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5-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2(1*H*)-quinolinones and 3,4-dihydro-2(1*H*)-quinolinones: Dual-acting 5-HT$_1$ receptor antagonists and serotonin reuptake inhibitors. Part 3

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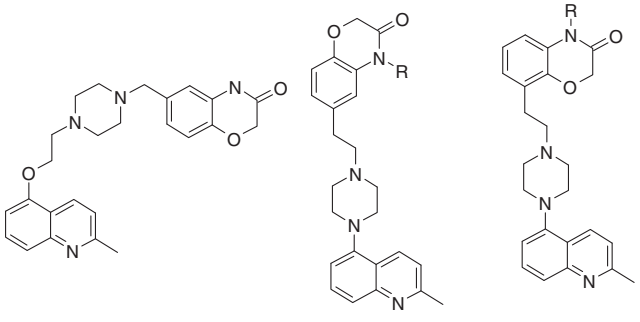
ABSTRACT

5-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2(1*H*)-quinolinones and 3,4-dihydro-2(1*H*)-quinolinones have been identified with different combinations of 5-HT$_1$ autoreceptor antagonist and hSerT potencies and excellent rat PK profiles. The availability of tool compounds with a range of profiles at targets known to play a key role in the control of synaptic 5-HT levels will allow exploration of different pharmacological profiles in a range of animal behavioral and disease models.

Over the past decades, a wealth of preclinical and clinical evidence has confirmed a link between extracellular levels of serotonin (5-HT) and a plethora of psychiatric indications, in particular anxiety and depression.[1] In fact, enhanced serotonergic neurotransmission has become the unifying mechanism of action of modern day antidepressants and the selective serotonin reuptake inhibitors (SSRIs) have become established as the most effective antidepressant agents in current clinical use.[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] This is consistent with preclinical neurochemical studies which have demonstrated that SSRIs such as paroxetine require chronic dosing (14–21 days) in order to enhance extracellular 5-HT levels in guinea pig dentate gyrus and rat frontal cortex but have no effect after acute administration.[4]

5-HT$_1$ autoreceptors are located on both the cell bodies (5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes) and nerve terminals (5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes) of 5-HT neurons.[5] They are widely distributed in the brain and in addition to serotonin transporters (SerT) are known to have a major role in the control of synaptic 5-HT levels.[5] Blockade of 5-HT$_{1A/B/D}$ autoreceptors, with or without concomitant SerT inhibition, rapidly increases brain 5-HT levels and consequently should provide a fast onset of antidepressant/anxiolytic action relative to current therapies.[6]

We have previously reported potent 5-HT$_{1A/B/D}$ receptor antagonists both with and without additional hSerT reuptake inhibitory activity including **1** (SB-649915) (Fig. 1).[7] More recently, we disclosed a series of 6-[2-(4-aryl-1-piperazinyl)ethyl]-2*H*-1,4-



1 (SB-649915)

2 R = H (SB-744185)
3 R = Me

4 R = H
5 R = Me

Figure 1.

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benzoxazin-3(4*H*)-ones (such as **2**,**3**)[8,9] and a series of 8-[2-(4-aryl-1-piperazinyl)ethyl]-2*H*-1,4-benzoxazin-3(4*H*)-ones (such as **4**,**5**)[10] which had improved selectivity over hERG potassium channels (Fig. 1). This article reports additional SAR in the latter series which has resulted in the identification of novel series of 5-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2(1*H*)-quinolinones (**10**–**24**) and 3,4-dihydro-2(1*H*)-quinolinones (**6**–**9**) which possess high 5-HT_{1A} and 5-HT_{1D} antagonist potencies with varying levels of 5-HT_{1B} and hSerT potencies. In addition, these compounds have good levels of selectivity over hERG together with good rat PK properties and thus represent interesting tool compounds to explore the therapeutic potential of different pharmacological profiles in a variety of disease indications.

