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Communications to the Editor

(*R*)-3,*N*-Dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide: The First Selective 5-HT$_7$ Receptor Antagonist

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The 5-HT$_7$ receptor is the most recent addition to the burgeoning family of 5-HT receptors.[1] 5-HT$_7$ receptors have been cloned from rat,[2−4] mouse,[5] guinea pig,[6] and human[7] cDNA and exhibit a high degree of interspecies homology (approximately 95%) but a low sequence homology with other 5-HT receptors (<40%). The pharmacological profile of this receptor is unique yet consistent across species. Thus, high 5-HT$_7$ receptor affinity is observed for 5-CT, 5-HT, 5-MeOT, and methiothepin, moderate affinity for 8-OHDPAT, clozapine, and ritanserin, and low affinity for pindolol, sumatriptan, and buspirone. Recent data have demonstrated the existence of four 5-HT$_7$ splice variants in humans and three in rat.[8] Preliminary pharmacological comparison of the long (5-HT$_{7a}$) and short (5-HT$_{7b}$) forms of the receptor have revealed no substantial differences in receptor binding affinity.[9] 5-HT$_7$ receptors are positively coupled to adenylate cyclase when expressed in cell lines,[2−4,7] native guinea pig hippocampus,[6] and cultured vascular smooth muscle cells.[10] No selective ligands for the 5-HT$_7$ receptor, agonists or antagonists, have been reported.

The greatest abundance of 5-HT$_7$ mRNA is found in the brain where it is discretely located within thalamus, hypothalamus, and various limbic and cortical regions.[2,4,7,11] Autoradiographic techniques confirm that the distribution of 5-HT$_7$ receptor binding sites in rat and guinea pig brain matches, to a large extent, the mRNA distribution.[11−13]

The biological functions of the 5-HT$_7$ receptor are poorly understood. It has been proposed that the 5-HT$_7$ receptor may be involved in the pathophysiology of sleep disorders,[6,14] depression,[14,15] and schizophrenia,[16] although the evidence for this is very preliminary. In the periphery, 5-HT$_7$ receptor stimulation causes relaxation of the blood vessels in monkey,[17] dog,[18] and rabbit.[19] Clearly the therapeutic utility of 5-HT$_7$ receptor ligands awaits the discovery of selective agonists and antagonists. We now report our preliminary findings on the identification of a novel series of selective 5-HT$_7$ receptor antagonists.

High-throughput screening of the SmithKline Beecham Compound Bank against the cloned human 5-HT$_7$ receptor identified the sulfonamide **1**,[20] which showed modest affinity (pK_i 7.2) for the 5-HT$_7$ receptor *and* an indication of selectivity over a range of other receptors. Since compound **1** contains two asymmetric centers and is therefore a mixture of four compounds, we prepared and evaluated the four individual enantiomers for 5-HT$_7$ receptor affinity (**2**−**5** in Table 1).[21] From these results it appears that the *R*,*R* stereochemistry is important for highest 5-HT$_7$ receptor affinity. It can be seen that the *R* chirality at center a is essential for 5-HT$_7$ receptor affinity, whereas the chirality at center b is less important. From an SAR study around compound **2**, it was found that the chiral center in the piperidine ring could be removed by moving the methyl substituent to the 4-position (removal of the 3-methyl group resulted in a loss of affinity, as did addition of a second methyl group at the 3-position), thus simplifying the structure considerably. Moving the methyl substituent to the 2-position also resulted in a loss of affinity. Additionally, it was found that a wide range of aromatic nuclei could replace the naphthalene ring in **2**, while retaining 5-HT$_7$

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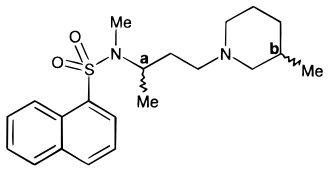
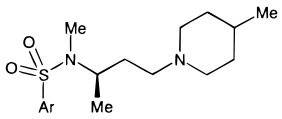
Table 1. 5-HT_7 Receptor Affinities for Compound **1** and Its Enantiomers[a]



compd	stereochemistry a	stereochemistry b	pK_i 5-HT_7[b]
1	*R*, *S*	*R*, *S*	7.2
2	*R*	*R*	6.9
3	*R*	*S*	6.2
4	*S*	*R*	5.8
5	*S*	*S*	<5.0

[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. [b] Binding affinity (cloned human receptors expressed in HEK 293 cells; [^{3}H]-5-CT).

