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3,4-Dihydro-2*H*-benzoxazinones as dual-acting 5-HT$_{1A}$ receptor antagonists and serotonin reuptake inhibitors

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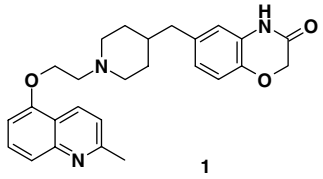
Abstract—Investigation of halogen substitution in lead compound **1** has led to the identification of analogues which combine high affinity for 5-HT$_{1A}$ receptors and potent serotonin reuptake inhibitory activity. Several compounds show an improved selectivity over 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and a superior pharmacokinetic profile in the rat.

Drugs which selectively inhibit the reuptake of serotonin (SSRIs) and therefore elevate 5-HT levels in the brain are the most effective antidepressant agents in use. Although they offer several advantages over the older tricyclic antidepressants, they still suffer several limitations. They are only effective in approximately 70% of the depressed population and induce side effects such as nausea and sexual dysfunction.[1] In addition, several weeks of treatment with SSRIs are required before the onset of antidepressant activity.[2] The latency to therapeutic onset is thought to be due to the time taken to desensitise 5-HT$_{1A}$ autoreceptors and SSRIs only acutely elevate brain 5-HT levels after this desensitisation process has occurred.[3] Therefore, a combined SSRI-5-HT$_{1A}$ receptor antagonist should have a faster onset of action than an SSRI alone. Indeed, in preclinical studies, co-administration of a 5-HT$_{1A}$ antagonist with an SSRI results in an immediate increase in CNS 5-HT levels[4] and also shortens the onset of anxiolytic activity in a rat model of anxiety.[5]

Furthermore, pindolol (a 5-HT$_{1A}$ receptor antagonist) has been reported to accelerate the antidepressant action of SSRIs in several clinical trials.[6]



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A series of 3,4-dihydro-2*H*-benzoxazinones **1** have already been described (*inter alia*) as 5-HT$_{1A}$ receptor ligands with potent 5-HT reuptake inhibition.[7] However, further profiling highlighted significant affinity for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and only moderate in vivo metabolic stability (CLb 50 mL/min/kg) in rat. This *Letter* now describes the further optimization of the pharmacological and pharmacokinetic profiles of this series of compounds.

In an attempt to rationalise the in vivo clearance data, in vitro metabolic studies were carried out. Metabolite identification using rat microsomes suggested that both the quinoline and benzoxazinone ring systems in **1** were potentially vulnerable to oxidation and prompted the preparation of halogenated analogues. Halogenated quinoline and benzoxazinone analogues were prepared according to Schemes 1 and 2.[8] Reaction of di- and

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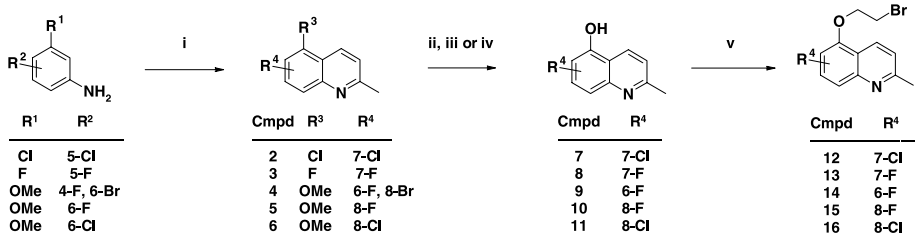
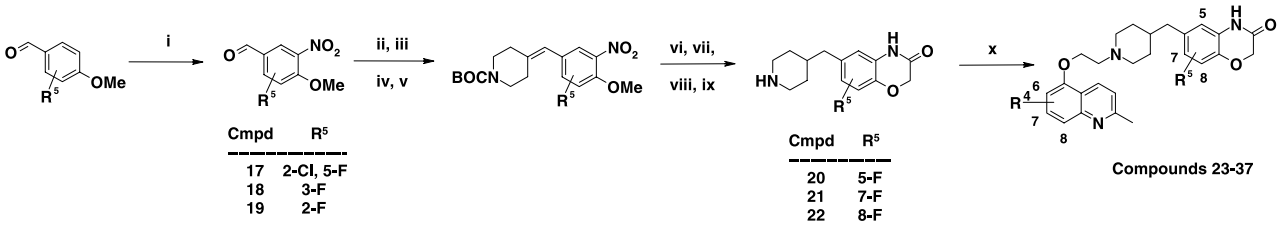


Scheme 1. Reagents and condition: (i) crotonaldehyde, 5 N HCl, reflux (22–86%); (ii) NaOMe, MeOH (38–79%), then BBr_3, CH_2Cl_2 (51–82%); (iii) H_2, Pd/C, EtOH then BBr_3, CH_2Cl_2 (48–78%); (iv) BBr_3, CH_2Cl_2 (51–82%); (v) $BrCH_2CH_2Br$, K_2CO_3, MEK, 85 °C (71–81%).



