Discovery of Potent, Orally Bioavailable, Selective 5-HT$_{1A/B/D}$ Receptor Antagonists

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Abstract: 5-HT$_1$ receptor antagonists have been discovered with good selectivity over the 5-HT transporter. This is the first report of highly potent, selective ligands for the 5-HT$_{1A/B/D}$ receptors with low intrinsic activity, which represent a useful set of molecules for further understanding the roles of the 5-HT$_1$ receptor subtypes and providing new approaches for the treatment of depression.

Of the biological systems that have been investigated as targets for the treatment of depression and related psychiatric diseases, there has been, and continues to be, a considerable focus in targeting pathways that are involved in the regulation of synaptic 5-hydroxytryptamine (5-HT) levels. Indeed, there is a large body of preclinical and clinical evidence that indicates a link between reduced synaptic 5-HT concentrations and depression, and consequently intervention to bring about an elevation of 5-HT levels should alleviate those symptoms associated with the disease.[1] The most established approach to this is demonstrated by block of 5-HT reuptake through inhibition of the 5-HT transporter (SerT) giving rise to the successful group of antidepressants, the SSRIs.[a]

Although SSRIs are an effective means of elevating synaptic 5-HT levels and thus of treating depression, there still exist a number of challenges to be met. Specifically, improvements are sought in the responder rate, the latency to onset of action, and the side effect profile, including nausea and sexual dysfunction. In particular, to address the latency to therapeutic onset that typically requires several weeks of treatment, studies have been conducted to investigate the role of 5-HT$_1$ receptors in this process. This work has led to the hypothesis that the latency to therapeutic onset of SSRI action is attributable to time required for the 5-HT$_1$ receptors to desensitize,[2] and consequently antagonism of one or more of these 5-HT$_1$ autoreceptors alone or in combination with inhibition of SerT may offer alternative antidepressant drugs.[3] Recent publications from our group in this field have described the identification of selective 5-HT$_{1D}$ antagonists,[4] mixed 5-HT$_{1A}$ antagonist/SSRIs,[5,6] and 5-HT$_{1A/B/D}$ ligands,[7] and clearly there exists within this chemotype the ability to identify a range of distinct pharmacological profiles. In particular, to complement the existing profiles studied in vivo, we were keen to prepare further analogues of **1** (SB-649915,[6] Figure 1) to explore the substitution pattern around the phenyl ring present in the benzoxazinone group with the aim of identifying potent, selective 5-HT$_1$ receptor antagonists, i.e., molecules with pK_i > 8 against the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors and importantly low intrinsic activity and good selectivity over other receptors and transporters, including the serotonin transporter (SerT). Compounds of this profile should thus be expected to be free of the side effects associated with the SSRIs (vide supra).

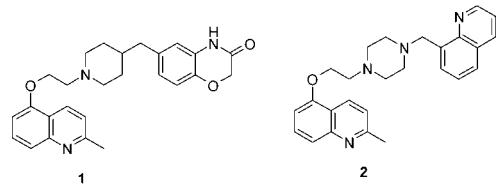
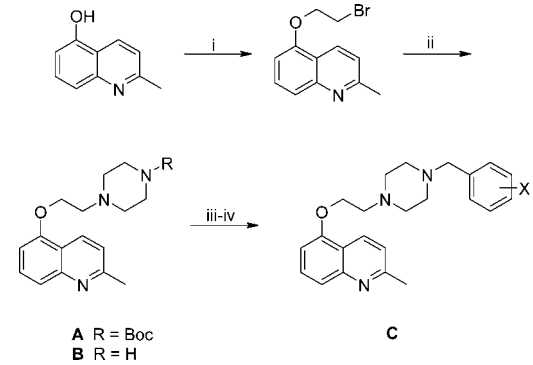


Figure 1. Structures of **1**, a dual 5-HT$_{1A}$ receptor antagonist and 5-HT reuptake inhibitor, and **2**, a selective 5-HT$_{1D}$ receptor antagonist.

Scheme 1[a]



[a] Reagents and conditions: (i) $BrCH_2CH_2Br$, K_2CO_3, butan-2-one, 80 °C, 91%; (ii) 1-Boc-piperazine, K_2CO_3, DMF, 70 °C, 37%; (iii) 1 M HCl in Et_2O, EtOH, 91%; (iv) X-PhCHO, $NaBH(OAc)_3$, dichloroethane, 45−75%.

