Discovery of the First Potent, Selective 5-Hydroxytryptamine$_{1D}$ Receptor Antagonist

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Abstract: A series of 5-(piperidinylethyloxy)quinoline 5-hydroxytryptamine$_{1D}$ (5-HT$_{1D}$) receptor antagonists have been discovered from elaboration of the series of dual 5-hydroxytryptamine$_1$−selective serotonin reuptake inhibitors (5HT$_1$−SSRIs) reported previously. This is the first report of highly potent, selective antagonists for the 5-HT$_{1D}$ receptor, which represents an extremely useful set of pharmacological tools for further understanding the roles of the 5-HT$_1$ receptor subtypes.

The 5-hydroxytryptamine (5-HT) family of receptors comprises 14 distinct subtypes that have been extensively studied and categorized into seven main families (5-HT$_1$−5-HT$_7$) on the basis of their operational, structural, signal transduction pathways and pharmacological attributes.[1,2] The largest subtype group, the 5-HT$_1$ receptors, has been further divided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, and 5-HT$_{1F}$ receptors, which show approximately 40−60% homology between members. Literature concerning the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors is further complicated by their nomenclature because 5-HT$_{1D}$ receptors were formerly classified as 5-HT$_{1D\alpha}$ and 5-HT$_{1B}$ receptors as 5-HT$_{1D\beta}$.[3] Significantly, the rat orthologue of the human 5-HT$_{1B}$ receptor has a key single amino acid modification leading to differential pharmacology.[4] The 5-HT$_{1B}$ receptor is located principally in the striatum, frontal cortex, and basal ganglia,[5] whereas the 5-HT$_{1D}$ receptor is found most abundantly in the hippocampus, cortex, caudate putamen, and nucleus accumbens.[6]

Considerable research effort has been expended on the 5-HT$_1$ receptor family, and a number of reviews have covered the potential therapeutic applications of agonists and antagonists of these receptors.[7,8] In brief, central 5-HT release is regulated by inhibitory 5-HT autoreceptors, including 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors, and potentially, antagonism of these receptors could result in acute increases in extracellular 5-HT.[9,10] Consequently antagonists of the 5-HT$_{1B}$ receptor, or mixed 5-HT$_{1B/D}$ ligands (Figure 1), have been studied in detail alone and in combination with other serotonergic agents for their potential utility in depression and anxiety.

Despite the high level of interest in the members of the 5-HT receptor family, including many known 5-HT$_{1D}$ agonists,[13] there is little known directly about the potential utility of a 5-HT$_{1D}$ receptor antagonist in part because of the lack of suitable, selective molecules, although recent publications describing combined 5-HT$_{1D}$ antagonist/5-HT reuptake blockers are providing increased evidence for the role of the 5-HT$_{1D}$ receptor as a presynaptic autoreceptor.[14,15]

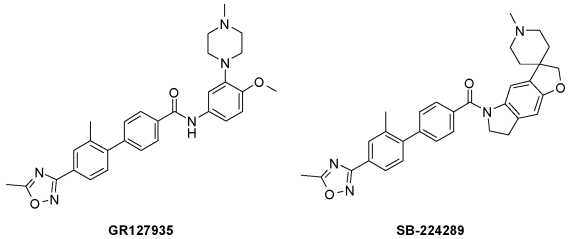


Figure 1. Structures of GR127935[11] (mixed 5-HT$_{1B/D}$ ligand) and SB-224289,[12] the first selective 5-HT$_{1B}$ tool compound.

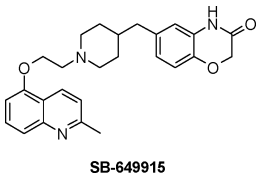
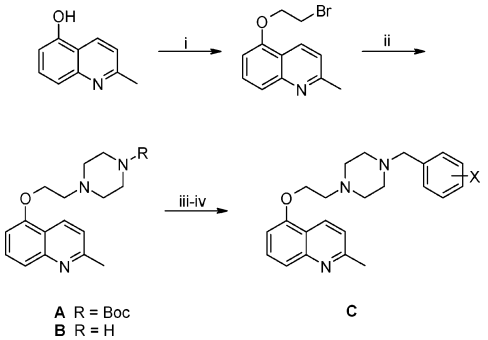


Figure 2. Structure of SB-649915, a dual 5-HT$_{1A}$ receptor antagonist and 5-HT reuptake inhibitor.