The synthesis of the new compounds are shown in Schemes 1–3.[11] The *N*-methylated 2(1*H*)-quinolinones (**11**, **19**, **21** and **23**) and the *N*-H-2(1*H*)-quinolinone (**10**) were prepared starting from the 5-quinolinol triflate **25** via two alternative synthetic approaches (Scheme 1). The *N*-methyl analogs were accessed from **25** by Suzuki coupling with allyltributylstannane followed by N-oxide formation with mCPBA, trifluoroacetic anhydride (TFAA) mediated rearrangement and N-methylation to afford **27**. Oxidative cleavage of the pendant vinyl with osmium tetraoxide followed by reductive amination with the appropriate piperazinyl quinoline, completed the synthesis. The *N*-unsubstituted 2(1*H*)-quinolinone (**10**) was also prepared from **25** via the 5-quinolinone triflate **29**. Heck coupling with 2-(ethenyloxy)-*N*,*N*-dimethylethanamine gave **30** which was hydrolyzed under acidic conditions to the aldehyde **31** which was converted into the desired final compound **10** by reductive amination as above.

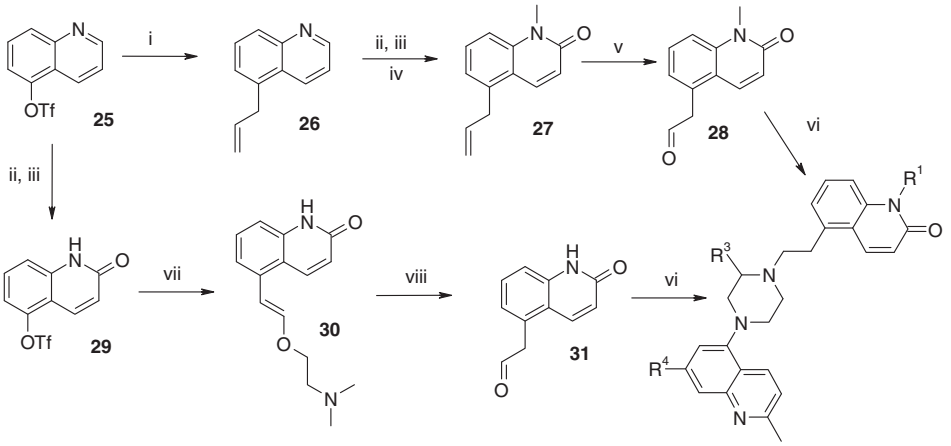
The 6-methyl 2(1*H*)-quinolinones (**12**, **13**, **16**, **17**, **20**, **22** and **24**) were prepared according to the same general approaches as those described in Scheme 1 starting from 5-bromo-6-methyl-2(1*H*)-quinolinone (**32**) (Scheme 2). 5-Bromo-6-methyl-2(1*H*)-quinolinone (**32**) itself was prepared from 5-bromo-6-methylquinoline by N-oxidation and TFAA mediated rearrangement as described above.

The 6-F and 6-Cl-2(1*H*)-quinolinone analogs (**14** and **15**) were also prepared by the same route as described in Scheme 1 for **11** via the triflate derived from the corresponding 6-halo-5-methoxy-2(1*H*)-quinolinone (**42**) (Scheme 3). Intermediates **42** were prepared by acid-catalyzed cyclization of **38** which were themselves accessed from the corresponding 4-halo-3-methoxyaniline by organolithiation and reaction with DMF followed by Wittig reaction.[12] The corresponding 3,4-dihydro-2(1*H*)-quinolinones (**6**–**9**) were prepared similarly following an initial catalytic hydrogentation step on **38** (Scheme 3).

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 by filtration assay.[13] The affinities of the compounds for hERG potassium channels, stably expressed in Chinese hamster ovary (CHO) cells, were assessed by displacement of [^{3}H]-dofetilide binding by proximity assays.[11] Compounds were screened against human-5-$HT_{1A/B/D}$ receptors in a dose response GTPgS functional assay using Scintillation Proximity Detection (LEADSeeker™) in both agonist and antagonist mode, that is, in absence and presence of 5-HT, respectively.[11] Compounds with intrinsic activity (IA) in the agonist mode $\geqslant$0.4 relative to 5-HT were classified as partial or full agonists and only the agonist data is reported (pEC_{50}) whereas compounds with IA <0.4 in the agonist mode were classified as antagonists and data from the antagonist mode is reported as a functional pK_i (f-pK_i).

