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Studies on a series of potent, orally bioavailable, 5-HT$_1$ receptor ligands

Simon E. Ward,* Christopher N. Johnson, Peter J. Lovell, Claire M. Scott, Paul W. Smith, Geoffrey Stemp, Kevin M. Thewlis, Antonio K. Vong and Jeannette M. Watson

Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

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Abstract—A series of 5-(piperidinylethyloxy)quinoline 5-HT$_1$ receptor ligands have been studied by elaboration of the series of dual 5-HT$_1$-SSRIs reported previously. These new compounds display a different pharmacological profile with potent affinity across the 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and selectivity against the serotonin transporter. Furthermore, they have improved pharmacokinetic profiles and CNS penetration.

Over the past decades, a body of pre-clinical and clinical evidence has highlighted a clear link between extracellular levels of 5-HT and a number of psychiatric indications, including those associated with depression. Indeed, the mode of action of a number of anti-depressant treatments is presumed to be effective through increasing synaptic 5-HT concentrations. These synaptic 5-HT levels are regulated not only by the presence of the 5-HT transporter which is the pharmacological target of the selective serotonin re-uptake inhibitors (SSRIs) but also by the 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ autoreceptors that are present in the region of the cell body and on nerve terminals and are widely distributed across the brain.[1]

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, particularly in mood disorders such as depression and anxiety.[2,3] This work is of particular significance, since SSRIs, which are the most prescribed group of anti-depressants, are still limited in their therapeutic profile. In addition to their incomplete spectrum of efficacy and potential side effects (e.g., sexual dysfunction and nausea), they exhibit a latency to therapeutic onset which is believed to be attributable to the activation of the aforementioned 5-HT$_1$ autoreceptors. The latency to therapeutic onset for SSRIs is thought to be attributed to the time taken for the 5-HT$_1$ autoreceptors to desensitize. Indeed, electrophysiological and electrochemical studies in rats have supported this hypothesis.

Consequently, a drug which either combined SSRI activity with antagonism of one or all of the 5-HT$_1$ autoreceptors, or selectively antagonized these autoreceptors could result in acute increases in extracellular 5-HT.[4,5] Previous publications from this group have described the development of novel compounds containing the 5-(piperidinylethyloxy)-quinoline and benzoxazinone units as either mixed SSRI-5-HT$_1$ receptor antagonists.[6,7] or selective 5-HT$_{1D}$ receptor antagonists.[8]

In particular SB-649915 (Fig. 1) was a member of these 3,4-dihydro-2H-benzoxazinones which displayed high affinity for the three 5-HT$_1$ autoreceptors as well as potent 5-HT reuptake inhibitory activity. In addition, this molecule possesses good oral bioavailability in rat and is moderately brain penetrant.

Continued studies in this area have sought to improve both the pharmacokinetic profile and CNS penetration of this molecule as well as further explore the structure–activity relationships. In particular, we sought to identify a potent 5-HT$_{1ABD}$ receptor ligand with low

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* Corresponding author. Tel.: +44 0 1279 622894; fax: +44 0 1279 627728; e-mail: simon.e.ward@gsk.com

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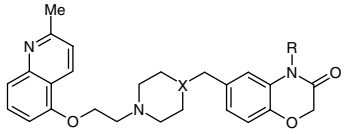
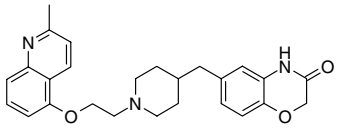
Table 1. Receptor binding affinity (pK_i[a]) for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and SerT for novel compounds



