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# 4-Aminoethylpiperazinyl aryl ketones with 5-HT$_{1A}$/5-HT$_7$ selectivity

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ABSTRACT

The well-known 5-HT$_{1A}$/5-HT$_7$ selectivity issue was tackled by a new series of 4-aminoethylpiperazinyl aryl ketones (**1a**–**1l**) specifically designed to distinguish the two hydrophobic sites centered at the anchoring salt bridge. The 4-aminoethylpiperazinyl aryl ketones showed a wide spectrum of activity and selectivity for the 5-HT receptors depending on the type of the hydrophobic groups attached at the aryl piperazinyl ketone scaffold. Docking study of the most active compounds against 5-HT$_7$R and 5-HT$_{1A}$R revealed that both receptors have two hydrophobic pockets around the anchoring salt bridge. These two binding sites are perpendicular to each other in 5-HT$_7$R but parallel in 5-HT$_{1A}$R, and this observation is well matched with the previous report which claimed that 5-HT$_7$R affinity arises from bent conformation of the bound ligand whereas an extended one is best suited for 5-HT$_{1A}$R selectivity. Also, as these pockets have different size and shape, inhibitory activity as well as selectivity of the 4-aminoethylpiperazinyl aryl ketones against 5-HT$_7$R and 5-HT$_{1A}$R seemed to be determined by combination of two hydrophobic substituents attached at both ends of the title compounds.

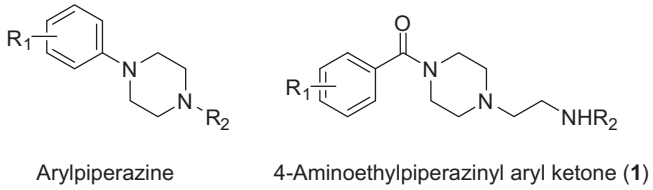


## 1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter, exerts its effects on the central and peripheral nervous system through interaction with various serotonergic receptor subtypes, classified into seven families (5-HT$_1$ to 5-HT$_7$). All 5-HT receptors with an exception of 5-HT$_3$ receptor (a ligand-gated ion channel) are G protein-coupled receptors (GPCRs), and dysfunction of this system has been implicated in cardiovascular, digestive, and psychiatric disorders.[1] Due to their important physiological role, many of the 5-HT receptors have become beneficial targets for drug discovery, and several drug candidates have reached clinical trial or market.[2,3]

5-HT$_{1A}$ receptor (5-HT$_{1A}$R) is a GPCR involved in several cognitive, behavioral, and developmental functions, and selective partial agonists of 5-HT$_{1A}$R such as Gepirone, Tandospirone, and Zalospirone, have been developed as anxiolytics and antidepressants.[4–6] The more recently discovered 5-HT$_7$ receptor (5-HT$_7$R) displays a low degree of homology (40%) with other serotonin GPCRs, but it shares a similar pharmacological profile with 5-HT$_{1A}$R.[7,8] As a result, a selectivity issue is encountered for these two receptor subtypes. With lack of crystallographic structure of 5-HT receptors, there have been attempts to address the problem of 5-HT$_7$R versus 5-HT$_{1A}$R selectivity through new sets of compounds with structural modifications at different pharmacophoric elements present in serotonergic ligands such as arylpiperazines (Fig. 1).[8–10]

In this study, based on the analysis of docking poses of the arylpiperazines to 5-HT$_{1A}$R as well as 5-HT$_7$R, we installed a carbonyl as well as an aminoethyl group as a hydrogen bond acceptor and donors around the piperazine ring to come up with a novel serotonergic ligand, 4-aminoethylpiperazinyl aryl ketone (**1**, Fig. 1), with an interesting 5-HT$_{1A}$/5-HT$_7$ selectivity profile. Herein we report a proof-of-concept study of the 4-aminoethylpiperazinyl aryl ketones which showed a wide spectrum of selectivity for the 5-HT receptors depending on the type of the hydrophobic groups attached at the




Arylpiperazine 4-Aminoethylpiperazinyl aryl ketone (**1**)

**Figure 1.** Structures of arylpiperazine and 4-aminoethylpiperazinyl aryl ketone (**1**).

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aryl piperazinyl ketone scaffold. Also, docking study of each ligand was performed to provide a rational explanation for the structure–activity relationship as well as observed selectivity.

## 2. Results and discussion

### 2.1. Design of the aryl piperazinyl ketones

Arylpiperazines are efficient building blocks used for design of ligands for the monoamine neurotransmitter receptors and they have been thoroughly studied as ligands for 5-$HT_{1A}$R[11] and 5-$HT_7$R.[9] Ligand-based construction of a pharmacophore model[8–10,12–14] confirmed that the arylpiperazines include an ideal geometry of two essential features required for a good affinity profile: a basic amine (protonated at physiological pH)[12,15] and a hydrophobic feature. However, due to lack of crystallographic structure, specific binding modes of arylpiperazine ligands to 5-$HT_{1A}$R and 5-$HT_7$R have been ambiguous. In order to overcome this problem, there have been many efforts to generate comparative model structures of the serotonergic receptors.[16–21] The model structures of 5-$HT_{1A}$R and 5-$HT_7$R have also been prepared and binding modes of ligands such as arylpiperazines were elucidated.[8–10,14,16] In an attempt to get a better understanding of the selective binding interactions involved in 5-$HT_{1A}$/5-$HT_7$ receptors and arylpiperazine ligands, we have constructed homology models of 5-$HT_{1A}$as well as 5-$HT_7$ receptors through the SwissModel server and docked the representative arylpiperazine ligands, MP349 (a ligand for 5-$HT_{1A}$R)[22] and UCM-5600 (a ligand for 5-$HT_7$R),[9] respectively, to the corresponding receptor structures (Fig. 2).

Binding modes of the arylpiperazine ligands to 5-$HT_{1A}$R and 5-$HT_7$R (right panels, Fig. 2) were in accordance with those previously reported[8–10,14,16] in which ionic interactions between the protonated amino groups of the ligands and Asp residues of the receptors (Asp116 for 5-$HT_{1A}$R and Asp162 for 5-$HT_7$R) constitute a main essential interaction. The hydrophobic groups attached at both ends of the piperazine moiety were found in hydrophobic pockets composed of amino acid residues with aromatic and/or aliphatic side chains [Thr196/Phe362 and Phe112/Val89/Val85/Leu90/Tyr390 for 5-$HT_{1A}$R (Fig. 2a); Val163/Tyr239/Phe344 and Phe158/Trp148/Thr141 for 5-$HT_7$R (Fig. 2b)]. Taken together, the binding interactions of the arylpiperazine ligands with the receptors of 5-$HT_{1A}$R as well as 5-$HT_7$R can commonly be characterized as bidirectional hydrophobic interactions anchored at the salt bridge, and the similar binding mode of the arylpiperazines to these two serotonergic receptors was conceived to contribute to the difficulty of designing novel arylpiperazine derivatives with high 5-$HT_{1A}$/5-$HT_7$ selectivity.

However, it was worth to note that, at the vicinity of a charged amine atom of the bound arylpiperazine ligands, different amino acid residues that can serve as hydrogen bond donors/acceptors

