Design, Synthesis, and Structure—Activity Relationships of Highly Potent 5-HT₃ Receptor Ligands

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ABSTRACT: The 5-HT₃ receptor, a pentameric ligand-gated ion channel (pLGIC), is an important therapeutic target. During a recent fragment screen, 6-chloro-N-methyl-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine (1) was identified as a 5-HT₃,R hit fragment. Here we describe the synthesis and structure—activity relationships (SAR) of a series of (iso)quinoline and quinazoline compounds that were synthesized and screened for 5-HT₃,R affinity using a [³H]granisetron displacement assay. These studies resulted in the discovery of several high affinity ligands of which compound 22 showed the highest affinity (pKᵢ > 10) for the 5-HT₃ receptor. The observed SAR is in agreement with established pharmacophore models for 5-HT₃ ligands and is used for ligand—receptor binding mode prediction using homology modeling and in silico docking approaches.

1. INTRODUCTION

5-HT₃ receptor antagonists like alosetron and granisetron are in clinical use to prevent emesis during chemotherapy-induced, radiotherapy-induced and postoperative nausea and vomiting and to alleviate the effects of irritable bowel syndrome (IBS).1−12 Recent studies have also indicated that the 5-HT₃ receptor is involved in depression13 and may play a role in a range of other indications such as schizophrenia, anxiety, substance abuse and addiction, bulimia and pruritus. Moreover, the 5-HT₃ receptor is thought to modulate analgesia, inflammation and cognitive processes.14 The 5-HT₃ receptor belongs to the Cys-loop receptor family of ion channels,5 which include nicotinic acetylcholine (nACh), GABAₐ and glycine receptors. All of these receptors consist of five subunits that surround a central ion conducting pore.6 At present, no high resolution structures are available for Cys-loop receptors, but the availability of crystal structures of the closely related acetylcholine binding protein (AChBP)7 has significantly improved our understanding of the extracellular domain. Together with the results from mutagenesis studies,8−15 it is now acknowledged that the ligand binding site is situated in the extracellular domain at the interface of two subunits. The principal subunit contributes three loops (A−C), while the complementary side contributes three beta-sheets (“loops” D−F) from the adjacent subunit (Figure 1a). 5-HT₃ receptors can be homomeric (all A subunits) or heteromeric (A + B to E), but the exact subunit composition/stoichiometry of the latter type is not yet clear.16,17 As early as 1989, a ligand-based pharmacophore for 5-HT₃ receptor ligands was constructed.18 During the last two decades, this pharmacophore has been continuously re-

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2. CHEMISTRY

Quinazolines and (iso)quinolines were either obtained from our in-house compound library or were synthesized as described below. 2,4-Dichloroquinazoline (2) was reacted with methylamine to yield the corresponding 4-substituted intermediate (5) which was directly used without further purification in the substitution reaction with N-methylpiperazine to form compound 21 (R² = NMe, R³ = H) (Scheme 1).

2,4,6-Trichloroquinazoline (3) was reacted with tributyltin hydride and tetrakis(triphenylphosphine)palladium(0) to yield 2,6-dichloroquinazoline (4) which in turn was substituted with N-methylpiperazine under microwave irradiation to yield 11 (R² = H, R³ = 6-Cl).

Starting from 2-aminobenzoic acid (23), 2-thioxo-2,3-dihydroquinazolin-4(1H)-one (24) was synthesized using a procedure described in literature. This intermediate is allowed to react with methyl iodide to create a more reactive intermediate (25) that is used in the subsequent substitution reaction with N-methylpiperazine which results in the formation compound 26 (Scheme 2).

Compounds 30 and 31 (Scheme 3) were synthesized by reacting commercially available 2,4-dibromoquinoline (27) with either ammoniumhydroxide or methylamine followed by coupling of N-methylpiperazine. This synthesis route yielded in the first step mixtures of both the 2- as well as the 4-substituted regio isomers (for 28 and 29). Since these two regioisomers were difficult to separate, it was decided to use them as regioisomeric mixtures in the substitution reaction with N-methylpiperazine. The obtained regioisomeric mixtures (for 30 and 31) could be separated by column chromatography. Both compound 30 and 31 were obtained as pure regioisomers whose identity was confirmed by 2D 1H NMR (NOESY, see Supporting Information, Figure S2).

2-Methoxynaphtalene (32) was substituted with N-methylpiperazine using n-butyllithium as the base to create compound 33 in a good yield (Scheme 4).

Scheme 1. Preparation of Quinazoline Compounds 6–22

Scheme 2. Preparation of 2-(4-Methylpiperazin-1-yl)quinazolin-4(3H)-one (26)

Scheme 3. Preparation of Quinoline Compounds 30 and 31

Figure 1. (a) Extracellular domains of two adjacent subunits of the 5-HT₃ receptor. (b) Pharmacophore features for 5-HT₃ receptor antagonists. A, B and C indicate respectively an aromatic part, a basic moiety and a hydrogen bond acceptor. (c) Structure of hit fragment VUF10434 (1).
Scheme 4. Preparation of 1-Methyl-4-(naphthalen-2-yl)piperazine (33)*

![Chemical structure](image)

**Reagents:** (a) N-Methylpiperazine, n-BuLi, 0 °C, then 2-methoxynaphthalene (32), THF, rt.

To investigate the role of the position of the heteroatom in the aromatic ring, 34 was reacted with deprotonated N-methylpiperazine in order to obtain 35 (Scheme 5).

