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6-[2-(4-Aryl-1-piperazinyl)ethyl]-2*H*-1,4-benzoxazin-3(4*H*)-ones: Dual-acting 5-HT$_1$ receptor antagonists and serotonin reuptake inhibitors

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ABSTRACT

Investigation of a series 6-[2-(4-aryl-1-piperazinyl)ethyl]-2*H*-1,4-benzoxazin-3(4*H*)-ones has led to the discovery of potent 5-HT$_{1A/1B/1D}$ receptor antagonists with and without additional SerT affinity. Modulation of the different target activities gave compounds with a range of profiles suitable for further in vivo characterization.

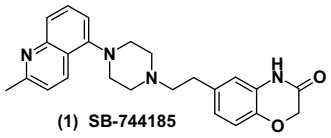
Over the past decades, a large body of pre-clinical and clinical evidence has confirmed a link between extracellular levels of serotonin (5-HT) and a number of psychiatric indications, including anxiety and depression.[1] In fact, the most effective antidepressant agents in current clinical use are the selective serotonin reuptake inhibitors (SSRIs) which inhibit serotonin transporters (SerT) and consequently elevate synaptic 5-HT levels.[2] Despite the success of SSRIs, one undesirable characteristic is a long latency to therapeutic onset which is hypothesized to be due to the requirement for desensitisation of 5-HT$_1$ autoreceptors to maintain increased 5-HT levels.[3]

5-HT$_1$ autoreceptors are located on both cell bodies (5-HT$_{1A}$ and 5-HT$_{1D}$ receptor subtypes) and nerve terminals (5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes). They are widely distributed in the brain and in addition to SerT are known to have a major role in the control of synaptic 5-HT levels.[4] For instance neurochemical studies have demonstrated that acute co-administration of SSRIs with 5-HT$_{1A}$ and/or 5-HT$_{1B/1D}$ receptor antagonists gave a greater increase in extracellular brain 5-HT levels with respect to SSRIs alone.[4,5] Interestingly, microdialysis studies also revealed that concomitant blockade of 5-HT$_1$ autoreceptors in the absence of a SerT component resulted in an immediate increase in central 5-HT levels.[5]

From the above, it follows that blockade of 5-HT$_{1A/1B/1D}$ autoreceptors, with or without concomitant SerT inhibition, should rapidly increase brain 5-HT to therapeutically effective levels and provide a fast onset of antidepressant/anxiolytic action relative to current therapies. This hypothesis is supported by a meta-analysis of clinical trials data which suggested that the 5-HT$_{1A}$ autoreceptor antagonist (±)-pindolol hastens the therapeutic response to SSRIs.[6]

Previous publications from this group have described the development of novel compounds containing the 5-(piperidinylethyloxy)quinoline and benzoxazinone units as both mixed SerT/5-HT$_{1A/1B/1D}$ receptor antagonists or 'selective' 5-HT$_{1A/1B/1D}$ receptor antagonists.[7] We also recently disclosed a new class of quinolinyl-piperazinyl ethylbenzoxazinones, in particular SB-744185 (**1**), as a highly potent, orally bioavailable and brain penetrant mixed SSRI-5-HT$_{1A/1B/1D}$ receptor antagonists (Fig. 1).[8]

This letter reports further exploration of the structure–activity relationships (SAR) in this novel series. In one aspect, we sought



(1) SB-744185

Figure 1. Structure of SB-744185 (**1**) a dual 5-HT$_{1A/1B/1D}$ receptor antagonist and SerT inhibitor.

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to modulate the different target activities in order to identify 5-$HT_{1A/1B/1D}$ receptor antagonists with varying levels of selectivity between the different 5-HT_1 autoreceptors and the serotonin transporter for detailed comparison of their in vivo profiles in terms of efficacy versus side-effects.

The novel compounds[9] (Table 1) were prepared as described below. The N-methylated analogue **2** was prepared by alkylation of **1** with MeI using sodium hydride in DMF in 55% yield. The ethyl analogue **3** was prepared by N-alkylation of 6-(2-chloroethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one with ethyl iodide followed by coupling with 2-methyl-5-(1-piperazinyl)quinoline.