The work that had focused on identification of a selective 5-HT$_{1D}$ receptor antagonist and led to the discovery of **2** (SB-714786,[4] Figure 1) had clearly demonstrated that replacement of the benzoxazinone group with a simply substituted phenyl led to a loss in affinity for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors and afforded potent, selective 5-HT$_{1D}$ antagonists. This work was continued by broadening the initial array run to explore reductive alkylation products from starting piperazine **B**, which could mimic the properties of the parent benzoxazinone to recover affinities for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors. For this initial work outlined in Scheme 1, 1,2-dibromoethane was reacted sequentially with 5-hydroxy-2-methylquinoline 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 **C**.

Consistent with previous observations, the results of this array demonstrated that high affinity for the 5-HT$_{1D}$ receptor can be maintained even for the unsubstituted phenyl derivative **3** and that this modification also resulted in a loss of affinity for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors and the 5-HT transporter. Interestingly, substitution of this phenyl ring with the constituent functional groups present in the parent **3**, namely, the *m*-acetamide **4** or *p*-methoxy **5**, resulted in some restoration of affinities for the 5-HT$_{1A}$ receptor, particularly for the acetamide (Table 1).

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[a] Abbreviations: SSRI, selective serotonin reuptake inhibitor; SerT, 5-HT transporter; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; PSA, polar surface area.

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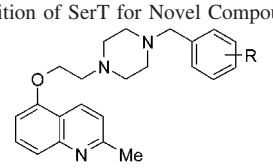
Table 1. Receptor Binding Affinity (pK_i[a]) for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and Functional Inhibition of SerT for Novel Compounds



compd[b]	*o*	*m*	*p*	5-HT_{1A} [**D**][c]	5-HT_{1A} [**W**][d]	5-HT_{1B}	5-HT_{1D}	SerT
1				9.5	8.6	8.0	8.8	8.0
2				6.5	ND	6.7	9.1	6.5
3	H	H	H	7.6	6.7	6.6	9.0	6.1
4	H	NHAc	H	8.7	6.7	7.7	9.3	5.9
5	H	H	OMe	8.3	7.3	6.3	7.9	6.4
6	H	OMe	H	7.9	ND	6.8	8.6	6.2
7	H	H	NHAc	6.8	ND	5.9	6.7	5.8
8	NHAc	H	H	ND	5.9	5.8	8.3	5.0
9	H	NMeAc	H	ND	6.6	7.3	9.5	6.0

[a] Radioligand binding assay to determine affinity at human recombinant 5-HT receptors and functional [^{3}H]5-HT uptake assays in rat cortical synaptosomes to determine potency for SerT. Each determination lies within 0.3 log units of the mean with a minimum of three replicates. ND = not determined. [b] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS. [c] Radioligand used was [^{3}H]8-OH-DPAT [**D**]. [d] Radioligand used was [^{3}H]WAY100635 [**W**]. Radioligand for 5-$HT_{1B/D}$ is [^{3}H]5-HT.

Table 2. Receptor Radioligand Binding Affinity for Human Recombinant 5-HT_{1A} Receptor (pK_i[a])

compd	5-HT_{1A} [**D**][b]	5-HT_{1A} [**W**][c]	ΔpK_i	5-HT_{1A} IA[d]
WAY-100635	9.8	9.1	0.7	0
1	9.5	8.6	0.9	0.1
4	8.7	6.7	2.0	0.5
8-OH-DPAT	9.1	5.4	3.7	0.9
5-HT	8.9	5.0	3.9	1

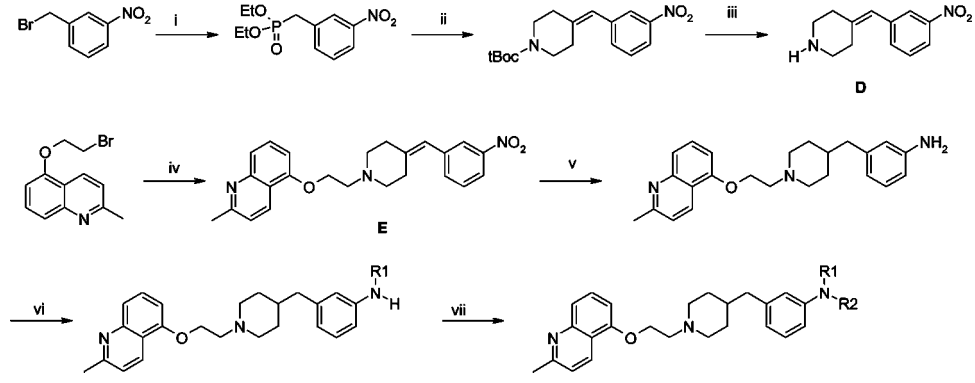
[a] Each determination lies within 0.3 log units of the mean with a minimum of three replicates. [b] Radioligand used was [^{3}H]8-OH-DPAT [**D**]. [c] Radioligand used was [^{3}H]WAY100635 [**W**]. [d] IA = intrinsic activity.