Scheme 5. Preparation of 3-(4-Methylpiperazin-1-yl)quinoline (35)*

![Chemical structure](image)

**Reagents:** (a) NaNH₂, t-BuOH, THF, 0 °C, 40 °C, then 3-bromoquinoline (34), 0 °C to rt.

For the synthesis of compounds 39 and 40 a similar approach was used as for the synthesis of compounds 30 and 31. First, the commercially available 1,3-dichloroisquinoline (36) was reacted with either ammoniumhydroxide or methylamine to yield 37 or 38 respectively as the single 1-substituted regioisomers. Both intermediates were then used without purification in the reaction with N-methylpiperazine to yield 39 and 40 as the single regioisomers (Scheme 6).

Compound 41 was reacted with a mixture of potassium nitrate and sulfuric acid that in situ yields the reactive nitric acid. This yielded a mixture of regioisomers with the 5- and 8-nitrocompounds (42, 43) as the main products. These regioisomers were separated by column chromatography and each individual regioisomer was subsequently reacted with N-methylpiperazine to yield nitro analogs 44 and 45. These intermediates in turn were reduced to the corresponding anilines (46, 47) with palladium on carbon and hydrogen gas (Scheme 7).

Scheme 6. Preparation of isoquinoline compounds 39 and 40*

![Chemical structure](image)

**Reagents:** (a) NH₃ in MeOH, 120 °C; (b) MeNH₂, DiPEA, EtOH, 100 °C; (c) N-methylpiperazine, 220 °C.

3. BIOCHEMICAL EVALUATION AND SAR STUDIES

Target compounds were evaluated using competition with the 5-HT₃-specific radioligand [³H]granisetron and most compounds displayed high affinity (expressed here as pKᵢ) for the 5-HT₃A receptor (Tables 1–5). With the quinazoline scaffold of compound 1 as a starting point, the role of the basic moiety as a key feature in the 5-HT₃ pharmacophore first was explored. A series of N-methylhomopiperazine replacements were investigated (Table 1) by introducing several other cyclic amines. The N-methylpiperazine analogue 6 shows a 16-fold increase in affinity. All other compounds, including rigid tertiary amine 7, rigid secondary amines (6, 9) and flexible tertiary amine 10, show reduced affinity for the 5-HT₃A receptor. Thus, for this series of compounds, the N-methyl piperazine moiety at the R₁ position results in the highest affinity.

Next, keeping the N-methylpiperazine moiety at the R₁ position, we explored the SAR associated with the R₂ position (Table 2). Removal of the substituent in the R₂ position (i.e., R₂ = H), leading to compound 11, results in a ~10-fold reduction in affinity. The same reduction in affinity is observed for the removal or addition of a methyl group on the aniline nitrogen atom (compounds 12, 13). Interestingly, the ~500-fold lower affinity of compound 48 (Table 2) shows that the aniline nitrogen atom N3 of compound 48b is no longer capable of interacting as a HBA.

Compounds that contain more bulky groups at position R₂, including saturated ring systems (15) and aromatic moieties (17), all show lower affinities than the lead compound (Table 2). Introduction of a large polar group results in higher affinity. The SAR at both the R₁ and R₂ position follow similar trends, as observed for the histamine H₁R affinities (Figure 2) and consequently nitrogen atom N3 of compound 48b is no longer capable of interacting as a HBA.

In Table 3, the effect of the 6-chloro atom on the 5-HT₃ receptor affinity is shown. Replacement of the 6-chloro atom by a hydrogen atom results in a ~5-fold reduction in affinity (compare 6 and 21 respectively) when R₂ = NMe. Replacement of R₁ = 6-Cl by R₁ = H results in analogs that have a higher affinity for the 5-HT₃A receptor when R₂ = H, OH or NH₂ (the affinity increases ~5, ~10, and ~100 fold, respectively). Interestingly, for the H₁R the latter modification leads to a reduction in affinity of more than 10-fold, marking a clear difference in SAR for 5-HT₃ and H₁R receptors. Ultimately, the SAR for these receptors is different, as can be deduced by comparing the different affinities of compounds 6, 8–10, 12–20, 22 and 48–52 which have been synthesized, evaluated...
for H₄R affinity and published by our group earlier. Importantly, compound 22 is now identified as a 5-HT₃ ligand with subnanomolar affinity and ~40 000 fold selectivity for the 5-HT₃A receptor over the H₄R.

