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Synthesis of potent and selective serotonin 5-HT$_{1B}$ receptor ligands

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Abstract—A series of serotonin 5-HT$_{1B}$ ligands were synthesized and evaluated for their potency and selectivity against other 5-HT receptor subtypes. Many of these new compounds displayed high affinity and selectivity for the 5-HT$_{1B}$ receptor and compound **6c** was found to have the in vitro binding profile necessary for development as a PET radioligand.
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The serotonin 5-HT$_{1B}$ receptors are autoreceptors that regulate the release of serotonin (5-HT) from the 5-HT terminals. Historically, these receptors were included in the class of 5-HT$_{1D}$ receptors. The human 5-HT$_{1D}$ receptors have been divided into 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$,[1] which were then renamed 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors, respectively.[2] The 5-HT$_{1B}$ is the predominant form of the 5-HT$_{1B/D}$ receptor family in the human brain, accounting for about 90% of the receptor population formerly designated as 5-HT$_{1D}$.[3,4] Over the last few years, biological and pharmacological studies have noted the importance of this receptor subtype and implicated the involvement of 5-HT$_{1B}$ receptors in depression, aggressive behavior, suicide, substance abuse, and alcoholism.[5–12] For example, studies in 5-HT$_{1B}$ knockout animals have shown that transgenic mice devoid of the 5-HT$_{1B}$ receptors displayed enhanced aggressiveness, increased cocaine intake, and heightened susceptibility to alcohol over-consumption.[6–8] Since the 5-HT$_{1B}$ receptor is an autoreceptor located on the nerve terminal and stimulation of this receptor inhibits the release of serotonin from the neurons, antagonism of the 5-HT$_{1B}$ receptor would, in theory, produce an immediate increase in the extracellular 5-HT concentrations, an effect induced by prolonged treatment with the most widely used antidepressants: the selective serotonin reuptake inhibitors (SSRIs). Therefore, it was proposed that 5-HT$_{1B}$ antagonists could probably function as fast-acting antidepressants or as an adjunct treatment of depression with SSRIs.[13,14] Along with the report of enhanced cocaine intake in 5-HT$_{1B}$ knockout mice, Koob et al. have reported that stimulation of 5-HT$_{1B}$ receptor potentiated cocaine reinforcement in rats, thus implicating the involvement of this receptor subtype in cocaine abuse.[15] Furthermore, biological and genetic studies have implicated the 5-HT$_{1B}$ receptors and the receptor genes in alcoholism, especially antisocial alcoholism, further underscoring the importance of the 5-HT$_{1B}$ receptor.[11,16]

Despite the recent advance in elucidating the role of the 5-HT$_{1B}$ receptor in the CNS and in psychiatric diseases, there is still a lack of specific pharmacological tools to probe this receptor's functions in vivo. Selective agonists and antagonists for the 5-HT$_{1B}$ receptor, among them GR127935 and SB-224289 (Fig. 1) are only recently available. GR127935 is a high affinity ligand (K_i 0.14 nM for the cloned human 5-HT$_{1B}$ (h5-HT$_{1B}$), 0.70 nM for h5-HT$_{1D}$, and 70 nM for h5-HT$_{1A}$ receptors).[17] However, this compound has been shown in some tests to be a partial agonist. SB-224289 was reported to be a 5-HT$_{1B}$ full antagonist with a 26-fold selectivity for the h5-HT$_{1B}$ over h5-HT$_{1D}$ receptor.[18,19] Nevertheless, its affinity for the h5-HT$_{1B}$ receptor (K_i 10 nM) seems to be relatively low. Yet another compound (**1**, Fig. 1) was reported to be a full antagonist with a K_i of 2 nM for the 5-HT$_{1B}$ receptor. However, in our hands this compound also displayed partial agonist activity. In view of the importance of the 5-HT$_{1B}$ receptor in psychiatric diseases, we aim to develop a

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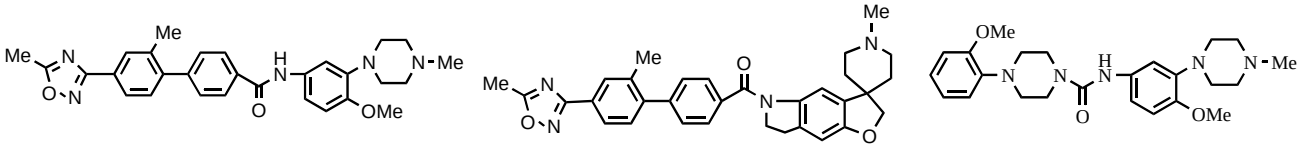


GR127935 **SB-224289** **1**

Figure 1. Structural representation of GR127935, SB-224289, and compound **1**.

