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Identification of a potent and selective 5-HT$_{1B}$ receptor antagonist

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Abstract—An SAR study around the mixed 5-HT$_{1ABD}$ receptor antagonist SB-272183 found that introduction of *cis*-2,6-dimethyl substitution onto the piperazine ring was a key structural change, which imparted a combination of both excellent selectivity over the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors and low intrinsic activity. This led to the identification of the selective 5-HT$_{1B}$ receptor antagonist SB-616234.

The 5-hydroxytryptamine (5-HT) family of receptors comprises 14 distinct sub-types, which 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 family comprises the 5-HT$_1$ receptors, which have been subdivided further into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptors showing approximately 40–60% homology between members. Indeed, much interest within central nervous system (CNS) research has been directed towards this family since 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors have been identified as autoreceptors and are thus considered potential targets for the modulation of central serotonergic function.[3,4]

Within the CNS, 5-HT$_{1B}$ receptors are located both pre- and postsynaptically. The presynaptic 5-HT$_{1B}$ receptors are located on nerve terminals and in somatodendritic regions (cell body or raphe) of the 5-HT neurone.[5] As the 5-HT$_{1B}$ receptor is negatively coupled to adenylate cyclase, in both cases activation of these presynaptic 5-HT$_{1B}$ receptors serves to inhibit 5-HT release.[5,6] The location of 5-HT$_{1B}$ receptors on 5-HT nerve terminals has resulted in a number of studies (including those carried out on human brain tissue[6]), which has led to the suggestion that blockade of this receptor will have the effect of relieving the autoinhibitory action of 5-HT at the terminal 5-HT autoreceptor and hence result in an increase in extracellular levels of 5-HT.[7–9] Chronic blockade of the 5-HT uptake carrier by selective serotonin re-uptake inhibitors (SSRIs) is also known to increase extracellular 5-HT levels, and this is hypothesized to result in the antidepressant and anxiolytic activity of this class of drugs in the clinic. It is therefore postulated that 5-HT$_{1B}$ receptor antagonists, by virtue of increasing 5-HT levels, will have the same net effect as SSRIs and hence may offer clinical utility in treatment of mood disorders.[5,9] Consequently, selective antagonists of the 5-HT$_{1B}$ receptor or mixed 5-HT$_{1B/1D}$ ligands have been studied alone or in combination with other serotonergic agents for their potential utility in depression and anxiety. A number of reviews have covered the potential therapeutic applications of agonists and antagonists of these receptors.[10,11]

Here, we describe part of our research in the 5-HT area in which we were interested in identifying a selective antagonist for the 5-HT$_{1B}$ receptor. Past literature concerning 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors is complicated by changes in nomenclature; 5-HT$_{1D}$ receptors were formerly classified as 5-HT$_{1D\alpha}$ and 5-HT$_{1B}$ receptors as 5-HT$_{1D\beta}$.[12] Most of the initial work in the 5-HT$_{1B}$ area was carried out using mixed 5-HT$_{1B/1D}$ ligands, such as GR127935,[13] which demonstrated silent antagonism in a rat glial cell line, as determined by the inhibition of forskolin-stimulated cAMP formation by 5-HT.[14] However, such compounds showed partial agonism in a human recombinant functional assay ([^{35}S]GTPγS binding).[15] Our earlier studies provided SB-224289 as the first truly selective 5-HT$_{1B}$ antagonist[16] and although this com-

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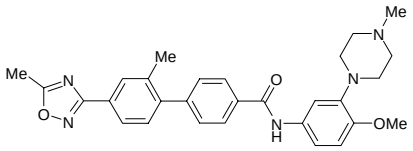
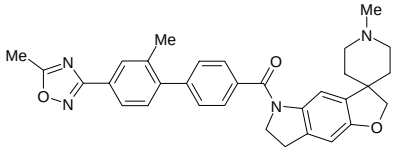
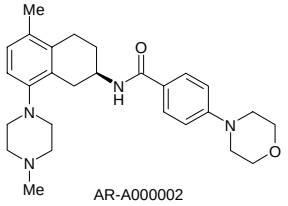


GR127935



SB-224289



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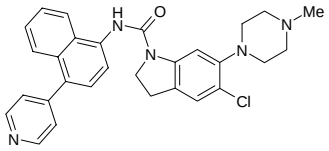


SB-272183

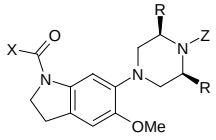
pound proved unsuitable for development it did serve as a very useful tool for investigating the pharmacology of this receptor. Recently, AR-A00002 has been reported as a selective 5-HT_{1B} receptor antagonist, although this also shows partial agonism in a [^{35}S]GTPγS assay.[17]

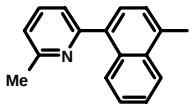
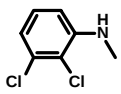
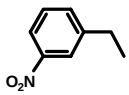
The starting point for our investigation came from SAR studies and data mining around the previously reported potent 5-HT_{1ABD} receptor antagonist SB-272183.[18] From these investigations, we identified similar structures in which *cis*-2,6-dimethyl substitution of the piperazine ring afforded improved selectivity for the 5-HT_{1B} receptor, as shown in Table 1.