The role of the H-bond acceptor moiety was explored using the ligands in Table 4. As already illustrated by the pharmacophore model in Figure 1B, a distance of ~5 Å between the basic moiety and the H-bond acceptor is essential. This is in line with our present findings, as the distance between these pharmacophore features in the minimal global energy conformation of our new compounds is calculated to be ~5 Å. The highest affinity compounds are those where the heteroaromatic nitrogen atom is positioned next to the N-methylpiperazine group. These are compounds 49 (N1 = 4.96 Å, N3 = 4.97 Å), 51 (4.71 Å) and 52 (4.75 Å), with the highest affinity compound (51) having a pKᵢ > 9. The affinity of compound 50 is 3−10 fold lower than compounds 49, 51 and 52; here the distance of N1 to the basic nitrogen atom is 4.79 Å while the distance for N4 is 6.42 Å. The distance between the basic nitrogen atom and the H-bond acceptor in compound 35 is 6.53 Å, and affinity drops >250 fold when compared to (iso)quinolines 51 and 52. The importance of this pharmacophore feature is supported by the fact that compound 33, which has no H-bond acceptor in the ring, shows similar affinity as compound 35.

For quinazolines (Table 3) R² = NH₂ in combination with R³ = H results in a significant increase in affinity (22). Therefore, the same derivatization strategy was applied to the corresponding (iso)quinolines (Table 5). Here, 4-NMe substitution of the quinoline scaffold (31) gives a ∼30-fold drop in affinity. In addition, the 4-NH₂ analogue (30) also shows a loss of affinity.

Table 1. 5-HT₃A Receptor Binding Affinities (pKᵢ) of Compounds 1, 6−10

| # | R¹ | pKᵢ \(^a\) |
|---|-----|---------|
| 1 | | 7.74 ± 0.45 |
| 6 | | 8.95 ± 0.05 |
| 7 | | 6.33 ± 0.31 |
| 8 | | 5.63 ± 0.10 |
| 9 | | 5.45 ± 0.15 |
| 10 | | 4.93 ± 0.13 |

\(^a\)Determined by radioligand competition using [³H]granisetron.

Table 2. 5-HT₃A Receptor Binding Affinities (pKᵢ) of Compounds with Different Substituents at R²

| # | R² | pKᵢ \(^a\) |
|---|-----|---------|
| 6 | H | 8.95 ± 0.05 |
| 11 | H | 8.13 ± 0.27 |
| 48 | OH | 6.24 ± 0.24 |
| 12 | NH₂ | 7.92 ± 0.09 |
| 13 | | 8.18 ± 0.04 |
| 14 | | 6.78 ± 0.18 |
| 15 | | 4.78 ± 0.13 |
| 16 | | 7.86 ± 0.39 |
| 17 | | 7.10 ± 0.22 |
| 18 | | 8.57 ± 0.15 |
| 19 | | 7.83 ± 0.06 |
| 20 | | 8.55 ± 0.12 |

\(^a\)Determined by radioligand competition using [³H]granisetron.

Figure 2. Tautomers of compound 48.
Although to a lesser extent. In order to explore the effect of the aniline moiety when positioned on the second aromatic ring of the quinoline moiety, two additional compounds were synthesized. The compound that has the aniline functionality at the 5-position of the quinoline scaffold resulted in compound 46. The 5-HT3AR affinity dropped ∼1000-fold and ∼100-fold when compared to compounds 51 and 30, respectively. The 8-aniline (47) derivative shows a comparable affinity to compound 46. Finally, a similar approach for the isoquinoline scaffold results in compounds 40 and 39. Here, addition of a 2-NMe moiety (compound 40) results in a ∼5-fold lower affinity. The 2-NH2 analogue (compound 39), however, shows a 140-fold decrease in affinity when compared to the parent isoquinoline compound 52.

To reassure that the most active compound (22) has no cross-target affinities, this compound was subjected to a broader pharmacological screening panel at a concentration of 1000 times its $K_i$ for 5-HT3AR. Compound 22 shows no affinity for other closely related receptors, except for nACh (α7) and 5-HT3B receptors for which 31% and 43% inhibition is observed respectively (Table 6 and Supporting Information, Tables S1–S3).

Table 3. Effect of $R^1 = Cl$ or $R^1 = H$ on the 5-HT3AR Affinity

| #  | $R^2$ | $R^3$ | $pK_i$ | $pK_i$ |
|----|-------|-------|-------|-------|
| 11 | H     | Cl    | 8.13 ± 0.27 |
| 49 | H     | H     | 8.87 ± 0.10 |
| 48 | OH    | Cl    | 6.24 ± 0.24 |
| 26 | OH    | H     | 7.33 ± 0.12 |
| 12 | NH₂   | Cl    | 7.92 ± 0.09 |
| 22 | NH₂   | H     | 10.29 ± 0.15 |
| 6  | NMe   | Cl    | 8.95 ± 0.05 |
| 21 | NMe   | H     | 8.53 ± 0.05 |

“Determined by radioligand competition using [3H]granisetron.

Table 4. 5-HT3A Receptor Binding Affinities ($pK_i$) of Compounds 33, 35 and 49–52

| #  | Structure | $pK_i$ |
|----|-----------|-------|
| 49 | ![Structure](image) | 8.87 ± 0.10 |
| 50 | ![Structure](image) | 8.33 ± 0.24 |
| 51 | ![Structure](image) | 9.34 ± 0.11 |
| 52 | ![Structure](image) | 8.79 ± 0.14 |
| 35 | ![Structure](image) | 6.39 ± 0.14 |
| 33 | ![Structure](image) | 6.27 ± 0.08 |

“Determined by radioligand competition using [3H]granisetron.