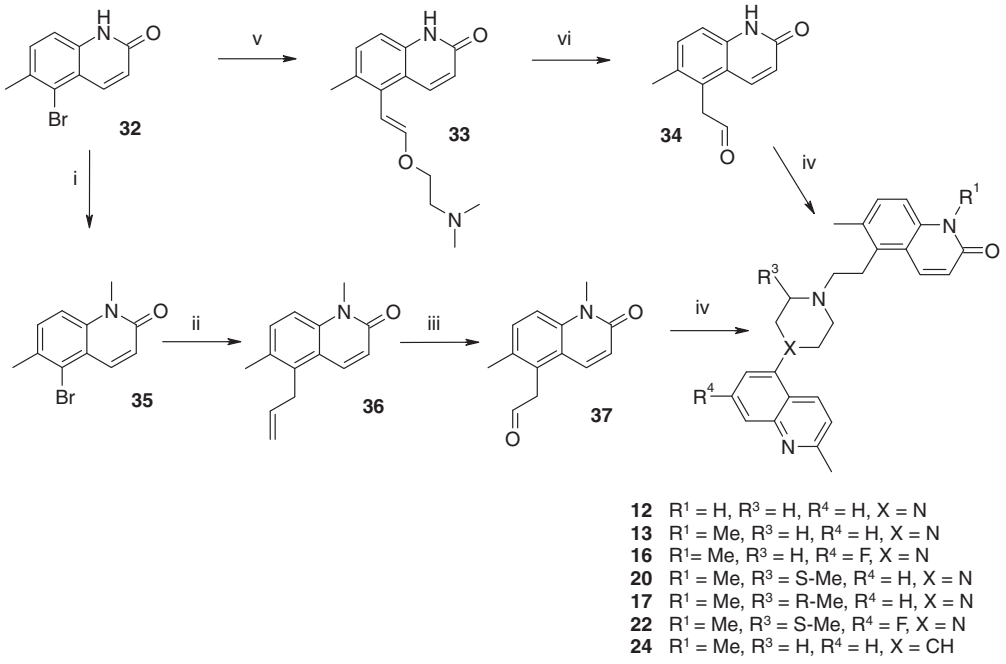
Replacement of the oxygen in the benzoxazinone ring of **4** and **5** with methylene to afford the corresponding 3,4-dihydro-2(1*H*)-quinolinones (**6** and **7**) maintained similar overall profiles with very potent 5-$HT_{1A/B/D}$ antagonist potencies, good selectivity over hERG and moderate hSerT potency in the case of the N-methyl analog **7** (Table 1). Interestingly, the introduction of a methyl substituent (R^2) into the 6-position (**8** and **9**) of the quinolinone ring selectively reduced 5-HT_{1B} potency affording potent 5-$HT_{1A/D}$ antagonists along with modest hSerT potency. On further profiling both these compounds demonstrated excellent rat PK properties combining low blood clearance and good half-lives with good oral bioavailability and brain exposure (Table 3).

Replacement of the CH_2O in the benzoxazinone ring of **4** with vinyl to afford the corresponding 2(1*H*)-quinolinone **10** maintained a similar profile with very potent 5-$HT_{1A/B/D}$ antagonist potencies combined with low to moderate hSerT potency but with significantly reduced hERG binding affinity (Table 2). A similar result was obtained in the case of the N-methyl analog **11** relative to the corresponding benzoxazinone **5** although in this case some 5-

