5–6 rats. Significantly different from vehicle + saline-treated group: **$P<0.01$; from vehicle + mCPP treated group: †$P<0.05$; ††$P<0.01$ by Newman-Keuls test and 1-way ANOVA.

SB 206553 was a potent inhibitor of the hypolocomotor response to mCPP, a putative model of central 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor function (Kennett & Curzon, 1988; Kennett *et al.*, 1994b) with an estimated $ID_{50}$ of 5.5 mg $kg^{-1}$, p.o. which compares with an $ID_{50}$ of 19.2 mg $kg^{-1}$, p.o. for SB 200646A (Kennett *et al.*, 1994b). SB 206553 was 20 fold more potent in this model when administered in an i.v. bolus injection. However, this may in part be explained by the use of a solubilising vehicle, unlike the simple suspension used for oral dosing studies. Like SB 200646A, SB 206553 increased total interaction in the social interaction test. At the two highest doses tested an increase in locomotion was also seen. However, as the positive control chlordiazepoxide had a larger effect in the same study, this is unlikely to indicate a stimulant action. Indeed, SB 206553 alone had no effect on locomotion in the mCPP hypolocomotion study, nor did it increase unpunished responding in the Geller-Seifter procedure as might have been expected of a stimulant (Geller & Seifter 1960). The effect of SB 206553 in the social interaction test is therefore consistent with anxiolysis (File & Hyde, 1978).

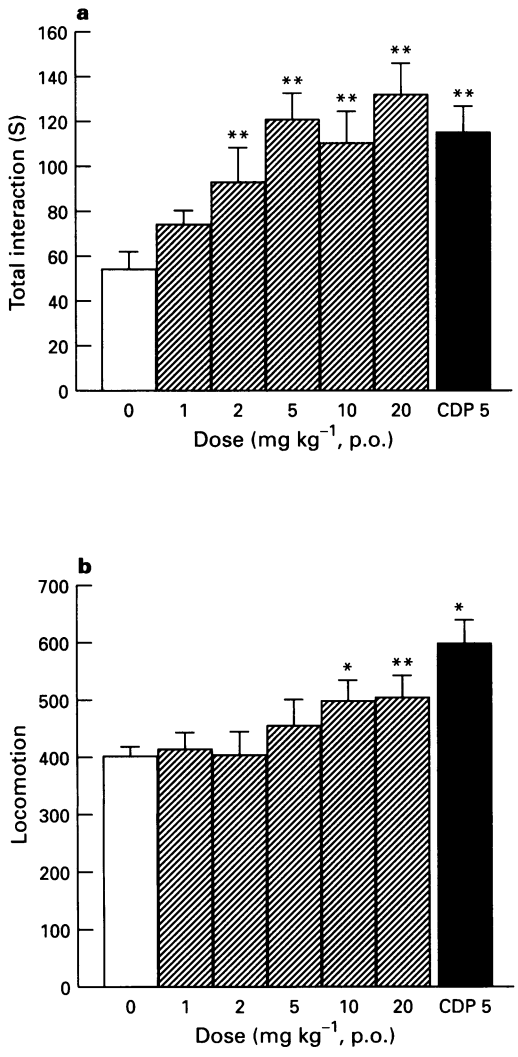


**Figure 5** Effect of SB 206553 (p.o. 1 h pretest) on a rat 15 min social interaction test under high light unfamiliar conditions. All data expressed as means ± s.e.mean, $n = 12-16$. Significantly different from vehicle-treated group: $^{*}P<0.05$; $^{**}P<0.01$ by Dunnett's test and 1-way ANOVA.

SB 200646A, SB 206553 was a potent inhibitor of the hypolocomotor response to mCPP, a putative model of central 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor function (Kennett & Curzon, 1988; Kennett *et al.*, 1994b) with an estimated $ID_{50}$ of 5.5 mg $kg^{-1}$, p.o. which compares with an $ID_{50}$ of 19.2 mg $kg^{-1}$, p.o. for SB 200646A (Kennett *et al.*, 1994b). SB 206553 was 20 fold more potent in this model when administered in an i.v. bolus injection. However, this may in part be explained by the use of a solubilising vehicle, unlike the simple suspension used for oral dosing studies. Like SB 200646A, SB 206553 increased total interaction in the social interaction test. At the two highest doses tested an increase in locomotion was also seen. However, as the positive control chlordiazepoxide had a larger effect in the same study, this is unlikely to indicate a stimulant action. Indeed, SB 206553 alone had no effect on locomotion in the mCPP hypolocomotion study, nor did it increase unpunished responding in the Geller-Seifter procedure as might have been expected of a stimulant (Geller & Seifter 1960). The effect of SB 206553 in the social interaction test is therefore consistent with anxiolysis (File & Hyde, 1978).