Table 5. 5-HT3A Receptor Binding Affinities ($pK_i$) of Different (Methyl)aniline Compounds

| #  | Structure | $pK_i$ |
|----|-----------|-------|
| 21 | ![Structure](image) | 8.53 ± 0.05 |
| 22 | ![Structure](image) | 10.29 ± 0.15 |
| 31 | ![Structure](image) | 7.87 ± 0.11 |
| 30 | ![Structure](image) | 8.84 ± 0.27 |
| 40 | ![Structure](image) | 8.23 ± 0.24 |
| 39 | ![Structure](image) | 6.66 ± 0.31 |
| 46 | ![Structure](image) | 6.69 ± 0.11 |
| 47 | ![Structure](image) | 6.48 ± 0.11 |

“Determined by radioligand competition using [3H]granisetron.

Table 6. Cross-target Pharmacology of Compound 22 at a Concentration of 0.1 μM

| target | radioligand | % inhibition of control specific binding ± s.e.m.a |
|--------|-------------|-----------------------------------------------|
| GABA1 (α1,β2,γ2) | [3H]muscimol | <15 |
| Glycine | [3H]strychnine | <15 |
| nACh (α4/β2) | [3H]cytisine | <15 |
| nACh (α7) | [3H]epibatidine | 31 ± 1 |
| 5-HT3A | [3H]5-HT3A | <15 |
| 5-HT3B | [3H]5-HT3B | <15 |
| 5-HT1A | [3H]8-OH-DPAT | <15 |
| 5-HT1B | [3H]5-HT1B | <15 |
| 5-HT1D | [3H]serotonin | <15 |
| 5-HT2A | [125I]DOI | <15 |
| 5-HT2B | [125I]DOI | <15 |
| 5-HT6 | [3H]LSD | <15 |
| 5-HT7 | [3H]LSD | <15 |

“Results are expressed as percent inhibition of control specific binding obtained in the presence of compound 22. Results showing an inhibition <15% are considered non binding and are displayed as <15 (see Supporting Information for more details). aHuman receptor. bRat receptor.
Although protein structural information of cys-loop receptors is very limited, the emerging crystallographic data on AChBP structures, in combination with 5-HT₃R site-directed mutagenesis studies allow some preliminary considerations with respect to protein−ligand interactions. A homology model of the 5-HT₃A receptor binding site was constructed using the tropisetron bound AChBP crystal structure (PDB ID: 2WNC) as a template.³⁹ The derived binding orientation of tropisetron is in agreement with published site-directed mutagenesis studies. The basic tropane moiety of tropisetron interacts with the carbonyl backbone of W183 and is positioned in an aromatic cavity consisting of W183, Y234, and Y90 of the principle subunit, and W90 of the complementary subunit. The basic nitrogen atom of tropisetron is positioned at 4.5 Å from E129, a residue that is critical for both serotonin and granisetron binding. The indole ring of tropisetron is in close proximity to R92, which can interact with this moiety through a cation−π interaction, and has been previously identified to interact with the indazole ring of granisetron.¹⁵,¹⁶ Finally, five water molecules from the template cocrystal structure (wat₁–wat₅) were included; these form a structural water network that interacts with both the carbonyl moiety of tropisetron and the receptor.³⁹ We found that these water molecules have a high level of conservation across the AChBP structures that are cocrystallized with small antagonists (Figure 3a).⁴⁴ Compounds were docked into the homology model with GOLD using standard settings and the resulting poses were scored with GOLDscore.⁴⁶

From the observed docking poses of compounds 22 and 35 (Figure 3c–d) and other novel ligands (data not shown) we speculate that the basic moiety of these ligands interacts with the same residues as observed for the basic moiety of the reference ligand tropisetron (Figure 3b). In the binding model, heteroaromatic HBA (N3) of the quinazoline ring of compound 22 can interact with a structural water molecule (wat₂) in the binding site (Figure 3c) similar to the carbonyl oxygen atom of tropisetron. This interaction is also possible for other ligands where the distance between the basic nitrogen atom and the HBA is ~5 Å. Therefore, this protein−ligand model is able to accommodate the classic ligand-based 5-HT₃R pharmacophore (Figure 1b) by suggesting that the hydrogen bond acceptor feature (C) of the ligands binds to the protein via conserved water molecules, thereby explaining the subtle differences in SAR with respect to this pharmacophore feature.

Figure 3. AChBP-based homology model of the human 5-HT₃A receptor binding site (protein carbon atoms and cartoon representation in white). (a) Overlay of the waters participating in the structural water network in several different AChBP crystal structures (red: 2WNC; blue: 2BYR; cyan: 2PGZ; green: 2BYS; yellow: 2XYT). (b–d) Binding poses for the 5-HT₃ receptor antagonists. (b) Tropisetron (gold sticks). (c) Compound 22 (green sticks). (d) Compound 35 (green sticks).
Future crystallization studies of 5-HT3R ligands with AChBP might lead to additional insights with respect to binding mode, although the ultimate proof via crystallization studies of the actual 5-HT3R remains a scientific challenge. Nevertheless, careful SAR studies and ligand-based design approaches in combination the use of AChBP derived structural information and site-directed mutagenesis might lead to insights that enable structure-based drug design approaches that have proven to be efficient for water-soluble drug targets.