Scheme 2. Reagents and condition: (i) HNO_3, AcOH (58–89%); (ii) $NaBH_4$, MeOH (79–99%); (iii) NBS, PPh_3 (48–76%); (iv) $P(OEt)_3$; (v) *N*-Boc-4-piperidone, *t*-BuOK, THF (74–88% over two steps); (vi) LiCl, DMF (34–57%); (vii) H_2, Pd/C, EtOH (78–99%); (viii) chloroacetylchloride (63–74%); (ix) trifluoroacetic acid, CH_2Cl_2 (76–99%); (x) diisopropylethylamine, isopropanol, reflux (39–74%).

tri-substituted anilines with crotonaldehyde gave the corresponding quinolines **2**–**6**. The 5-halo substituent in both **2** and **3** could be selectively displaced with sodium methoxide and then reacted with BBr_3 to afford 7-halo quinolinols **7** and **8**. Similarly, the 8-halo quinolinols **10** and **11** were derived from methoxy precursors **5** and **6**, respectively. Regioselective synthesis of the 6-fluoroquinoline analogue **9** however required the use of a blocking group; an *ortho*-bromo substituent was used to control the Skraup reaction as this could be readily removed by hydrogenation. Alkylation of phenols **7**–**11** was achieved using 1,2 dibromoethane to afford the coupling partners **12**–**16**. The synthesis of halogenated benzoxazinones (Scheme 2) required construction of the parent ring system. Nitration of substituted anisoles gave compounds **17**–**19**. Reduction of the aldehyde to the benzyl alcohol allowed formation of the benzyl bromide using NBS and subsequent transformation into the benzyl phosphonate. Wadsworth–Emmons coupling with *N*-Boc-4-piperidone ensued in high yield and was followed by a straightforward three-step sequence for the construction of the oxazinone ring. Removal of the Boc-protecting group gave piperidines **20**–**22** which were coupled with **12**–**16** to afford target compounds **23**–**37**.

All compounds were evaluated using the displacement of tritiated WAY100635 from human cloned 5-HT_{1A} receptors expressed in CHO cells.[9] The potency at the 5-HT reuptake site (serotonin transporter, SerT) was assessed by measuring the inhibition of reuptake of tritiated 5-HT into rat cortical synaptosomes.[10]

Halogenation at either the 6- or 8-position of the quinoline ring gave a 10-fold reduction in potency at 5-HT_{1A} receptors (Table 1) when compared to **1**. In contrast, 5-HT_{1A} affinity could be maintained with a small substituent at the quinoline 7-position, but more interestingly this modification introduced high selectivity over 5-HT_{1B} and 5-HT_{1D} receptors. These selectivity data could be rationalised by docking **1** into our 5-HT_1 receptor homology models (Fig. 1).[11] These suggest that a 7-F or 7-Cl substituent could be accommodated by the 5-HT_{1A} receptor and would reside

Table 1. 5-HT_1 receptor binding affinities, SerT potency[a,b,c]

Compound	R^4	5-HT_{1A} pK_i[a]	5-HT_{1B} pK_i[a]	5-HT_{1D} pK_i[a]	SerT pK_i
1	H	8.6	8.0	8.8	8.1
23	8-F	7.7	6.1	7.1	8.1
24	8-Cl	7.4	6.8	7.2	7.9
25	7-F	9.1	<6.0	6.4	7.8
26	7-Cl	8.8	5.9	6.1	8.0
27	7-CN	7.7	6.0	6.6	7.0
28	6-F	7.3	7.5	8.6	8.0

[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean.
[b] Receptors and radioligands used in binding assays: 5-HT_{1B} human cloned receptors in CHO cells, [^{3}H]5-HT; 5-HT_{1D} human cloned receptors on CHO cells, [^{3}H]5-HT.
[c] All new compounds gave satisfactory NMR, MS and analytical data.