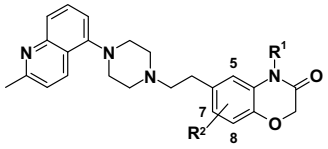
The 8- and 7-fluoro analogues **4** and **5** were prepared according to Scheme 1 from the key appropriately substituted fluorobenzoxazinone **24**. The 7-fluorobenzoxazinone **24b** was commercially available, while the corresponding 8-fluorobenzoxazinone **24a** was prepared by alkylation of 2-fluoro-6-nitrophenol **22** with methyl bromoacetate to give **23** which was then subject to nitro reduction and concomitant cyclization. The 8- or 7-fluorobenzoxazinone **24** was converted to **25** by Friedel–Crafts acylation with chloroacetyl chloride followed by carbonyl reduction to give the key chloroethyl derivative **26** which was alkylated with 2-methyl-5-(1-piperazinyl)quinoline.

The 5-fluoro analogue **6** was prepared as shown in Scheme 2. Thus, treatment of chloro-4-fluoro-2-methoxybenzene **27** with chloroacetyl chloride and aluminum chloride gave acylation and concomitant O-demethylation to afford the phenol ethanone **28**. Carbonyl reduction to **29**, followed by nitration to **30** and subsequent hydrogenation gave the amino phenol **31** which was acylated with chloroacetyl chloride and cyclized to the key chloroethyl 5-F-benzoxazinone **32** which was finally alkylated with 2-methyl-5-(1-piperazinyl)-quinoline to afford **6**.

The analogues **7**–**10** (Table 2) containing piperazine modification were prepared by alkylation of the appropriately substituted 2-methyl-5-piperazinyl-quinolines with 6-(2-chloroethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one. The 2-methyl-5-piperazinyl-quinolines were themselves prepared by Buchwald amination of 2-methylquinolin-5-yl trifluoromethanesulfonate with the appropriate unsubstituted or mono BOC protected piperazine. Substituted quinolines **11**–**16** (Table 3) were similarly prepared via triflation of the appropriately substituted 2-methyl-5-hydroxyquinoline and Buchwald amination with BOC-piperazine.[10] Compounds **17**–**21** (Table 4) were prepared as above by alkylation of the appropriate commercially available or known arylpiperazines with 6-(2-chloroethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one.

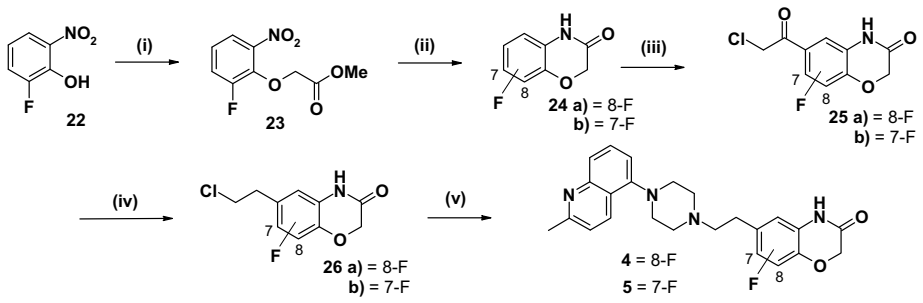
The affinities of the compounds for h5-HT_{1A}, h5-HT_{1B}, and h5-HT_{1D} receptors, stably expressed in CHO cells, were evaluated by displacement binding using [^{3}H]-WAY100635 (5-HT_{1A}) and [^{3}H]-5-HT (5-HT_{1B} and 5-HT_{1D}), respectively, as radioligands. The affinities of the compounds for the reuptake site of the hSerT, stably expressed in epithelial pig kidney (LLCPK) cells, were assessed by displacement of [^{3}H]-citalopram binding.[9] The intrinsic activities

Table 1
Binding affinities (pK_i[a]) for human 5-$HT_{1A/1B/1D}$ and SerT and intrinsic activity (IA): substitution of the benzoxazinone[b]

