Bioorganic & Medicinal Chemistry Letters 18 (2008) 5581–5585

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel 5-HT$_{1A/1B/1D}$ receptors antagonists with potent 5-HT reuptake inhibitory activity

Halina T. Serafinowska [a,*], Frank E. Blaney [b], Peter J. Lovell [a], Giancarlo G. Merlo [c], Claire M. Scott [a], Paul W. Smith [a], Kathryn R. Starr [a], Jeannette M. Watson [a]

[a] Neurosciences CEDD, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[b] Department of Computational and Structural Chemistry, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[c] Neurosciences CEDD, GlaxoSmithKline, Medicines Research Centre, GlaxoSmithKline, Via A. Fleming 4, Verona 37135, Italy

ARTICLE INFO

Article history:
Received 1 July 2008
Revised 8 August 2008
Accepted 29 August 2008
Available online 3 September 2008

Keywords:
Potent 5-HT$_{1A/1B/1D}$ receptors antagonists
Potent 5-HT reuptake inhibitory activity
6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2*H*-1,4-benzoxazin-3(4*H*)-one
5-HT transporter model
5-HT$_{1A}$ receptor model

ABSTRACT

Novel 2-methyl-5-quinolinyl-1-piperazinylalkyl-3,4-dihydro-2*H*-1,4-benzoxazin-3-ones showing high affinities for the 5-HT$_{1A/1B/1D}$ receptors coupled with potent 5-HT reuptake inhibitory activity have been discovered. This is the first report describing docking of the lead compound 6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2*H*-1,4-benzoxazin-3(4*H*)-one **1**, into a model of the 5-HT transporter and the 5-HT$_{1A}$ receptor model.

One of the major drawbacks of selective serotonin reuptake inhibitors (SSRIs) in the pharmacological treatment of depression is latency in the onset of clinically meaningful effects. It has been hypothesized that this is associated with 5-HT$_{1A}$, and possibly 5-HT$_{1B}$, autoreceptor activation resulting in a negative control of serotonergic neurotransmission.[1,2] Consequently, prolonged dosing of an SSRI is required to desensitize the 5-HT$_1$ autoreceptors and to bring about restoration of serotonergic firing rate. A number of strategies have been investigated to address this issue.[3] In particular, approaches based on inhibition of serotonin reuptake in conjunction with antagonism of 5-HT$_1$ autoreceptors offer advantages over the current antidepressants in terms of a faster onset of therapeutic effect and improved efficacy. This is supported by studies demonstrating that the 5-HT$_{1A}$ antagonism enhances the antidepressant-like effects of SSRIs in animal models of social interaction, resident-intrude and schedule-induced polydipsia.[4–6]

In addition, clinical trials using a 5-HT$_{1A}$ receptor ligand pindolol in combination with SSRIs, suggest that improvements in the latency to therapeutic onset can be achieved.[7–9]

The terminal 5-HT$_{1B}$ receptors also control 5-HT release into the synapse and their blockade has been shown to enhance the effects of SSRIs on serotonin levels in vivo.[10–12]

The role of central 5-HT$_{1D}$ receptors is less documented, although, various reports on combined 5-HT$_{1D}$ antagonist/5-HT reuptake inhibitors provide increased evidence that they act as presynaptic autoreceptors.[13]

We have recently described a potent serotonin reuptake inhibitor **2** (SB-649915) incorporating 5-HT$_{1A/1B/1D}$ autoreceptors antagonist properties.[14,15] SB-649915 was found active in animal models of anxiety, substantially reducing the latency to onset of anxiolytic activity.[16] Here, we wish to report further studies in this series of compounds which have led to the discovery of molecule **1** displaying a highly increased activity at 5-HT$_1$ autoreceptors[17] coupled with potent inhibition of the 5-HT reuptake site.