**CONCLUSION**

In summary, optimization of fragment hit (1) has led to the identification of several novel 5-HT3 receptor ligands with (sub)nanomolar affinities that are comparable to some of the most potent 5-HT3 ligands described to date. These ligands match the known pharmacophore descriptors for 5-HT3 ligands. We found that the N-methylpiperazine group at the R1 position is favorable, and that a hydrogen bond acceptor at the reaction mixture was concentrated under reduced pressure to a solid. 1H NMR (500 MHz, CDCl3) δ ppm 7.49–7.44 (m, 3H), 7.44 (m, 1H), 6.11 (br s, 1H), 3.22 (d, J = 4.9, 3H).

6-Chloro-2-(3-(dimethylamino)azetidin-1-yl)-N-methylquinazolin-4-amine (7). Methylamine in ethanol (33%, w/v, 0.11 mL, 0.86 mmol) and DIPEA (0.61 mL, 3.5 mmol) were added to a suspension of 2,4,6-trichloroquinazoline (3) (200 mg, 0.86 mmol) in EtOAc (2 mL) and stirred at rt until TLC indicated complete conversion. Then N,N-Dimethyl-3-azetidinamine dihydrochloride (150 mg, 0.87 mmol) was added and the resulting suspension was heated by microwave irradiation at 120 °C for 30 min. The resulting mixture was diluted with water and extracted with EtOAc. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude oil was purified over SiO2 (EtOAc/MeOH/Et2N (90/5/5, v/v/v)) to yield 1.89 mg of 7 (2.29 mmol, 91%) as a beige solid.

1H NMR (500 MHz, CDCl3) δ ppm 7.47–7.38 (m, 3H), 5.54 (br s, 1H), 4.23–4.16 (m, 2H), 4.06–3.99 (m, 2H), 3.24–3.14 (m, 1H), 3.11 (d, J = 4.8 Hz, 3H), 2.22 (s, 6H); 13C NMR (126 MHz, CDCl3) δ ppm 160.56, 159.70, 150.40, 132.90, 127.22, 125.65, 120.25, 111.31, 55.99, 54.31, 41.91, 28.01; LCMS: ret. time 2.06 min, purity >99%, [M + H+]292.06; HRMS m/z: [M + H+]2 calculated for C18H17ClN3; 292.1323, found: 292.1312.

6-Chloro-2-(4-(methylpiperazin-1-yl)quinazoline (11). Tributyltin hydride (312 mg, 1.07 mmol) was added dropwise to a round-bottom flask containing 2,4,6-trichloroquinazoline (3) (250 mg, 1.07 mmol) in dry toluene (5 mL). Then tetrakis (triphenylphosphine)-palladium(0) (60 mg, 0.05 mmol) was added and the reaction mixture was stirred at 100 °C for 1 h. Next, toluene was removed under reduced pressure, the residue was dissolved in DCM (5 mL) and hydrolyzed with a saturated solution of potassium fluoride. The mixture was stirred vigorously for 30 min, filtered over a pad of Celite and washed with DCM. The aqueous phase was extracted with DCM and the combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified over SiO2 (Hept/DCM = 50/50 to 0/100, v/v) yielding 30 mg of 4. This crude intermediate was then added to a microwave tube containing N-methylpiperazine (1 mL, 9.0 mmol) and EtOAc (3 mL). The resulting mixture was heated at 140 °C for 30 min under microwave irradiation. The solvent and excess of N-methylpiperazine were removed under reduced pressure and the residue was purified over SiO2 (KPNH (DCM/EtOAc = 90/10 to 60/40, v/v)) yielding 20 mg of 11 (0.08 mmol, 7% over two steps) as a yellow solid. 1H NMR (500 MHz, CDCl3) δ ppm 8.92 (s, 1H), 7.62 (d, J = 2.2 Hz, 1H), 7.59–7.56 (m, 1H), 7.50 (d, J = 9.0 Hz, 1H), 4.02–3.83 (m, 4H), 2.56–2.47 (m, 4H), 2.36 (s, 3H);13C NMR (126 MHz, CDCl3) δ 150.47, 159.23, 150.89, 143.80, 127.40, 127.37, 126.01, 119.87, 55.01, 43.72, 43.92; LCMS: ret. time: 2.07 min, purity 97%, [M + H+]220.04; HRMS m/z: [M + H+]2 calculated for C14H12N3; 220.1058, found: 220.1048.