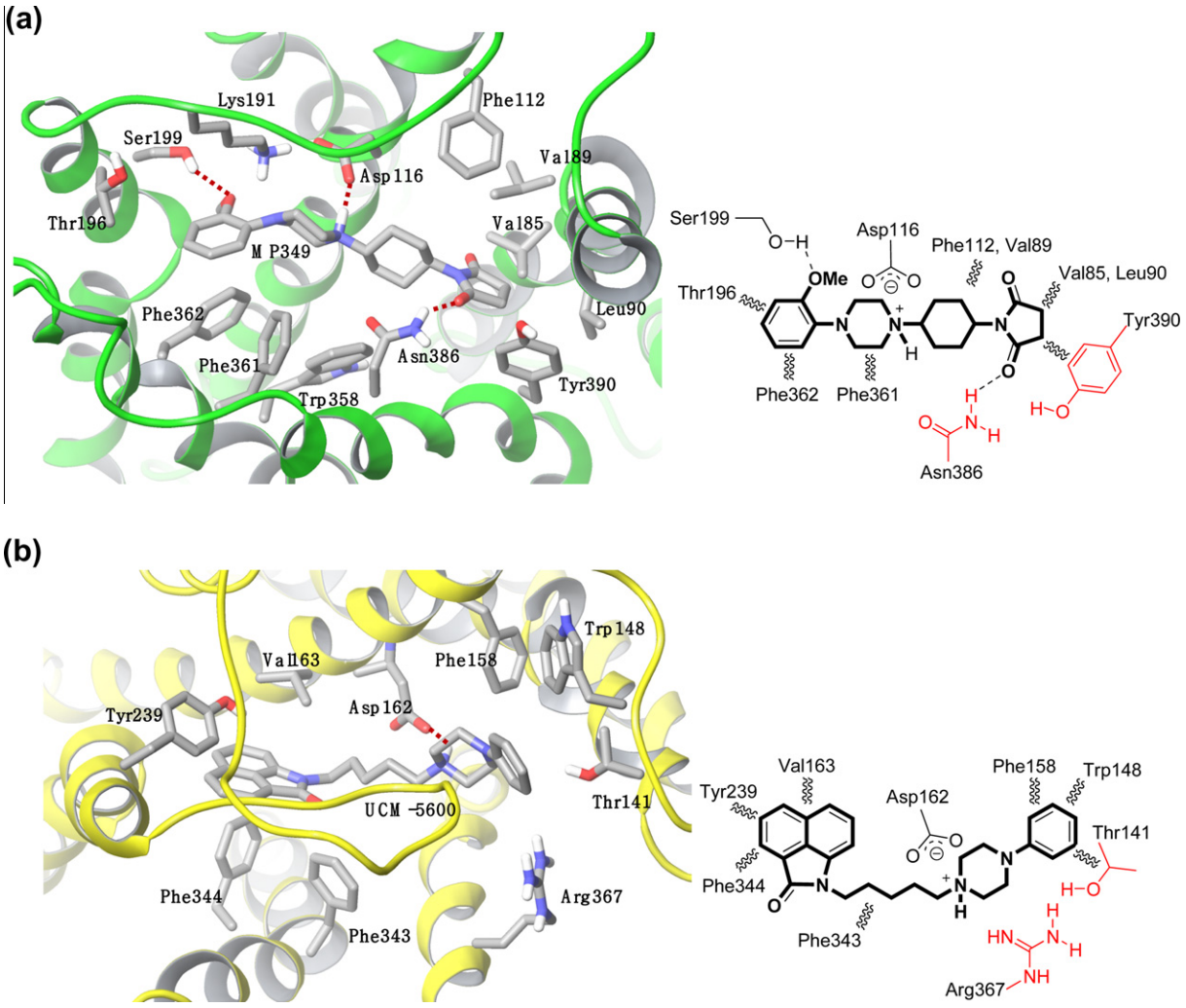



**Figure 2.** Binding modes of (a) MP349 to 5-$HT_{1A}$ receptor and (b) UCM-5600 to 5-$HT_7$ receptor. Right panels are two dimensional illustrations of the binding mode analyses. Hydrogen bonds are indicated as dotted lines.

are located in 5-$HT_{1A}$R (Asn386 and Tyr390, Fig. 2a) and 5-$HT_7$R (Arg367 and Thr141, Fig. 2b), respectively. Based on this observation, we reasoned that introduction of a hydrogen bond donor or acceptor around the ionizable piperazine moiety would help differentiate the bidirectional hydrophobic interactions anchored at the salt bridge and thereby increase 5-$HT_{1A}$/5-$HT_7$ selectivity. The title compound of this study, 4-aminoethylpiperazinyl aryl ketone (**1**, Fig. 3), was thus designed via installation of the fourth pharmacophoric element, carbonyl and aminoethyl group as a hydrogen bond acceptor and donor, at both ends of the piperazine core structure.

## 2.2. Chemistry

A straightforward synthesis of the title compounds, aryl piperazinyl ketones (**1a**–**1l**), is summarized in Scheme 1. Commercially available 1-Boc-piperazine (**2**) was reacted with four acyl chlorides ($R_1$COCl) to give the corresponding aryl piperazinyl ketones **3a**–**3d** in 66–98% yields. After deprotection of the Boc protecting group under acidic conditions, ethylamino functionality was introduced to the resulting ketones**4** through substitution with *N*-(2-bromoethyl)-phthalimide followed by *N*-dephthalation with hydrazine hydrate. Reductive amination of the free amines **6**, thus obtained, with three aldehydes ($R_2$CHO), followed by deprotection as needed, provided the title compounds **1a**–**1l** in 8–74% yields.

## 2.3. Biological activity

Newly synthesized compounds **1a–1l** were assessed for in vitro binding affinity at human serotonergic 5-$HT_{1A}$ and 5-$HT_7$ receptors by radioligand binding assays, using [$^3$H]LSD in transfected CHO-K1 cells and [$^3$H]-8-OH-DPAT in transfected HEK-293 cells, respectively.[23,24] All compounds were assayed as hydrochloride salts. The competitive inhibition assays were performed at a fixed dose of 10 μM, and the %-inhibition data were shown in Table 1.

### 2.3.1. Binding affinity to 5-$HT_{1A}$R

The 4-aminoethylpiperazinyl aryl ketones showed broad inhibitory spectrum of ligand binding to one serotonin receptor, 5-$HT_{1A}$R (Table 1). The compounds with 4-chloro and 3,4-dichloro moieties (**1a**, **1b**, **1e**, **1f**, **1i** and **1j**) showed only marginal activities against 5-$HT_{1A}$R. The benzimidazole-substituted derivatives **1k** and **1l** were most active. It is noteworthy that these two compounds have $R_1$-aromatic substituents such as chlorine atom or trifluoromethyl group at both 3 and 5 positions.

### 2.3.2. Binding affinity to 5-$HT_7$R

Inhibitory activity of 4-aminoethylpiperazinyl aryl ketones against 5-$HT_7$R was more heavily dependent on $R_2$ group rather than $R_1$ group (Table 1). Thus, regardless of $R_1$ substituent, the *tert*-butyl-substituted ketones (**1e**–**1h**) showed no inhibition at all while benzimidazole-substituted ketones (**1i**–**1l**) were moderately potent. On the other hand, the aromatic $R_1$ substituent played a critical role in determining activity of the isoxazole-substituted ketones (**1a**–**1d**), and only the 4-chlorophenyl ketone (**1a**) showed significant activity with 93.6%-inhibition.

### 2.3.3. 5-$HT_{1A}$/5-$HT_7$ selectivity

Regarding receptor selectivity of the title compounds, three important features were identified. First, complete inactivity of the *tert*-butyl-substituted derivatives (**1e**–**1h**) against 5-$HT_7$R regardless of the aromatic substituents in $R_1$ indicates that the *tert*-butyl group associated in the aryl piperazinyl ketone scaffold cannot be accommodated by the 5-$HT_7$R. Even though the *tert*-butyl-substituted derivatives (**1e**–**1h**) were only moderately potent against 5-$HT_{1A}$R, their complete inactivity toward 5-$HT_7$R provides a possibility to discover 5-$HT_{1A}$R-selective inhibitors through extensive structure–activity relationship study of the $R_1$ substituent. Second, the receptor selectivity is lost upon replacement of the *tert*-butyl group with a benzimidazole to result in better inhibitory activity of the corresponding aryl piperazinyl derivatives (**1i**–**1l**) against 5-$HT_{1A}$R and 5-$HT_7$R. Once again, the specific role of the *tert*-butyl group in discriminating the 5-$HT_7$R is of great interest. Third, unlike other series of the aryl piperazinyl ketones, the isoxazole-substituted derivatives (**1a**–**1d**) showed $R_1$-dependent inhibitory activity against 5-$HT_7$R, and compound **1a** was the only example which showed significant selectivity for 5-$HT_7$R. In other words, this compound must have recognized the ligand binding site of 5-$HT_7$R more specifically than others, which indicates that analysis of its binding mode would provide valuable information for designing 5-$HT_7$R-selective inhibitors.