Our efforts were focused on modifications of the central 2-ethoxypiperidine linker in **2** (Fig. 1) to investigate a correlation between the potency at the 5-HT reuptake site and affinity for 5-HT$_{1A/1B/1D}$ receptors. The potential binding mode of **2** in a 5-HT$_{1A}$ receptor model has been previously described.[18] While the primary binding site of the compound's basic nitrogen is assumed to be the highly conserved aspartate on TM3, the models did suggest that movement of this aspartate was possible and that some alternative binding subpockets were accessible which might better

* Corresponding author. Tel.: +44 1279 627683; fax: +44 1279 622790.
E-mail address: halina.serafinowska@gsk.com (H.T. Serafinowska).

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doi:10.1016/j.bmcl.2008.08.110

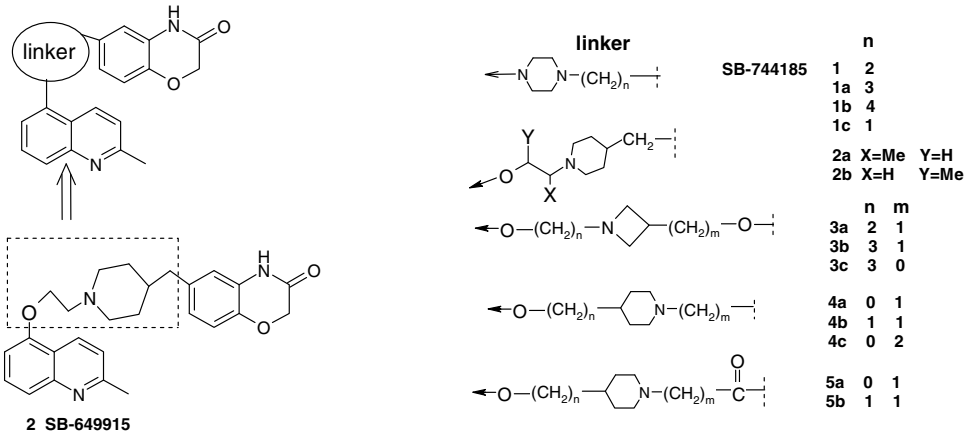


Figure 1. Linker replacements investigated.

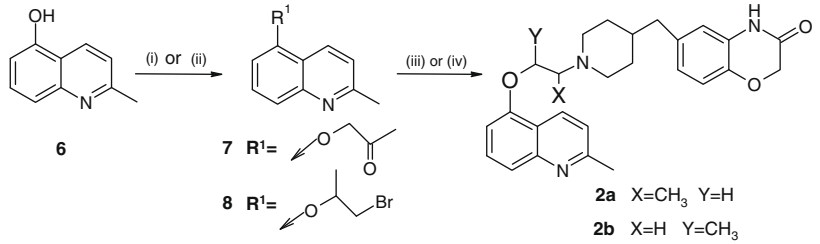
accommodate alternative linker lengths and conformations. Our desire to increase affinity across all three $5HT_1$ autoreceptor subtypes, also suggested that this could be best achieved by binding the ligands into pockets where the residues were conserved across these subtypes. To help our understanding of the interactions of the compounds with the 5-HT reuptake site, a model of the transporter protein was built based on its homology with the leucine bacterial transporter from aquifex aeolicus.[19]

A set of targets was designed to probe the binding pockets with a range of alternative linkers and to identify structural features ensuring high affinity at $5\text{-}HT_1$ sites and the 5-HT reuptake site.