Transposition of the *m*-acetamide group to the ortho **8** and para **7** positions resulted in a reduction in affinity for all 5-HT receptors, although interestingly the *p*-methoxy group could be transposed to the meta position **6** with comparable binding affinities to the para substituted analogue. Furthermore, it was clear that selectivity over the 5-HT transporter (SerT) could be more easily achieved, as all groups replacing the benzoxazinone showed considerably reduced SerT affinity, including those such as the *m*-acetamide or *p*-methoxy just described. Given the superiority for the *m*-acetamide over the other substituents investigated, the role of the secondary amide was investigated further by preparation of tertiary amide **9**. This modification had limited impact on the pharmacological profile, confirming the key interaction is presumably as a hydrogen bond acceptor rather than donor, which is in line with previous studies on an alkylated series of benzoxazinones.[7]

During the course of this work, the binding assay for the 5-HT_{1A} receptor was revised from displacement of [^{3}H]8-OH-DPAT (a 5-HT_{1A} agonist) to displacement of [^{3}H]WAY-100635 (a 5-HT_{1A} antagonist). It has been well reported that h5-HT_{1A} receptors that have been expressed in cultured cell lines can exist in low and high agonist affinity states, with the overall receptor density being considerably higher in the recombinant versus native tissue environment. Furthermore, the difference between the measured pK_i from displacement of a 5-HT_{1A} agonist such as [^{3}H]8-OH-DPAT and a 5-HT_{1A} antagonist such as [^{3}H]MPPF or [^{3}H]spiperone can be used to predict the functional efficacy at h5HT_{1A} receptors expressed in HEK293 cells.[8] As such and in order to ensure we were targeting molecules with low intrinsic activity against the 5-HT_{1A} receptor, we generated data for the displacement of an antagonist radioligand and compared the results to those previously obtained for displacement of an agonist radioligand (Table 2).

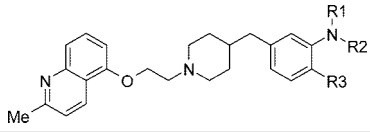
Despite the high binding affinities for the acetamide **4**, it was clear that its level of intrinsic activity for the 5-HT_{1A} receptor was too high. As such, it was decided to continue exploration in the piperidine core series, which had previously shown lower levels of intrinsic activity.[6] For this exploration (Scheme 2), the key intermediate **D** was prepared by Horner−Wadsworth−Emmons reaction of the phosphonate ester derived from 3-nitrobenzyl bromide. This key intermediate could then be coupled with the quinolinyl fragment to afford intermediate **E**, which required reduction of alkene and nitro functionalities to generate the aniline which could be derivatized further as shown. Gratifyingly, this modification had the desired effect, and the direct piperidine analogue **10** showed not only lower intrinsic activity but also higher measured affinity for displacement of the antagonist radioligand in line with our earlier set of data.[7] It is noteworthy, however, that although affinities for the 5-HT_{1A} and 5-HT_{1D} receptors are generally improved in this investigation, the target 5-HT_{1B} receptor affinity of $pK_i > 8$ proved routinely difficult to achieve (Table 3). Standard exploration of the amide functionality encompassing the size of the alkyl group (**11** and **12**) and the introduction of an additional substituent on the nitrogen (**13** and **14**) showed limited differentiation from the parent acetamide **10**. Increasing the alkyl size to ethyl **11**

Scheme 2[a]



[a] Reagents and conditions: (i) $P(OEt)_3$, toluene, reflux 24 h, (68%); (ii) *N*-Boc-piperid-4-one, NaH, THF, room temp, 4 h (100%); (iii) 1 M HCl in Et_2O, 72 h (85%); (iv) **D**, K_2CO_3, DMF, 100 °C, 16 h, 78%; (v) 10% Pd on C, H_2, 0.5 M KOH in EtOH, 24 h, 54%; for amides, (vi) R^1COCl or $(R^1CO)_2O$, NaH, THF, 0 °C (60−100%); for sulfonamides, (vi) R^1SO_2Cl or $(R^1SO_2)_2O$, NEt_3, CH_2Cl_2 (65−90%); for ureas, (vi) RNCO, NEt_3, toluene, 60 °C (48−59%); (vii) NaH, THF, R^2I, 0 °C (63−74%).