## 2.4. Binding mode analysis

Structure–activity relationship as well as 5-$HT_{1A}$/5-$HT_7$ selectivity of the aryl piperazinyl ketones described above need to be interpreted by specific binding modes in order to provide information for designing receptor-selective serotonergic ligands. For this purpose, docking study of the aryl piperazinyl ketones (**1a**–**1l**) to ligand binding sites of 5-$HT_{1A}$R and 5-$HT_7$R was performed with Glide 4.0 implemented in Maestro 7.5 (Schrödinger, Inc.). During docking, an H-bond constraint between the charged amino group of each ligand and Asp116 (5-$HT_{1A}$R) or Asp162 (5-$HT_7$R) was imposed in order to avoid spurious results. Interestingly, under this constraint, the two most active compounds against 5-$HT_{1A}$R (**1k** and **1l**, Table 1) and the most active one against 5-$HT_7$R (**1a**, Table 1) were successfully docked to the target receptor but none of others provided docking poses. These three derivatives maintained the same binding modes even without the H-bond constraint, but others showed nonspecific binding modes at the periphery of the receptor under

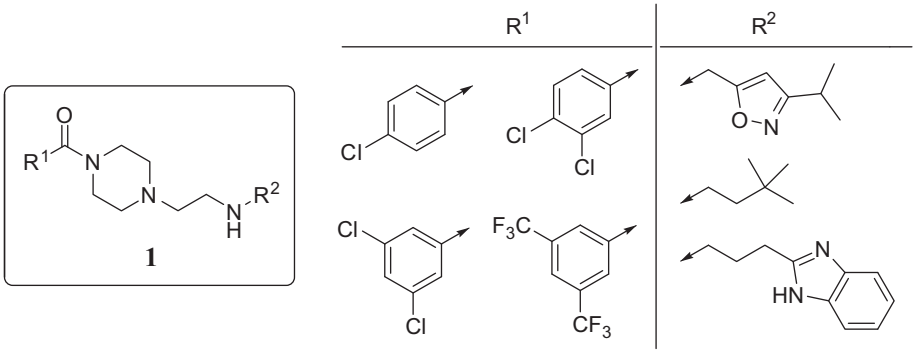
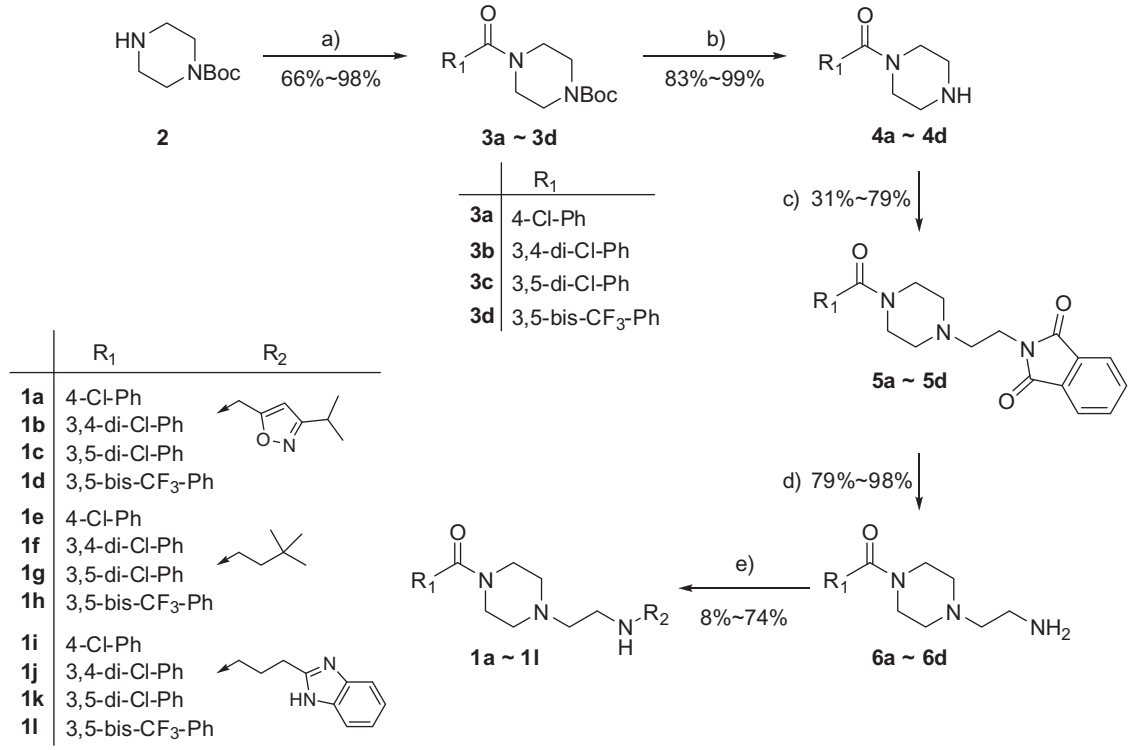
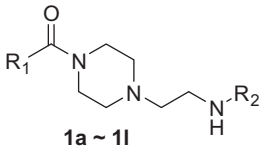



**Figure 3.** Structures of the 4-aminoethylpiperazinyl aryl ketones (**1**) prepared in this study.

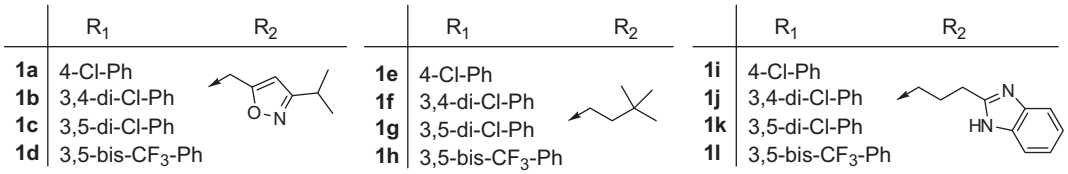



**Scheme 1.** Synthesis of the 4-aminoethylpiperazinyl aryl ketones (**1a**–**1l**). Reagents and conditions: (a) $R_1COCl$, DIPEA, $CH_2Cl_2$, rt; (b) 30% TFA in $CH_2Cl_2$, rt; (c) *N*-(2-bromoethyl)-phthalimide, $K_2CO_3$, $CH_3CN$, reflux; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux; (e) $R_2CHO$, $NaBH(OAc)_3$, MeOH, rt, then, if needed, 30% TFA in $CH_2Cl_2$, rt.

**Table 1**
%-Inhibition data of the 4-aminoethylpiperazinyl aryl ketones (**1a**–**1l**) against 5-$HT_{1A}$ and 5-$HT_7$ receptors



1a ~ 1l




| Compds | %-Inhibition at 10 μM | | Compds | %-Inhibition at 10 μM | | Compds | %-Inhibition at 10 μM | |
|---|---|---|---|---|---|---|---|---|
| | 5-$HT_{1A}$ | 5-$HT_7$ | | 5-$HT_{1A}$ | 5-$HT_7$ | | 5-$HT_{1A}$ | 5-$HT_7$ |
| **1a** | 56.5 | 93.6 | **1e** | 36.7 | −0.6 | **1i** | 47.5 | 36.8 |
| **1b** | 76.4 | 16.1 | **1f** | 65.2 | 2.0 | **1j** | 49.5 | 56.2 |
| **1c** | 43.2 | −0.2 | **1g** | 33.7 | −0.6 | **1k** | 90.1 | 62.7 |
| **1d** | 13.0 | −0.3 | **1h** | −4.4 | −0.3 | **1l** | 87.7 | 45.4 |

the non-constrained docking conditions. In particular, due to large hydrophobic surface and hydrogen bonding capacity, the benzimidazole-substituted derivatives (**1i**–**1l**) were more susceptible for nonspecific binding, which provides a reasonable explanation for their moderate inhibitory activity and low selectivity against the two serotonergic receptors. Thus, the docking results were in accordance with the biological activity data and analysis of the detailed docking poses was anticipated to delineate subtle differences in the ligand binding sites of 5-$HT_{1A}$R and 5-$HT_7$R.

As expected, hydrophobic interaction of the two substituents ($R_1$ and $R_2$) played the key role in distinguishing the two serotonergic receptors, 5-$HT_{1A}$R and 5-$HT_7$R. The binding mode of aryl piperazinyl ketone **1a** to 5-$HT_7$R (Fig. 4a) shows the four key pharmacophoric elements are all in action. Thus, in addition to the critical ionic interaction of N2-nitrogen of the piperazine with Asp162, the keto carbonyl group forms a hydrogen bond with Arg367 (5-$HT_7$R) and the two substituents, 4-chlorophenyl ($R_1$) and 3-isopropylisoxazole ($R_2$), were located in the hydrophobic pockets, R-I and R-II composed of Leu232/Cys231/Cys155/Trp148 (for $R_1$) and Ile159/Phe343/Phe344/Leu370/Val163/Thr244/Ser243 (for $R_2$), respectively (Fig. 4).The binding site for 4-chlorophenyl $R_1$ substituent is flat and narrow disallowing any substituent on the aromatic