Compound	Structure[b]		pK_i				
	X	R	5-HT_{1A} [D][c]	5-HT_{1A} [W][d] (I.A.)[e]	5-HT_{1B} (I.A.)	5-HT_{1D} (I.A.)	SerT
SB-649915	CH	H	9.5	8.6 (0.1)	8.0 (0.5)	8.8 (0.5)	8.0
1	CH	Me	9.4	8.2 (0)	8.2 (0.5)	8.6	7.4
2	CH	Et	9.6	8.5 (0.1)	8.4 (0.4)	8.8 (0.6)	6.9
3	CH	*i*-Pr	9.2	8.3 (0)	7.4 (0.6)	8.4	7.0
4	N	H	8.8	7.2 (0)	7.0 (0.6)	8.0	7.6
5	N	Me	8.8	8.0 (0)	8.1 (0.8)	9.3	7.0
6	N	Et	8.9	7.4 (0.1)	7.6 (0.4)	9.0 (0.3)	5.7
7	N	*n*-Pr	9.6	7.6 (0.1)	7.5 (0.4)	8.7 (0.5)	6.8
8	N	*n*-Bu	8.9	ND	7.5	8.5	6.8
9	N	*i*-Bu	8.7	ND	7.5	8.6	5.8
10	N	Bn	8.4	ND	7.2	8.2	6.5
11	N	Phenethyl	8.9	7.5	7.6	8.7	ND

[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.
[b] All compounds were characterised 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].
[e] I.A., intrinsic activity; ND, not determined.



SB-649915

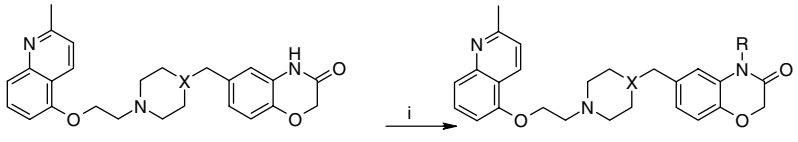
Figure 1. Structure of SB-649915, a dual 5-HT_1 receptor antagonist and 5-HT re-uptake inhibitor.

functional activity and with selectivity over the serotonin transporter which could be profiled in relevant animal models of both efficacy and side effects to allow comparison to the dual 5-HT_1 receptor antagonist 5-HT re-uptake inhibitor previously described.

The initial focus of our work centred on exploration of the benzoxazinone moiety, and in particular on understanding the role of the secondary lactam nitrogen. Previous studies[7] suggested the presence of the amide N–H might be crucial for 5-HT reuptake inhibition activity, and so a range of analogues were prepared by derivatisation of the lead (Scheme 1) with a view to obtaining potent 5-HT_1 antagonists which are selective against the serotonin transporter (Table 1).

All compounds prepared were screened against h5-HT_{1A} receptors expressed in CHO cells using displacement of either [^{3}H]-WAY100635 (a 5-HT_{1A} antagonist) or [^{3}H]-8-OH-DPAT (a 5-HT_{1A} agonist) and against h5-HT_{1B} and h5-HT_{1D} receptors expressed in CHO cells using displacement of [^{3}H]-5-HT.

Potency for the 5-HT re-uptake site was assessed by measurement of the inhibition of [^{3}H]-5-HT uptake into rat cortical synaptosomes, with data expressed as pK_i. 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 ten point half log serial dilution was used to generate a concentration response for each compound. The results of this investigation demonstrated that substitution on the lactam nitrogen had a limited impact against all receptor affinities, even for the largest substituents investigated **8**–**11**. The selectivity for 5-$HT_{1A/B/D}$ over the serotonin transporter increased for alkyl modifications to a maximum of 50-fold. The best overall new profile was achieved for compound **2**



A X = CH
B X = N

1-3 X = CH
4-11 X = N

Scheme 1. Typical reagents and conditions: (i) K_2CO_3 or NaH, DMF, 0 °C then RBr/RI 25 °C, 4 h.