Three significant findings emerged from this initial investigation. First, the biaryl amide LHS could be replaced by a substituted phenyl urea or phenylacetamide and maintain good 5-HT_{1B} affinity. Second, compounds **2**, **7** and **9** all showed comparable 5-HT_{1B} affinity to the corresponding piperazine analogues (**1**, **6**, and **8**), whereas they showed a consistent reduction in 5-HT_{1D} affinity together with a marked reduction in affinity at the 5-HT_{1A} receptor. A highly significant third finding was the effect on intrinsic agonist activity of introducing the *cis*-dimethyl substitution, as evaluated in the [^{35}S]GTPγS functional assay,[19] which was shown to be significantly reduced at both the 5-HT_{1B} and 5-HT_{1D} receptors. Thus, compound **2** was a very encouraging early lead with a good 5-HT_{1B}/5-HT_{1D} selectivity, low 5-HT_{1B} intrinsic activity, and it also showed >100-fold selectivity over a range of other 5-HT and dopamine receptors. Although this compound subsequently proved to have in vitro metabolic liability, it prompted further SAR investigations around these molecules.

As N-demethylation of **2** was indicated as one possible source of instability a small investigation was conducted on this compound. Increasing the size of the *N*-alkyl

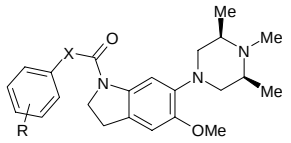
Table 1. Receptor binding affinity (pK_i)[a], intrinsic activity[19] and intrinsic clearance for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}



Compound	X	R	Z	Binding affinity pK_i (intrinsic activity)			Selectivity		Intrinsic clearance (liver microsomes) ml/min/g	
				5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1B}:5-HT_{1A}	5-HT_{1B}:5-HT_{1D}	Rat	Human
SB-272183				8.4	8.4	9.0	1	0.25		
1		H	Me	7.9	8.7 (0.9)	7.6 (0.9)	6	12	—	—
2		Me	Me	6.3	8.5 (0.3)	7.0 (inv)[b]	160	30	46	34
3		Me	H	6.0	7.3	6.9	21	2.8	12	14
4		Me	Et	6.3	8.4	6.9	110	28	45	47
5		Me	*n*-Pr	7.1	7.5	6.8	2	4.7	—	—
6		H	Me	7.3	8.4 (0.6)	7.9 (0.7)	13	3	—	—
7		Me	Me	5.8	8.1 (0.1)	7.1 (0.1)	200	10	3.0	3.0
8		H	Me	6.8	8.1	7.5	20	4	—	—
9		Me	Me	5.7	7.8 (0.1)	6.6 (0.2)	125	15	—	—

[a] Radioligand binding assay from cloned human 5-HT receptors.
[b] Inverse agonism.

Table 2. Receptor binding affinity (pK_i)[a] and intrinsic activity[19] of substituted benzamides, phenylacetamides, ureas and carbamates for 5-HT_{1B} and 5-HT_{1D}



Compound	X	R	5-HT_{1B} pK_i (intrinsic activity)	5-HT_{1D} pK_i
10	Bond	2-Cl, 3-Cl	7.9	6.6
11	Bond	3-Cl	7.7	6.6
12	CH_2	2-F, 3-Cl	8.7 (0.1)	7.0
13	CH_2	2-CF3, 3-F	8.5 (0.1)	6.7
14	CH_2	2-F, 3-CF3	8.8 (0.1)	6.9
15	NH	2-F, 3-Cl	8.1 (0.1)	6.7
16	NH	2-F, 3-CF3	8.4 (0)	6.8
17	NH	2-Cl, 3-CF3	8.6 (0)	7.3
18	O	2-F, 3-CF3	8.6 (0.1)	7.5
19	O	2-Cl, 3-CF3	8.6 (0.15)	7.6

[a] Radioligand binding assay from cloned human 5-HT receptors.

