Communications to the Editor

5-Chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046): A Potent, Selective, and Orally Bioavailable 5-HT$_6$ Receptor Antagonist

Steven M. Bromidge,*,† Anthony M. Brown,‡ Stephen E. Clarke,§ Kathy Dodgson,‡ Tracey Gager,‡ Helen L. Grassam,† Phil M. Jeffrey,§ Graham F. Joiner,† Frank D. King,† Derek N. Middlemiss,‡ Stephen F. Moss,† Helen Newman,‡ Graham Riley,‡ Carol Routledge,‡ and Paul Wyman†

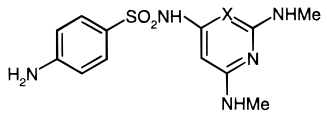
Departments of Medicinal Chemistry, Neuroscience Research, and Drug Metabolism and Pharmacokinetics, SmithKline Beecham Pharmaceuticals, Discovery Research, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, England

Received September 21, 1998

The 5-hydroxytryptamine (5-HT, serotonin) superfamily of receptors currently consists of 7 classes (5-HT$_1$−5-HT$_7$) that embrace 14 human subclasses.[1] The most recent addition is the 5-HT$_6$ receptor which was first cloned from rat striatal mRNA in 1993 by two independent groups.[2] The human 5-HT$_6$ receptor, cloned in 1994 by Kohen et al.,[3] is a 440-amino acid polypeptide with seven transmembrane spanning domains typical of G-protein-coupled receptors. Within the transmembrane region, the human 5-HT$_6$ receptor shows 96% identity to its rat homologue, but only 30−40% homology to other human 5-HT receptors. The 5-HT$_6$ receptor is positively coupled to adenylyl cyclase.[2]

In the rat, the 5-HT$_6$ receptor mRNA has its highest abundance in the nucleus accumbens, striatum, cerebral cortex, olfactory tubercle, and hippocampus.[2,4] Although the biological functions of the 5-HT$_6$ receptor are poorly understood, the distribution, together with its high affinity for several therapeutically important antipsychotic and antidepressant agents, suggests a possible role for this receptor in the treatment of schizophrenia and depression.[2a,5] Most atypical antipsychotic drugs, which lack extrapyrimidal side effects, bind with very high affinity to the 5-HT$_6$ receptor. In fact, the prototypic atypical antipsychotic agent, clozapine, exhibits greater affinity for the 5-HT$_6$ receptor than for any other receptor subtype. Recent in vivo experiments demonstrated that administration of antisense oligonucleotides, directed at 5-HT$_6$ receptor mRNA, elicited a behavioral syndrome in rats consisting of yawning, stretching, and chewing which could be dose-dependently blocked by the muscarinic antagonist atropine.[6] This study implies that 5-HT$_6$ receptors may modulate cholinergic neurotransmission and hence 5-HT$_6$ receptor antagonists may be useful for the treatment of memory dysfunction.

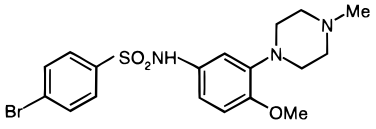
Unfortunately, further pharmacological evaluation of the function of the 5-HT$_6$ receptor has been hampered by the lack of selective ligands. Recently, the first selective 5-HT$_6$ antagonists, Ro 04-6790 and Ro 63-0563, were reported.[7] These compounds were found to have moderate affinity for the rat 5-HT$_6$ receptor but were poorly brain penetrant (<1%). However, when Ro 04-6790 was administered intraperitoneally to rats, sufficient brain levels were achieved to evoke a statistically significant effect on stretching similar to that seen following treatment with antisense oligonucleotides.



X = N **Ro 04-6790** 5-HT$_6$ pK_i 7.3

X = CH **Ro 63-0563** 5-HT$_6$ pK_i 7.9

High-throughput screening of the SmithKline Beecham Compound Bank against the cloned human 5-HT$_6$ receptor in HeLa cell membranes, using [^{3}H]lysergic acid diethylamide as radioligand,[2a] identified the bisaryl sulfonamide **1.** This compound (4-bromo-*N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]benzenesulfonamide) showed excellent affinity for the 5-HT$_6$ receptor (pK_i 8.3) and greater than 50-fold selectivity over a number of other key receptors including 10 other 5-HT receptor subtypes (Table 1). The sulfonamide **1** was further tested in a commercial screening package (Cerep) and has been found to have no appreciable affinity for a total of over 50 receptors, enzymes, or ion channels so far tested.



