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3,4-Dihydro-2*H*-benzoxazinones are 5-HT$_{1A}$ receptor antagonists with potent 5-HT reuptake inhibitory activity

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Abstract—Starting from a high throughput screening hit, a series of 3,4-dihydro-2*H*-benzoxazinones has been identified with both high affinity for the 5-HT$_{1A}$ receptor and potent 5-HT reuptake inhibitory activity. The 5-(2-methyl)quinolinyloxy derivative combined high 5-HT$_{1A/1B/1D}$ receptor affinities with low intrinsic activity and potent inhibition of the 5-HT reuptake site (pK_i 8.2). This compound also had good oral bioavailability and brain penetration in the rat.

Abnormalities in brain serotonin (5-HT) neurotransmission are thought to be the underlying cause of mood disorders, such as depression and anxiety.[1] Synaptic 5-HT levels are under the control of 5-HT transporters (SerT), located presynaptically, and 5-HT autoreceptors that reside on cell bodies (5-HT$_{1A}$ receptor subtype) and on nerve terminals (5-HT$_{1B}$ and/or 5-HT$_{1D}$ receptor subtype).[2] Despite the success of selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression and anxiety, one potential downside is a long latency to therapeutic onset.[3] The lack of clinical efficacy following acute administration of SSRIs has been hypothesised to be due to indirect 5-HT$_{1A}$ autoreceptor activation resulting in an inhibitory effect on 5-HT cell firing and net synaptic 5-HT levels. Consequently, SSRIs only effectively elevate 5-HT levels following desensitisation of 5-HT$_{1A}$ autoreceptors after chronic dosing.[4] Therefore, concomitant blockade of 5-HT$_{1A}$ receptors should theoretically enhance 5-HT levels above that of an SSRI alone.[5] This has been confirmed in microdialysis studies which demonstrated that co-administration of the selective 5-HT$_{1A}$ antagonist WAY-100635 with an SSRI resulted in an immediate increase in central 5-HT levels.[6] Furthermore, in the rat High-Light Social Interaction model of anxiety, the onset of anxiolytic activity of paroxetine was accelerated from 21 to 7 days by co-administration with WAY-100635.[7] Therefore, a combined SSRI/5-HT$_{1A}$ receptor antagonist should have a reduced latency to onset of therapeutic effects relative to SSRIs.[5] This hypothesis is also supported by the recent meta-analysis of clinical trials data which suggests that the 5-HT$_{1A}$ autoreceptor antagonist (±)-pindolol hastens the therapeutic response to SSRIs.[8]

In order to identify compounds with such a dual profile, high throughput screening was carried out using a [^{35}S]-GTPγS binding assay with CHO cells expressing human

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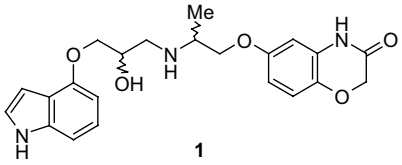
(h) 5-HT_{1A} receptors. Compounds with good antagonist potency were then tested for blockade of [^{3}H]-5-HT reuptake into rat cortical synaptosomes. From this approach, the diastereomeric mixture of 3,4-dihydro-2*H*-benzoxazinones **1**[9] was identified as a lead. This letter describes the optimisation of benzoxazinones related to **1**.