Scheme 1[a]



[a] Reagents and conditions: (i) $BrCH_2CH_2Br$, K_2CO_3, butan-2-one, 80 °C; (ii) 1-Boc-piperazine, K_2CO_3, DMF, 70 °C; (iii) 1 M HCl in Et_2O, EtOH; (iv) X-PhCHO, $NaBH(OAc)_3$, dichloroethane.

Previous publications have described the development of novel selective serotonin reuptake inhibitors (SSRIs) with additional 5-HT$_{1A}$ receptor antagonist properties as potentially therapeutically useful compounds that should have a shorter latency to onset for antidepressant action.[16] In particular, the compounds described (Figure 2) show potent activity against 5-HT$_1$ receptors and inhibition of the serotonin transporter. Continued SAR studies in this area have led to the identification of a range of compounds with specific activities against the 5-HT$_1$ receptor subtypes and the 5-HT transporter. Herein, we describe a series of piperazines that constitute the first report of highly selective 5-HT$_{1D}$ receptor antagonists as valuable pharmacological tools.

The general synthetic approach employed is outlined in Scheme 1. 1,2-Dibromoethane was reacted sequentially with 5-hydroxy-2-methylquinoline[17] and then 1-(*tert*-butoxycarbonyl)piperazine to afford intermediate **A**. Deprotection of this product afforded key intermediate **B**, which was further elaborated by reductive alkylation to afford a set of substituted benzylamines

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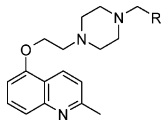
Table 1. Receptor Binding Affinity (pK_i [a]) for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and SerT for Novel Compounds[b]



compd	R	pK_i 5-HT_{1A}	pK_i 5-HT_{1B}	pK_i 5-HT_{1D}	pK_i SerT	selectivity 5-HT_{1D}/5-HT_{1B}
SB-649915		8.6	8.0	8.8	8.4	6
1	6-benzoxazinonyl	7.2	7.0	8.0	7.6	10
2	3-NH_2, 4-OH-phenyl	<5.9	6.6	8.8		158
3	3-NO_2, 4-OH-phenyl	6.1	6.4	8.0	6.0	40
4	3-NO_2, 4-OMe-phenyl	6.4	6.5	7.7		16
5	3-NO_2, 4-F-phenyl	6.3	6.6	8.1	6.5	32
6	3-NO_2, 4-Me-phenyl	7.4	7.1	8.7	7.3	40
7	3-NO_2, 4-Cl-phenyl	7.2	6.7	8.3	6.7	40
8	3,4-dichlorophenyl	<5.0	5.7	5.6	6.3	1
9	2,6-dichlorophenyl	<5.0	<5.0	<5.0	5.9	
10	2,5-dichlorophenyl	7.1	6.2	8.4	5.9	158
11	5-indolyl	6.0	6.8	8.5		50
12	5-quinolyl	6.4	6.6	8.6		100
13	5-quinazolyl	6.4	6.5	8.6		126
14 (SB714786)	8-quinolyl	6.5	6.7	9.1	6.5	251
15	7-indolyl	6.4	6.4	8.5	6.4	126
16	4-benzimidazolyl	6.8	7.2	9.6	6.1	251

[a] Radioligand binding assay from cloned human receptors for 5-HT receptors and rat cortical synaptosomes for SerT. Each determination lies within 0.3 log units of the mean. [b] All compounds were characterized, and purity was assessed using ^{1}H NMR and LC−MS.

C, affording easy access to explore the chemical space[16] (previously thought to be important for selectivity) occupied by the benzoxazinone moiety in Figure 2.

All compounds prepared were screened against h5-HT_{1A} receptors expressed in Chinese hamster ovary (CHO) cells using displacement of [^{3}H]WAY100635 and against h5-HT_{1B} and h5-HT_{1D} receptors expressed in CHO cells using displacement of [^{3}H]-5-HT. Affinity for the 5-HT reuptake site was assessed by measurement of the inhibition of [^{3}H]-5-HT into rat cortical synaptosomes, with data expressed as pK_i (Table 1). The intrinsic activity of the compounds was determined using a [^{35}S]GTPγS binding assay in cells expressing the h5-HT_{1A}, h5-HT_{1B}, or h5-HT_{1D} receptors, with data reported relative to the maximum response elicited by the endogenous agonist 5-HT. A 10-point half-log serial dilution was used to generate a concentration response for each compound.

Historically, no selective antagonists of the 5-HT_{1D} receptor have been reported, although mixed 5-HT_{1D} antagonist/5-HT reuptake blockers and selective agonists are well documented. Most commonly, compounds with low or no intrinsic activity for the 5-HT_{1D} receptor are also high-affinity ligands for the closely related 5-HT_{1B} receptor as previously described.