1

Compound **1** was evaluated in a functional model of 5-HT$_6$ receptor activation in which 5-HT-stimulated adenylyl cyclase activity was determined by measuring the conversion of [α-^{33}P]ATP to [^{33}P]cAMP in HeLa cells expressing the cloned human 5-HT$_6$ receptor.[9] In this system, 5-HT elicited a dose-dependent 3−5-fold increase over basal cAMP levels which was surmountably antagonized by clozapine, methiothepin, amitriptyline, and **1**. In the presence of compound **1**, the 5-HT concentration−response curve had the same maximal response but was shifted rightward in a parallel manner (Figure 1) with an apparent pK_b of 7.8 ± 0.2 (n = 3), which is in agreement with the binding affinity. In

* To whom correspondence should be addressed.
† Department of Medicinal Chemistry.
‡ Department of Neuroscience Research.
§ Department of Drug Metabolism and Pharmacokinetics.

10.1021/jm980532e CCC: $18.00
Published on Web 01/05/1999

Table 1. Receptor Binding Profile of Compounds **1**, **2**, and **15**[a]

	affinity (pK_i)		
	1	**2**	**15**
5-HT_{1A}	6.6	6.3	6.4
5-HT_{1B}	6.4	6.1	6.1
5-HT_{1D}	6.6	6.7	6.6
5-HT_{1E}	5.8	5.6	<5.0
5-HT_{1F}	6.5	6.6	<6.0
5-HT_{2A}	5.9	6.0	<5.6
5-HT_{2B}	6.2	6.0	<5.4
5-HT_{2C}	6.0	6.3	5.7
5-HT_4	5.6	5.5	5.4
5-HT_6	**8.3 ± 0.2 (*n* > 10)**	**9.2 ± 0.1 (*n* = 3)**	**8.9 ± 0.2 (*n* = 3)**
5-HT_7	5.6	5.5	5.4
adrenergic α_{1B}	5.6	5.7	5.7
dopaminergic D_2	5.4	6.1	5.6
dopaminergic D_3	6.1	6.7	6.3

[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. Receptors and radioligands used in binding assay: 5-HT_{1A} (human cloned receptors in HEK 293 cells, [^{3}H]-8-OH-DPAT); 5-HT_{1B} (human cloned receptors in CHO cells, [^{3}H]-5-HT); 5-HT_{1D} (human cloned receptors in CHO cells, [^{3}H]-5-HT); 5-HT_{1E} (human cloned receptors in CHO cells, [^{3}H]-5-HT); 5-HT_{1F} (human cloned receptors in CHO cells, [^{3}H]-5-HT); 5-HT_{2A} (human cloned receptors in HEK 293 cells, [^{3}H]ketanserin); 5-HT_{2B} (human cloned receptors in HEK 293 cells, [^{3}H]-5-HT); 5-HT_{2C} (human cloned receptors in HEK 293 cells, [^{3}H]mesulergine); 5-HT_6 (human cloned receptors in HeLa cells, [^{3}H]LSD); 5-HT_7 (human cloned receptors in HEK 293 cells, [^{3}H]-5-CT); D_2 (human cloned receptors in CHO cells, [^{125}I]iodosulpride); D_3 (human cloned receptors in CHO cells, [^{125}I]iodosulpride).

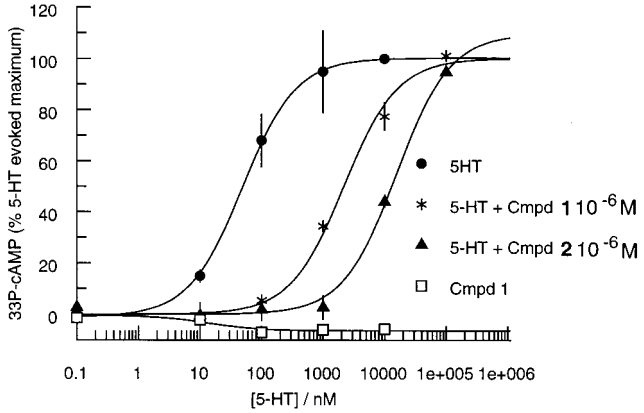


Figure 1. Effect of compounds **1** and **2** on 5-HT-stimulated adenylyl cyclase activity in membranes from HeLa cells transfected with the human 5-HT_6 receptor. Data points represent the mean of duplicate determinations from a typical experiment which was repeated at least twice.

addition **1** showed no evidence of intrinsic activity in this system as demonstrated by the lack of effect on basal formation of cAMP with compound alone. Thus, **1** possesses the profile of a competitive antagonist.