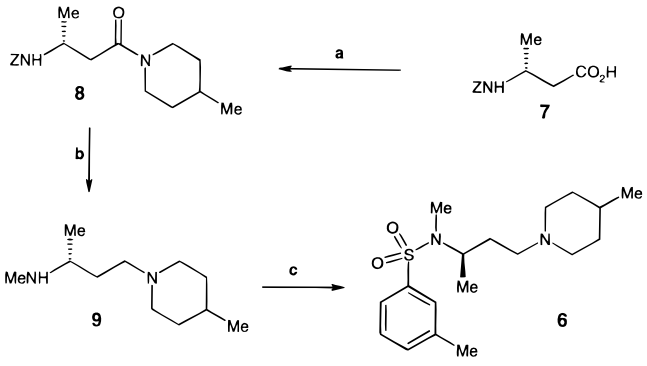
Table 2. Investigation of Alternative Aromatic Ring Systems



compd	Ar	pK_i 5-HT_7[a,b]
6	3-methylphenyl	7.5
10	1-naphthyl	7.5
11	3,4-dichlorophenyl	7.5
12	3,4-dibromophenyl	7.7
13	4,5-dibromo-2-thienyl	7.8

[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. [b] Binding affinity (cloned human receptors expressed in HEK 293 cells; [^{3}H]-5-CT).

Scheme 1[a]



Z= benzyloxycarbonyl

[a] Reagents: (a) $(COCl)_2$, DMF, CH_2Cl_2, 4-methylpiperidine, Et_3N (93%); (b) $LiAlH_4$, THF (78%); (c) 3-methylbenzenesulfonyl chloride, diisopropylethylamine, CH_2Cl_2 (40%).

receptor affinity[21] as illustrated in Table 2. This resulted in the identification of the 3-methylphenylsulfonamide **6**, SB-258719 as the first 5-HT_7 receptor antagonist with 100-fold selectivity over a range of other receptors.

Compound **6** (SB-258719) was synthesized as shown in Scheme 1. The homologated alanine derivative **7** was prepared according to the literature procedure.[22] The chiral integrity of **7** was shown to be >95% (*R*) by NMR, by conversion to diastereomeric amide derivatives, and by comparison with the (*S*) enantiomer. Conversion of **7** to the acid chloride followed by reaction with 4-methylpiperidine yielded the amide **8**. Treatment of **8** with lithium aluminum hydride effected the reduction of both the amide and the carbamate functionalities to afford in good yield the key chiral amine **9**, which was coupled with 3-methylbenzenesulfonyl chloride to give the desired sulfonamide **6**.

Table 3. Receptor Binding Profile of **6**[a]

receptor	affinity (pK_i)	receptor	affinity (pK_i)
5-HT_{1A}	<5.1	5-HT_{2C}	<4.8
5-HT_{1B}	<5.3	5-HT_4	<5.0
5-HT_{1D}	5.5	5-HT_6	<4.8
5-HT_{1E}	<4.8	5-HT_7	7.5 ± 0.04 (n = 6)
5-HT_{1F}	<5.2	adrenergic α_{1B}	<4.8
5-HT_{2A}	<4.8	dopaminergic D_2	5.4
5-HT_{2B}	<5.3	dopaminergic D_3	5.4