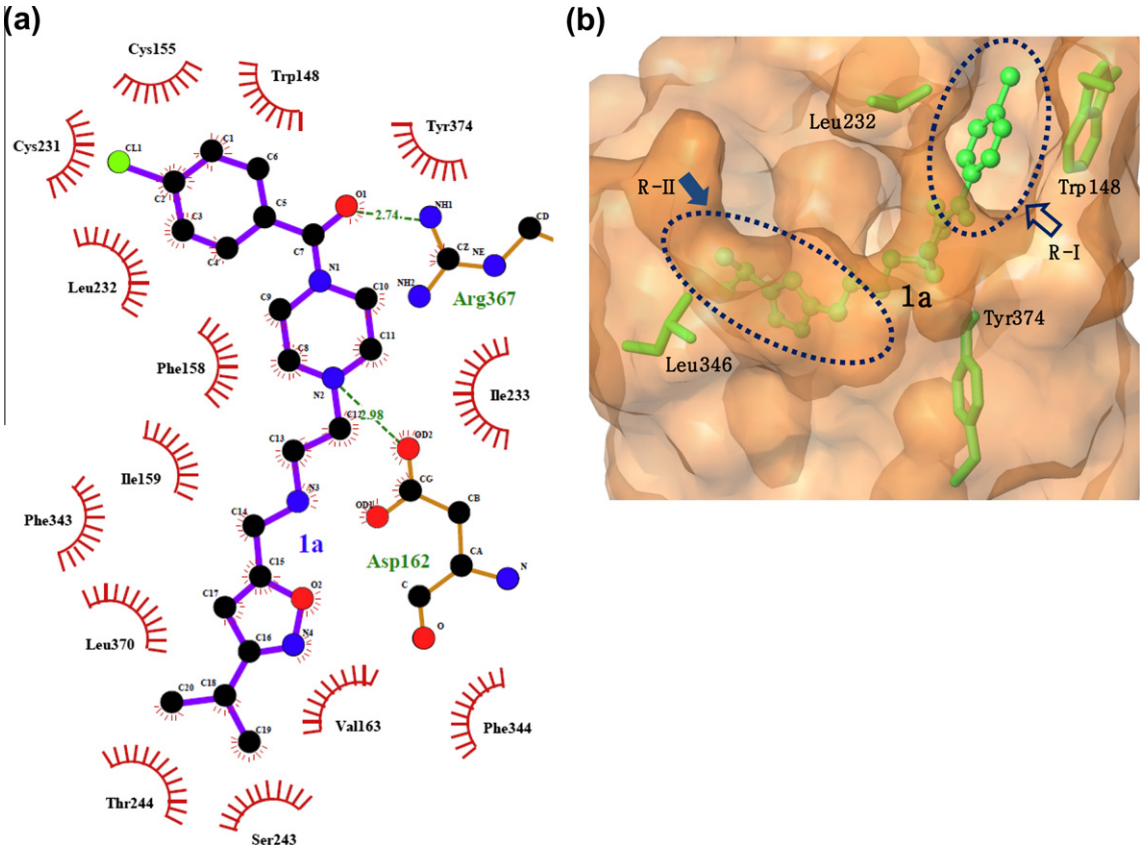



**Figure 4.** Binding mode of the compound **1a** to 5-HT$_7$R. (a) Schematic 2D plot showing intermolecular interactions. (b) Surface representation to show hydrophobic pockets (dotted circles) perpendicular to each other. Two hydrophobic binding sites are indicated as dotted circles and the $R_1$- and $R_2$-binding site are noted by R-I and R-II, respectively. The 2D schematic plots were created with the program LIGPLOT.[25]

ring other than at 4-position (Fig. 4a), which is in match with the dramatic decrease in the binding affinity of **1b**–**1d** in comparison with **1a** (Table 1). The $R_2$-binding site of 5-HT$_7$R is more globular than the $R_1$-binding site but not big enough to accommodate bulky or large substituent such as *tert*-butyl or benzimidazole (Fig. 4a). Thus, among the three $R_2$-substituents introduced in this study, relatively small 3-isopropylisoxazole fits in this pocket and the lack of inhibitory activity of the *tert*-butyl substituted derivatives (**1e**–**1h**, Table 1) as well as peripheral binding of the benzimidazole-substituted derivatives (**1i**–**1l**) to 5-HT$_7$R can be associated with this feature of the $R_2$-binding site. Surface representation of the binding mode (Fig. 4b) shows that these two hydrophobic binding sites (R-I and R-II, Fig. 4b) are perpendicular to each other and adequate for binding of a bent conformation of the ligand **1a**. This observation is reminiscent of the report by Lepailleur et al.[8] which claimed that 5-HT$_7$R affinity arises from bent conformation of the phenylpyrrole derivatives whereas an extended one is best suited for 5-HT$_{1A}$R selectivity.

The binding modes of **1k** and**1l** to 5-HT$_{1A}$R were almost identical to each other, and the schematic 2D plot showing intermolecular interactions of the representative ligand**1k** with 5-HT$_{1A}$R was similar to that of **1a** (Fig. 5a). The piperazinyl N2-nitrogen of **1k**was in ionic interaction with Asp116 and the two substituents ($R_1$ and $R_2$) were located in two hydrophobic pockets, R-I′ and R-II′, composed of Ala365/Lys191 (for $R_1$) and Asp82/Val85/Cys119/Cys120/Asn392/Ser123 (for $R_2$) in Fig. 5. However, surface representation of the binding mode of **1k** (Fig. 5b) is significantly different from that of **1a** (Fig. 4b) in that the two hydrophobic binding sites (R-I′ and R-II′, Fig. 5b) are parallel rather than perpendicular. Thus, an optimal ligand conformation for binding to 5-HT$_{1A}$R is expected to be an extended one, which, once again, is consistent with the report by Lepailleur et al.[8] Also, the docking pose of the ligand **1k** bound in 5-HT$_{1A}$R has a flipped conformation in comparison to that of the ligand **1a** in 5-HT$_7$R, resulting in its 3,5-dichlorophenyl ring ($R_1$) located at R-I′ corresponding to the $R_2$-binding site (R-II) of 5-HT$_7$R (Figs. 4b and 5b). Like R-II in 5-HT$_7$R, the hydrophobic pocket R-I′ has a globular shape and this structural feature allows selective binding of the aromatic $R_1$ with substituents at 3 and/or 5 position rather than 4 position. Potent inhibitory activity of **1k** and **1l** against 5-HT$_{1A}$R among the benzimidazole-substituted derivatives (Table 1) might be related with the substituent-specific binding event at R-I′.

On the other hand, while the $R_1$-binding site in 5-HT$_7$R (R-I, Fig. 4b) is formed by a shallow opening between Leu232 and Trp148, the corresponding site in 5-HT$_{1A}$R composed of Cys187 and Trp102 is closed (Fig. 5b). The lack of second hydrophobic pocket in 5-HT$_{1A}$R is compensated by a new binding site for the $R_2$-substituent (R-II′) near Tyr390 (Fig. 5b). This site has a structure of narrow cleft flanked by hydrophobic side chains (Fig. 5a).Therefore, it is unlikely for this site to accommodate bulky $R_1$ or $R_2$ substituent with a branched chain and this provides a reasonable explanation for lack of specific docking poses of the 3-isopropyloxazole- (**1a**–**1d**) as well as *tert*-butyl-substituted (**1e**–**1h**) aryl piperazinyl ketones at 5-HT$_{1A}$R and thereby their moderate to low inhibitory activity against this receptor. And the benzimidazole-substituted aryl piperazinyl ketones (**1k** and **1l**) with relatively long carbon chain show potent activity with depending on the $R_1$ substitutents.

## 3. Conclusion

The 4-aminoethylpiperazinyl aryl ketones showed a wide spectrum of activity and selectivity for the 5-HT receptors depending on

