Communications to the Editor

A Novel, Potent, and Selective 5-HT$_7$ Antagonist: (*R*)-3-(2-(2-(4-Methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970)

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Introduction. The human 5-HT$_7$ receptor is a recently discovered member of the seven-transmembrane G-protein-coupled receptor superfamily.[2] The most abundant isoform 5-HT$_{7(a)}$ consists of a 445-amino acid polypeptide with a relatively short third intracellular loop and a long carboxy terminus. 5-HT$_7$ receptors have been cloned from rat,[3−5] mouse,[6] guinea pig,[7] and human[8] cDNA. Sequence alignments show a high degree of interspecies homology (95%) but a low overall homology (<40%) with other 5-HT receptors. The 5-HT$_7$ receptor is positively coupled to adenylyl cyclase through G_s when expressed in cell lines.[3−5,8] The greatest abundance of 5-HT$_7$ receptor mRNA is found in the brain where it is localized in the thalamus, hypothalamus, and various limbic and cortical regions in rats,[3,5] humans,[8] and guinea pigs.[9] Autoradiography studies using [^{3}H]5-CT confirm that the distribution of 5-HT$_7$ binding sites in rat and guinea pig brain matches, to a large extent, the mRNA distribution.[9−11] Although the biological functions of the 5-HT$_7$ receptor are poorly understood, receptor localization, combined with some preliminary pharmacological studies in rat, suggests that 5-HT$_7$ receptors play a role in mediating 5-HT-induced phase shifts of neuronal activity in the suprachiasmatic nucleus of the hypothalamus.[4] These data suggest that 5-HT$_7$ receptors might be linked to the control of circadian rhythms.[12]

Further pharmacological evaluation has been hampered by the lack of selective ligands. However, we recently reported the synthesis and biological activity of the sulfonamide **1** (Chart 1), the first potent 5-HT$_7$ receptor antagonist with 100-fold selectivity over a wide range of receptors.[1] More recently a series of tetrahydrobenzindoles have been reported as potent 5-HT$_7$ receptor antagonists (e.g. **3**, pK_i 8.7) although selectivity over 5-HT$_2$ receptors was only 50-fold.[13] In this communication we report the further optimization of **1** by conformational restraint of the side chain which has led to more potent and selective compounds exemplified by **15**.

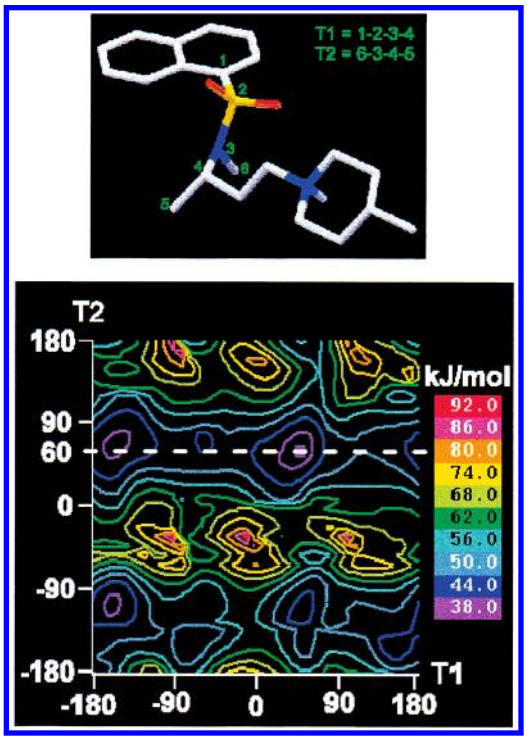
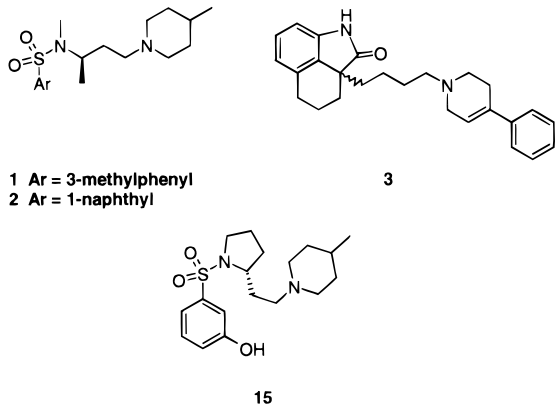


Figure 1. Ramachandran plot. Rotation about the N(Me)−C(Me) bond (T2) reveals an energy minima when the two methyl groups are orientated gauche (60°).