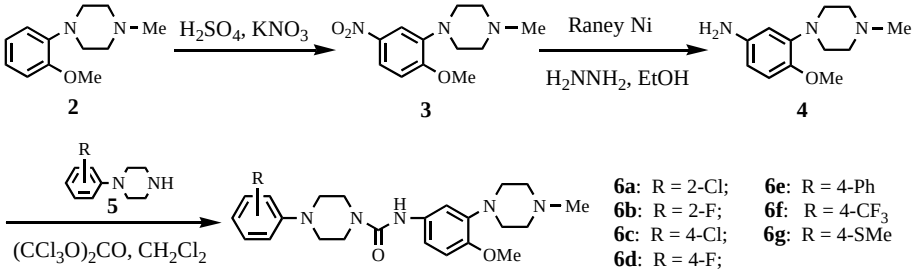
radioligand for the positron emission tomography (PET) imaging studies of the 5-HT_{1B} receptor in vivo. To this end, we have synthesized a series of compounds in search of a high affinity 5-HT_{1B} antagonist for potential development as PET radiotracer.

The synthetic program was initiated using compound **1** as a lead.[20] Based on this lead compound, a number of derivatives bearing different functional groups were prepared and screened in comprehensive in vitro binding assays. The new compounds **6a**–**g** were prepared according to the synthetic pathway outlined in Scheme 1. Briefly, nitration of 1-methyl-4-(2-methoxyphenyl)piperazine (**2**) gave compound **3**, which was then reduced with Raney nickel and hydrazine to the aniline **4**. The reaction of **4** with triphosgene and coupling of the resulting carbamyl chloride with various monosubstituted phenylpiperazines (**5**) provided the final products **6a**–**g**. The monosubstituted phenylpiperazines, if not commercially available, were prepared by the palladium-catalyzed aromatic amination of substituted phenylbromides with piperazine.[21] Monosubstituted phenylpiperazines **5e**–**g** were obtained in 32–75% yield. The desired compounds **6a**–**g** were prepared in 45–82% yield, following purification by column chromatography, salt formation with fumaric acid, and recrystallization from acetone. In addition, we have also prepared compound **11** to elucidate further the structure–activity relationship of this class of compounds (Scheme 2). All new compounds were fully characterized by ^{1}H NMR, MS, HRMS, and/or elemental analysis.

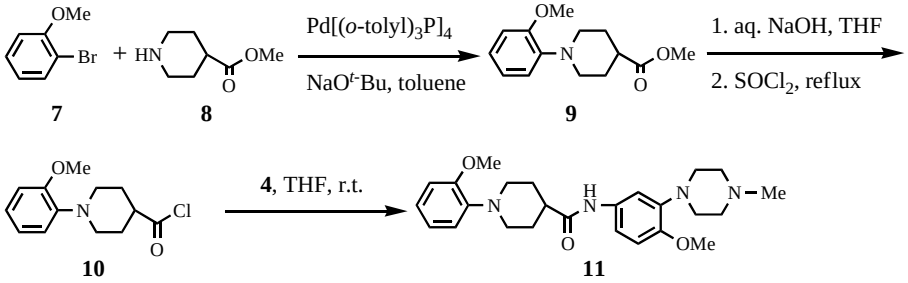
The newly synthesized compounds were tested in receptor binding assays in vitro. Binding to the 5-HT_{1B} and 5-HT_{1D} receptors was carried out by displacement of the radioligand [^{3}H]5-carboximidotryptamine (5-CT) at the cloned human 5-HT_{1B} and 5-HT_{1D} receptors with the test compounds. In addition, binding affinities of these compounds at various cloned human 5-HT receptors, including 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_6 and 5-HT_7, were also determined, as previously described.[22] Selected binding data are given in Table 1.

As is evident from Table 1, all the test compounds demonstrated high affinity for the serotonin 5-HT_{1B} receptor. Compounds **6a**, **6c**, and **11** also displayed high affinity (K_i < 100 nM) for the 5-HT_{1D} receptor. In addition, compounds **6a**–**d**, **6f**, and **6g** had greater than 13-fold selectivity for 5-HT_{1B} over the other 5-HT receptor subtypes. All compounds display negligible affinities (K_i > 10 μM) for the following cloned receptors: dopamine D_1, D_2, D_3, D_4 and D_5, κ, δ, and μ-opiod, histamine-H_1, muscarinic M_1, M_2, M_3, M_4 and M_5, nicotinic acetylcholine receptors, and the PCP site.