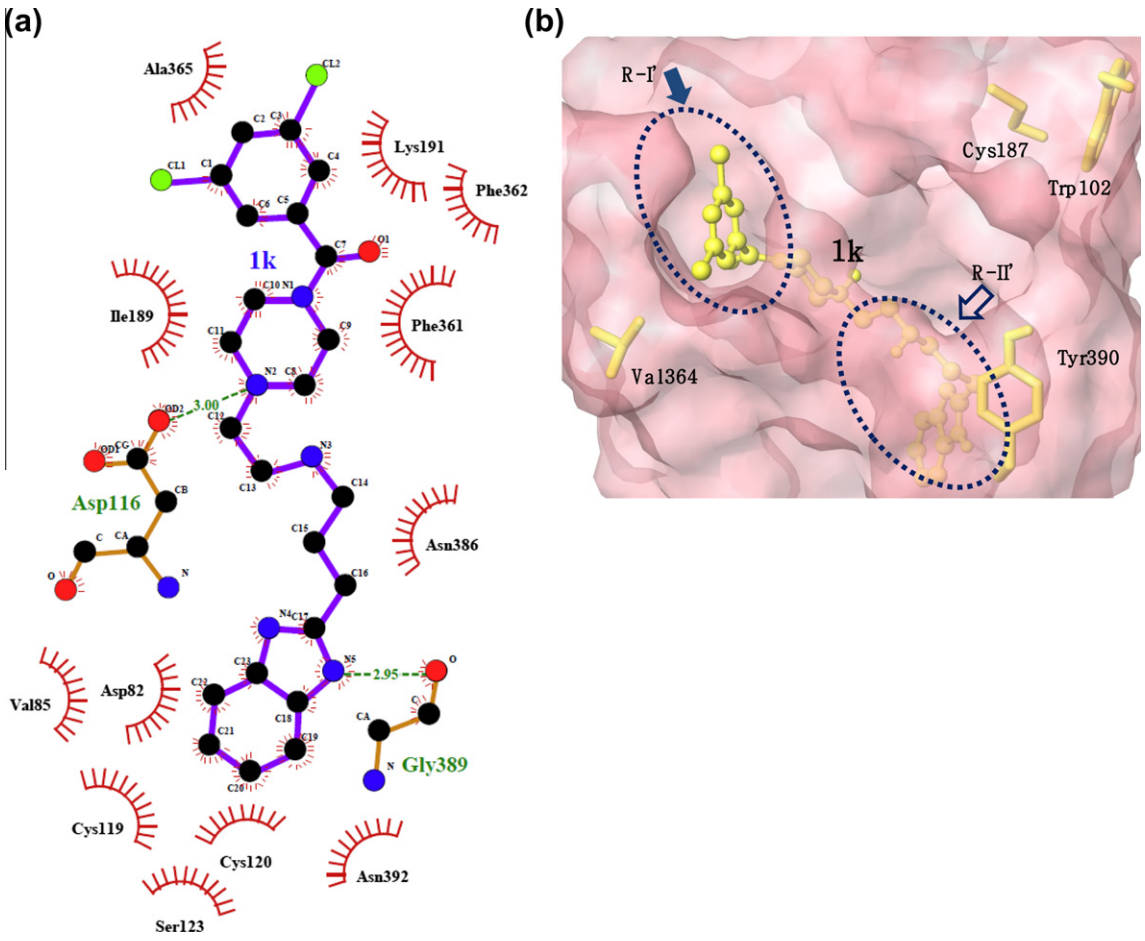



**Figure 5.** Binding mode of **1k** to 5-$HT_{1A}$R. (a) Schematic 2D plot showing intermolecular interactions. (b) Surface representation to show hydrophobic pockets (dotted circles) perpendicular to each other. Two hydrophobic binding sites are indicated as dotted circles and the $R_1$- and $R_2$-binding site are noted by R-I′ and R-II′, respectively.

the type of the hydrophobic groups attached ($R_1$ and $R_2$) at the aryl piperazinyl ketone scaffold. The title compounds with the benzimidazole-substituted derivatives (**1k** and **1l**) showed the most activity against 5-$HT_{1A}$R receptor and, the compound **1a** with an isoxazole substituent showed the best binding affinity against 5-$HT_7$ receptor.

In order to interpret the seemingly complicated activity as well as selectivity profile, a docking study was performed. Analysis of binding modes of **1a** and **1k** to 5-$HT_7$R and 5-$HT_{1A}$R, respectively, revealed that both receptors have two hydrophobic pockets around the anchoring salt bridge and the hydrophobic substituents ($R_1$ and $R_2$) of **1a** and **1k** bind to these pockets. It is noteworthy that these two binding sites are perpendicular to each other in 5-$HT_7$R but parallel in 5-$HT_{1A}$R, and this observation is well matched with the previous report which claimed that 5-$HT_7$R affinity arises from bent conformation of the bound ligand whereas an extended one is best suited for 5-$HT_{1A}$R selectivity. Also, as these pockets have different size and shape, appropriate combination of $R_1$ and $R_2$ is crucial for selective binding to each receptor.

Through synthesis, biological evaluation, and docking study of 4-aminoethylpiperazinyl aryl ketones against 5-$HT_7$R and 5-$HT_{1A}$R, we have shown that their inhibitory activity as well as selectivity were maneuvered by interplay of two hydrophobic substituents attached at both ends of the title compound. Based on this information, extensive structure–activity relationship study is in progress and will be published elsewhere.

## 4. Experimental

### 4.1. Chemistry

#### 4.1.1. General remarks

Nuclear magnetic resonance spectra were recorded at 400 or 300 MHz for $^1$H NMR and at 100 or 75 MHz for $^{13}$C NMR with tetramethylsilane as internal standard. Chemical shift are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Coupling constants are reported in hertz (Hz). The chemical shifts are reported as parts per million ($\delta$) relative to the solvent peak.

#### 4.1.2. Synthesis of *tert*-butyl 4-(aryloyl)piperazine-1-carboxylate (3a–3d)

*General procedure: To* a solution of 1-Boc-piperazine **2** (5.37 mmol) in $CH_2Cl_2$ was added DIPEA (10.74 mmol) and acyl chloride (8.06 mmol). The reaction mixture was stirred for 24 h. After reaction, the mixture was diluted with satd $NaHCO_3$, extracted with $CH_2Cl_2$, dried $MgSO_4$. The crude compound was purified by column chromatography ($SiO_2$, Hexane/EtOAc = 2:1) to afford the desired compound **3a**–**3d** as yellow oil in 66–99% yield.

**4.1.2.1. Synthesis of *tert*-butyl 4-(4-chlorobenzoyl)piperazine-1-carboxylate (3a).** Reaction of the compound **2**with 4-chlorobenzyoyl chloride according to the procedure described above afforded **3a** in 66% yield as yellow oil:$^1$H NMR (300 MHz, $CDCl_3$)

δ 7.42 (dd, *J* = 6.5, 2.1 Hz, 2H), 7.36 (dd, *J* = 6.5, 2.2 Hz, 2H), 3.46–3.69 (m, 8H), 1.48 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 169.51, 154.51, 136.05, 133.80, 128.87, 128.61, 80.41, 43.67, 28.35.

**4.1.2.2. Synthesis of *tert*-butyl 4-(3,4-dichlorobenzoyl)piperazine-1-carboxylate (3b).** Reaction of the compound **2** with 3,4-dichlorobenzoyl chloride according to the procedure described above afforded **3b** in 82% yield as yellow oil:$^1$H NMR (300 MHz, $CDCl_3$) δ 7.51–7.53 (m, 2H), 7.24–7.28 (m, 1H), 3.36–3.82 (m, 8H), 1.49 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 168.13, 162.91, 135.21, 134.42, 133.18, 130.73, 129.32, 126.37, 80.52, 28.35.

**4.1.2.3. Synthesis of *tert*-butyl 4-(3,5-dichlorobenzoyl)piperazine-1-carboxylate (3c).** Reaction of the compound **2** with 3,5-dichlorobenzyoyl chloride according to the procedure described above afforded **3c** in 99% yield as yellow oil:$^1$H NMR (300 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.29 (s, 2H), 3.45–3.75 (m, 8H), 1.48 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 167.55, 154.46, 138.23, 135.55, 130.02, 125.51, 80.56, 28.35.

**4.1.2.4. Synthesis of *tert*-butyl 4-(3,5-bis-trifluoromethylbenzoyl)piperazine-1-carboxylate (3d).** Reaction of the compound **2**with 3,5-bis-trifluoromethylbenzoyl chloride according to the procedure described above afforded **3d** in 99% yield as yellow oil: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.87 (s, 2H), 3.30–3.80 (m, 4H), 2.80–3.00 (m, 4H), 2.01 (s, 1H).

### 4.1.3. Synthesis of aryl(piperazin-1-yl)methanone (4a–4d)

*General procedure:* To a solution of **3a**–**3d** (3.5 mmol) in $CH_2Cl_2$ was added 30% TFA, and the resulting mixture was stirred at rt for 5 h. After reaction, the mixture was made basic with saturated $NaHCO_3$ solution. This mixture was extracted with $CH_2Cl_2$, dried over $MgSO_4$, and concentrated under reduced pressure to give the desired compounds **4a**–**4d** as white solids. These compounds were used for the next reaction without further purification (83–99% yield).

**4.1.3.1. Synthesis of (4-chlorophenyl)(piperazin-1-yl)methanone (4a).** Reaction of **3a** with 30% TFA in $CH_2Cl_2$according to the procedure described above afforded **4a** in 83% yield as white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.33–7.41 (m, 4H), 3.40–3.71 (m, 4H), 2.85–2.90 (m, 4H), 1.79 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 169.31, 135.70, 134.26, 128.75, 128.57, 46.20.

**4.1.3.2. Synthesis of (3,4-dichlorophenyl)(piperazin-1-yl)methanone (4b).** Reaction of **3b** with 30% TFA in $CH_2Cl_2$according to the procedure described above afforded **4b** in 97% yield as white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.48–7.52 (m, 2H), 7.23–7.28 (m, 1H), 3.40–3.73 (m, 4H), 2.85–2.90 (m, 4H), 2.02 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 167.90, 135.63, 134.04, 133.01, 130.61, 129.26, 126.36, 46.12.