Table 3. Receptor Binding Affinity (pK_i[a]) for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and SerT for Novel Aniline Derivatives



compd[b]	R1	R2	R3	5-HT_{1A}[c]	5-HT_{1B}	5-HT_{1D}	SerT
10	COMe	H	H	7.6 (0.3)	7.7 (0.3)	9.3 (0.5)	6.3
11	COEt	H	H	7.6 (0.2)	7.8 (0.3)	9.2 (0.3)	6.1
12	CO^{i}Pr	H	H	8.2 (0)	7.3 (0.3)	8.9 (0.4)	6.3
13	COMe	Me	H	7.6 (0)	7.4 (0.3)	8.9 (0.6)	7.1
14	COMe	Et	H	8.3 (0)	7.0 (0.3)	8.8 (0.6)	6.1
15	$CH_2CH_2CH_2CO$		H	8.2 (0)	7.7 (0.4)	8.7 (0.5)	6.7
16	$COCH_2NMe_2$	H	H	7.8 (inv)	8.2 (0.2)	9.0 (0.4)	6.4
17	CONHEt	H	H	7.5 (0)	7.6 (0.2)	9.6 (0.3)	6.6
18	SO_2Me	H	H	8.6 (0)	8.7 (0.3)	9.3 (0)	6.0
19	SO_2Me	Me	H	7.9 (0)	7.9 (0)	8.8 (0.5)	6.0
20	$SO_2{}^i$Pr	H	H	8.6 (0)	8.9 (0.3)	9.4 (0.4)	6.4
21	$CH_2CH_2CH_2SO_2$		H	8.1 (0)	7.9 (0.5)	8.8 (0.4)	6.1
22	SO_2Me	H	OMe	6.9	7.4	8.2	6.2

[a] Radioligand binding assay to determine affinity at human recombinant 5-HT receptors and functional [^{3}H]5-HT uptake assays in rat cortical synaptosomes to determine potency for SerT. Each determination lies within 0.3 log units of the mean with a minimum of three replicates. Values in parentheses are intrinsic activity values. [b] All compounds were characterized and purity was assessed using ^{1}H NMR and LCMS [c] Radioligand used was [^{3}H]WAY100635.

and then isopropyl **12** led to a slight increase in affinity for the 5-HT_{1A} receptor which was offset by a comparable decrease in 5-HT_{1B} receptor binding. Similarly, although substitution on the nitrogen of the acetamide was clearly tolerated, increasing the size of the alkyl group from methyl to ethyl (from **13** to **14**) again led to a slight improvement in 5-HT_{1A} affinity and a comparable decrease in that for 5-HT_{1B}. Interestingly, this position of the molecule could also tolerate introduction of a basic group, as demonstrated by dimethylamine **16**. The extension of these investigations into pyrrolidinone **15** gave a good overall profile that we hoped would also lead to a good pharmacokinetic profile. A wider investigation of the aniline derivatives showed no clear advantage for urea **17**, although replacement of the amide with a sulfonamide **18** finally gave us the improvement in 5-HT_{1B} affinities which allowed us to identify molecules that now met our primary in vitro criteria. As for the amides, larger alkyl groups **20** and substitution on the sulfonamide nitrogen **19** were tolerated and gave molecules with pK_i greater than our target of 8, although the formation of the cyclic sultam **21** led to a drop in the 5-HT_{1B} affinity. Surprisingly, reintroduction of a *p*-methoxy substituent giving **22** to mimic the functionality present in the benzoxazinone led to a marked decrease in binding affinities relative to **18**.

A number of these molecules underwent pharmacokinetic evaluation in rat to assess suitability for use as tool compounds in vivo.[10] From the data presented (Table 4) it was clear that the original acetamide analogue **10** and the secondary amide analogues **12**−**14** as well as tertiary amide **15** gave similar pharmacokinetic profiles with modest blood clearance and elimination half-lives and brain to blood ratios consistent with free passage across the blood−brain barrier.