SB 206553 also had an anxiolytic-like profile in the rat Geller-Seifter test (Geller *et al.*, 1962) where it increased punished responding but had little effect on unpunished responding. Chlordiazepoxide similarly increase punished responding, but caused small increases in unpunished responding as well at some doses. The ability of SB 206553 to increase punished responding in the marmoset conflict test is consistent with its action in the Geller-Seifter procedure. However, in the marmoset, the compound markedly reduced unpunished responding, unlike the benzodiazepine diazepam. There is no evidence from the rat to suggest that the compound has sedative properties, at least over the dose-range studied and the origins of this effect are unclear although they are unlikely to be related to either 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor antagonism as SB 200646A did not lower unpunished responding in previous studies under identical conditions (Kennett *et al.*, 1995). It is conceivable that confounding properties may produce false positives in any one model of anxiety. Thus, an increase in appetite or decrease in pain sensitivity might interfere with the Geller-Seifter test, while altered body odour is reported to affect the social interaction test (Higgins *et al.*, 1991). However, the anxiolytic-like efficacy of SB 206553 in three separate models and two different species is strong evidence that the compound is, indeed, anxiolytic.

In previous studies, SB 200646A, had equal efficacy to chlordiazepoxide, given at a dose of 5 mg $kg^{-1}$, p.o. in the social interaction test (Kennett *et al.*, 1994a), but had approximately one third of the efficacy of this dose of chlordiazepoxide in the Geller-Seifter procedure. In the present study, the maximal response to SB 206553 in the social interaction test was also similar to that observed after 5 mg $kg^{-1}$, p.o. chlordiazepoxide, but for reasons that are currently unclear,

**Table 4** Effect of SB 206553 and chlordiazepoxide on behaviour in a rat Geller-Seifter conflict test

| *Treatment* (p.o. 1 h before test) | n | *% change in no of lever presses from mean score on two preceding days after vehicle-treatment* (mean ± s.e.mean) *Unpunished* (VI) | *Punished* (FR) |
|---|---|---|---|
| SB 2-6553 1 mg $kg^{-1}$ | 6 | +5.1 ± 2.4 | −3.9 ± 9.7 |
| 2 mg $kg^{-1}$ | 6 | +9.6 ± 4.4 | +68.1 ± 29.8* |
| 5 mg $kg^{-1}$ | 6 | +11.9 ± 6.2* | +80.8 ± 30.1** |
| 10 mg $kg^{-1}$ | 6 | −12.3 ± 19.3 | +257.0 ± 158* |
| 20 mg $kg^{-1}$ | 6 | −2.4 ± 7.0 | +176.5 ± 55.7** |
| 40 mg $kg^{-1}$ | 6 | −13.6 ± 13.1 | +225.3 ± 124.3** |
| Chlordiazepoxide 1 mg $kg^{-1}$ | 9 | −1.7 ± 8.2 | +20.5 ± 19.9 |
| 2.5 mg $kg^{-1}$ | 12 | +23.4 ± 4.0** | +168.5 ± 59.6** |
| 5 mg $kg^{-1}$ | 16 | +29.7 ± 10.6* | +335.9 ± 101.3** |

Significantly different from mean number of lever presses on two days preceding challenge with SB 206553 or chlordiazepoxide: $^{*}P<0.05$; $^{**}P<0.01$ by two-way ANOVA (treatment × subjects). Significant $F$ values, SB 206553 2 mg $kg^{-1}$, FR, $F(1,11)=8.0$, $P<0.05$, 5 mg $kg^{-1}$, VI, $F(1,11)=5.9$, $P<0.05$, FR $F(1,11)=14.1$, $P<0.01$, 10 mg $kg^{-1}$, FR, $F(1,11)=6.3$, $P<0.05$, 20 mg $kg^{-1}$, FR, $F(1,11)=20.2$, $P<0.01$, 40 mg $kg^{-1}$, FR, $F(1,11)=9.6$, $P<0.01$, chlordiazepoxide, 2.5 mg $kg^{-1}$, VI, $F(1,23)=24.8$, $P<0.01$, FR, $F(1,23)=22.0$, $P<0.01$, 5 mg $kg^{-1}$, VI, $F(1,31)=7.5$, $P<0.01$, FR, $F(1,31)=24.0$, $P<0.01$.