Chart 1. 5-HT$_7$ Antagonists



Results and Discussion. A key finding from our earlier SAR study was the importance of the chiral center in the flexible side chain in compounds such as **1** and **2**. Conformational analysis of this side chain using MACROMODEL[14] revealed that all bonds are relatively free to rotate apart from the S−N and N(Me)−C(Me) bonds. A Ramachandran plot (Figure 1) showing rotation around these two bonds in **2** was constructed and reveals an energy minimum when the two methyl

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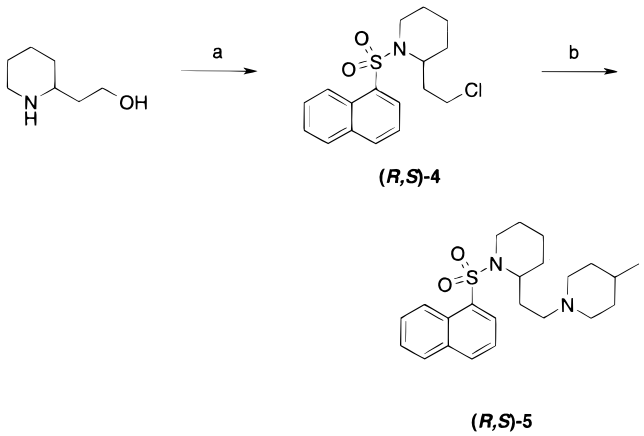
Scheme 1[a]



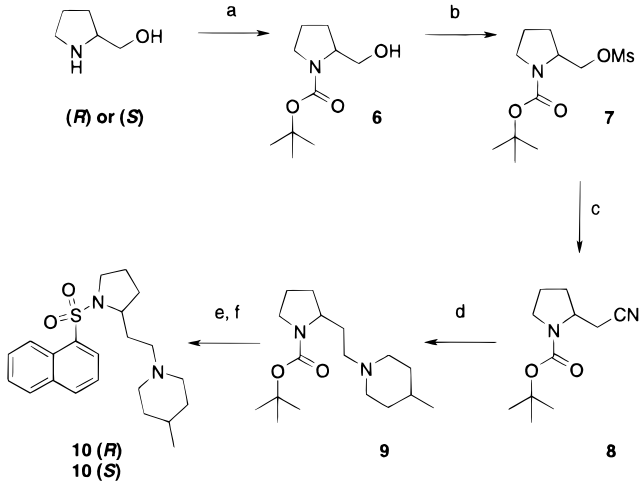
[a] Reagents: (a) naphthalene-1-sulfonyl chloride, diisopropylethylamine, CH_2Cl_2 (53%); (b) 4-methylpiperidine, NaI, K_2CO_3, CH_3CN (88%).

groups are orientated gauche (T2 = 60°) with respect to each other, which may represent the binding conformation. This suggested the synthesis of analogues in which both methyl groups have been tied together into a ring. Therefore we targeted analogues incorporating both 2-pyrrolidinylethyl and 2-piperidinylethyl side chains. Docking of **5** into our 5-HT_7 receptor homology model, constructed on the basis of recent electron microscopy studies by Baldwin,[15] predicted that (*R*)-**5** would have the lower binding energy.

Synthesis of the six-membered constrained side chain (Scheme 1) started with reaction of piperidine-2-ethanol with 2 equiv of naphthalene-1-sulfonyl chloride to give **4**. Displacement of the chloride with 4-methylpiperidine using sodium iodide to catalyze the reaction gave the racemic sulfonamide (*RS*)-**5**, in good overall yield. Preparation of the individual enantiomers (*R*)-**5** and (*S*)-**5** was achieved using the same route, starting with *(R)*- and *(S)*-piperidine-2-ethanol,[16] respectively.