**4.1.3.3. Synthesis of (3,5-dichlorophenyl)(piperazin-1-yl)methanone (4c).** Reaction of **3c** with 30% TFA in $CH_2Cl_2$according to the procedure described above afforded **4c** in 97% yield as white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.28 (s, 2H), 3.35–3.95 (m, 6H), 2.90–3.00 (m, 4H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 167.37, 138.33, 135.50, 129.90, 125.48, 45.50.

**4.1.3.4. Synthesis of (3,5-bis-trifluoromethylphenyl)(piperazin-1-yl)methanone (4d).** Reaction of **3d** with 30% TFA in $CH_2Cl_2$ according to the procedure described above afforded **4d** in 99% yield as white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.87 (s, 2H), 3.30–3.80 (m, 4H), 2.80–3.00 (m, 4H), 2.01 (s, 1H).

### 4.1.4. Synthesis of 2-(2-(4-arylpiperazin-1-yl)ethyl)isoindoline-1,3-dione (5a–5d)

*General procedure:* To a solution of piperazine **4a**–**4d** (2.9 mmol) obtained above in acetonitile was added $K_2CO_3$ (5.8 mmol) and *N*-(2-bromoethyl)-phthalimide (4.4 mmol). The reaction mixture was refluxed for 24 h. After reaction, the mixture was diluted with $H_2O$ and then extracted with EtOAc three times. The combined organic layer was dried over $MgSO_4$. The crude product was purified by column chromatography ($SiO_2$, Hexane/EtOAc/$CH_2Cl_2$ = 1:1:1) to afford the desired compound **5a**–**5d** as white powders in 31–79% yield.

**4.1.4.1. Synthesis of 2-(2-(4-(4-chlorobenzoyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (5a).** Reaction of **4a** with *N*-(2-bromoethyl)-phthalimide in the presence of $K_2CO_3$ in acetonitile according to the procedure described above afforded **5a** in 31% yield as white powder:$^1$H NMR (300 MHz, $CDCl_3$) δ 7.84–7.87 (m, 2H), 7.72–7.76 (m, 2H), 7.31–7.40 (m, 4H), 3.81–3.85 (m, 2H), 3.68–3.71 (m, 2H), 3.33–3.36 (m, 2H), 2.50–2.69 (m, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 169.16, 168.35, 135.74, 134.21, 133.95, 132.16, 128.73, 128.61, 123.25, 55.57, 35.09.

**4.1.4.2. Synthesis of 2-(2-(4-(3,4-dichlorobenzoyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (5b).** Reaction of **4b** with *N*-(2-bromoethyl)-phthalimide in the presence of $K_2CO_3$ in acetonitile according to the procedure described above afforded **5b** in 69% yield as white powder:$^1$H NMR (300 MHz, DMSO) δ: 7.82–7.90 (m, 4H), 7.65–7.71 (m, 2H), 7.37 (dd, *J* = 8.2, 1.9 Hz, 1H), 3.71 (t, *J* = 6.4 Hz, 2H), 3.45–3.55 (m, 2H), 3.13–3.24 (m, 2H), 2.57 (t, *J* = 6.4 Hz, 2H), 2.41–2.51 (m, 4H).

**4.1.4.3. Synthesis of 2-(2-(4-(3,5-dichlorobenzoyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (5c).** Reaction of **4c** with *N*-(2-bromoethyl)-phthalimide in the presence of $K_2CO_3$ in acetonitile according to the procedure described above afforded **5c** in 65% yield as white powder: $^1$H NMR (300 MHz, DMSO) δ 7.87 (dd, *J* = 5.4 Hz, 3.0 Hz, 2H), 7.74 (dd, *J* = 5.4 Hz, 3.0 Hz, 2H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.25–7.28 (m, 2H), 3.84 (t, *J* = 6.3 Hz, 2H), 3.69 (s, 2H), 3.34 (s, 2H), 2.51–2.71 (m, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 168.35, 138.65, 135.37, 133.97, 132.15, 129.73, 125.50, 123.26, 55.53, 35.07.

**4.1.4.4. Synthesis of 2-(2-(4-(3,5-bis-trifluoromethylbenzoyl)piperazin-1-yl)ethyl)isoindoline-1,3-dione (5d).** Reaction of **4d** with *N*-(2-bromoethyl)-phthalimide in the presence of $K_2CO_3$ in acetonitile according to the procedure described above afforded **5d** in79% yield as white powder: $^1$H NMR (300 MHz, DMSO) δ: 7.94 (s, 1H), 7.85–7.88 (m, 4H), 7.72–7.77 (m, 2H), 3.84 (t, *J* = 6.2 Hz, 2H), 3.71–3.76 (m, 2H), 3.30–3.35 (m, 2H), 2.70 (t, *J* = 6.2 Hz, 2H), 2.50–2.65 (m, 4H).

### 4.1.5. Synthesis of (4-(2-aminoethyl)piperazin-1-yl)arylmetha none (6a–6d)

*General procedure:* To a solution of the phthalimide obtained above (0.91 mmol) in EtOH was added hydrazine monohydrate (2.73 mmol). The mixture was refluxed at 60 °C for 2 h and then cooled at rt. After reaction, EtOH was removed, and then filtered. The organic solvent was evaporated to give the desired compound **6a**–**6d** as yellow oil in 79–91% yield, which was used for the next step without further purification.

**4.1.5.1. Synthesis of (4-(2-aminoethyl)piperazin-1-yl)(4-chlorophenyl)methanone (6a).** Reaction of **5a** with hydrazine monohydrate in EtOH according to the procedure described above afforded **6a** in 92% yield as yellow oil: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.33–7.41 (m, 4H), 3.76–3.78 (m, 2H), 3.41–3.43 (m, 2H), 2.79–2.83

(m, 2H), 2.44–2.51 (m, 6H), 1.67 (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.19, 135.76, 134.15, 128.75, 128.61, 60.91, 53.48, 38.64.

**4.1.5.2. Synthesis of (4-(2-aminoethyl)piperazin-1-yl)(3,4-dichlorophenyl)methanone (6b).** Reaction of **5b** with hydrazine monohydrate in EtOH according to the procedure described above afforded **6b** in 91% yield as yellow oil:$^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.47–7.50 (m, 2H), 7.23 (d, $J$ = 8.2, 1.9 Hz, 1H), 3.35–3.75 (m, 4H), 2.81 (t, $J$ = 5.8 Hz, 2H), 2.40–2.50 (m, 6H), 2.24 (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.78, 135.53, 134.08, 132.98, 130.60, 129.26, 126.38, 60.63, 53.38, 42.36, 38.48.

**4.1.5.3. Synthesis of (4-(2-aminoethyl)piperazin-1-yl)(3,5-dichlorophenyl)methanone (6c).** Reaction of **5c** with hydrazine monohydrate in EtOH according to the procedure described above afforded **6c** in 79% yield as yellow oil: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.42 (s, 1H), 7.28 (s, 2H), 3.35–3.80 (m, 4H), 2.82 (t, $J$ = 5.9 Hz, 2H), 2.45–2.55 (m, 6H), 1.86 (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 167.20, 138.61, 135.39, 129.76, 125.50, 60.65, 38.56.

**4.1.5.4. Synthesis of (4-(2-aminoethyl)piperazin-1-yl)(3,5-bis(trifluoromethyl)phenyl)methanone (6d).** Reaction of **5d** with hydrazine monohydrate in EtOH according to the procedure described above afforded **6d** in 81% yield as yellow oil:$^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (s, 1H), 7.87 (s, 2H), 3.80–3.85 (m, 2H), 3.40–3.50 (m, 2H), 2.81 (t, $J$ = 5.9 Hz, 2H), 2.47–2.62 (m, 6H), 1.65 (s, 2H).

**4.1.6. Synthesis of aryl(4-alkylaminoethyl)piperazin-1-yl)meth anone (1a–1l)**

*General procedure: To* a solution of an aldehyde (0.76 mmol) in MeOH was added the amine **6a**–**6d** prepared above (0.84 mmol). The mixture was stirred for 2 h, and then $NaBH(OAc)_3$ (2.28 mmol) was added. After 6 h of stirring, the solvent was removed. The reaction mixture was diluted with $H_2O$, added saturated $NaHCO_3$ solution, extracted with $CH_2Cl_2$, dried $MgSO_4$. The crude compound was purified by column chromatography to afford the desired compound **1a**–**1h** as an oil in 8–74% yield.