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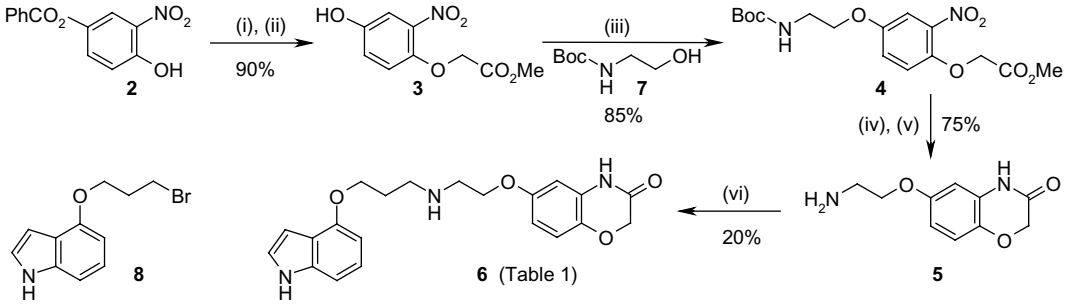
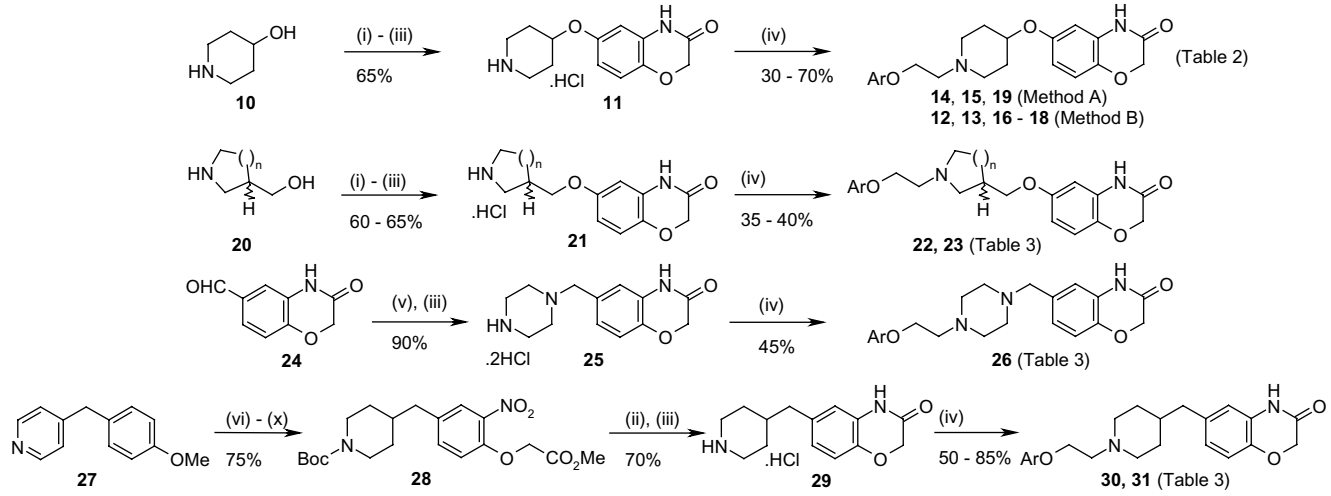
Novel compounds **6**, **12–19**, **22**, **23**, **26**, **30** and **31** were prepared according to Schemes 1 and 2. Alkylation of nitrophenol **2** with methyl bromoacetate was followed by debenzoylation using sodium methoxide in methanol to give **3** (Scheme 1), then Mitsonobu reaction with *N*-Boc-ethanolamine **7** gave **4**. Nitro group reduction and concomitant cyclisation was followed by acid-mediated removal of the *N*-Boc group to give **5**, which was alkylated with 4-(3-bromopropyloxy)indole[10] to give **6**, albeit in low yield. The remaining compounds were prepared according to Scheme 2. Phenol **3** underwent Mitsonobu reaction with *N*-Boc protected cyclic aminoalcohols **10** and **20**, then reductive cyclisation and Boc removal as described above gave benzoxazinones **11** and **21**, respectively. Improved yields for the final stage were obtained either by using the appropriate aryloxyethyl bromide[11] in the presence of excess diisopropylethylamine as base (Method A), or by employing reductive alkylation conditions using the appropriate aryloxyacetaldehyde[12] (Method B). The piperazine analogue **26** was prepared by reductive amination of aldehyde **24**[13] with 1-Boc-piperazine, followed by deprotection to give **25**, then subsequent alkylation according to Method A. Demethylation of **27** was followed by pyridine ring reduction, nitration, Boc protection and alkylation with methyl bromoacetate, to give nitroester **28**. Reductive cyclisation as previously described gave key intermediate **29** from which methylene-linked benzoxazinones **30** and **31** were prepared according to Method A. All final compounds were purified by chromatography and isolated as their hydrochloride salts.

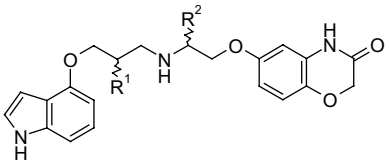


Scheme 1. Reagents and conditions: (i) $BrCH_2CO_2Me$, K_2CO_3, acetone, reflux, 18 h; (ii) NaOMe, MeOH, 20 °C, 1 h; (iii) **7**, Bu_3P, 1,1′-(azodicarbonyl)dipiperidine, 20 °C, 6 h; (iv) Pd–C, cyclohexene, EtOH, reflux, 2 h; (v) CF_3CO_2H, CH_2Cl_2, 20 °C, 18 h; (vi) **8**, $NaHCO_3$, EtOH, reflux, 20 h.