The starting points for the syntheses of the chiral five-membered analogues were *(R)*- and *(S)*-pyrrolidinol (Scheme 2). BOC protection of each pyrrolidinol allowed transformation of the primary alcohols into the corresponding mesylates **7**. Displacement with sodium cyanide in DMF gave the nitriles **8**, which were transformed into the protected side chains **9** by hydrogenation over Pt catalyst in the presence of 4-methylpiperidine. Removal of the BOC protection using TFA and reaction with naphthalene-1-sulfonyl chloride gave the target sulfonamides **10** in good overall yield.

Scheme 2[a]



[a] Reagents: (a) BOC anhydride, THF/H_2O, K_2CO_3 (84%); (b) methanesulfonyl chloride, Et_3N, CH_2Cl_2 (100%); (c) NaCN, DMF (78%); (d) 4-methylpiperidine, H_2, PtO_2 (47%); (e) trifluoroacetic acid, CH_2Cl_2 (100%); (f) naphthalene-1-sulfonyl chloride, diisopropylethylamine, CH_2Cl_2 (60%).

The receptor binding affinity of the racemate (*RS*)-**5** for the 5-$HT_{7(a)}$ receptor (Table 1) was comparable to that of the unconstrained analogue **2**. (*R*)-**5** has a pK_i of 7.8 for the 5-$HT_{7(a)}$ receptor which is 25-fold greater than that of (*S*)-**5** demonstrating that the *R* enantiomer has greater activity at the 5-HT_7 receptor, as predicted by modeling. This was confirmed in the more constrained five-membered series where similar differences were seen between the *R* and *S* enantiomers. (*R*)-**10** demonstrated a slightly higher 5-$HT_{7(a)}$ receptor affinity (pK_i 8.0) compared to (*R*)-**5,** indicating that the pyrrolidine ring constrains the side chain in a more optimum conformation for 5-HT_7 binding.

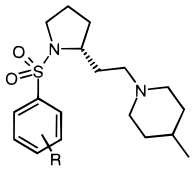
Using the optimized *(R)*-pyrrolidinylethyl side chain, further investigations into the effect of aromatic substitution on 5-HT_7 receptor affinity and selectivity were carried out. In general the SAR for the constrained five-membered side chain paralleled that already seen for the unconstrained side chain.[1] Lipophilic substituents in both the 3- and 4-positions of the aromatic ring are well-tolerated, with **11**−**13** all giving highly potent and selective compounds (Table 2). Suprisingly, introduction of a polar 3-hydroxy group to afford **15** resulted in a compound with the highest 5-$HT_{7(a)}$ receptor affinity (pK_i 8.9). Cross-screening data showed that **15** also has an excellent selectivity profile (>250-fold) over 13 other receptors apart from the 5-HT_{5A} receptor (50-fold).

Table 1. Receptor Binding Profiles in Radioligand Binding Assay[a,b]

	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_4	5-HT_6	**5-$HT_{7(a)}$**	adren α_{1b}	dopam D_2	dopam D_3
(*RS*)-5	–	<5	5.7	<5	<5	<5.5	5.4	<5	–	–	**7.4**	–	–	–
(*R*)-5	5.8	<5.5	6.3	<5	<5	<5.6	5.4	<5	–	–	**7.8**	<6	6.4	6
(*S*)-5	–	–	–	–	–	–	–	–	–	–	**6.4**	–	–	–
(*R*)-10	6.0	5.4	6.2	<5	<5	5.8	5.7	6.0	–	–	**8.0**	5.6	6.2	5.9
(*S*)-10	–	–	–	–	–	–	–	–	–	–	**6.4**	–	–	–

[a] All values represent the mean of at least two determinations carried out using cell lines stably expressing the cloned receptors. Each determination lies within 0.2 log unit of the mean. [b] Receptors and radioligands used in binding assays: 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_{5A} (human cloned receptors in HEK 293 cells; [^{3}H]5-CT); 5-$HT_{7(a)}$ (human cloned receptors in HEK 293 cells; [^{3}H]5-CT); D_2 (human cloned receptors in CHO cells; [^{125}I]iodosulpiride); D_3 (human cloned receptors in CHO cells; [^{125}I]iodosulpiride).