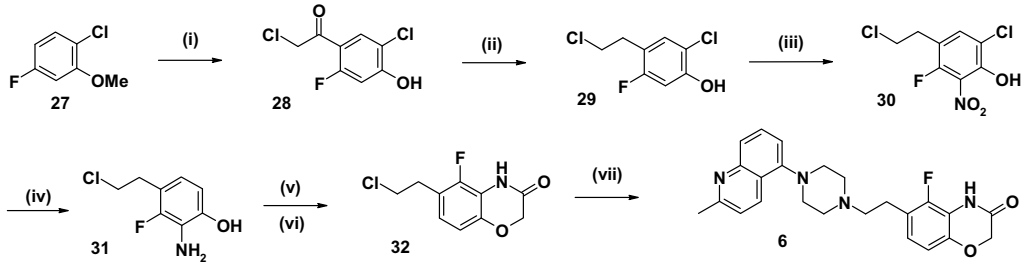


Compound	R^1	R^2	pK_i			
			5-HT_{1A} (IA)	5-HT_{1B} (IA)	5-HT_{1D} (IA)	SerT
1	H	H	9.6 (0.2)	9.3 (0.1)	9.8 (0.0)	8.8
2	Me	H	9.4 (0.3)	9.1 (0.2)	9.5 (0.0)	7.7
3	Et	H	9.3 (0.0)	9.0 (0.0)	9.4 (0.0)	7.1
4	H	8-F	8.9 (0.0)	8.8 (0.3)	9.7 (0.0)	8.9
5	H	7-F	9.6 (0.2)	9.4 (0.1)	9.6 (0.0)	9.1
6	H	5-F	9.6 (0.3)	8.9 (0.2)	9.4 (0.0)	8.3

[a] Each pK_i determination lies within 0.3 log units of the mean with a minimum of 3 replicates.
[b] Compounds were characterized and purity assessed using ^{1}H NMR and LC–MS.

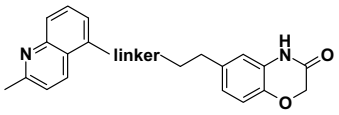


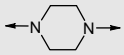
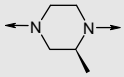
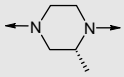
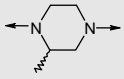
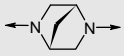
Scheme 1. Reagents and conditions: (i) $BrCH_2CO_2Me$, K_2CO_3, acetone, reflux, 5 h (93%); (ii) iron powder, acetic acid, rt, 14 h (71%); (iii) $ClCH_2COCl$, $AlCl_3$, 80 °C, 5 h (50–65%); (iv) triethylsilane, TFA, 0 °C to rt, overnight (72–91%); (v) 2-methyl-5-(1-piperazinyl)quinoline, NaI/Na_2CO_3, NMP, 120 °C, 12 h (24–43%).



Scheme 2. Reagents and conditions: (i) $ClCH_2COCl$, $AlCl_3$, 70 °C, 3 h (38%); (ii) triethylsilane, TFA, 0 °C to rt, 2 h (42%); (iii) $cHNO_3$, glacial acetic acid, 0 °C, 45 min (70%); (iv) H_2, 10% Pd/C, EtOH, 1 atmosphere/rt, 24 h (100%); (v) $ClCH_2COCl$, $NaHCO_3$, THF, 0 °C, 30 min; (vi) K_2CO_3, butan-2-one, reflux, 1 h (71%); (vii) 2-methyl-5-(1-piperazinyl)quinoline, NaI/Na_2CO_3, NMP, 120 °C, 3 h (46%).