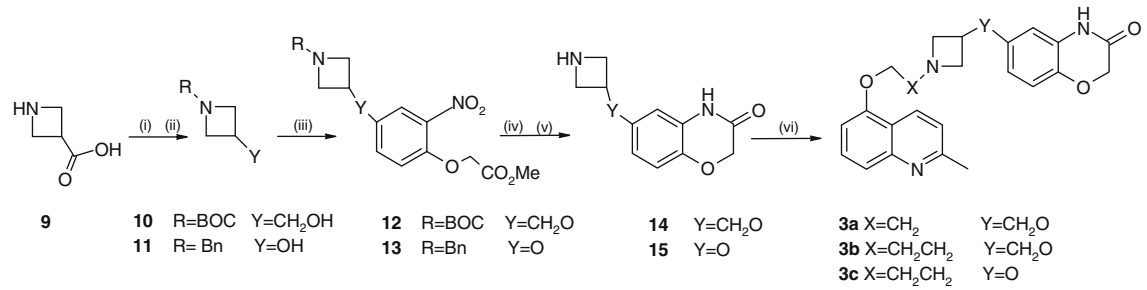
The initial study targeted analogues of **2**, substituted with a methyl group at the 1-, or 2-position of the 2-ethoxy sidechain (Scheme 1). Our previous data[15] suggested that similar structural manipulations were beneficial to affinity for the $5\text{-}HT_{1A}$ receptor. Compound **2a** was prepared by alkylation of 2-methyl-5-hydroxyquinoline **6** with 1-bromo-2,2-dimethoxypropane followed by reductive amination of the resulting ketone **7** with 6-(4-piperidinylmethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one.[15] The enantiomers of **2a** were separated by chiral chromatography but their absolute stereochemistry was not determined.

The isomeric 2-substituted analogue **2b** was constructed by coupling of **6** with 1-bromo-2-propanol to give compound **8** which in turn was used in alkylation of 6-(4-piperidinylmethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one.[15]

We also examined a 1,3-di-substituted azetidine system as a replacement of the piperidine moiety in **2** (Scheme 2). The synthesis of compounds **3a–c** involved transformation of 3-azetidinecarboxylic acid **9** into 3-hydroxymethylazetidine **10**, followed by a Mitsunobu reaction of **10** or **11**[20] with methyl([(4-hydroxy-2-nitrophenyl)oxy]acetate to give products **12** and **13**. Subsequent cyclisation of **12** or **13** to the corresponding 3,4-dihydro-2*H*-benzoxazinone was effected with Pd/C and ammonium formate. Deprotection of the azetidine nitrogen and



Scheme 1. Reagents and conditions: (i) 1-bromo-2,2-dimethoxypropane, K_2CO_3, 2-butanone, 100 °C (53%); (ii) Ph_3P, DIAD, 1-bromo-2-propanol, THF, rt (16%); (iii) sodium triacetoxyborohydride, DCE, acetic acid, 6-(4-piperidinylmethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one, rt (29%); (iv), $^iPr_2EtN$, 6-(4-piperidinylmethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one, isopropanol (9%).



Scheme 2. Reagents and conditions: (i) $(BOC)_2O$, THF, NaOH aq, K_2CO_3 aq; (ii) EtOCOCl, Et_3N, $LiBH_4$, THF (45% over two steps, **10**); (iii) Ph_3P, DIAD, methyl-[(4-hydroxy-2-nitrophenyl)oxy]acetate, rt (30–62%); (iv) Pd/C, NH_4CO_2H, MeOH, rt (88–92%); (v) 1 N HCl/Et_2O, MeOH or Pd/C, H_2, (90% for **14**; crude product-**15**); (vi) 2-methyl-5-bromoethoxyquinoline or 2-methyl-5-bromopropoxyquinoline, $^iPr_2EtN$, isopropanol, reflux, 17 h (33–38% for **3a–b**; low yield for **3c**).

alkylation of the resulting intermediates **14** and **15** with 2-methyl-5-bromoalkoxyquinolines[15] furnished target compounds **3a–c** in 33–38% overall yield.

To explore further the chemical space occupied by the basic nitrogen, a selection of piperidine and piperazine templates was investigated (Scheme 3). The key intermediates, quinolines linked to the 4-position of a piperidine moiety via an oxygen atom (**17**) or a methyloxy group (**18**) were prepared from **6** by a sequence of coupling, displacement and deprotection methods.