Table 2. P450 inhibition profile for compounds

Compound	P450 IC_{50} (<20 μM)[a]	c log D[b]
5 (*N*-Me)	All >20 μM	2.5
6 (*N*-Et)	1A2 18 μM; 2C9 14 μM	3.1
7 (*N*-^{n}Pr)	2C9 18 μM	3.6
8 (*N*-^{n}Bu)	1A2 4 μM; 2C9 12 μM; 2C19 16 μM	4.1
10 (*N*-Bn)	1A2 11 μM; 2C9 7 μM; 2C19 11 μM; 2D6 15 μM; 3A4 12 μM	4.3
11	1A2 17 μM; 2C9 4 μM; 2C19 5 μM; 2D6 14 μM; 3A4 9 μM	4.7

[a] P450 isoform inhibition IC_{50} for CYP1A2, 2C9, 2C19, 2D6, 3A4 ®Gentest. Data only shown for values <20 μM.
[b] log D@pH7.4 (ACDlabs software).[9]

Table 3. Rat pharmacokinetic profile for compounds

Compound	Cli rat; hum[a]	CLb[b] (ml/min/kg)	V_{ss} (L/kg)	$t_{1/2}$[b] (h)	Fpo[c]	Br:Bl[c]
SB-649915	3.4; 1.2	50	4.4	1.3	40	0.4
1	4.9; 0.9	57	4.2	1.3	35	1.3
2	7.7; 2.8	31	2.5	1.6	ND	ND

ND, not determined.
[a] Intrinsic clearance in microsomes (ml/min/g liver).
[b] In vivo data determined by 1 mg/kg iv study in rat.
[c] Oral bioavailability and brain:blood (based on total brain concentration) ratio determined by additional 3 mg/kg oral rat PK study.

(*N*-ethyl) with good 5-$HT_{1A/B/D}$ receptor affinities and approximately 30-fold selectivity over the serotonin transporter.

Furthermore, consistent with the general observation for increasing lipophilicity, we observed an increase in inhibition of selected P450 enzyme isoforms with increasing size of alkyl substituent (Table 2). Indeed for the largest substituent, *N*-phenethyl **11**, we observed potent inhibition for all isoforms tested.

The most encouraging compounds were those with small alkyl substituents which were profiled further in rat pharmacokinetic studies (Table 3). From this analysis and despite the data generated in microsomes, a reduction in the rate of blood clearance was observed with **2**, which may be rationalised by presumed increases in plasma protein binding in line with increased lipophilicity.

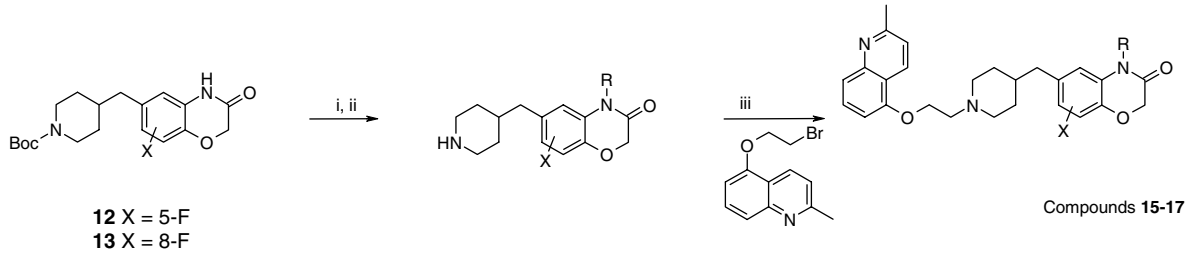
In addition to the changes in clearance and P450 inhibition profile, this lipophilicity also increased the CNS penetration leading to brain:blood ratio >1 for **1** compared to 0.4 for SB-649915 (Fpo from 3 mg/kg dose = 35–40% for both compounds), but also gave additional unwanted pharmacological activities such as inhibition of α_1, H_2 and M_1 receptors (CEREP screen inhibition data at 1 μM).

From these studies it was apparent that substitution of the lactam nitrogen alone could only afford a limited improvement in terms of both selectivity and pharmacokinetics, and further modification of the benzoxazinone skeleton was required.

Previous data[7] indicated that SB-649915 underwent oxidative metabolism of the benzoxazinone, which could be blocked by fluorine substitution into the aromatic rings. This strategy was adopted for the most promising compounds **1** and **2** above. Analogues were prepared (Scheme 2) using intermediates **12** and **13** previously described[7] via alkylation, deprotection and coupling with the quinolinyloxy fragment.