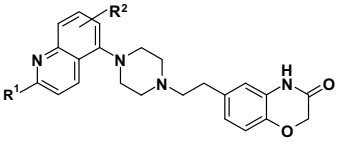
Table 2
Binding affinities (pK_i[a]) for human 5-$HT_{1A/1B/1D}$ and SerT and intrinsic activity (IA): substitution of the piperazine ring[a]



Compound	Linker	pK_i			
		5-HT_{1A}[d] (IA)[e]	5-HT_{1B} (IA)	5-HT_{1D} (IA)	SerT
1		9.6 (0.2)	9.3 (0.1)	9.8 (0.0)	8.8
7		8.5 (0.0)	8.1 (0.0)	8.8 (0.0)	9.4
8		8.8 (0.0)	8.7 (0.0)	9.3 (0.0)	9.1
9		9.1 (0.2)	9.1 (0.1)	9.5 (0.0)	8.0
10		6.9 (0.8)	6.9 (0.2)	7.6 (0.0)	8.4

[a] See Table 1.

Table 3
Binding affinities (pK_i[a]) for human 5-$HT_{1A/1B/1D}$ and SerT and intrinsic activity (IA): substitution of the quinoline ring[a]



Compound	R^1	R^2	pK_i			
			5-HT_{1A}[d] (IA)[e]	5-HT_{1B} (IA)	5-HT_{1D} (IA)	SerT
1	Me	H	9.6 (0.2)	9.3 (0.1)	9.8 (0.0)	8.8
11	Me	8-F	8.4 (0.2)	8.6 (0.0)	9.5 (0.0)	8.4
12	Me	8-Cl	8.6 (0.7)	9.3 (0.3)	9.7 (0.0)	8.8
13	Me	7-F	9.5 (0.0)	9.2 (0.0)	9.7 (0.0)	9.1
14	Me	7-Cl	9.9 (0.4)	9.5 (0.0)	9.8 (0.0)	9.1
15	Me	7-Me	9.2 (1.2)	9.1 (0.3)	9.4 (0.3)	7.8
16	Ph	H	7.0 (0.4)	7.5 (0.2)	7.9 (0.0)	9.8

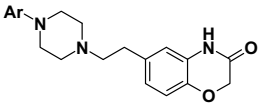
[a] See Table 1.

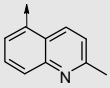
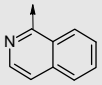
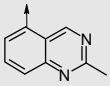
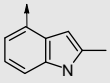
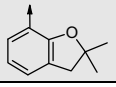
of the compounds at h5-HT_{1A}, h5-HT_{1B} and h5-HT_{1D} receptors were determined using a [^{35}S]-GTPγS binding assay, with data reported relative to the maximum response elicited by 5-HT.[9]

The initial focus of this work centered on exploration of the benzoxazinone moiety (Table 1). Our previous studies[7] suggested the presence of the secondary lactam N–H is crucial for SerT activity and so the alkylated analogues **2** and **3** were prepared. N-substitution with methyl or ethyl maintained the impressive sub-nanomolar 5-$HT_{1A/1B/1D}$ receptor affinities and low intrinsic activities with the anticipated reduction in SerT affinities relative to **1** resulting in at least 25- and 80-fold selectivity for 5-$HT_{1A/1B/1D}$ receptors over SerT for **2** and **3**, respectively. The effects of introducing fluoro substituents into available positions on the benzoxazinone aromatic ring was also explored (Table 1). While the 8-fluoro **4** and 7-fluoro **5** analogues had profiles almost identical to **1**, the 5-fluoro analogue **6** maintained high 5-$HT_{1A/1B/1D}$ receptor affinities with a modest reduction in SerT.

A limited exploration of the effects of substitution of the piperazine was also undertaken (Table 2). 3-Methyl substitution (**7** and **8**)

Table 4
Binding affinities (pK_i[a]) for human 5-$HT_{1A/1B/1D}$ and SerT and intrinsic activity (IA): replacement of the quinoline ring[a]



Compound	Ar	pK_i			
		5-HT_{1A}[d] (IA)[e]	5-HT_{1B} (IA)	5-HT_{1D} (IA)	SerT
1		9.6 (0.2)	9.3 (0.1)	9.8 (0.0)	8.8
17		8.8 (1.0)	9.2 (0.5)	9.6 (0.4)	9.1
18		9.4 (0.8)	9.4 (0.7)	9.6 (0.7)	8.0
19		8.5 (0.6)	9.2 (0.2)	9.5 (0.0)	8.7
20		9.3 (0.3)	9.4 (0.4)	9.7 (0.4)	8.2
21		9.7 (1.2)	9.2 (0.6)	9.8 (0.5)	8.6

[a] See Table 1.