Subsequent reductive alkylation of **17** or **18** with 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-6-carbaldehyde provided target compounds **4a**, **4b**. Reaction of **17** with mesylate **27**, which was prepared as shown in Scheme 4, gave **23**. Cyclisation of **23** to the corresponding 3,4-dihydro-2*H*-benzoxazinone **4c** was achieved by reduction of the 2-nitro group (Scheme 3).

Alkylation of **17** or **18** with 6-chloroacetyl-3,4-dihydro-2*H*-benzoxazinone afforded the keto analogues **5a**, **5b**.

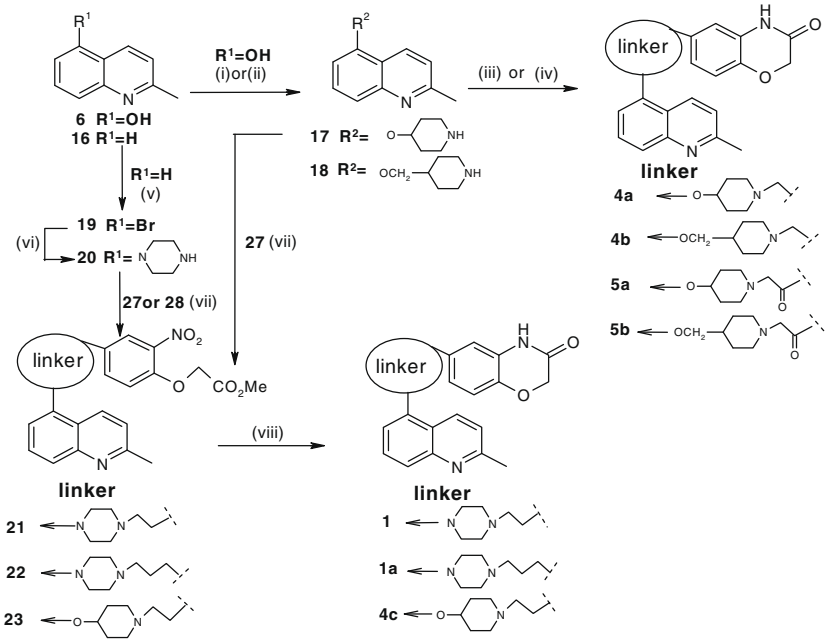
The synthesis of title compounds **1** and **1a** entailed a prior preparation of 5-piperazine-2-methylquinoline **20**. Thus, reaction of **16** with bromine in the presence of aluminium chloride gave the desired 5-bromo isomer **19** in 30% yield. A palladium catalysed cross-coupling of **19** with piperazine afforded intermediate **20** which was then converted into **1** or **1a** employing the synthetic sequence described already for the preparation of **4c**.

Mesylates **27** and **28**, used in the displacement reactions were obtained by a three-step synthesis (Scheme 4) which involved a cross-coupling reaction[21] of **24** with tri-*n*-butylvinyltin or tri-

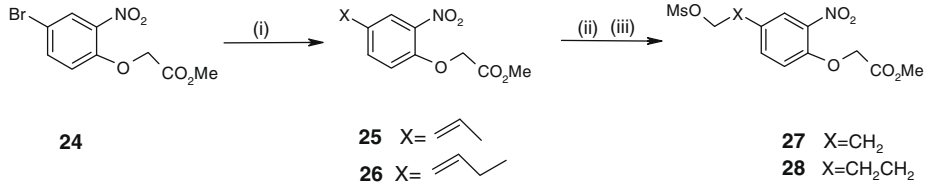
Table 1
5-HT_1 receptor binding[a,b] affinities, SerT potency and IA[d]