**Table 5** Effect of SB 206533 and diazepam on behaviour in a marmoset conflict test

| *Treatment* (p.o. 1 h before test) | n | *% change in no. of lever presses from mean score on two preceding days after vehicle treatment* (mean ± s.e.mean) *Unpunished* (VI) | *Punished* (FR) |
|---|---|---|---|
| SB 206553 10 mg kg$^{-1}$ | 4 | −20.1 ± 10 | −30.7 ± 7.9 |
| 15 mg kg$^{-1}$ | 5 | −45.3 ± 9.8* | +320.2 ± 197.9* |
| 20 mg kg$^{-1}$ | 6 | −42.3 ± 10.8** | +134.9 ± 79.9* |
| Diazepam 2 mg kg$^{-1}$ | 6 | +51.6 ± 19.9** | +439.5 ± 250.2** |

Significantly different from mean number of lever presses on two days preceding challenge with SB 206553 or diazepam: *$P<0.05$, **$P<0.01$ by two-way ANOVA (treatment × subjects). Significant $F$ values, SB 206553 15 mg kg$^{-1}$, VI, $F(1,9)=5.5$, $P<0.05$, FR, $F(1,9)=5.33$, $P<0.05$, 20 mg kg$^{-1}$, VI, $F(1,11)=20.5$, $P<0.01$, FR, $F(1,11)=7.2$, $P<0.05$; diazepam 2 mg kg$^{-1}$, VI, $F(1,11)=15.0$, $P<0.01$, FR, $F(1,11)=11.5$, $P<0.01$.

SB 206553 appears to have produced a greater maximal response than SB 200646A in the Geller-Seifter test. This observation is strengthened by the comparable response to chlordiazepoxide in the present and previous (Kennett *et al.*, 1995) studies. Which, if any, of the paradigms is most predictive of clinical efficacy is, as yet, uncertain.

The potency of SB 206553 in the two rat models of anxiety was similar to the compound's potency in the mCPP-induced hypolocomotion model of rat or 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor function. As the compound has 100 fold or greater selectivity for 5-$HT_{2C}$/5-$HT_{2B}$ receptors over all other receptors tested, including the benzodiazepine, $GABA_A$ and TBPS sites which are known to be associated with anxiety (Ticku, 1991; Macksay, 1993), the anxiolytic-like profile in the tests is therefore likely to be mediated by one of the two sites. This is entirely consistent with results from studies with SB 200646A (Kennett *et al.*, 1994b) and with earlier studies with non-selective 5-$HT_2$ receptor antagonists such as mianserin, ICI 169,369 and LY 53857 (Kennett *et al.*, 1994a). The potency of the compound in marmosets was lower. This may be accounted for by species differences in metabolism or absorption or by the action of the drug which reduced unpunished responding.

At the present time, it is not possible to discern whether the *in vivo* actions of SB 206553 are 5-$HT_{2C}$ or 5-$HT_{2B}$ receptor-mediated. mCPP is an agonist at both sites (Clineschmidt *et al.*, 1985; Schoeffter & Hoyer, 1989; Brown *et al.*, 1990; 1991; Baxter *et al.*, 1994) and the currently available antagonists are non-selective (Baxter *et al.*, 1995). The low levels of 5-$HT_{2B}$ receptor mRNA in mouse (Loric *et al.*, 1992) and human (Schmuck *et al.*, 1994) brains and the extremely low levels in the rat (Pompeiano *et al.*, 1994; Flanigan *et al.*, 1995) argue in favour of mediation by the 5-$HT_{2C}$ site. However, 5-$HT_{2B}$ receptor mRNA is reported to be concentrated in the rat hippocampus (Flanigan *et al.*, 1995) while the receptor protein has been reported to be concentrated in the rat lateral amygdala as well (Duxon *et al.*, 1995). Both these areas of the brain have long been associated with the control of anxiety (Kuhar, 1986; Higgins *et al.*, 1991). Indeed, infusion of mCPP into the hippocampus, although not the central amygdaloid nucleus, has been reported to induce anxiogenic-like effects in rats (Whitton & Curzon, 1990). Resolution of this issue therefore awaits the development of more selective ligands.

In conclusion, SB 206553 is the most potent and selective mixed 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist reported to date. Like its forerunner, SB 200646A, SB 206553 blocks mCPP-induced hypolocomotion and, at similar doses, has an anxiolytic-like profile in three models of anxiety which is therefore likely to be mediated by 5-$HT_{2C}$/5-$HT_{2B}$ receptor blockade. This is consistent with the anxiolytic efficacy of several non-selective 5-$HT_2$ receptor antagonists such as mianserin and ritanserin in man (Ceulemans *et al.*, 1985 and see Kennett, 1993) and with evidence that the widely observed anxiogenic effects of mCPP in man (see, Kennett, 1993) are likely to be accounted for by its agonist action at one of these two sites (Kennett *et al.*, 1994b). The results therefore suggest that 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonists such as SB 206553 are likely to anxiolytic in man.

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