**EXPERIMENTAL SECTION**

**Chemistry.** Chemicals and solvents were purchased from Aldrich and used as received. Unless indicated otherwise, all reactions were carried out under an inert atmosphere of dry N2. TLC analyses were performed with Merck F254 alumina silica plates using UV visualization or staining. Column purifications were carried out automatically using the Biotage equipment. All HRMS spectra were recorded on Bruker microTOF mass spectrometer using ESI in positive ion mode. 1H NMR spectra were recorded on a Bruker 250 (250 MHz) or a Bruker 500 (500 MHz) spectrometer. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, br = broad, m = multiplet), and coupling constants (Hz). Chemical shifts are reported in ppm with the natural abundance of deuterium in the solvent as the internal reference (DCl3 in CDCl3: δ 7.26 and CH3OH in CH3OD: δ 3.31, (CH3)2SO in (CD3)2SO: δ 2.50). 13C NMR spectra were recorded on a Bruker 500 (126 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent resonance resulting from incomplete deuteration as the internal reference (CDCl3: δ 77.16, CH3OD: δ 49.00, (CD3)2SO: δ 39.52). Systematic names for molecules according to IUPAC rules were generated using the Chemdraw AutoNom program. Purity was determined using a Shimadzu HPLC/MS workstation with a LC-20AD pump system, SPD-M20A diode array detector, and a LCMS-2010 EV liquid chromatography mass spectrometer. The column used was an Xbridge C18 5 μm column (100 mm × 4.6 mm). Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 230 nm. Solvents used were the following: solvent B = MeCN 0.1% Formic Acid; solvent A = water 0.1%. The analysis was conducted using a flow rate of 1.0 mL/min, start 5% B, linear gradient to 90% B in 4.5 min, then 1.5 min at 90% B, linear gradient to 5% B in 0.5 min and then 1.5 min at 5% B, total run time of 8 min. Compounds 6, 8–10, 12–20, 22 and 48–52 were synthesized by our group as described by Smits et al. 33–37

2-Chloro-N-methylquinazolin-4-amine (5). Methanamine in ethanol (40%, w/v, 0.24 mL, 2.76 mmol) and DIPEA (357 mg, 2.76 mmol) were added to a suspension of 2,4-dichloroquinazoline (2) (500 mg, 2.51 mmol) in EtOH (20 mL) and stirred at rt. After 4.5 h the reaction mixture was concentrated under reduced pressure to a volume of 1 mL. The resulting mixture was diluted with water (25 mL) and extracted with EtOAc. The combined organic extracts were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to yield 444 mg of 5 (2.29 mmol, 91%) as a white solid. 1H NMR (250 MHz, CDCl3) δ ppm 7.86–7.61 (m, 3H), 7.44 (m, 1H), 6.11 (br s, 1H), 3.22 (d, J = 4.9, 3H).

2-Thioxo-2,3-dihydroquinazolin-4(1H)-one (24). A suspension of anthranilic acid (23) (10.0 g, 73.0 mmol) in thionyl chloride (40.0
N-Methyl-2-(4-methylpiperazin-1-yl)quinolin-4-amine (31). Methanamine in MeOH (40%, w/v, 0.6 mL, 7.00 mmol) was added to a microwave tube containing 1,4-dibromoquinoline (27) (100 mg, 0.35 mmol), DiPEA (50 mg, 0.38 mmol) and EtOAc (2 mL). The resultant mixture was heated at 100 °C under microwave irradiation for 6 h. The reaction mixture was concentrated under reduced pressure, dissolved in N-methylpyridine (5 mL) and heated at 160 °C under microwave irradiation for 1 h. Then the mixture was diluted with H2O (10 mL) and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure and purified over SiO2 (EtOAc/MeOH/Et3N = 96/2/2, v/v/v) yielding 46 mg of 31 (0.18 mmol, 51%) as a white solid. 1H NMR (500 MHz, CDCl3) δ ppm 7.65 (d, J = 8.4 Hz, 1H), 7.52–7.43 (m, 2H), 7.20–6.93 (m, 1H), 5.92 (s, 1H), 4.89 (s, 1H), 3.79–3.69 (m, 4H), 3.00 (d, J = 5.0 Hz, 3H), 2.58–2.51 (m, 4H), 2.35 (s, 3H); 13C NMR (126 MHz, CDCl3) δ ppm 158.99, 151.51, 148.11, 129.21, 127.30, 121.08, 118.92, 115.39, 85.14, 55.21, 46.27, 45.25, 30.06. LCMS ret. time 1.83 min, purity 97%, [M + H]+ 257.05; HRMS m/z: [M + H]+ calcd for C13H17N4O: 245.1397, found: 245.1401.

1-Methyl-4-(naphthalen-2-yl)piperazine (32). A solution of n-butyllithium in hexanes (2.5 N, 8.8 mL, 22.0 mmol) was added dropwise to a stirred solution of N-methylpyridine (2.0 mL, 18.1 mmol) in THF while maintaining a temperature around 0 °C. The reaction mixture was kept at 0 °C for another 30 min and subsequently stirred at rt for 1 h. Then 2-methoxynaphthalene (32) (3.16 g, 20.0 mmol) was added and the mixture was stirred at rt for another 16 h. The reaction mixture was poured over an aqueous HCl solution (10%, 100 mL), basified with an aqueous solution of NaOH (2.5 M) and extracted with DCM. The combined organic extracts were washed with anhydrous sodium sulfate, filtered, concentrated under reduced pressure and purified over SiO2 (EtOAc/MeOH/NH4OH = 95/5, v/v/v) yielding 5.86 g (17.0 mmol, 97%) as a white solid. 1H NMR (500 MHz, CDCl3) δ ppm 7.97 (d, J = 2.8 Hz, 1H), 8.08–7.97 (m, 1H), 7.72 (m, 1H), 7.60–7.45 (m, 2H), 7.40 (d, J = 2.8 Hz, 1H), 3.48–3.32 (m, 4H), 2.79–2.65 (m, 4H), 2.46 (s, 3H); 11C NMR (63 MHz, CDCl3) δ ppm 144.96, 144.79, 142.99, 126.98, 126.84, 126.92, 126.59, 126.39, 116.73, 54.87, 49.14, 46.13. LCMS: ret. time 2.18 min, purity 99%, [M + H]+ 227.95; HRMS m/z: [M + H]+ calcd for C13H17N2: 227.1543, found: 227.1536.