Compound[c]	Affinity (pK_i)				IA 1A/1B/1D[d]
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	SerT	
1	9.6	9.3	9.7	8.4	0.2/0.1/0
1a	8.6	7.3	8.0	7.6	0/0.1/0
1b[22]	9.2	8.2	7.9	8.2	
1c[22]	6.2	<5.0	6.9	6.1	
2	8.6	8.0	8.8	8.1	0.1/0.5/0.5
2a[f]	7.3	7.4	6.6	ND[e]	
2a[g]	7.0	7.0	6.3	ND	
2b	6.4	6.7	7.1	6.6	
3a	7.3	7.3	8.1	7.2	0.3/0.5/0.5
3b	7.1	6.5	6.8	ND	
3c	6.8	6.4	6.6	ND	
4a	<5.0	<5.6	5.9	6.7	
4b	<5.8	<5.0	5.9	7.5	
4c	6.5	7.6	8.2	7.0	
5a	<5.0	<5.3	5.7	ND	
5b	6.3	6.2	6.6	6.1	

[a] All values represent the mean of at least three determinations, with each determination lying within 0.3 log unit of the mean.
[b] Receptors and radioligands used in binding assays; 5-HT_{1A} (human cloned receptors in CHO cells, [^{3}H]-5-HT; 5-HT_{1B} (human cloned receptors in CHO cells, [^{3}H]-5-HT; 5-HT_{1D} (human cloned receptors in CHO cells, [^{3}H]-5-HT; [^{3}H]-5-HT uptake into rat cortical synaptosomes.
[c] All new compounds gave satisfactory NMR and LC/MS data.
[d] IA, intrinsic activity.
[e] ND, not done.
[f] Enantiomer 1, unknown stereochemistry.
[g] Enantiomer 2, unknown stereochemistry.



Scheme 3. Reagents and conditions: (i) NaH/1-BOC-piperidine-4-mesylate, DMF, rt then 1 N HCl/Et_2O, MeOH, rt (75%, **17**); (ii) Ph_3P, DIAD, 1-BOC-4-hydroxymethylpiperidine, rt then 1 N HCl/Et_2O, MeOH, rt (51%, **18**); (iii) **17** or **18**, 3-oxo-3,4-dihydro-2-1,4-benzoxazine-6-carbaldehyde, sodium triacetoxyborohydride, DCE, rt (50–55%, **4a–b**); (iv) **17** or **18**, 6-chloroacetyl-2*H*-1,4-benzoxazin-3(4*H*)-one, ^{i}Pr$_2$EtN, isopropanol, reflux, 4 h (71–59%, **5a–b**); (v) Br_2, $AlCl_3$, DCE (30%, **19**); (vi) piperazine, Pd(AcO)$_2$, BINAP, $Cs(CO_3)_2$, dioxane, 100 °C, 2.5 days (49%, **20**); (vii) **27** or **28**, ^{i}Pr$_2$EtN or K_2CO_3, NaI, DMF, 80 °C (13–69%, **21–23**); (viii) iron, acetic acid, (23–71%, **1**, **1a**, **4c**).



Scheme 4. Reagents and conditions: (i) tri-*n*-butylvinyltin or tri-*n*-butylallyltin, Pd(Ph_3P)$_4$, toluene, rt (34–64%), (ii) BH_3/THF, 5 °C followed by sodium perborate tetrahydrate, rt (23–46%); (iii) MesCl, Et_3N, CH_2Cl_2 (87%).

n-butylallyltin, hydroboration of **25**–**26** followed by mesylation of the resulting alcohols.

All compounds prepared were evaluated using published procedures.[14,15] Data expressed as pK_i are shown in Table 1.

Introduction of a methyl group into the 1- or 2-position of the 2-ethoxy sidechain in **2** resulted in more than a 10-fold reduction in potency at 5-$HT_{1A/1B/1D}$ receptors and the 5-HT reuptake site. Interestingly, the individual enantiomers of **2a** possessing the methyl group at the 1-position had similar activity profiles at 5-HT_1 receptors whereas compound **2b** as a racemic mixture, containing the methyl group at the 2-position, showed good potency only at the 5-HT_{1D} receptor.

Replacement of the piperidine moiety in **2** with an azetidine system provided compound **3a** with an encouraging overall activity profile (5-HT_1/SerT pK_i >7.2). Disappointingly, further investigation of the nature of this sidechain resulted in less potent analogues **3b–c**.