**4.1.6.1. Synthesis of (4-chlorophenyl)(4-(2-((3-isopropylisoxazol-5-yl)methylamino)ethyl)piperazin-1-yl)methanone (1a) .** Reaction of **6a** with 3-isopropylisoxazole-5-carbaldehyde according to the procedure described above afforded **1a** in 74% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.34–7.41 (m, 4H), 6.03 (s, 1H), 3.91 (s, 2H), 3.76–3.78 (m, 2H), 3.42–3.43 (m, 2H), 3.03–3.07 (m, 1H), 2.73–2.77 (m, 2H), 2.40–2.56 (m, 4H), 1.90 (s, 1H), 1.28 (d, $J$ = 6.9 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.94, 169.35, 169.19, 135.79, 134.11, 128.77, 128.62, 99.86, 57.44, 45.49, 45.02, 26.48, 21.78.

**4.1.6.2. Synthesis of (3,4-dichlorophenyl)(4-(2-((3-isopropyliso xazol-5-yl)methylamino)ethyl)piperazin-1-yl)methanone (1b) .** Reaction of **6b** with 3-isopropylisoxazole-5-carbaldehyde according to the procedure described above afforded **1b** in 55% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.47–7.50 (m, 2H), 7.23 (dd, $J$ = 8.2, 1.9 Hz, 1H), 6.02 (s, 1H), 3.88 (s, 2H), 3.70–3.75 (m, 2H), 3.40–3.45 (m, 2H), 2.99–3.06 (m, 1H), 2.72–2.76 (m, 2H), 2.40–2.56 (m, 6H), 1.88 (s, 1H), 1.27 (d, $J$ = 7.0 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.93, 169.33, 167.74, 135.55, 134.08, 132.99, 130.59, 129.28, 126.39, 99.85, 57.51, 45.48, 45.00, 26.47, 21.75.

**4.1.6.3. Synthesis of (3,5-dichlorophenyl)(4-(2-((3-isopropylisoxazol-5-yl)methylamino)ethyl)piperazin-1-yl)methanone (1c).** Reaction of **6c** with 3-isopropylisoxazole-5-carbaldehyde according to the procedure described above afforded **1c** in 33% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.41 (s, 1H), 7.27 (s, 2H), 6.03 (s, 1H), 3.90 (s, 2H), 3.73–3.77 (m, 2H), 3.38–3.42 (m, 2H), 3.02–3.07 (m, 1H), 2.73–2.77 (m, 2H), 2.38–2.57 (m, 6H), 1.28 (d, $J$ = 7.0 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 170.92, 169.35, 167.18, 138.57, 135.40, 129.77, 125.50, 99.87, 57.49, 45.45, 45.00, 26.48, 21.76.

**4.1.6.4. Synthesis of (3,5-bis(trifluoromethyl)phenyl)(4-(2-((3-isopropylisoxazol-5-yl)methylamino)ethyl)piperazin-1-yl)methanone (1d).** Reaction of **6d** with 3-isopropylisoxazole-5-carbaldehyde according to the procedure described above afforded **1d** in10% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (s, 1H), 7.87 (s, 2H), 6.03 (s, 1H), 3.91 (s, 2H), 3.80–3.82 (m, 2H), 3.40–3.42 (m, 2H), 3.01–3.07 (m, 1H), 2.74–2.77 (m, 2H), 2.40–2.58 (m, 6H), 1.28 (d, $J$ = 7.0 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.80, 169.35, 166.98, 137.82, 132.15 (q, $J$ = 33.5 Hz), 127.45, 123.55, 122.86 (q, $J$ = 271.4 Hz), 99.88, 57.45, 52.72, 45.43, 44.97, 26.47, 21.93.

**4.1.6.5. Synthesis of (4-chlorophenyl)(4-(2-(3,3-dimethylbutylamino)ethyl)piperazin-1-yl)methanone (1e).** Reaction of **6a** with 3,3-dimethylbutanalaccording to the procedure described above afforded **1e** in 8% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.33–7.41 (m, 4H), 3.34–3.77 (m, 4H), 2.50–2.61 (m, 10H), 1.35–1.40 (m, 2H), 0.90 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 169.21, 135.79, 134.09, 128.81, 128.76, 56.68, 51.10, 50.07, 39.73, 29.79, 29.49.

**4.1.6.6. Synthesis of (3,4-dichlorophenyl)(4-(2-(3,3-dimethylbutylamino)ethyl)piperazin-1-yl)methanone (1f).** Reaction of **6b** with 3,3-dimethylbutanalaccording to the procedure described above afforded **1f** in 61% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.48–7.51 (m, 2H), 7.24 (d, $J$ = 8.1 Hz, 1H), 3.70–3.75 (m, 2H), 3.40–3.45 (m, 2H), 2.73 (t, $J$ = 6.0 Hz, 2H), 2.59–2.65 (m, 2H), 2.44–2.56 (m, 6H), 1.39–1.44 (m, 2H), 0.91 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.75, 135.59, 134.07, 132.98, 130.59, 129.28, 126.39, 57.89, 53.47, 46.51, 46.18, 44.11, 29.82, 29.60.

**4.1.6.7. Synthesis of (3,5-dichlorophenyl)(4-(2-(3,3-dimethylbutylamino)ethyl)piperazin-1-yl)methanone (1g).** Reaction of **6c** with 3,3-dimethylbutanalaccording to the procedure described above afforded **1g** in 55% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.42 (s, 1H), 7.28 (s, 2H), 3.75–3.80 (m, 2H), 3.38–3.42 (m, 2H), 2.73 (t, $J$ = 5.9 Hz, 2H), 2.42–2.65 (m, 8H), 1.39–1.44 (m, 2H), 0.92 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.18, 138.63, 135.39, 129.74, 125.50, 57.91, 46.53, 46.20, 44.15, 29.82, 29.60.

**4.1.6.8. Synthesis of (3,5-bis-trifluoromethylphenyl)(4-(2-(3,3-dimethylbutylamino)ethyl)piperazin-1-yl)methanone (1h) .** Reaction of **6d** with 3,3-dimethylbutanalaccording to the procedure described above afforded **1h** in63% yield as yellow oil after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (s, 1H), 7.87 (s, 2H), 3.80–3.83 (m, 2H), 3.38–3.42 (m, 2H), 2.65–2.75 (m, 2H), 2.50–2.63 (m, 8H), 1.39–1.44 (m, 2H), 0.91 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.97, 137.86, 132.13 (q, $J$ = 33.8 Hz), 127.45, 123.50, 122.86 (q, $J$ = 271.3 Hz), 57.87, 46.51, 46.19, 44.14, 29.81, 29.58.

**4.1.6.9. Synthesis of (4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)propylamino)ethyl)piperazin-1-yl)(4-chlorophenyl)methanone (1i).** Reaction of **6a** with *tert*-butyl 2-(3-oxopropyl)-1*H*-benzo[d]imidazole-1-carboxylate according to the procedure described above afforded *tert*-butyl 2-(3-(2-(4-(4-chlorobenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxyl atein 28% yield after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.29–7.37 (m, 5H), 6.83–6.91 (m, 2H), 6.65–6.67 (m, 1H), 3.21–3.62 (m, 10H), 2.46–2.65 (m, 6H), 1.96–2.11 (m, 2H), 1.47 (s, 9H).

To a solution of the *tert*-butyl 2-(3-(2-(4-(4-chlorobenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylate obtained above (105.9 mg, 0.2 mmol) in $CH_2Cl_2$ was added 30% TFA, and the resulting solution was stirred at rt for 5 h. After reaction, the mixture was made basic with saturated $NaHCO_3$ solution. This mixture was extracted with $CH_2Cl_2$ three times and the combined organic layer was dried over $MgSO_4$. The crude compound was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 10:1→$CHCl_3$/MeOH/$H_2O$/$NH_4OH$ = 70:30:3:3) to give the desired product (37 mg, 0.087 mmol, 44% yield): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.45–7.50 (m, 2H), 7.31–7.40 (m, 4H), 7.15–7.20 (m, 2H), 3.40–3.73 (m, 4H), 3.03 (t, $J$ = 6.4 Hz, 2H), 2.79–2.88 (m, 4H), 2.55–2.62 (m, 6H), 1.98–2.06 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.24, 155.06, 140.40, 138.64, 135.91, 135.84, 128.82, 128.58, 122.18, 115.00, 56.75, 49.22, 45.86, 28.03, 26.29.

**4.1.6.10. Synthesis of (4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)propylamino)ethyl)piperazin-1-yl)(4-chlorophenyl)methanone (1j) .** Reaction of **6b** with *tert*-butyl 2-(3-oxopropyl)-1*H*-benzo[*d*]imidazole-1-carboxylate according to the procedure described above afforded *tert*-butyl 2-(3-(2-(4-(3,4-dichlorobenzoyl)piperazin-1-yl)ethylamino) propyl)-1*H*-benzo[*d*]imidazole-1-carboxylatein 25% yield after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.48–7.51 (m, 2H), 7.39 (s, 1H), 7.23 (dd, $J$ = 8.2, 2.0 Hz, 1H), 6.87–6.96 (m, 2H), 6.69 (dd, $J$ = 7.7, 1.5 Hz, 1H), 4.10–4.14 (m, 2H), 3.65 (t, $J$ = 6.7 Hz, 2H), 3.40–3.50 (m, 4H), 2.48–2.69 (m, 8H), 1.95–2.01 (m, 2H), 1.51 (s, 9H).