Judging from the binding data, it is clear that substituents as diverse as fluorine, methoxy, thiomethyl, and trifluoromethyl, placed at either the *ortho* or *para* position



Scheme 1. Synthetic route for compounds **6a**–**g**.



Scheme 2. Synthetic route for compound **11**.

Table 1. In vitro binding affinities of compounds **6a–g** and **11** at selected 5-HT receptors

Compound	K_i (nM)[a]						
	5-HT_{1B}	5-HT_{1D}	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_6	5-HT_7
1	12.4 ± 1.1	105.9 ± 20.1	641 ± 147	6739 ± 1146	1937 ± 794	2410 ± 409	1412 ± 762
6a	11.3 ± 0.9	34.7 ± 12.1	335 ± 74	5988 ± 958	1241 ± 521	469 ± 80	342 ± 195
6b	18.1 ± 2.7	103.1 ± 39.2	315 ± 85	>10,000	1077 ± 409	841 ± 404	>10,000
6c	1.82 ± 0.35	6.51 ± 1.69	52.5 ± 14.2	1008 ± 60	267.0 ± 98.8	5089 ± 1272	>10,000
6d	38.3 ± 9.0	168.2 ± 40.4	1079 ± 291	4451 ± 490	>10,000	>10,000	>10,000
6e	13.7 ± 1.4	209.2 ± 14.6	405 ± 182	171 ± 19	6.50 ± 2.47	1429 ± 286	>10,000
6f	28.4 ± 2.2	345.1 ± 65.6	382 ± 187	614 ± 117	2833 ± 3395	2863 ± 830	>10,000
6g	16.7 ± 4.1	328.7 ± 62.5	882 ± 432	1335 ± 280	1145 ± 1054	>10,000	>10,000
11	9.68 ± 0.74	15.4 ± 2.8	15.4 ± 2.8	>10,000	9954 ± 2688	nd	nd
GR127935	4.31 ± 0.50	12.3 ± 1.85	80.0 ± 42.4	42.7 ± 9.0	105 ± 70	>10,000	>10,000

nd, not determined.

[a] All assays were conducted in duplicate. Data are means ± SD of computer-derived estimates for $n = 4$ separate determinations.

of the phenylpiperazine moiety in the lead compound, are well-tolerated by the 5-HT_{1B} binding site and the resulting compounds all retain their high binding affinity for the 5-HT_{1B} receptor. Replacement of the methoxy group in the lead compound with a bulky substituent, such as the phenyl group, also results in a compound with high affinity and selectivity for the 5-HT_{1B} receptor (**6e**). However, strongly electron-withdrawing substituents, such as fluorine or trifluoromethyl group, placed at the 4-position of the phenyl ring (**6d** and **6f**) appear to diminish the compounds' affinities for 5-HT_{1B} and 5-HT_{1D} receptors.

Among the compounds synthesized, it is notable that the 4-chlorophenyl derivative (**6c**) displays a higher affinity for the 5-HT_{1B} receptor than both the lead and GR127935, a compound that has been shown in the literature to be the ligand with the highest affinity for the 5-HT_{1B} receptor. In our binding assays, compound **6c** is twice as potent as GR127935, and thus appears to be the most potent 5-HT_{1B} ligand reported to date.

In functional assays using the forskolin-stimulated cyclic AMP production test,[23] both compounds **1** (64% intrinsic activity) and GR127935 (29% intrinsic activity) displayed partial agonist activity. On the other hand, compound **11** was shown to be completely devoid of any intrinsic agonist activity, and thus appeared to be a full antagonist for the 5-HT_{1B} receptor.

Compounds with nanomolar or subnanomolar affinity at the target receptor are, in general, good candidates for development as in vivo radioligands for PET imaging. Compound **6c** fulfills this requirement. In addition, it possesses functional groups (*O*-methyl and *N*-methyl) that are amenable to labeling with a positron-emitting [^{11}C]methyl group and therefore is a suitable candidate for radiolabeling and development as potential PET radioligand.

In summary, a number of compounds were synthesized and evaluated in vitro for their binding affinity and selectivity for the serotonin 5-HT_{1B} receptor. One compound (**6c**) was found to be a high affinity ligand for the 5-HT_{1B} receptor and a suitable candidate for development as a PET radioligand to investigate the serotonin 5-HT_{1B} receptor in vivo. Another compound, **11**, was found to be a 5-HT_{1B} full antagonist devoid of any agonist activity.

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