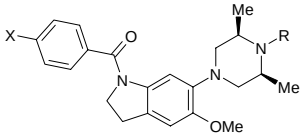
group to ethyl (**4**) retained 5-HT_{1B} affinity, but had no effect on intrinsic clearance. The *n*-propyl analogue **5** was less potent at the 5-HT_{1B} receptor. Removing the *N*-methyl group did reduce the intrinsic clearance, but the NH compound **3** also showed a reduced 5-HT_{1B} affinity.

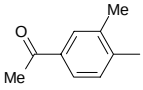
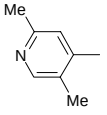
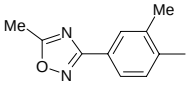
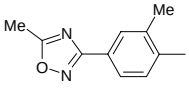
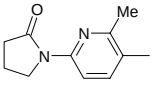
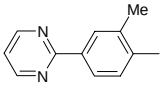
Therefore, investigation was focused on the LHS. A series of substituted benzamides, phenylacetamides, ureas and carbamates was prepared and the effect on 5-HT_{1B} affinity, selectivity and intrinsic activity examined. It was rapidly found that optimal affinity was obtained with substitution by a 2- and/or 3-position electron-withdrawing group. Table 2 shows a representative set of compounds, which also indicates that very low intrinsic activity at the 5-HT_{1B} receptor was maintained.

This study revealed that the benzamides **10** and **11** were less active at the 5-HT_{1B} receptor. Phenylacetamides **12**–**14** generally had a slightly greater affinity than the corresponding ureas **15**–**17**, whereas the carbamates **18** and **19** had good 5-HT_{1B} affinity but generally these compounds showed lower selectivity over the 5-HT_{1D} receptor. Where the corresponding NH piperazine analogues were prepared, these showed reduced 5-HT_{1B} affinity (data not shown).

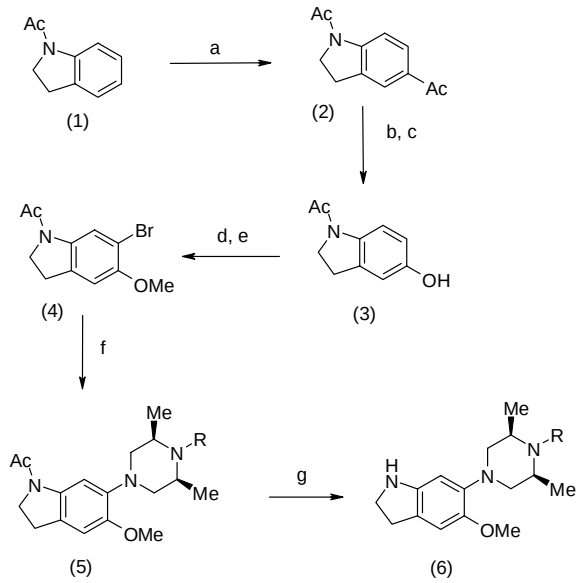
Compounds **14** and **16** were profiled further in vivo, but both of them failed to meet all the requirements for progression. Although the simple benzamides (e.g., **10**)

Table 3. Receptor binding affinity (pK_i)[a], intrinsic activity[19] and human intrinsic clearance (CLi) of biaryl LHSs for 5-HT_{1B} and 5-HT_{1D}



Compound	X	R	5-HT_{1B} pK_i (intrinsic activity)	5-HT_{1D} pK_i	CLi human
20		Me	9.2 (0)	7.8	—
21		H	8.5 (inv)[b]	6.4	1.9
22		H	8.1 (0)	6.3	6.6
23		Me	8.3	7.8	—
24		H	8.5 (0)	6.6	1.0
25		H	8.3 (0)	6.4	0.9
26		H	8.3 (inv)[b]	6.6	0.6

[a] Radioligand binding assay from cloned human 5-HT receptors.
[b] Inverse agonism.



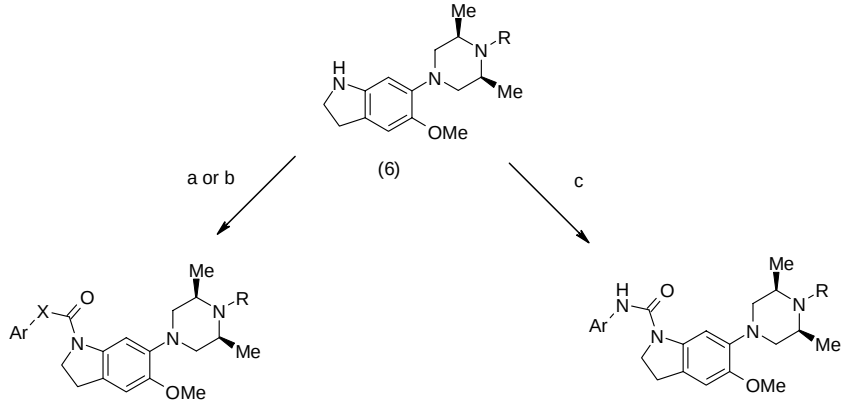
Scheme 1. Synthesis of indoline (**6**). Reagents: (a) AcCl, $AlCl_3$, DCM, 81%; (b) AcOOH, 90%; (c) NaOH, 100%; (d) NBS, AcOH, 64%; (e) MeI, K_2CO_3, DMF, 92%; (f) *cis*-2,6-dimethylpiperazine (R = H, Me), BINAP, $Pd(OAc)_2$, Cs_2CO_3, dioxane, 63%; (g) 2 M HCl, 90%.