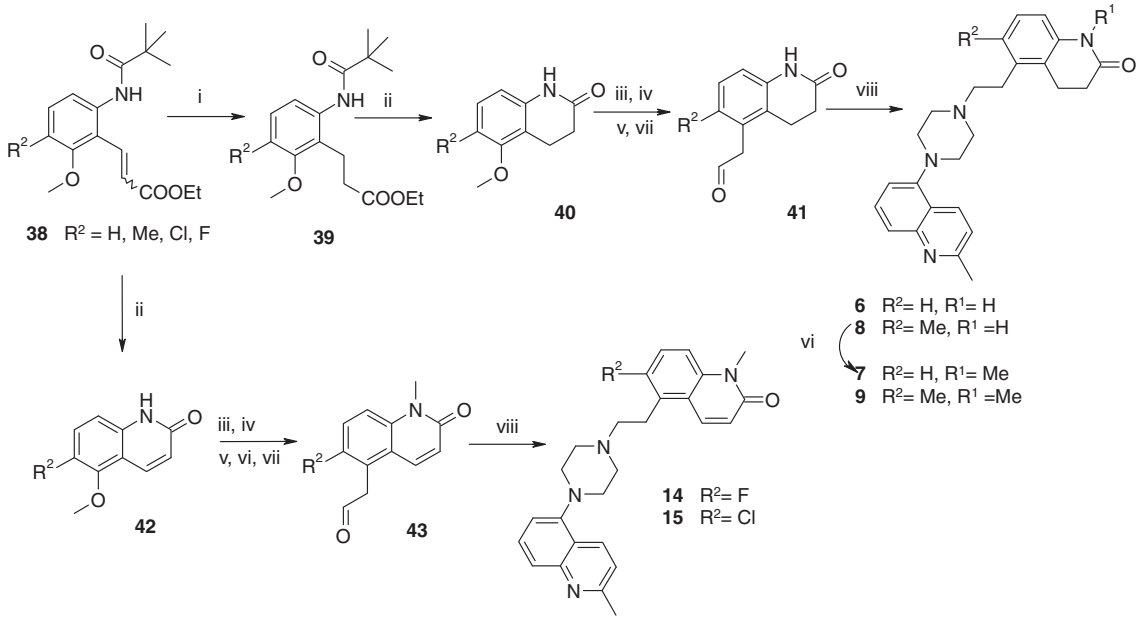


10 R^1 = H, R^3 = H, R^4 = H
11 R^1 = Me, R^3 = H, R^4 = H
19 R^1 = Me, R^3 = S-Me, R^4 = H
21 R^1 = Me, R^3 = S-Me, R^4 = F
23 R^1 = Me, R^3 = di-Me, R^4 = H

Scheme 1. Reagents and conditions: (i) allyltributylstannane, $Pd(PPh_3)_4$, LiCl, DMF, 100 °C, 1.5 h (95%); (ii) mCPBA, DCM, 0 °C, 2 h (100%); (iii) TFAA, DMF, 0 °C–rt, overnight (85%); (iv) NaH, MeI, DMF, 0 °C–rt, 1 h (72%); (v) OsO_4, $NaIO_4$, THF/H_2O (2:1), 10 min (65%); (vi) 7-R^4-2-Me-5-(3-R^3-1-piperazinyl)quinoline, $NaBH(OAc)_3$, 1,2-DCE, rt, 6 h (39–78%); (vii) 2-(ethenyloxy)-*N*,*N*-dimethylethanamine, $Pd(OAc)_2$, PPh_3, TEA, DMF, 100 °C, overnight (58%); (viii) H_2SO_4 (24%), DCM/pentane (4:1), rt, 4 h, (100%).



Scheme 2. Reagents and conditions: (i) NaH, MeI, DMF, 0 °C–rt, 1 h (67%); (ii) allyltributylstannane, $Pd(PPh_3)_4$, LiCl, DMF, 100 °C, 1.5 h (94%); (iii) OsO_4, $NaIO_4$, THF/H_2O (2:1), 10 min (64%); (iv) 7-R^4-2-Me-5-(3-R^3-1-piperazinyl)quinoline, $NaBH(OAc)_3$, 1,2-DCE, rt, 6 h (27–67%); (v) 2-(ethenyloxy)-*N*,*N*-dimethylethanamine, $Pd(OAc)_2$, PPh_3, TEA, DMF, 100 °C, overnight (40%); (vi) H_2SO_4 (24%), DCM/pentane (4:1), rt, 4 h, (100%).



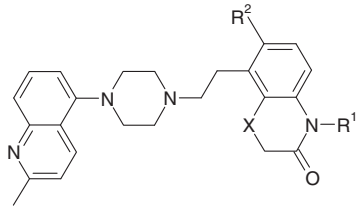
Scheme 3. Reagents and conditions: (i) H_2, Pd/C, EtOH, rt, 2 h (74–87%); (ii) HCl (10%), EtOH, reflux, overnight (59–63%); (iii) HBr (48% in H_2O), 130 °C, 2.5 h (100%); (iv) 1,1,1-trifluoro-*N*-phenyl-*N*-[(trifluoromethyl)sulfonyl]-methanesulfonamide, TEA, MeCN, rt, 7 h (73%); (v) allyltributylstannane, $Pd(PPh_3)_4$, LiCl, DMF, 100 °C, 1.5 h (62–89%); (vi) NaH, MeI, DMF, 0 °C–rt, 1 h (53–68%); (vii) OsO_4, $NaIO_4$, THF/H_2O (2:1), 10 min (52–64%); (viii) 2-Me-5-(1-piperazinyl)quinoline, $NaBH(OAc)_3$, 1,2-DCE, rt, 6 h (36–55%).