Linking the quinoline ring to the 4-position of a piperidine moiety via an oxygen atom or a methyloxy group proved detrimental to 5-HT_1 affinities. Thus, compounds **4a**, **4b** and **5a** were inactive, whereas the analogous **4c** and **5b** showed highly reduced potency at the 5-HT_{1A} receptor. In contrast, potency at the 5-HT reuptake site was less affected by these modifications, as the methoxy linked compound **4c** and its oxygen analogue **4b** displayed respectable inhibitory activities with pK_i values of 7.5 and 7.0, respectively.

The breakthrough modification was identified when the 2-ethoxypiperidine fragment in **2** was replaced with a piperazine system. Compound **1** (SB-744185) showed outstanding affinity for the 5-$HT_{1A/1B/1D}$ receptors, excellent 5-HT reuptake inhibitory activity and high selectivity over a range of monoamine receptors (5-HT_{2A} pK_i 5.9, 5-HT_{2B} pK_i 6.5, 5-HT_6 pK_i <5, 5-HT_{2C} pK_i 5.4 and D_2 pK_i 6.5). The distance between the quinoline ring and the 3,4-dihydro-2*H*-benzoxazinone moiety seemed crucial for the potency. Thus, a three carbon analogue **1a** showed a considerable reduction in affinity for all 5-HT_1 receptors but a smaller decrease in affinity for the 5-HT reuptake site. Rather surprisingly, the four carbon linker compound **1b**[22] provided a