Methyl-2-(4-methylpiperazin-1-yl)quinolin-4-amine (31). A solution of methanamine in MeOH (40%, w/v, 0.6 mL, 7.00 mmol) was added to a microwave tube containing 1,4-dibromoquinoline (27) (100 mg, 0.35 mmol), DiPEA (50 mg, 0.38 mmol) and EtOAc (2 mL). The resultant mixture was heated at 100 °C under microwave irradiation for 6 h. The reaction mixture was concentrated under reduced pressure, dissolved in N-methylpyridine (5 mL) and heated at 160 °C under microwave irradiation for 1 h. Then the mixture was diluted with H2O (10 mL) and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure and purified over SiO2 (EtOAc/MeOH/Et3N/NH4OH = 96/2/2/2, v/v/v/v) yielding 46 mg of 31 (0.18 mmol, 51%) as a white solid. 1H NMR (500 MHz, CDCl3) δ ppm 7.65 (d, J = 8.4 Hz, 1H), 7.52–7.43 (m, 2H), 7.20–6.93 (m, 1H), 5.92 (s, 1H), 4.89 (s, 1H), 3.79–3.69 (m, 4H), 3.00 (d, J = 5.0 Hz, 3H), 2.58–2.51 (m, 4H), 2.35 (s, 3H); 13C NMR (126 MHz, CDCl3) δ ppm 158.99, 151.51, 148.11, 129.21, 127.30, 121.08, 118.92, 115.39, 85.14, 55.21, 46.27, 45.25, 30.06. LCMS ret. time 1.83 min, purity 97%, [M + H]+ 257.05; HRMS m/z: [M + H]+ calcd for C13H17N4O: 245.1397, found: 245.1401.
irradiation. Then the excess N-methylpiperazine was removed under reduced pressure and the crude product was purified over SiO₂ (EtOAc/EtN = 98/2, v/v) to yield 102 mg of 39 (0.42 mmol, 21% over 2 steps) as a dark green oil. ¹H NMR (500 MHz, CDCl₃) δ ppm 7.60 (d, J = 8.2 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.44–7.40 (m, 1H), 7.17–7.12 (m, 1H), 6.22 (s, 1H), 4.97 (s, 2H), 3.69–3.32 (m, 4H), 2.65–2.56 (m, 4H) ppm 157.30, 148.39, 148.30, 135.54, 135.49, 128.82, 125.45, 124.89, 119.91; LCMS: ret. time 4.56 min, purity >99%, [M + H]+ 220.00. 2-Chloro-8-nitroquinoline (43): ¹H NMR (250 MHz, CDCl₃) δ ppm 7.93 (d, J = 9.1 Hz, 1H), 7.14 (d, J = 9.1 Hz, 1H), 7.07–7.00 (m, 2H), 6.94–6.89 (m, 1H), 3.87–3.76 (m, 4H), 2.88–2.77 (m, 4H), 2.54 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ ppm 157.30, 148.32, 142.21, 130.97, 130.11, 117.84, 113.00, 107.81, 107.14, 55.05, 46.36, 45.02; LCMS: ret. time 0.96 min, purity 99%, [M + H]+ 243.10; HRMS m/z: [M + H]+ calc for C₁₄H₁₉N₄: 243.1344, found: 243.1359.