To a solution of *tert*-butyl 2-(3-(2-(4-(3,4-dichlorobenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylate (100 mg, 0.18 mmol) obtained above in $CH_2Cl_2$ was added 30% TFA, and the resulting solution was stirred at rt for 5 h. After reaction, the mixture was made basic with saturated $NaHCO_3$ solution. This mixture was extracted with $CH_2Cl_2$ three times and the combined organic layer was dried over $MgSO_4$.The filtrate was concentrated under reduced pressure to give white solid (58 mg, 0.13 mmol, 70% yield): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.48–7.52 (m, 4H), 7.18–7.24 (m, 3H), 3.40–3.76 (m, 4H), 3.08 (t, $J$ = 6.3 Hz, 2H), 2.79–2.89 (m, 4H), 2.47–2.64 (m, 6H), 1.97–2.05 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 167.91, 155.25, 138.85, 135.50, 134.32, 133.15, 130.77, 129.38, 126.50, 122.06, 114.73, 57.25, 49.54, 45.86, 28.39, 26.72.

**4.1.6.11. Synthesis of(4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)propylamino)ethyl)piperazin-1-yl)(3,5-dichlorophenyl)methanone (1k).** Reaction of **6c** with *tert*-butyl 2-(3-oxopropyl)-1*H*-benzo[*d*]imidazole-1-carboxylate according to the procedure described above afforded *tert*-butyl 2-(3-(2-(4-(3,5-dichlorobenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylate in 13% yield after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.87–7.90 (m, 1H), 7.64–7.67 (m, 1H), 7.41 (s, 1H), 7.21–7.31 (m, 4H), 3.72 (s, 2H), 3.19–3.38 (m, 4H), 2.31–2.69 (m, 10H), 2.06 (s, 2H), 1.69 (s, 9H), 1.49–1.65 (m, 1H).

To a solution of *tert*-butyl 2-(3-(2-(4-(3,5-dichlorobenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylateobtained above (80.4 mg, 0.14 mmol) in $CH_2Cl_2$ was added 30% TFA, and the resulting solution was stirred at rt for 5 h. After reaction, the mixture was made basic with saturated $NaHCO_3$ solution. This mixture was extracted with $CH_2Cl_2$ three times and the combined organic layer was dried over $MgSO_4$.The crude compound was purified by column chromatography ($SiO_2$,$CH_2Cl_2$/MeOH = 10:1→$CHCl_3$/MeOH/$H_2O$/$NH_4OH$ = 70:30:3:3) to give the desired product (40.2 mg, 0.087 mmol, 62% yield): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.53–7.57 (m, 3H), 7.41–7.42 (m, 1H), 7.19–7.24 (m, 4H), 3.66 (s, 2H), 3.28 (s, 2H), 3.05 (t, $J$ = 6.8 Hz, 3H), 2.31–2.57 (m, 10H), 1.92–1.96 (m, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.22, 155.70, 138.45, 137.96, 135.45, 129.87, 125.44, 122.78, 115.11m 56.23, 53.54, 51.71, 50.41, 27.01, 25.95.

**4.1.6.12. Synthesis of (4-(2-(3-(1*H*-benzo[*d*]imidazol-2-yl)propylamino)ethyl)piperazin-1-yl)(3,5-bis(trifluoromethyl)phenyl)methanone (1l).** Reaction of **6d** with *tert*-butyl 2-(3-oxopropyl)-1*H*-benzo[*d*]imidazole-1-carboxylate according to the procedure described above afforded *tert*-butyl 2-(3-(2-(4-(3,5-*bis*-trifluoromethylbenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylatein 23% yield after column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 20:1): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85–7.98 (m, 5H), 7.38–7.67 (m, 2H), 3.44–3.79 (m, 4H), 3.31–3.63 (m, 6H), 2.89 (t, $J$ = 6.4 Hz, 2H), 2.49–2.67 (m, 4H), 1.99–2.16 (m, 2H), 1.49 (s, 9H).

To a solution of *tert*-butyl 2-(3-(2-(4-(3,5-*bis*-trifluoromethylbenzoyl)piperazin-1-yl)ethylamino)propyl)-1*H*-benzo[*d*]imidazole-1-carboxylate obtained above (102.2 mg, 0.16 mmol) in $CH_2Cl_2$ was added 30% TFA, and the resulting solution was stirred at rt for 5 h. After reaction, the mixture was made basic with saturated $NaHCO_3$ solution. This mixture was extracted with $CH_2Cl_2$ three times and the combined organic layer was dried over $MgSO_4$.The crude compound was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH = 10:1→$CHCl_3$/MeOH/$H_2O$/$NH_4OH$ = 70:30:3:3) to give the desired product (40.7 mg, 0.077 mmol, 48% yield): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.95 (s, 1H), 7.85 (s, 2H), 7.47–7.51 (m, 2H), 7.16–7.20 (m, 2H), 3.76 (s, 2H), 3.32–3.35 (m, 2H), 3.06 (t, $J$ = 6.3 Hz, 2H), 2.81–2.90 (m, 4H), 2.47–2.66 (m, 6H), 2.01–2.06 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.98, 155.04, 138.60, 137.67, 132.16 (q, $J$ = 33.8 Hz), 127.42, 123.59, 123.58 (q, $J$ = 270.8 Hz), 122.05, 114.58, 56.69, 49.25, 45.54, 28.10, 26.25.

## 4.2. Biological assay for synthesized compounds against 5-$HT_{1A}$R and 5-$HT_7$R

This screen was performed by the National Institute of Mental Health Psychoactive Drug Screening Program (PDSP). Radioligands were purchased by PDSP from Perkin–Elmer or GE Healthcare. Competition binding assays were performed using transfected or stably expressing cell membrane preparations as previously described[23,24] and are available online (http://pdsp.med.unc.edu). All experimental details are available online (http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf).

## 4.3. Docking study

### 4.3.1. Preparation of model structures

For homology modeling of human 5-$HT_{1A}$and 5-$HT_7$ serotonin receptors, the crystal structure of the seven helix bundle of $\beta_2$-adrenergic receptor (PDB code 2rh1) was used as a template.[26] Aminoacid sequences of the receptors (P08908 for 5-$HT_{1A}$R andP34969 for 5-$HT_7$R) were downloaded from the Uniprot database (http://www.uniprot.org) and the starting homology models of each receptor were generated using the SwissModel server (accessible from the program DeepView/SwissPdb-Viewer). Further modifications of the models were performed using components of Schrödinger Suite 2008. The models were subjected to energy minimization and

molecular dynamics simulations (MD) in order to obtain a stable, low-energy conformation. Energy minimization was performed using a conjugate gradient minimize (0.05 convergence criteria), the OPLS-AA force field, and GB/SA continuum water model. MD simulations were performed by pre-equilibration for 100 ps and simulation for 1 ns at 300 K with a 1-fs time step and SHAKE applied to all bonds to hydrogen. In the next step, induced fit docking (IFD) was involved for ligand-based optimization of the receptors.[27,28] For this purpose, the representative arylpiperazine ligands with high affinity for given receptors, MP349[22] for 5-$HT_{1A}$R and UCM-5600[9] for 5-$HT_7$R, were chosen. Receptor models found in the top-scored complexes were selected to serve as molecular targets in further docking studies.

### 4.3.2. Preparation of ligand structures

Three dimensional structures of 4-aminoethylpiperazinyl aryl ketones were sketched by 'build' module of Maestro 7.5 (Schrödinger, Inc.). Energy minimization was performed using a conjugate gradient minimize (0.05 convergence criteria), the OPLS-AA force field, and GB/SA continuum water model. A torsional scan along every rotatable bond was then performed for the minimized structures. 'Conformational search' module implemented in Maestro 7.5 was used with automatic setup.

### 4.3.3. Docking study

With the modeled structure, docking of 4-aminoethylpiperazinyl aryl ketones was carried out. We used the protein preparation utilities in Maestro 7.5 to assign the charge state of ionizable residues, add hydrogens, and carry out energy minimization. The ligands were then docked into the homology models using GLIDE 4.0 (http://www.schrodinger.com) with H-bond constraint between Asp116 (5-$HT_{1A}$R) or Asp162 (5-$HT_7$R) and the protonated nitrogens of the ligands. The default setting of the extreme precision mode of GLIDE was employed for the docking, and up to ten poses were saved for analysis.

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