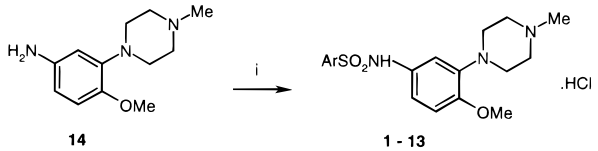
The cytochrome P450 inhibitory potential of **1** was determined using isoform-selective assays and heterologously expressed human CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Table 2) in order to assess the potential likelihood of drug interactions.[8] Low to moderate levels of inhibition were seen at several of the major human P450 enzymes with the highest level of inhibition seen against CYP3A4 (IC_{50} 6 μM). Pharmacokinetic studies at steady state in rats (n = 3, following 8 h iv infusion) demonstrated that **1** was moderately brain penetrant (25%) but was subject to rapid blood clearance (~60 mL/min/kg) resulting in low oral bioavailability

Table 2. Human Cytochrome P450 Inhibitory Potential of **1** and **15**

	IC_{50} (μM)				
compd	1A2	2C9	2C19	2D6	3A4
1	10	>100	46	11	6
15	66	>100	26	32	40

[a] The cytochrome P450 inhibitory potential was determined using isoform-selective assays and heterologously expressed human CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. IC_{50}'s were determined at the substrate K_m as has been previously described[12] [CYP1A2, caffeine N3-demethylation (500 μM); CYP2C9, tobutamide methylhydroxylation (100 μM); CYP2C19, *S*-mephenytoin 4-hydroxylation (100 μM); CYP2D6, bufuralol 1′-hydroxylation (10 μM); CYP3A4, total cyclosporin oxidation (1 μM)]. These values are the mean of duplicate determinations which did not vary by more than 10%.

Scheme 1. Synthesis of Bisaryl Sulfonamides **1**−**13**[a]

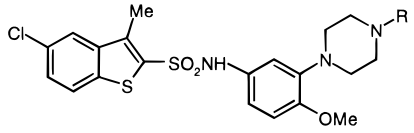


14 **1 - 13**

[a] Reagents: (i) $ArSO_2Cl$, acetone, rt, 18 h (65−92%).

(F_{po} = 12%, iv/po crossover study). The structure of **1** was deemed to be readily amenable to exploration of structure−activity relationships (SAR) by rapid parallel synthesis, and it was therefore selected as the starting point for a chemical program.

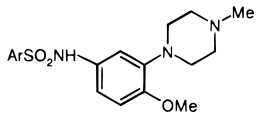
The SAR around the lead structure **1** was investigated by coupling 4-methoxy-3-(4-methylpiperazin-1-yl)-aniline[10] (**14**) in a parallel manner with commercially available sulfonyl chlorides containing a wide variety of aromatic nuclei (Scheme 1).[11] The binding results on a representative selection of compounds (**2**−**13**) are shown in Table 3. A range of affinities for the 5-HT_6 receptor were obtained with a number of analogues including monocyclic and bicyclic aromatics demonstrating improved binding profiles relative to **1**. The unsubstituted phenyl **10** with a pK_i of 8.0 at the 5-HT_6 receptor provides a baseline activity for comparison. Lipophilic substituents, in particular halogen, were beneficial to 5-HT_6 activity (e.g., **4, 5, 7**−**9**), whereas polar groups were detrimental, e.g., the 3-cyano (**11**) and 4-nitro (**12**) analogues. The polar imidazole **13** also showed very poor 5-HT_6 receptor affinity. The 5-chloro-3-methylbenzothiophene **2** was optimal in this study demonstrating sub-nanomolar 5-HT_6 receptor affinity and greater than 300-fold selectivity against a range of other receptors (totalling 13 subtypes). In the functional adenylyl cyclase assay, **2** was found to be a competitive antagonist with an apparent pK_b of 8.5 ± 0.2 (n = 3) (Figure 1). Several iodophenyl analogues (e.g., **4, 5, 8**) were also identified with excellent 5-HT_6 receptor affinity and selectivity. The use of [^{125}I]-**8** as a specific radioligand will be reported elsewhere.