Scheme 2. Reagents and conditions: (i) **3**, Ph_3P, DIAD, THF, 20 °C, 18 h; (ii) H_2, Pd–C, 3 bar, 50 °C, 18 h; (iii) HCl, Et_2O, 2-propanol, reflux, 18 h; (iv) $ArOCH_2CH_2Br$, *i*-Pr_2NEt, 2-propanol, reflux, 48 h (Method A), OR $ArOCH_2CHO$, $NaBH(OAc)_3$, CH_2Cl_2, 20 °C, 18 h (Method B); (v) 1-Boc-piperazine, $NaBH(OAc)_3$, 20 °C, 18 h; (vi) 48% aq HBr, reflux, 8 h; (vii) H_2, PtO_2, H_2SO_4, MeOH, 20 °C, 18 h; (viii) HNO_3, AcOH, 20 °C, 2 h; (ix) $(Boc)_2O$, NEt_3, H_2O, THF, 20 °C, 18 h; (x) $BrCH_2CO_2Me$, K_2CO_3, acetone, reflux, 18 h.

Table 1. 5-HT_{1A} receptor binding affinity (pK_i), 5-HT_{1A} intrinsic activity (IA), SerT potency (pK_i) and β_2 adrenergic receptor binding affinity (pK_i): individual diastereoisomers of **1** (**1a**–**d**) and **6**[a]



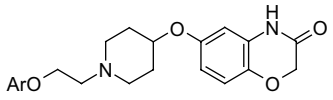
Compound[b]	R^1	R^2	5-HT_{1A}	IA	SerT	β_2[c]
1	OH	Me	9.1	0.3	7.3	9.2
1a	OH	Me	7.9	0.4	6.9	7.7
1b	OH	Me	8.7	0.3	7.1	8.6
1c	OH	Me	8.1	0.3	7.0	7.7
1d	OH	Me	9.3	0.2	6.5	8.8
6	H	H	8.8	0.6	7.1	6.0

[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean. IA values represent the mean of three experiments, each within 0.1 of the mean.
[b] All new compounds gave satisfactory analytical and/or mass spectral data.[15]
[c] Displacement of [^{125}I]-iodocyanopindolol from human cloned β_2 receptors expressed in CHO cells.

Compounds **1**, **6**, **12**–**19**, **22**, **23**, **26**, **30** and **31** were evaluated using displacement of [^{3}H]-8-OH-DPAT binding from h5-HT_{1A} receptors expressed in CHO cells, whilst potency for the serotonin transporter (SerT) was assessed by measuring the inhibition of reuptake of [^{3}H]-5-HT into rat cortical synaptosomes, with data expressed as pK_i values. Functional activity was measured using [^{35}S]-GTPγS binding in HEK293 cells expressing h5-HT_{1A} receptors, with intrinsic activity (IA) expressed relative to the 5-HT response (5-HT = 1). Data are shown in Tables 1–3.

Diastereomeric mixture **1** had high affinity in the 5-HT_{1A} receptor binding assay together with moderate SerT potency (Table 1), but also possessed high affinity for β_2 adrenergic receptors (pK_i 9.2). Separation of the four constituent diastereomers (**1a**–**d**) was carried out using chiral HPLC.[14] Encouragingly, 5-HT_{1A} and SerT activity did not reside in separate diastereomers, but β_2 activity unfortunately ran parallel with 5-HT_{1A} activity. The adrenergic activity is not surprising given the presence of an aryloxypropanolamine moiety in the molecules (cf. pindolol). Therefore, the simplified analogue **6** in which the hydroxy group is removed from the linker was prepared and found to retain both 5-HT_{1A} and SerT activity with greatly reduced β_2 affinity.