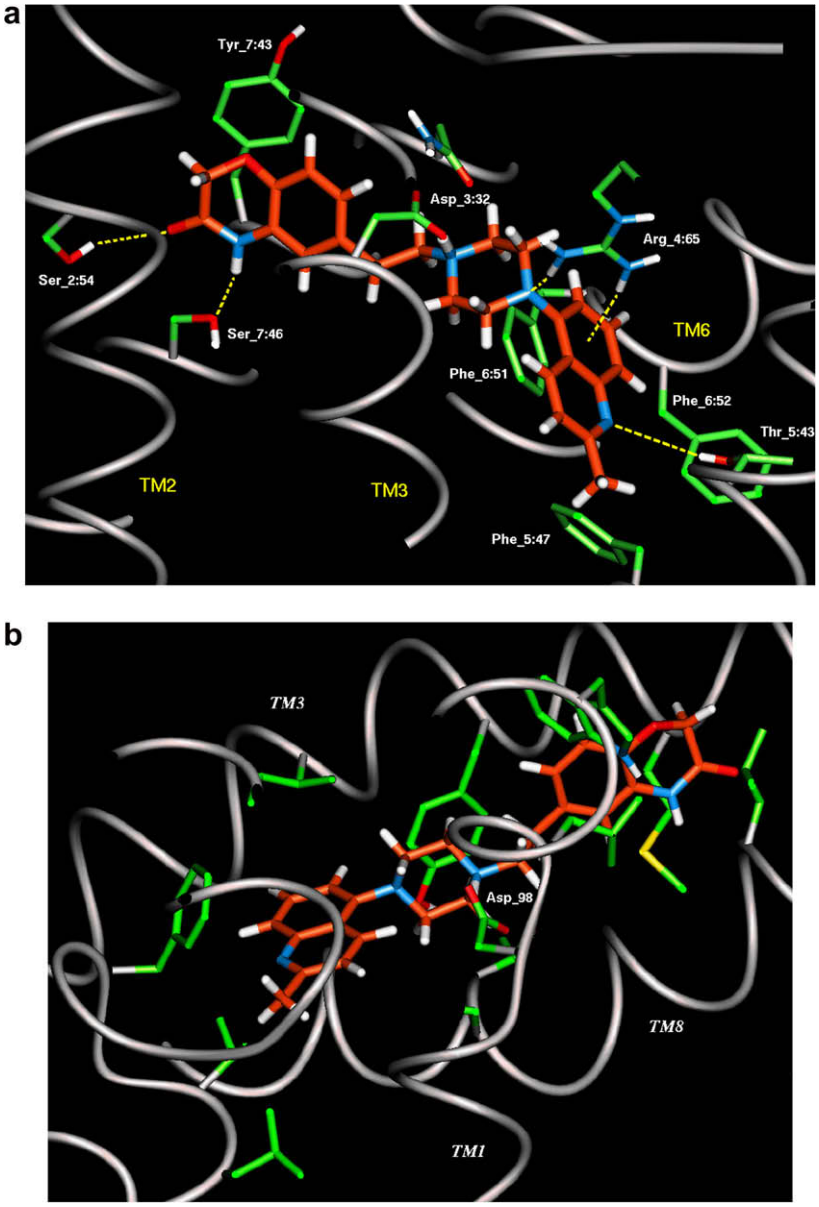


Figure 2. Compound 1 docked (a) into the 5-HT1A receptor model and (b) into a model of the 5-HT transporter. Some key interactions are shown as dashed lines in (a).

marked restoration of potency at the 5-$HT_{1A/1B}$ receptors and the 5-HT reuptake site however affinity for the 5-HT_{1D} receptor remained almost unchanged when compared to **1a**. Compound **1c** incorporating a one carbon sidechain[22] (Fig. 1) was virtually inactive.

We had reasoned that replacement of the flexible linker with a piperazine ring directly attached to the quinoline would completely restrict movement of the ligand with respect to its interaction with the TM3 aspartate. This indeed proved to be the case and docking of compound **1** into the 5-HT_{1A} receptor model revealed a binding mode which was somewhat different from that described previously for compound **2**.[18] It was found to sit in a more horizontal position with respect to the TM bundle axis. In addition to the salt bridge formed between the piperazine nitrogen and asp_3:32[23], the quinoline ring sat in the 'classic agonist pocket' forming a π–π stacking with Phe_6:51 and to a lesser extent Phe_6:52. The quinoline nitrogen itself was 2.9 Å from the hydroxyl of Thr_5:43 thus forming a weak hydrogen bond. At 1.8 Å an arginine at the N-terminal region of the second extracellular loop (Arg_4:65) formed a strong hydrogen bond to the piperazine's anilinic nitrogen. This arginine also had a π-cation interaction with the face of the quinoline ring. Finally, the 1,4-benzoxazinone ring formed H-bonds with Ser_2:54 (2.8 Å) and Ser_7:46 (1.8 Å) and a π–π interaction with Tyr_7:43. Interestingly, all these residues are conserved across the three receptors.

Docking of compound **1** into the 5-HT transporter model showed that the primary salt bridge interaction was with Asp_98 in TM1. This has been previously implicated from SDM experiments[24] with other monoaminergic transporters. The molecules generally need to be fairly linear but otherwise the interactions are largely hydrophobic and non-specific which would explain the relative lower increase in potency observed between **1** and **2** (see Fig. 2b).

Encouragingly, pharmacokinetic profiling in the rat revealed that compound **1** showed low in vivo clearance of 7 ml/min/kg, good brain exposure with C_{max} of 360 ng/mL, a B/B ratio of 1.0 and an estimated oral bioavailability of 72% (following oral administration at 3 mg/kg to the male rat).

In conclusion, extensive modifications of the 1-ethoxypiperidine linker in compound **2** have led to the discovery of novel, potent serotonin reuptake inhibitors displaying high affinities for the 5-$HT_{1A/1B/1D}$ receptors. Compound **1** has a considerably improved pharmacokinetic profile in comparison with **2**[18] and it is a suitable candidate for a further in vivo evaluation with the aim of identifying more effective antidepressant agents.

Acknowledgement

We are indebted to our colleagues from the DMPK Department at Psychiatry CEDD Harlow for providing in vivo data.

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