had shown reduced potency at 5-HT_{1B}, when a second aryl ring was introduced, this gave a significant increase in 5-HT_{1B} receptor affinity and afforded compounds, such as **20** (Table 3), with nanomolar potency and full antagonism in the [^{35}S]GTPγS functional assay. However, these compounds showed either insufficient 5-$HT_{1B/1D}$ selectivity, inhibited P450 isoforms (data not shown) or had high CLi in microsomes precluding further studies. Introduction of a further ring onto the biaryl side chain produced many analogues with excellent $5HT_{1B}$ affinity and selectivity. This SAR mimics that seen originally in the corresponding aniline series.[9] Significantly for compounds in this class, the N–H piperazine analogues had similar $5HT_{1B}$ affinity to the N–Me piperazines, yet retained their superior 5-$HT_{1B/1D}$ receptor selectivity and, therefore, offered examples avoiding potential metabolic instability due to N-demethylation. Consequently, a number of compounds were taken up for in vivo evaluation from this series and compound **24** (SB-616234) demonstrated to be the best overall profile for further studies.

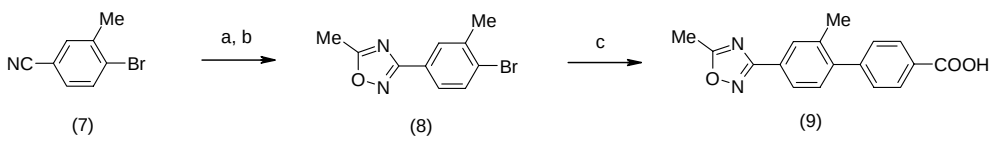
The synthesis of this series of indoline piperazines utilised (**6**) (R = H or Me) (Scheme 1) as a key intermediate. This was prepared from 1-acetylindoline (**1**), which was acetylated under Friedel–Crafts conditions to give the 5-acetyl analogue (**2**). Baeyer–Villiger oxidation gave the acetoxy intermediate, which readily hydrolysed to give the 5-hydroxy compound (**3**). Bromination of this material with *N*-bromosuccinimide followed by O-methylation, afforded (**4**) and the 1,2,6-trimethyl or 2,6-dimethyl piperazine could then be introduced to give (**5**). In the case of dimethylpiperazine, only one isomer was obtained.

Acidic hydrolysis then gave the NH indoline (**6**) and the final amides and phenylacetamides were prepared by either acylation with the appropriate acid chloride or via EDC/HOBt coupling with the acid. Carbamates were prepared from the chloroformates and the ureas were prepared by reaction with the appropriate isocyanate; those not commercially available were prepared in situ from the appropriate amine and triphosgene (Scheme 2).

The oxadiazole biphenyl acid (**9**) required for SB-616234 was prepared from the bromobenzonitrile (**7**) via reaction with hydroxylamine, followed by acetic



Scheme 2. Synthesis of amides, ureas and carbamates. Reagents: (a) ArXCOCl, Et_3N, DCM; (b) ArXCOOH, EDC, HOBt, DCM; (c) ArNCO, DCM.



Scheme 3. Synthesis of oxadiazole biphenyl acid. Reagents: (a) H_2NOH, MeOH, 90%; (b) Ac_2O, 100%; (c) 4-carboxybenzeneboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 74%.

anhydride to give (**8**). This was coupled with 4-carboxybenzeneboronic acid under Suzuki reaction conditions to afford (**9**). Other biaryl acids were prepared by similar reported procedures[20] (Scheme 3).

In summary, we have been able to identify 5-HT_{1B} receptor antagonists with increased selectivity and reduced intrinsic agonist activity through the introduction of *cis*-2,6-dimethyl substitution onto the piperazine ring of a mixed 5-$HT_{1A/1B/1D}$ ligand. Details of the in vitro and in vivo activity of SB-616234 (**24**) will be described in forthcoming papers.[21,22]

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