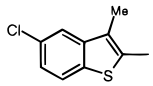
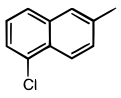
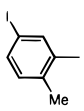
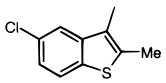
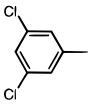
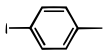
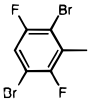
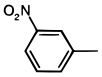
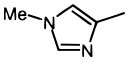


R = Me **2**

R = H **15 (SB-271046)**

Table 3. 5-HT_6 Receptor Binding Affinity and Selectivity[a] of Bisaryl Sulfonamides **2**−**13**



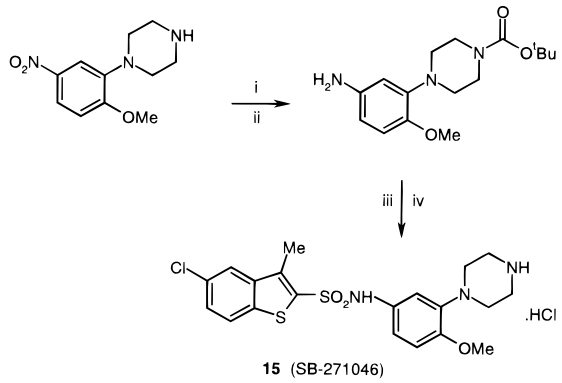
compd	Ar	pK_i 5-HT_6	selectivity vs 13 receptor subtypes
2		9.2	>300
3		9.1	>250
4		9.1	>200
5		9.0	>125
6		8.9	>300
7		8.7	>100
8		8.6	>160
9		8.5	>300
10		8.0	80
11		7.2	-
12		7.1	-
13		6.1	-

[a] Selectivity was determined against the 13 receptor subtypes detailed in Table 1.

Pharmacokinetic studies at steady state in rats following a 16-h infusion ($n = 4$) demonstrated that **2** was moderately brain penetrant (18%) and, in contrast to **1**, was subject to low blood clearance (12.5 mL/min/kg). However, in rats **2** was metabolically N-dealkylated to the corresponding NH-piperazine **15**. As significant levels of **15** were found in blood, it was synthesized and its biological profile assessed. Thus, 5-chloro-*N*-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-2-benzothiophenesulfonamide (**15**) (SB-271046) was prepared via the BOC-protected piperazine according to Scheme 2. The receptor binding profile of **15** is shown in Table 1. The 5-HT_6 receptor affinity, although slightly reduced relative to that of the *N*-methylpiperazine **2**, remains excellent (pK_i 8.9), and furthermore **15** also has good selectivity (>200-fold) against a total of over 50 receptors, enzymes, or ion channels.

In the functional adenylyl cyclase assay, **15** was found to be a competitive antagonist with a pA_2 of 8.7 which is in good agreement with its binding affinity (Figure 2). Linear regression analysis of Schild plot data revealed a correlation coefficient of unity and a slope of 1.04. In addition to an excellent binding profile, compound **15** demonstrated no significant inhibitory activity at the major human P450 enzymes (Table 2). Pharmacokinetic studies demonstrated that **15** was moderately brain penetrant (10%), subject to low blood clearance (7.7 mL/min/kg) with a good half-life in rats (4.8 ± 0.1 h), and had excellent oral bioavailability (>80%).

Scheme 2. Synthesis of 5-Chloro-*N*-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-2-benzothiophenesulfonamide **15**[a]



[a] Reagents: (i) $(BOC)_2O/K_2CO_3$, THF/H_2O, rt, 18 h (89%); (ii) H_2/10% Pd/C, EtOH, rt, 18 h (99%); (iii) 5-chloro-3-methylbenzo[*b*]thiophene-2-sulfonyl chloride/pyridine, DCM, rt, 18 h (97%); (iv) THF/cHCl (5:1), reflux, 2 h, recrystallization (EtOH/H_2O) (83%).