Table 2. Effect of Aromatic Substitution on 5-HT_7 Affinity and Selectivity[a,b]



	R	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_4	5-HT_{5A}	5-HT_6	**5-$HT_{7(a)}$**	adren α_{1b}	dopam D_2	dopam D_3
11	3,4-dichloro	6.5	5.8	6.2	5.6	<5.5	<6	<6	<6	<5.5	–	–	**8.4**	5.7	6.0	6.0
12	3-bromo	6.4	6.0	6.3	<5.6	<5.3	<5.3	<5.8	<5.3	<5.2	–	5.5	**8.7**	–	6.1	6.2
13	3-methyl	6.0	5.8	5.5	<5	<5	<5.3	<5.6	<5.3	<5	–	–	**8.5**	<5.5	5.8	5.9
14	3-methoxy	–	–	–	–	–	–	–	–	–	–	<5	**8.0**	–	–	–
15	3-hydroxy	<5	6.0	5.8	<5.2	<5.5	<5	5	<5	5.9	7.2	5.2	**8.9**	<5	6.5	5.6

[a,b] See Table 1 for details.

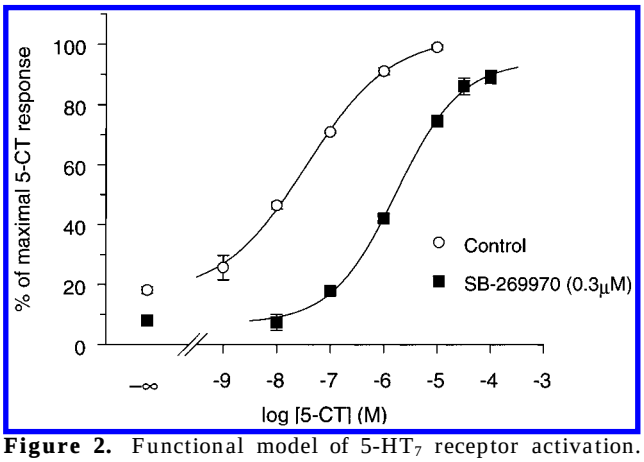


Figure 2. Functional model of 5-HT_7 receptor activation. Stimulation of adenylyl cyclase activity in human 5-HT_7/HEK293 membranes by 5-CT alone (control) and in the presence of SB-269970 (0.3 μM). Data points represent the mean ± SEM of at least three separate experiments each performed using duplicate determinations.

Furthermore, in a commercial screening package (Cerep) **15** was found to be over 100-fold selective against a total of 50 receptors, enzymes, or ion channels. Compound **15** shows a substantial increase in 5-HT_7 receptor affinity when compared to the methoxy derivative **14,** suggesting a favorable interaction between the hydroxyl group and the receptor. We were able to rationalize this finding by docking **15** into our 5-HT_7 homology model which revealed an additional hydrogen-bonding interaction site.

Compound **15** was 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-$HT_{7(a)}$ receptor. The nonselective 5-HT_7 receptor agonist 5-CT stimulated basal adenylyl cyclase activity with a pEC_{50} of 7.5 ± 0.1 (n = 3) (Figure 2). In the presence of compound **15,** the 5-CT concentration–response curve had the same maximal response but was shifted rightward in a parallel manner indicating competitive, surmountable antagonism of the response. The calculated pK_B of 8.3 ± 0.1 (n = 3) is in reasonable agreement with the receptor binding affinity. In addition, **15** produced a small apparent reduction in basal adenylyl cyclase activity in the absence of added 5-CT. This is consistent with inverse agonism which has previously been reported for **1** (SB-258719) and a number of nonselective 5-HT receptor antagonists.[17]

In conclusion, conformational analysis of the flexible side chain in **1** indicated a low-energy, potential binding conformation in which the two methyl groups adopt a gauche orientation. The design and preparation of analogues that mimic this conformation led to the discovery of **15**, a highly potent and selective 5-HT_7 receptor antagonist. These compounds are currently being used to evaluate the therapeutic potential of 5-HT_7 ligands.

Supporting Information Available: Experimental details for the synthesis of compounds **4**–**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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