Table 5
Rat pharmacokinetic profile for compounds **1**, **2**, and **19**

Compound	Cli rat; human[a] ml/min/g liver	CLb[b] ml/min/kg	Vss (L/kg)	$t_{1/2}$ (h)	Fpo (%)	Br:Bl
1	1.3; <0.5	5	2.0	5.1	68	1.6
2	0.8; 1.1	8	2.7	4.4	59	2.2
19	ND	4	1.1	2.8	67	0.3

[a] Intrinsic clearance in liver microsomes.
[b] In vivo data determined by 0.5 mg/kg iv and 1 mg/kg po administration in rat

reduced 5-$HT_{1A/1B/1D}$ affinities by about an order of magnitude while SerT affinity was maintained or even increased. The data suggest the (*S*)-enantiomer **7** has somewhat higher affinity for SerT than 5-$HT_{1A/1B/1D}$ receptors compared to the (*R*)-enantiomer **8**. In contrast, the racemic 2-methyl analogue **9** showed reduced SerT affinity relative to **1** but maintained sub-nanomolar 5-$HT_{1A/1B/1D}$ target affinities combined with low intrinsic activities. The bridged piperazine **10** suffered a drop in all target activities in particular for 5-$HT_{1A/1B/1D}$.

Introduction of substituents into the quinoline ring also revealed a range of interesting profiles and subtle effects on intrinsic activity (Table 3). 8-Fluoro substitution **11** afforded ~10-fold drop in 5-$HT_{1A/1B}$ affinities but maintained 5-HT_{1D} and SerT affinities. The corresponding 8-chloro analogue **12** had a similar profile but with increased intrinsic activity at 5-HT_{1A} indicative of partial agonism in this particular recombinant receptor system. In contrast, the 7-fluoro substitution **13** maintained high and balanced affinities across all four targets with zero intrinsic activities. The 7-chloro analogue **14** gave the highest 5-HT_{1A} affinity yet observed, but again accompanied by increased 5-HT_{1A} intrinsic activity. Interestingly, the corresponding 7-methyl analogue **15** showed a similar 5-$HT_{1A/1B/1D}$ affinity profile but even higher 5-HT_{1A} intrinsic

activity (IA = 1.2, i.e., full agonist) and a 10-fold drop in SerT affinity. Replacing the 2-methyl quinoline substituent with phenyl **16** gave the highest SerT activity yet achieved in conjunction with a dramatic drop in 5-$HT_{1A/1B/1D}$ affinities (~100-fold selectivity for SerT).

A selection of aryl replacements for the 5-quinoline were also introduced (Table 4). In general 5-$HT_{1A/1B/1D}$ sub-nanomolar affinities were maintained with good SerT affinity. Notably, significant increases in intrinsic activity at all three receptors were observed, particularly for 5-HT_{1A}, highlighting once again strict structural requirements for maintaining low intrinsic activity.

Having successfully identified compounds with a range of pharmacological profiles across 5-HT_1 autoreceptors and SerT, their suitability for progression to in vivo studies was investigated in rat pharmacokinetic studies. Several compounds such as **2** and **19** demonstrated good oral bioavailability combined with reasonable half-lives and high brain exposure (Table 5).

In conclusion, exploration of SAR of a series 6-[2-(4-aryl-1-piperazinyl)ethyl]-2*H*-1,4-benzoxazin-3(4*H*)-ones led to the discovery of highly potent 5-$HT_{1A/1B/1D}$ receptor antagonists both with and without additional SerT activity. Modulation of the balance between the different target affinities allowed us to identify tool compounds for in vivo comparison of efficacy versus side-effect profiles with the ultimate aim to identify faster acting antidepressants with reduced side-effect burden.

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