The in vitro pharmacology for the compounds tested was very encouraging (Table 4), with all compounds displaying high affinities for the 5-HT_1 receptors coupled with low intrinsic activity for the 5-HT_{1A} receptor. The intrinsic activity observed for the 5-HT_{1B} and 5-HT_{1D} receptors is higher, although it should be noted that partial agonism in the recombinant cell lines does not necessarily translate into partial agonism in native tissue (for comparison, GR 127935[10] data are included which has been characterised as a $5HT_{1B/D}$ antagonist in native tissue studies). Furthermore, **17** demonstrated over 100-fold separation of affinities between the serotonin transporter and the 5-$HT_{1A/B/D}$ receptors which represents the most selective compound profiled to date.

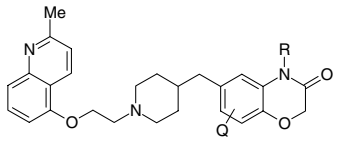
In vivo pharmacokinetic profiling of these compounds led to an improvement over earlier analogues with all three compounds showing lower blood clearance and



12 X = 5-F
13 X = 8-F

Scheme 2. Typical reagents and conditions: (i) K_2CO_3 or NaH, DMF, 0 °C then RBr/RI 25 °C, 4 h; (ii) trifluoroacetic acid, CH_2Cl_2; (iii) diisopropylethylamine, isopropanol, reflux.

Table 4. Receptor binding affinity (intrinsic activity with respect to 5-HT) for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and SerT potency for fluorinated compounds



Compound	Q	R	pK_i			
			5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	SerT
15	8-F	Me	8.1 (0)	9.1 (0.7)	9.4 (0.6)	7.3
16	5-F	Me	8.5 (0)	8.8 (0.4)	9.3 (0.6)	7.1
17	5-F	Et	8.6 (0.1)	8.8 (0.5)	8.9 (0.7)	6.7
GR 127935				(0.3)	(0.8)	

All compounds were characterised and purity was assessed using ^{1}H NMR and LCMS.

Table 5. Rat pharmacokinetic profile for fluorinated compounds

Compound	P450[a] (μM)	Clb[b] (ml/min/kg)	$t_{1/2}$[b] (h)	Fpo[c]	Br:Bl[c]
15	>19	35	1.3	ND	3.6
16	>16	34	2.0	65	2.7
17	>9	23	2.8	34	1.2

ND, not determined.

[a] P450 isoform inhibition IC_{50} for CYP1A2, 2C9, 2C19, 2D6, 3A4 ®Gentest.

[b] In vivo data determined by 1 mg/kg iv study in rat.

[c] Oral bioavailability and brain:blood (based on total brain concentration) ratio determined by additional 3 mg/kg oral rat PK study.

improved brain penetration and concentrations compared to SB-649915 (Table 5).

In summary compound **17** has high affinity for the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors with good selectivity against other targets (data not presented) including the serotonin transporter and as such constitutes the most selective 5-HT_{1ABD} receptor ligand reported to date. Compound **17** displays negligible intrinsic efficacy at 5-HT_{1A} receptors and moderate-high efficacy at 5-HT_{1B} and 5-HT_{1D} receptors, respectively. Further studies in native tissue are required to determine whether this degree of partial agonism at human recombinant 5-$HT_{1B/1D}$ receptors is predictive of intrinsic efficacy at the native receptor, following data previously discussed for GR 127935. Furthermore, partial agonists at the 5-HT_{1A} receptor are themselves strategies for the treatment of anxiety and depression disorders.[11] In addition, **17** shows excellent oral pharmacokinetics in rat with good brain penetration and so represents an attractive tool for characterisation in behavioural models to further understand the roles of the 5-HT_1 autoreceptors and the serotonin transporter with the ultimate aim to identify faster acting anti-depressants.

Acknowledgments

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