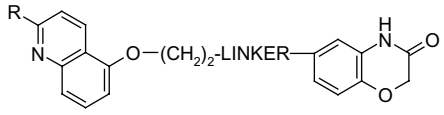
Table 2. 5-HT_{1A} receptor binding affinity (pK_i), 5-HT_{1A} intrinsic activity (IA) and SerT potency (pK_i): variation of LHS aryloxy moiety[a]

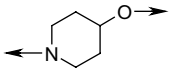
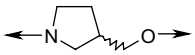
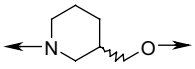
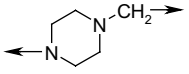
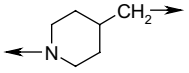
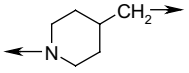


Compound[b]	Ar	5-HT_{1A}	IA	SerT
12	4-Indolyl	8.4	0.2	8.3
13	4-Indolyl(2-cyano)	8.8	0.3	8.1
14	1,2-Dihydrobenzo-[*b*]furanyl(2,2-dimethyl)	8.7	0.9	7.6
15	1-Naphthyl	8.6	0.4	7.0
16	1-Isoquinolinyl	8.1	0.8	7.3
17	4-Quinolinyl	7.0	ND	7.0
18	8-Quinolinyl	8.2	0.5	7.4
19	5-Quinolinyl	7.9	0.1	7.5

ND = not determined.
[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean. IA values represent the mean of three experiments, each within 0.1 of the mean.
[b] All new compounds gave satisfactory analytical and/or mass spectral data.[15]

Table 3. 5-HT_{1A} receptor binding affinity (pK_i), 5-HT_{1A} intrinsic activity (IA) and SerT potency (pK_i): 5-quinolinyloxy derivatives[a]



Compound[b]	R	Linker	5-HT_{1A}	IA	SerT
19	H		7.9	0.1	7.5
22	H		8.9	0.3	7.3
23	H		8.8	0.3	7.1
26	H		8.0	0.2	7.5
30	H		8.9	0.2	8.2
31	Me		9.5	0.2	8.2

[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean. IA values represent the mean of three experiments, each within 0.1 of the mean.
[b] All new compounds gave satisfactory analytical and/or mass spectral data.[15]

Although **6** had improved selectivity, this was at the cost of increased 5-HT_{1A} IA, and reduced SerT potency (target $pK_i \geqslant 8$). The effect of increased conformational constraint of the central basic linker was investigated, and the piperidinyloxy analogue **12** proved to be a key breakthrough (Table 2), exhibiting 5-HT_{1A} and SerT $pK_i > 8$ together with low 5-HT_{1A} IA. Although **12** showed >100-fold selectivity over β_2 adrenoceptors, it had <100-fold selectivity over a range of other monoamine receptors and poor in vitro metabolic stability in human and rat liver microsomes.[16] The SAR of the left-hand aryloxy moiety was next investigated, and from ca. 90 variations it was found that bicyclic aryl groups had the most promising profiles (**13**–**19**, Table 2). However, very few achieved $pK_i \geqslant 8$ at both 5-HT_{1A} and SerT, and most had similar or higher 5-HT_{1A} IA than **12**. 2-Cyanoindolyl **13** had $pK_i > 8$ at both target sites, but had high β_2 adrenoceptor binding

affinity (pK_i 7.3). Dihydrobenzofuran **14** had excellent 5-HT_{1A} affinity but very high IA. The encouraging profile of 1-naphthyl **15** prompted investigation of isoquinolinyl and quinolinyl derivatives **16**–**19**. 5-Quinolinyloxy **19** was an important lead, having the lowest 5-HT_{1A} IA. Consequently, replacement of the piperidinyloxy linker moiety by a range of alternative basic linkers was then investigated in the 5-quinolinyloxy series (Table 3).

Molecular modelling suggested that the 3-pyrrolidinylmethyl **22** and 3-piperidinylmethyl **23** would overlap with 4-piperidinyl **19**. Both compounds had improved 5-HT_{1A} affinity compared to **19** but with slightly higher IA. 4-Piperazinylmethyl **26** had a similar in vitro profile to **19**, but 4-piperidinylmethyl **30** represented a significant advance, having pK_i 8.9 at 5-HT_{1A} with low IA and, importantly, pK_i > 8 at the reuptake site. Encouragingly, microsomal metabolic stability of **30** was improved relative to **12** and, in a rat steady-state CNS penetration assay,[17] **30** showed good brain penetration (Brain:Blood = 0.8:1). However, the compound was rapidly cleared (CLb 115 mL/min/kg). In an attempt to rationalise this observation, the in vitro metabolic stability of **30** was determined in rat and human liver S9 fraction.[16] Intrinsic clearance was high (rat CLi 6 mL/min/g, human CLi 42 mL/min/g), suggesting the possible involvement of aldehyde oxidase (AO) in the metabolism of **30**. It has been previously shown that AO can oxidise quinolinyl groups at C-2,[16] so the corresponding 5-(2-methyl)quinolinyl derivative **31** (SB-649915) was prepared in which this potential site of metabolism is blocked. Gratifyingly, this compound was apparently stable to aldehyde oxidase (rat and human S9 CLi <0.6 mL/min/g) and had significantly reduced clearance in a rat PK study (CLb 42 mL/min/kg). The oral bioavailability of **31** was estimated at 45% and Brain:Blood ratio at steady-state was 0.4:1. In addition **31** had excellent 5-HT_{1A}/SerT activity (pK_i 9.5 and 8.2, respectively) and low 5-HT_{1A} IA (0.2).