2-(4-Methylpiperazin-1-yl)-5-nitroquinoline (44): 2-(4-Methylpiperazin-1-yl)-5-nitroquinoline (44) (206 mg, 0.76 mmol) was dissolved in methanol (50 mL). Pd/C 5 wt % (30 mg) was added to the solution and the resulting suspension was stirred overnight at rt under a hydrogen gas atmosphere. Hereafter, the mixture was filtered over Celite and concentrated under reduced pressure to obtain 46 (143 mg, 0.59 mmol, 81%) as a brown solid. ¹H NMR (500 MHz, CDCl₃) δ ppm 7.93–7.87 (m, 2H), 7.74 (d, J = 8.0, 1.3 Hz, 1H), 7.23–7.15 (m, 1H), 7.04 (d, J = 9.3 Hz, 1H), 3.85–3.78 (m, 4H), 2.56–2.48 (m, 4H), 2.35 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ ppm 157.56, 154.42, 139.34, 137.29, 131.48, 125.13, 124.17, 120.03, 110.60, 54.85, 46.16, 44.57; LCMS: ret. time 2.70 min, purity >99%, [M + H]+ 220.05; HRMS m/z: [M + H]+ calc for C₁₄H₁₉N₄: 220.0644, found: 220.0647. 5-HT₃AR. HEK293 cells stably expressing 5-HT₃AR were scraped into 1 mL of ice-cold HEPES buffer (10 mM, pH 7.4) and frozen. After thawing, they were washed with HEPES buffer and homogenized using a fine-bore syringe. Fifty microliters of membrane suspension was incubated in 0.5 mL HEPES buffer containing 0.7 nM [³H]granisetron (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestosterone (–Kᵢ) and dihydrotestoste...
where $L$ is the concentration of ligand present; $B_L$ is the binding in the presence of ligand concentration $L$; $B_{max}$ is the binding when $L = 0$; $B_{min}$ is the binding when $L = \infty$, $L_{50}$ is the concentration of $L$ which gives a binding equal to $(B_{max} + B_{min})/2$; and $nH$ is the Hill coefficient. $K_i$ values were estimated from IC50 values using the Cheng-Prusoff equation: $K_i = IC_{50}/(1+[L]/K_c)$ where $K_i$ is the equilibrium dissociation constant for binding of the unlabeled antagonist, $IC_{50}$ is the concentration of antagonist that blocks half the specific binding, $[L]$ is the free concentration of radioligand and $K_c$ is the equilibrium dissociation constant of the radioligand.

Non 5-HT3AR. Off-target binding of compound 22 was assessed at 13 additional targets (Table 6). With the exception of nACh ($\alpha 7$), all single point radioligand competition binding was performed by Cerep. The results are expressed as a percent inhibition of control specific binding (100-([measured binding]/[fixed binding])*100) in the presence of compound 22 (see Supporting Information, Tables S1–S3 for more details).

Homology Modeling. A model of the 5-HT3A receptor binding site was constructed by homology modeling using MOE (version 2010.10, Chemical Computing Group Montreal), based on the tropsinbound AChBP X-ray crystal structure determined at 2.20 Å resolution (PDB code: 2WNC). The sequence of the human 5-HT3A gene (Q7KZM7) was aligned with the Aplysia californica gene (Q8WSF8) using the "Protein Align" option in MOE (standard settings) and adjusted manually. The final sequence alignment is given in Supporting Information, Figure S2. Chains A and E of the original PDB structure were selected to serve as the template. Structural waters located in this binding pocket of the 2WNC crystal structure form a conserved protein–ligand H-bond interaction network in several other AChBP crystals containing small competitive antagonists (e.g., 2BYR, 2PG2, Z8YS, 2XST), and were included in the 5-HT3A receptor model. The template backbone, the ligand and the water molecules were fixed and 10 preliminary receptor models were constructed based on the template backbone. During this construction, the ligand and waters were considered as an additional restraint using the "Environment" option within MOE. The structural quality of the models was checked using the evaluation modules in MOE. During this evaluation the focus was on the binding site region of the model. The protein geometry of receptor atoms was evaluated for their bond lengths, bond angles, atom clashes and contact energies. Ramachandran plots were used to check the Phi and Psi angles of all residues. Model 1 was selected for further refinement, hydrogen atoms were added, partial atomic charges were calculated and the protein was minimized around the fixed ligand and static water molecules using the Amber99 force field in MOE.

Molecular Docking. Ligands were protonated according to physiological pH using the MMFF94 force field and MOE software. Relevant tautomers of the ligands were created and subsequently the three-dimensional structures were energy minimized and converted into mol2 files using Molecular Network’s Conina. Docking studies were performed with the docking program GOLD (Version 5.0, CCDC, Cambridge, UK), using default settings. The protein binding site was defined by a radius of 12 Å around W183 (atom NE1) of the principle subunit. A total of 30 dockings were set up for each ligand run with a root-mean-square deviation (rmsd) tolerance of 1.5 Å for early termination. Docking scores were calculated with the GoldScore scoring function.

ASSOCIATED CONTENT

Supporting Information

Overview of the 2D NOESY 1H NMR interactions found for compounds 30 and 31. The sequence alignment between the human 5-HT3A gene (Q7KZM7) and the Aplysia californica gene (Q8WSF8). Radioligand binding studies for the receptors shown in Table 6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS USED

AChBP, acetylcholine binding protein; pLGIC, pentameric ligand-gated ion channel; IBS, irritable bowel syndrome; 5-HT, 5-hydroxy tryptamine; nACh, nicotinic acetylcholine; GABA, y-aminobutyric acid; DOI, 2,5-dimethoxy-4-iodoamphetamine; 8-OH-DPAT, 8-hydroxy-2-(di-N-propylamino)-tetralin; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; DiPEA, diisopropyl ethyl amine; t-BuOH, tert-butoxide; n-BuLi, n-butyllithium; DCM, dichloromethane; Hept, heptanes; MeNH2, methylvamine; HBA, hydrogen bond acceptor; ESI, electron-spray ionization; UV, ultraviolet; LCMS, liquid chromatography mass spectrometry

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