$HT_{1A/D}$ partial agonism was apparent and the hSerT potency was increased.

Substitution of the 6-position (R^2) of the quinolinone ring with F, Cl, and Me (**14**, **15** and **16**, respectively) was well tolerated in terms of hSerT and 5-$HT_{1A/D}$ antagonist potencies (affording low intrinsic activity) but selectively reduced 5-HT_{1B} antagonist potency. Further enhancements in hSerT potency were achieved by introducing methyl substitution α to the basic piperazine nitrogen (**18**–**23**). Whereas the *R*-enantiomer **18** had subnanomolar hSerT and 5-$HT_{1A/B/D}$ antagonist potencies the *S*-enantiomer **19** demonstrated significantly reduced 5-HT_{1B} activity and increased hERG affinity. The dimethyl analog **23** maintained good 5-$HT_{1A/B}$ antagonist potencies with the highest level of hSerT potency yet achieved in this series (Table 2).

Introducing a fluoro substituent into the 7-position of the quinoline was also well tolerated (**18**, **21** and **22**) and in the case of **21** had a beneficial effect on hSerT relative to the corresponding unsubstituted analog **19**. Combination of the substitutions described above generally had additive effects on SAR (**17**, **20** and **22**). Replacement of the piperazine ring of **13** with piperidine **24** led to a 10-fold increase in hSerT and maintained 5-HT_{1A} potency whilst increasing selectivity over 5-HT_{1B} and for the first time over 5-HT_{1D}.

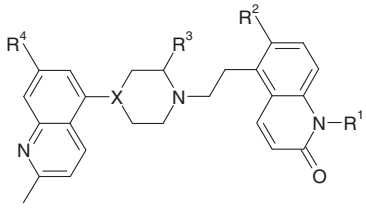
Table 1
Functional activity (f-pK_i or pEC$_{50}$)[a,b] for human 5-HT$_{1A/B/D}$ receptors with intrinsic activity (IA) and SerT and hERG binding affinities (pK_i)[b]: 5-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3,4-dihydro-2(1*H*)-quinolinones



Compd[c]	X	R^1	R^2	f-pK_i or *pEC$_{50}$[a,b]			pK_i[b]	
				5-HT$_{1A}$ (IA)	5-HT$_{1B}$ (IA)	5-HT$_{1D}$ (IA)	hSerT	hERG
4	O	H	H	9.6 (0.0)	9.0 (0.0)	10.3 (0.0)	6.5	5.6
5	O	Me	H	9.8 (0.0)	8.8 (0.0)	10.0 (0.0)	8.0	5.4
6	CH_2	H	H	9.4 (0.3)	9.2 (0.0)	10.0 (0.0)	6.6	5.5
7	CH_2	Me	H	9.2 (0.0)	*9.1 (0.0)	*10.1 (0.0)	7.9	5.4
8	CH_2	H	Me	9.1 (0.0)	*6.7 (0.4)	*8.3 (0.5)	7.3	5.4
9	CH_2	Me	Me	9.2 (0.0)	*6.4 (0.4)	9.0 (0.0)	7.6	6.1

[a] Data from the agonist mode (pEC$_{50}$)* are reported for those compounds with intrinsic activity (IA) ⩾ 0.4 in this mode (i.e., partial or full agonists), whereas, for those compounds with IA <0.4 in the agonist mode (i.e., antagonists) data from the antagonist mode is reported (f-pK_i).
[b] Each determination lies within 0.3 log units of the mean with a minimum of three replicates. See text for radioligands and assay details.
[c] Compounds were characterized and purity assessed using ^{1}H NMR and LCMS.