Compound **31** was further profiled against a range of monoamine receptors and transporters (5-HT, noradrenergic and dopaminergic) and was found to have significant affinity for 5-HT_{1B} and 5-HT_{1D} receptors (pK_i 8.1 and 8.7, respectively).[18] Selectivity was otherwise excellent, with p$K_i \leqslant 6.3$ at all other receptors and transporters tested.

Starting from screening hit **1**, which had high 5-HT_{1A} receptor affinity, moderate SerT potency and poor selectivity versus β_2 adrenergic receptors, compound **31** (SB-649915) has been identified as a high affinity 5-HT_{1A} antagonist combined with potent 5-HT reuptake inhibitory activity. Compound **31** has low 5-HT_{1A} intrinsic activity in the [^{35}S]-GTPγS binding assay using HEK293 cells expressing the h5-HT_{1A} receptor and has been shown to have no intrinsic activity as measured by GTPγS autoradiography[19] in a human dorsal raphe nucleus preparation. Compound **31** has an excellent selectivity profile, together with good oral bioavailability and brain penetration in the rat, and therefore represents a useful compound for the further in vivo investigation of the role of enhanced 5-HT transmission in the treatment of depression and anxiety.

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10. Prepared by treatment of 4-hydroxyindole with sodium hydride in *N*,*N*-dimethylformamide and subsequent reaction with 1,3-dibromopropane at 20 °C.
11. Prepared from the corresponding phenol by treatment with excess 1,2-dibromoethane and potassium carbonate in 2-butanone at reflux.
12. Intermediates for **12** and **13** were prepared according to the method described in: Sasai, H.; Yamada, Y. M. A.; Suzuki, T.; Shibasaki, M. *Tetrahedron* **1994**, *50*, 12313–12318. Intermediates for **16** and **17** were prepared from 1-chloroisoquinoline and 4-chloroquinoline, respectively, by reaction with the sodium salt of 2-hydroxyacetaldehyde dimethylacetal in *N*,*N*-dimethylformamide and subsequent acid hydrolysis. For **18**, (8-quinolinyloxy)-acetaldehyde was prepared by oxidative cleavage of 8-allyloxyquinoline with sodium periodate in the presence of osmium tetroxide.
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14. HPLC method: Chiralpak AD, 50/50/0.1 hexane/ethanol/triethylamine.
15. ^{1}H NMR spectra were recorded at 250 MHz in $CDCl_3$ as solvent. Compound **31**, mp 207–208 °C (dihydrochloride salt); δ_H 1.25–1.38 (2H, m), 1.49 (1H, m), 1.65 (2H, m), 2.14 (2H, m), 2.45 (2H, d, J = 7 Hz), 2.72 (3H, s), 2.94 (2H, t, J = 6 Hz), 3.05 (2H, m), 4.27 (2H, t, J = 6 Hz), 4.58 (2H, s), 6.56 (1H, d, J = 2 Hz), 6.73 (1H, dd, J = 7, 2 Hz), 6.78 (1H, d, J = 8 Hz), 7.86 (1H, d, J = 7 Hz), 7.23 (1H, d, J = 8 Hz), 7.50–7.64 (2H, m), 8.42 (1H, d, J = 8 Hz), 8.75 (1H, br s) (free base).
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17. CNS penetration at steady-state in rat. Compounds were dissolved in 2% (v/v) DMSO in 5% (w/v) dextrose aq and administered at a constant infusion rate over 12 h at a target dose rate of 0.3 mg free base/kg/h. Blood

samples were removed during the latter part of the infusion to confirm steady-state blood concentrations. Blood and brain samples were analysed by LC/MS/MS.
18. Displacement of [^{3}H]-5-HT from human cloned 5-HT_{1B} and 5-HT_{1D} receptors expressed in CHO cells.
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