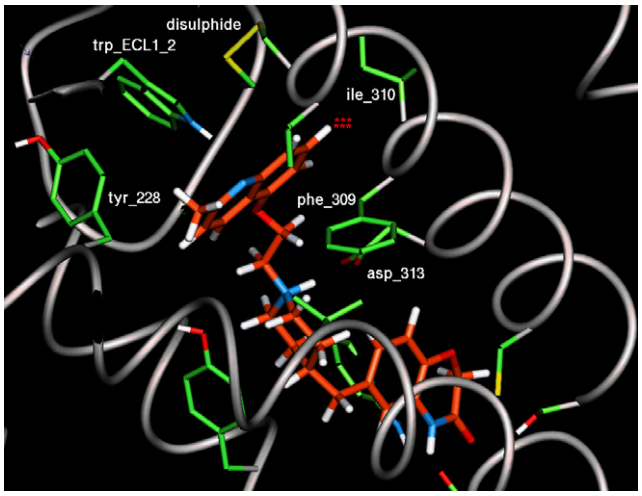


Figure 1. Compound **1** docked into a 5-HT_{1A} homology model.[11]

Table 2. Rat PK profile for halogenated analogues[a]

	Compound	Clb (mL/min/kg)	Fpo	Brain: blood ratio
	1	50	45	0.4
Halogenated quinolines	**25**	25	41	—
	26	25	56	0.2
Halogenated benzoxazinones	**29**	41		4.0
	30	36	33	—
	31	22		—
Dihalogenated analogues	**32**	27	39	6.4
	33	28	43	
	34	19	54	1.2
	35	18	60	
	36	25	88	0.4
	37	16	51	

[a] 3 mg/kg dose administered orally.

between residues Ile 310 and Phe 309. However, these residues are leucine and tryptamine, respectively, in 5-HT_{1B} and 5-HT_{1D} receptors which implies that the primary origins of selectivity are due to a steric interaction.

Encouragingly, PK profiling of both **25** and **26** in the rat revealed an increase in metabolic stability (Table 2), which substantiated our in vitro metabolism data. CNS penetration however remained poor and modification of the benzoxazinone now became the focus for further investigation.

In general, halogen substitution on the benzoxazinone ring (compounds **29**–**31**) was well tolerated with the 7-fluoro isomer **30** showing some enhancement in 5-HT_{1A} binding (Table 3). As expected, the electron-rich 8-position of the benzoxazinone ring showed the greatest susceptibility towards oxidation. Indeed, pharmacokinetic profiling of all three fluoro benzoxazinone isomers (Table 2) revealed that **31** achieved the greatest increase in metabolic stability. More importantly the 5-fluoro isomer **29** showed a dramatic 10-fold increase in brain to blood ratio compared to **1**, presumably due to a modification of polarity by a neighbouring group effect.

Further investigation now focused on the effects of combining halogenation in both of the quinoline and benzoxazinone rings. This strategy led to the identification of several highly potent and selective 5-HT_{1A} receptor antagonists **32**–**37** with potent 5-HT reuptake inhibition (Table 3). No significant improvements to metabolic stability were made with these analogues (Table 2). However, CNS penetration studies again revealed the beneficial effects of benzoxazinone substitution, in particular the 5-fluoro analogue **32** showed a dramatic increase in brain to blood ratio. Oral bioavailability in the rat was good for analogues **32**–**37** and would not present a limiting factor for subsequent in vivo experiments. The dihalogenated analogues were further profiled against a range

Table 3. Receptor binding affinities in radioligand binding assays[a,b]