The piperazine analogue **1** of SB-649915[16] showed reduced 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} affinities relative to the parent piperidine but allowed rapid exploration of the SAR, which led to the identification of selective 5-HT_{1D} receptor antagonists.

Interestingly, molecules **2**−**4**, which contained some features of the benzoxazinone but lacked the carbonyl group, maintained or increased affinity for the 5-HT_{1D} receptor but lost affinity for 5-HT_{1A} and 5-HT_{1B} receptors and the serotonin transporter. Further investigation of the nature of the group at the 4-position revealed that fluorine **5**, chlorine **7**, and methyl **6** were all tolerated for 5-HT_{1D} affinity, with the 4-methyl affording the highest 5-HT_{1A} and 5-HT_{1D} affinity. With the knowledge that the nitro group was not essential for activity, we prepared the 3,4-dichloro derivative **8**. Comparison of this compound with the 3-chloro, 4-nitro derivative **7** described above showed a significant loss in affinity for all 5-HT_1 receptors. Further dichloro derivatives were prepared in this exploration, and suprisingly, although the 2,6-dichlorophenyl **9** was also inactive, the 2,5-dichlorophenyl **10** provided a marked restoration of affinity for the 5-HT_{1A} and 5-HT_{1D} receptors.

Although the substituted phenyl derivatives had led to an improvement in selectivity for the 5-HT_{1D} receptor, the majority also led to a reduction in affinity for the 5-HT_{1D} receptor from parent benzoxazinone derivative **1**. Consequently we resumed exploration of this template by investigation of other bicyclic systems. Encouragingly, the 5-indolyl derivative **11** showed good 5-HT_{1D} receptor affinity and furthermore exhibited significantly improved selectivity over 5-HT_{1A} and 5-HT_{1B} receptors. From this lead, we widened our exploration of the aromatic ring systems to encompass derivatives **12**−**16** and saw a consistent pattern of high 5-HT_{1D} affinity with excellent selectivity over the other receptors screened. In particular, 4-benzimidazolyl derivative **16** has outstanding affinity for the 5-HT_{1D} receptor and selectivity against the 5-HT_{1B} receptor in this assay.

To assess the functional activity of these compounds, intrinsic activity measurements were determined as described. Because this [^{35}S]GTPγS binding assay was run in a recombinant cell line with high receptor density, it is anticipated that weak partial agonists identified from this screen would behave as antagonists in the native system.[18,19] Gratifyingly, all compounds that were assessed for their intrinsic activity had IA < 0.55, implying that all were antagonists or partial agonists.

From those compounds assayed, a proportion had no intrinsic activity. These are the first examples of potent, selective, full antagonists at the 5-HT_{1D} receptor to be

Table 2. In Vitro Cross-Screening Characterization (pK_i) of SB-714786[a]

5-HT									adrenergic		dopamine		
1E	1F	2A	2B	2C	4	5A	6	7	α1B	β2	D2	D3	D4
<5.0	5.7	<5.6	5.9	5.5	<5.3	5.5	<5.0	6.3	6.0	5.7	5.5	6.0	<5.0

[a] Radioligand binding assays from cloned human receptors. Each determination lies within 0.3 log units of the mean.

Table 3. Intrinsic Activity of Piperazines[a]

	IA		
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}
5-HT	1.0	1.0	1.0
14, SB-714786	0	0.3	0
15	0	0.1	0.5
16	0	0.3	0.5
SB-272183	0.44		
GR127935		0.47	0.86

[a] [^{35}S]GTPγS binding assay in the human receptor cell lines. Intrinsic activity of 0: full antagonist. Intrinsic activity of 1: full agonist. 5-HT, SB-27213, and GR127935 are standards.

reported. As can be seen (Table 3), compounds typically exhibited low or no intrinsic activity against the 5-HT_{1A} and 5-HT_{1B} receptors, with intrinsic activity varying from antagonist to partial agonist against the 5-HT_{1D} receptor. In particular, **14** (SB-714786) was found to have no or low intrinsic activity against all three receptors (Table 3). Further in vitro characterization of this compound across a range of other monoamine receptors has confirmed its exquisite selectivity for the 5-HT_{1D} receptor, with at least 250-fold separation over all of the 16 other receptors studied (Table 2).

In conclusion, we have identified a novel series of potent, selective 5-HT_{1D} receptor antagonists and partial agonists that are currently being used as tools to increase our understanding of the 5-HT_{1B} and 5-HT_{1D} receptor systems. Further chemical exploration around this template is also in progress and will be disclosed at a later date.

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