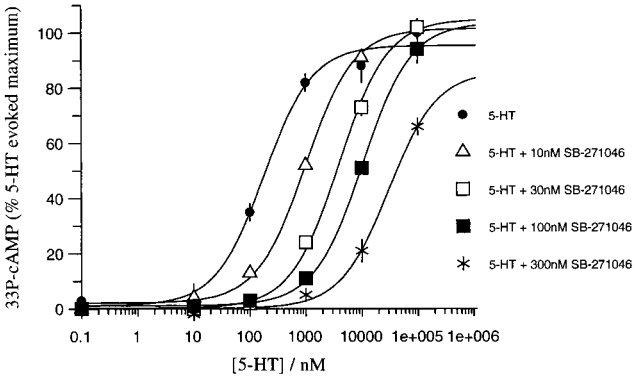


Figure 2. Effect of compound **15** (SB-271046) on 5-HT-stimulated adenylyl cyclase activity in membranes from HeLa cells transfected with the human 5-HT_6 receptor. Data points represent the mean of duplicate determinations from a typical experiment which was repeated at least twice.

In conclusion, a series of potent and selective *N*-methylpiperazines has been developed from a high-throughput screening lead **1**. The benzothiophene **2** which was the most potent compound from this series was metabolically demethylated in vivo. Consequently, the NH-piperazine benzothiophene **15** was prepared and found to be a high-affinity, selective, and orally bioavailable 5-HT_6 receptor antagonist. Compound **15** is currently being further evaluated for its therapeutic potential.

Supporting Information Available: Experimental Section containing the synthesis of compounds **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hoyer, D.; Martin, G. 5-HT receptor classification and nomenclature: towards a harmonisation with the human genome. *Neuropharmacology* **1997**, *36*, 419−428. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev*. **1994,** *46*, 157−204.

(2) (a) Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol*. **1993**, *43*, 320−327. (b) Ruat, M.; Traiffort, E.; Arrang, J.-M.; Tardivel-Lacombe, J.; Diaz, J.; Leurs, R.; Schwartz, J.-C. A novel serotonin (5-HT_6) receptor: Molecular cloning, localisation and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun*. **1993**, *193*, 269−276.

(3) Kohen, R.; Metcalf, M. A.; Druck, T.; Huebner, K.; Sibley, D. R.; Hamblin, M. W. Cloning and chromosomal localization of a human 5-HT_6 serotonin receptor. *Soc. Neurosci. Abstr*. **1994**, *20***,** 476.8.

(4) Ward, R. P.; Hamblin, M. W.; Lachowicz, J. E.; Hoffman, B. J.; Sibley, D. R.; Dorsa, D. M. Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience* **1995**, *64***,** 1105−1111. Gerard, C.; El Mestikawy, S.; Lebrand, C.; Adrien, J.; Ruat, M.; Traiffort, E.; Hamon, M.; Martres, M.-P. Quantitative RT-PCR distribution of serotonin 5-HT_6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse* **1996**, *23*, 164−173.

(5) Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptor. *J. Pharmacol. Exp. Ther*. **1994**, *268*, 1403−1410.

(6) Bourson, A.; Borroni, E.; Austin, R. H.; Monsma, F. J.; Sleight, A. J. Determination of the role of the 5-HT_6 receptor in the rat brain: A study using antisense oligonucleotides. *J. Pharmacol. Exp. Ther*. **1995**, *274*, 173−180. Sleight, A. J.; Monsma, F. J.; Borroni, E.; Austin, R. H.; Bourson, A. Effects of altered 5-HT_6 expression in the rat: Functional studies using antisense oligonucleotides. *Behav. Brain Res*. **1996**, *73***,** 245−248.

(7) Sleight, A. J.; Boess, F. G.; Bös, M.; Levet-Trafit, B.; Riemer, C.; Bourson, A. Characterization of Ro 04−6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT_6 receptors. *Br. J. Pharmacol*. **1998**, *124*, 556−562.

(8) Lewis, D. F. V. *Cytochromes P450: Structure, Function and Mechanism;* Taylor and Francis Publishing: London, 1996.

(9) Salomon, Y. Adenylate Cyclase Assay. *Adv. Cyclic Nucleotide Res*. **1979**, *10*, 35−55.

(10) Oxford, A. W.; Mitchell, W. L.; Bradshaw, J.; Clitherow, J. W. EP Patent 533267, March 24, 1993.

(11) Bromidge, S. M.; King, F. D.; Wyman, P. A. WO Patent 98/27081, June 25, 1998.

(12) Baldwin, S. J.; Bloomer, J. C.; Smith, G. J.; Ayrton, A. D.; Clarke, S. E.; Chenery, R. J. Ketoconazole and Sulfaphenazole as the respective selective inhibitors of P453A and 2C9. *Xenobiotica* **1995**, *25*, 261−270.

JM980532E