Table 2
Functional activity (f-pK_i or pEC$_{50}$)[a,b] for human 5-HT$_{1A/B/D}$ receptors with intrinsic activity (IA) and SerT and hERG binding affinities (pK_i)[b]: 5-{2-[(2*R*)-2-methyl-4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2(1*H*)-quinolinones



Compd[c]	R^1	R^2	R^3	R^4	X	f-pK_i or *pEC$_{50}$[a,b]			pK_i[b]	
						5-HT$_{1A}$ (IA)	5-HT$_{1B}$ (IA)	5-HT$_{1D}$ (IA)	hSerT	hERG
10	H	H	H	H	N	9.2 (0.0)	8.7 (0.0)	*9.9 (0.0)	6.7	<4.5
11	Me	H	H	H	N	10.5 (0.4)	*9.1 (0.0)	*9.0 (0.7)	8.6	5.0
12	H	Me	H	H	N	9.0 (0.0)	*6.3 (0.4)	8.6 (0.0)	7.0	4.6
13	Me	Me	H	H	N	9.2 (0.0)	*7.2 (0.4)	8.7 (0.0)	7.9	5.4
14	Me	F	H	H	N	9.4 (0.0)	*7.5 (0.0)	9.4 (0.0)	7.9	4.5
15	Me	Cl	H	H	N	8.4 (0.0)	*6.5 (0.5)	8.4 (0.0)	7.8	5.3
16	Me	Me	H	F	N	8.9 (0.0)	<6.0	8.7 (0.0)	8.0	5.6
17	Me	Me	*R*-Me	H	N	8.9 (0.0)	<6.0	8.6 (0.0)	8.4	5.1
18	Me	H	*R*-Me	F	N	9.0 (0.0)	9.1 (0.0)	9.9 (0.0)	9.3	5.0
19	Me	H	*S*-Me	H	N	8.5 (0.0)	6.4 (0.0)	8.3 (0.0)	8.6	5.3
20	Me	Me	*S*-Me	H	N	8.4 (0.0)	<6.0	7.7 (0.0)	9.3	5.8
21	Me	H	*S*-Me	F	N	9.0 (0.0)	6.5 (0.0)	8.9 (0.0)	9.3	5.2
22	Me	Me	*S*-Me	F	N	8.4 (0.0)	<6.0	<6.0	9.0	5.9
23	Me	H	di-Me	H	N	8.8 (0.3)	<6.0	9.3 (0.0)	9.6	5.6
24	Me	Me	H	H	CH	8.9 (0.0)	<6.0	7.4 (0.0)	8.9	5.7

[a–c] See Table 1.

In an attempt to rationalize the observed SAR, a number of compounds were docked into the homology model of 5-HT$_{1A}$ receptor previously built in house.[9] In these models the primary interaction of the compounds is with Asp13 on helix 3, which makes a charge interaction with their tertiary amine moiety (Fig. 2). Another key interaction involves the quinoline of the ligands and Trp20 in helix 6.[14] The docking solutions obtained for **24** seem to suggest that the methyl group at position 6 on the quinolone can be accommodated near Phe9 in helix 3, which corresponds to a Trp in 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. Furthermore, this fragment is close to Ile10 in helix 3 and to Asn8 in helix 7, which is replaced by a Thr in 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. Finally, this group is also close to the ECL2 residues which are not conserved among the three receptors. In light of these results, the reduced affinity/potency showed by **24** at the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors might be related to the greater steric hindrance of the Trp in 5-HT$_{1B}$ and 5-HT$_{1D}$ with respect to Phe in 5-HT$_{1A}$ and contacts with the ECL2 residues preventing **24** from favorably interacting with 5-HT$_{1B}$ and 5-HT$_{1D}$ binding site residues.

In general all the quinolinones had low to moderate hERG binding affinity suggesting low risk of potential QT effects in vivo. On the basis of their interesting and varied pharmacological profiles a range of analogs were tested in rat PK studies to access their utility as biological tool compounds and potential for further progression (Table 3). In general all the *N*-methylquinolinones tested had good oral bioavailability (12–88%) moderate clearance and moderate brain exposure based on brain to blood ratios.