Compound	R^5	R^4	pK_i 5-HT									pK_i	pK_i dopamine	
			1A	1B	1D	SerT	2A	2B	2C	6	7	$\alpha_{1\beta}$	D_2	D_3
1	H	H	8.6	8.0	8.8	8.1	5.5	5.8	<5.1	<5	6.7	5.6	5.9	6.2
29	5-F	H	8.6	8.6	9.1	7.8	—	—	—	—	—	—	—	—
30	7-F	H	9.3	7.7	8.5	8.1	—	—	—	—	—	—	—	—
31	8-F	H	8.3	9.1	9.4	8.2	—	—	—	—	—	—	—	—
32	5-F	7-F	8.8	6.4	7.0	7.6	5.7	—	5.6	<5	6.9	5.9	5.9	6.1
33	5-F	7-Cl	8.6	5.8	6.2	7.2	5.4	5.8	5.4	<5	6.9	6.4	5.9	6.0
34	7-F	7-F	9.4	6.0	6.6	8.1	5.7	6.0	5.9	<6	7.4	6.1	5.7	6.3
35	7-F	7-Cl	8.6	5.7	6.1	7.8	<5	<6	<5.3	5.1	7.4	5.6	5.5	5.7
36	8-F	7-F	8.4	6.5	7.1	7.2	<5	<5.1	<5	<5.2	7.1	<6	<5	5.4
37	8-F	7-Cl	8.0	6.2	6.2	7.2	5.5	5.5	5.6	<5	7.1	5.8	5.6	5.9

[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean.

[b] Receptors and radioligands used in binding assays: 5-$HT_{1A/1B/1D}$ receptors as above; 5-HT_{2A} (human cloned receptors in HEK 293 cells; [^{3}H]-ketanserin); 5-HT_{2B} (human cloned receptors in HEK 293 cells; [^{3}H]5-HT); 5-HT_{2C} (human cloned receptors in HEK 293 cells; [^{3}H]-mesulergine); 5-HT_6 (human cloned receptors in HeLa cells; [^{3}H]-LSD); 5-$HT_{7(a)}$ (human cloned receptors in HEK 293 cells; [^{3}H]-5-CT); D_2 (human cloned receptors in CHO cells; [^{125}I]-iodosulpiride); D_3 (human cloned receptors in CHO cells; [^{125}I]-iodosulpiride).

of monoamine receptors (Table 3) and generally were found to be highly selective, though modest affinity was seen for 5-HT_7 receptors.

A decision to further profile compounds **34** and **35** was based on their excellent 5-HT_{1A} and SerT activities, 100-fold selectivity over 5-HT_{1B} and 5-HT_{1D} receptors and promising PK profiles. The functional activity of compounds **34** and **35** was measured using GTPγS binding in HEK293 cells expressing 5-HT_{1A} receptors, with intrinsic activity (IA) being expressed relative to the 5-HT response (5-HT = 1).[9] Neither compound displayed 5-HT_{1A} receptor agonist activity, giving IA values of zero.

In vitro radioligand binding studies in native tissue from several species (rat, guinea pig, marmoset) with **34** and **35** showed nanomolar affinity ($pK_i > 9$) for the high agonist affinity state of 5-HT_{1A} receptors. A minimal difference was observed for the low agonist binding affinity state which is consistent with both compounds showing low intrinsic activity.

Ex vivo binding studies with both **34** and **35** showed a dose dependent displacement of both [^{3}H]WAY100635 and [^{3}H]8-OH DPAT binding in rat cortex. Approximately 50% inhibition of binding of both radioligands was observed with **34** after a 1 mg/kg oral dose, suggesting good in vivo occupancy of 5-HT_{1A} receptors. This was further supported in a 5-HT_{1A} receptor pharmacodynamic model[12] in which **35** attenuated 8-OH DPAT-induced locomotor activity (LMA) in rats with an ED_{50} of 3 mg/kg following oral dosing. There were no effects on LMA per se up to 10 mg/kg.

Ex vivo [^{3}H]5-HT uptake studies show that both compounds also inhibited uptake in rat cortical synaptosomes with an ED_{50} of 3–5 mg/kg, demonstrating similar in vivo occupancy of SerT.

Similarly, **34** potently inhibited 8-OH DPAT-induced LMA with an $ED_{50} < 1$ mg/kg, which is consistent with **34** showing a 10-fold greater affinity for 5-HT_{1A} receptors.

In conclusion, halogenation of both the quinoline and benzoxazinone rings present in **1** has led to the discovery of highly potent and dual-acting 5-HT_{1A} receptor antagonists and serotonin reuptake inhibitors. The dihalogenated analogues display an excellent selectivity profile over a range of monoamine receptors, in particular 5-HT_{1B} and 5-HT_{1D} subtypes, which can be attributed to halogen substitution at the quinoline 7-position. Halogen substitution also has a marked effect on the pharmacokinetic profile in the rat and has afforded compounds with improved metabolic stability and CNS penetration which were suitable for in vivo evaluation.

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