Although the sulfonamides had been advantageous for the primary *in vitro* potencies, we suspected that their increased polarity would be detrimental with respect to their levels of CNS penetration. Indeed, comparison of the polar surface areas (PSA) of acetamide **10** and sulfonamide **18** showed an increase from 54 to 72 which translated, *in vivo*, into a reduction in brain/blood ratio from 1.1 to 0.2 (although brain Cmax levels remained high due to improved blood concentrations). Accordingly, the less polar tertiary sulfonamide **21** gave a reduction in PSA to 63 and a corresponding increase in brain/blood ratio to 0.9.

Further affinity profiling of **18** and **21** across the various 5-HT subunits (Table 5) indicated that they had highest affinity for the 5-$HT_{1A/B/D}$ receptors, moderate affinity for 5-HT_7 and D_2, and little or no affinity against the others, and as such, these represented our first compounds that had potent affinity for the 5-$HT_{1A/B/D}$ receptors with approximately 1000-fold selectivity against SerT.

With these discoveries, we proceeded to evaluate this pharmacological and pharmacokinetic profile in vivo with the aim of not only establishing an in vivo pharmacological effect but also further exploring the 5-HT_{1B} receptor, which, unlike the 5-HT_{1A} and 5-HT_{1D} receptors, showed an intrinsic activity greater than 0 in the recombinant cell line. In order to fulfill both requirements, we investigated the activity of the first analogue prepared, **18**, in a 5-HT_{1B} pharmacodynamic model that involves administration of a 5-$HT_{1B/D}$ agonist, SKF99101 (15 mg/kg, ip) to induce an increase in seizure threshold in the maximal electroshock threshold test in the rat.[9] Gratifyingly, oral administration of **18** demonstrated a clear, dose-dependent reversal of the agonist induced increase in seizure threshold in line with that achieved for the selective 5-HT_{1B} receptor antagonist SB-224289, with an approximate ED_{50} of 1 mg/kg with exposures in line with data observed from the rat PK screen. This result is particularly significant, as it confirms **18** was acting as a 5-HT_{1B} antagonist in vivo.

This work has therefore allowed us to generate a new profile, achieving potent affinities across the 5-$HT_{1A/B/D}$ receptors. Furthermore, we have achieved an appropriate pharmacokinetic profile in rat to allow for demonstration of functional antagonism

Table 4. Pharmacokinetic and in Silico Profiles of Aniline Derivatives in Rat[a]

compd	R1	R2	CL_b ((mL/min)/kg)	$t_{1/2}$ (h)	CNS br/bl	V_{ss} (L/kg)	brain C_{max} (ng/g)	clogD pH 7.4	PSA
10	COMe	H	47	0.6	1.1	1.2	221	3.6	54
12	CO^{i}Pr	H	45	0.9	2.3	1.8	175	4.5	54
13	COMe	Me	37	1.2	ND	2.2	190	3.6	46
14	COMe	Et	34	1.1	ND	1.9	234	4.1	46
15	$CH_2CH_2CH_2CO$		35	1.3	1.2	1.5	410	3.9	46
18	SO_2Me	H	38	0.6	0.2	1.6	346	3.5	72
21	$CH_2CH_2CH_2SO_2$		39	0.9	0.9	0.9	287	3.4	63

[a] In vivo data determined by 1 mg/kg iv study in rat. Brain C_{max} and brain/blood ratio determined by additional 3 mg/kg oral rat PK study (br/bl from whole brain AUC concentration measurements). ND = not determined.

Table 5. In Vitro Cross-Screening Characterization (pK_i) of **18** and **21** at h5-HT Receptors and Representative Other Monoaminergic Receptors[a]

	1A	1B	1D	1E	1F	2A	2B	2C	4	5A	6	7	SerT	D2	α1B	β2
18	8.6	8.7	9.3	5.6	6.3	5.6	5.7	<6	<5	5.9	<5	7.7	5.8	7.2	6.7	6.3
21	8.1	7.9	8.8	<5	6.0	<5.2	<6	<5.4	5.1	5.8	5.8	7.8	6.4	6.3	6.8	5.8

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

in vivo. These molecules and this pharmacological profile are now being further investigated for their potential to deliver a differentiated antidepressant profile.

Supporting Information Available: Experimental procedures and data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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