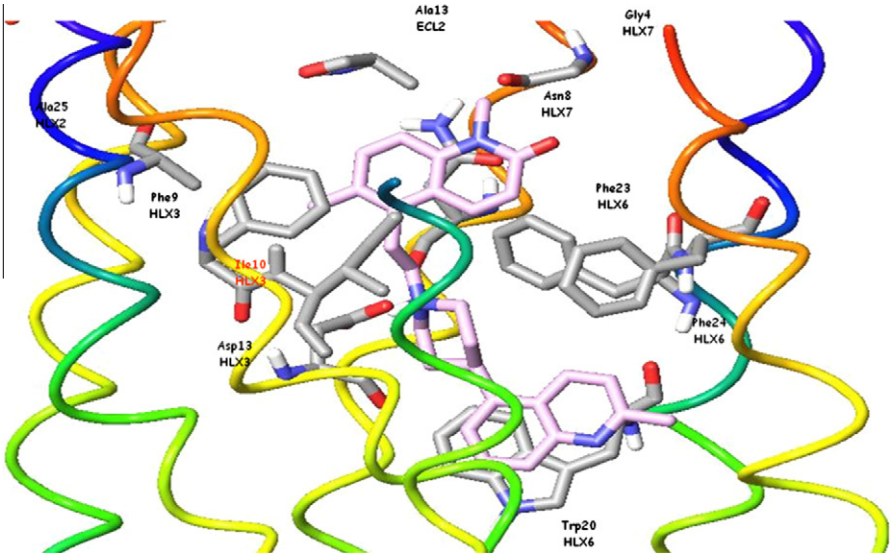


Figure 2. Docking of **24** in 5-HT$_1$ receptor models.

Table 3
Rat pharmacokinetic profile for compounds **1**–**4**, **13**, **14** and **16**

Compd	Cli rat; hum[a] liver (ml/min/g)	CLb[b] (ml/min/kg)	V_{ss} (L/kg)	$t_{½}$ (h)	Fpo %	Br:Bl
4	0.7; 1.0	8	3.4	5.2	63	0.7
5	1.0; 2.4	29	1.7	1.0	26	1.5
8	<0.5; <0.5	7	3.0	5.4	73	0.8
9	1.6; 1.3	9	2.0	3.0	83	0.4
11	0.8; 1.2	32	2.1	1.3	37	1.1
13	2.2; 1.5	28	2.3	1.1	88	0.5
16	2.3; 1.6	46	2.4	0.7	82	ND
17	3.8; 1.2	21	2.1	1.5	31	0.4
19	0.7; 1.5	32	1.2	0.6	12	1.3
20	1.9; <0.5	46	2.7	1.2	35	1.7
21	0.7; 0.8	34	2.0	1.6	34	1.2
23	8.0; 3.8	34	0.8	0.6	27	1.1
24	2.0; 0.9	31	3.4	1.4	82	0.8

[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.

Thus 3,4-dihydro-2(1*H*)-quinolinones and 2(1*H*)-quinolinones (**6**–**24**) have been identified with a range of different pharmacological profiles at hSerT and 5-HT$_1$ autoreceptors combined with good rat PK properties. The availability of tool compounds with a range of profiles at targets known to play a key role in the control of synaptic 5-HT levels will allow the exploration of different permutations of activity in a variety of animal behavioral and disease models. The results of these studies and the further optimization of these series will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.085.

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14. The 3D structure of **24** was generated starting from its Daylight SMILE which was converted into SD 2D file format with the use of smi2mol Daylight routine and then transformed into SD 3D with the use of Corina [Accelrys]. ESP charges were calculated with the use of MOPAC 6.0 [available within Sybyl, Tripos]. Docking experiments were carried out within the receptor substructure defined by the residues lying within a 15 Å radius sphere centred on Asp13 in helix 3. Coordinates were allowed to change upon energy minimization. The remaining receptor model residues surrounding the previous ones were kept rigidly frozen in place throughout all the simulations. **24** was manually docked in the binding site described above and the energy of the complex obtained was minimized with the use of AMBER* force field as implemented in Batchmin V9.1 [Schrodinger], distance dependent electrostatic model ($\varepsilon = 2r$), VDW cut-off = 7 Å, electrostatic cut-off = 20 Å, charges from structure file. 10K steps of PR Conjugate Gradient (PRCG) were used to minimize the complex; convergence was set on gradient (0.05 kJ mol^{-1} Å^{-1}).