[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} (cloned human receptors in HEK 293 cells; [^{3}H]-8-OH-DPAT); 5-HT_{1B} (cloned human receptors in CHO cells; [^{3}H]-5-HT); 5-HT_{1D} (cloned human receptors in CHO cells; [^{3}H]-5-HT); 5-HT_{1E} (cloned human receptors in CHO cells; [^{3}H]-5-HT); 5-HT_{1F} (cloned human receptors in CHO cells; [^{3}H]-5-HT); 5-HT_{2A} (cloned human receptors in HEK 293 cells; [^{3}H]-ketanserin); 5-HT_{2B} (cloned human receptors in HEK 293 cells; [^{3}H]-5-HT); 5-HT_{2C} (cloned human receptors in HEK 293 cells; [^{3}H]mesulergine); 5-HT_4 (guinea pig hippocampus; [^{125}I]SB-207710); 5-HT_6 (cloned human receptors in HeLa cells; [^{3}H]LSD); 5-HT_7 (cloned human receptors in HEK 293 cells; [^{3}H]5CT); D_2 (cloned human receptors in CHO cells; [^{125}I]iodosulpride); D_3 (cloned human receptors in CHO cells; [^{125}I]iodosulpride).

As can be seen from Table 3, compound **6** has a pK_i of 7.5 for the 5-HT_7 receptor and at least 100-fold selectivity over a range of other receptors. Particularly noteworthy is the lack of affinity at the 5-HT_{1A} receptor. Thus, compound **6** represents the first reported ligand which has selectivity for the 5-HT_7 receptor.

Compound **6** was also evaluated in a functional model of 5-HT_7 receptor activation by examination of adenylyl cyclase activity in HEK 293 cells stably expressing the human 5-HT_7 receptor. Adenylyl cyclase activity was determined by measuring the conversion of [^{33}P]ATP to [^{33}P]cAMP, which was isolated using the method of Salomon.[23] The 5-HT_7 receptor agonist 5-carboxamidotryptamine (5-CT) stimulated basal adenylyl cyclase activity with a pEC_{50} of 7.7 ± 0.1 (n = 3). Compound **6** did not stimulate basal activity on its own, thus indicating no agonist activity, but produced a surmountable antagonism of the 5-CT response (Figure 1) with a calculated pK_B of 7.0 ± 0.1 (n = 3). Thus compound **6** possesses a profile consistent with competitive antagonism at the human 5-HT_7 receptor.

In conclusion, the chiral aryl sulfonamide **6** is the first example of a selective 5-HT_7 receptor antagonist discovered from a high-throughput screening lead. It has been shown that the *R* stereochemistry in the side chain is important for high 5-HT_7 receptor affinity and that a 4-methylpiperidine substituent is preferred to the 3-isomer. The availability of selective tools such as **6** should enable the biological role of 5-HT_7 receptors in the central nervous system and in the periphery to be elucidated.

Supporting Information Available: Experimental procedures, including analytical and spectral data, for the prepa-

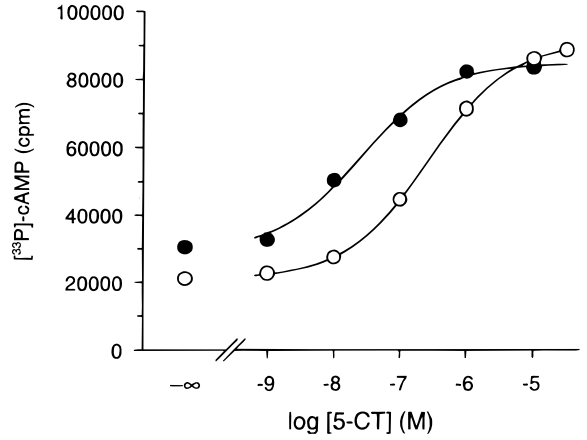


Figure 1. Effect of compound **6** on 5-CT-stimulated adenylyl cyclase activity. Stimulation of adenylyl cyclase activity in human 5-HT_7/HEK 293 membranes by 5-CT alone (●) and in the presence of 1 μM compound **6** (○). Data points represent the mean of duplicate determinations from a typical experiment which was repeated twice.

ration of **6** (3 pages). Ordering information is given on any current masthead page.

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