Synthesis and SAR of *N*-Chlorophenyl Substituted Piperazinylethyl-aminomethylpyrazoles as 5-HT$_{3A}$ Inhibitors

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The 5-HT$_{3A}$ receptors are one of ligand-gated ion channels and are known to be involved in visceral pain, anxiety, or anticancer agent-induced nausea and vomiting. In present study, we designed novel skeletons based on the developed 5-HT$_3$ receptor antagonists and evaluated their effects on 5-HT$_{3A}$ receptor channel currents ($I_{5\text{-}HT}$) of a series of pyrazole derivatives having *N*-chlorophenylpiperazine functionality (**6-9**). We found that most of *N*-*p*-chlorophenyl substituted piperazinyl-pyrazole derivatives (**7b**, **7c**, **7e** and **7h**) exhibited the high potency for the inhibition of $I_{5\text{-}HT}$, whereas the compound without chloride (**6**) or with *m*-chlorophenyl group (a serious of **8** and **9**) showed the low potency. These results indicate that *p*-chlorophenyl group is might play an important role for increasing the inhibitory potency on $I_{5\text{-}HT}$.

Key Words: 5-HT$_3$ receptor, 5-HT$_{3A}$ receptor channel activity, Novel 5-HT$_3$ receptor channel current blockers, Chlorophenyl substituted piperazinylethylaminomethylpyrazoles

Introduction

Serotonin (5-Hydroxytryptamine, 5-HT) is an important neurotransmitter playing the diverse functions in intracellular communications. 5-HT receptors are classified into over the 8 different receptor subtypes according to structural or pharmacological differences.[1] While most of serotonin receptors are GTP-binding protein coupled receptors, the 5-HT$_3$ subtypes are the only ionotropic receptors.[2] Activation of this receptor mediates fast depolarizing responses and is permeable for the monovalent and divalent cations. The 5-HT$_3$ receptors consist of homomeric pentamers.[3,4,5]

5-HT$_3$ receptors are mainly expressed in brain stem and spinal cord in central nervous system (CNS) and in intestine in peripheral nervous system (PNS).[6] In CNS, 5-HT$_{3A}$ receptors are known for nausea and vomiting, whereas are known for visceral pain such as irritable bowel syndrome in PNS.[7,8,9,10,11] In the early 1990s the introduction of 5-HT$_3$ receptor antagonists into markets was perceived great success to attenuate side effects related with chemotherapy-induced emesis. It is well known that nausea and vomiting induced by chemotherapy of cancer patients result from activation of 5-HT$_{3A}$ receptors in the brain stem. Therefore, several 5-HT$_3$ antagonists are currently available and show efficacy in the control of the emesis induced by anticancer chemotherapy. For example, tropisetron, ondansetron, granisetron, dolasetron, and palonsetron are in markets and bemesetron (MDL-72222), and renzapride are underdeveloped state in phase II or Phase III. The palonosetron and indisetron were launched in 2003 and 2004, respectively.[12,13,14] (Fig. 1).

Recent studies show that 5-HT$_3$ receptor antagonist were also focused on other CNS related beneficial effects such as pain, anxiety, cognitive function, anti-inflammatory and immune modulatory properties.[12]

In present study, we designed novel skeletons based on known 5-HT$_3$ antagonists for the development of novel 5-HT$_{3A}$ antagonists. Thus, we developed novel target molecule (**6-9**), called *N*-chlorophenyl substituted piperazinylethylaminomethylpyrazole, which is contained piperazine ring, chlorophenyl and pyrazole group (Fig. 2). Each group was reconstructed from chlorophenyl group of MDL-72222, piperazine moiety of leriseron and MCI-225, and pyrazol moiety of indisetron (Fig. 1). Here, we first report novel syntheses of *N*-chlororoaryl substituted piperazinylethylaminomethyl pyrazole derivatives **6-9** (Fig. 2) and *in vitro* evaluations against 5-HT$_{3A}$ receptor channel activities at single cell level. We also mention the structure-activity relationships of the synthesized compounds.

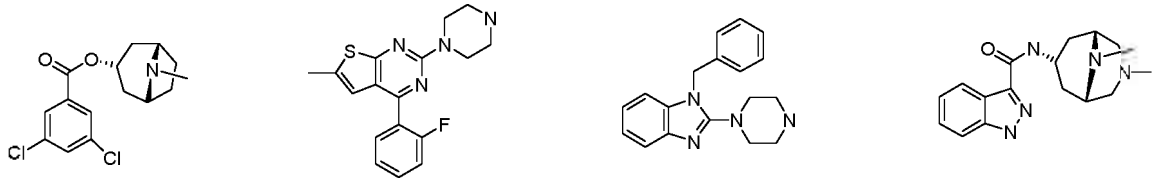


Figure 1. Examples of recently developed 5-HT$_{3A}$ antagonists.

Results and Discussion

Chemistry. The core structure of the target compound was piperazinylethylaminomethylpyrazole which connected to pyrazole and piperazine through alkylamine chain (Fig. 2). The chlorophenyl group was introduced to each ring as substituents. The synthesis of target compound was outlined in Scheme 1.

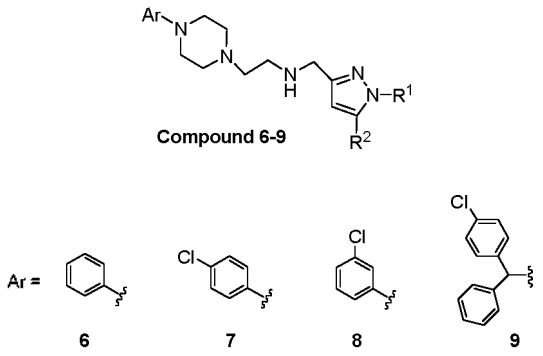
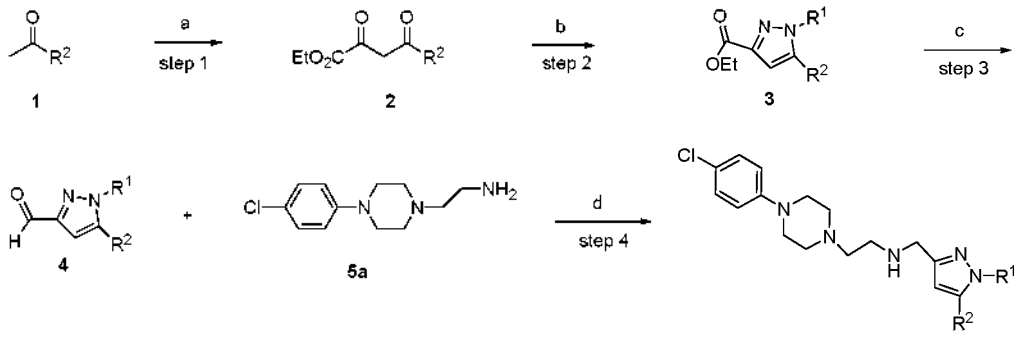
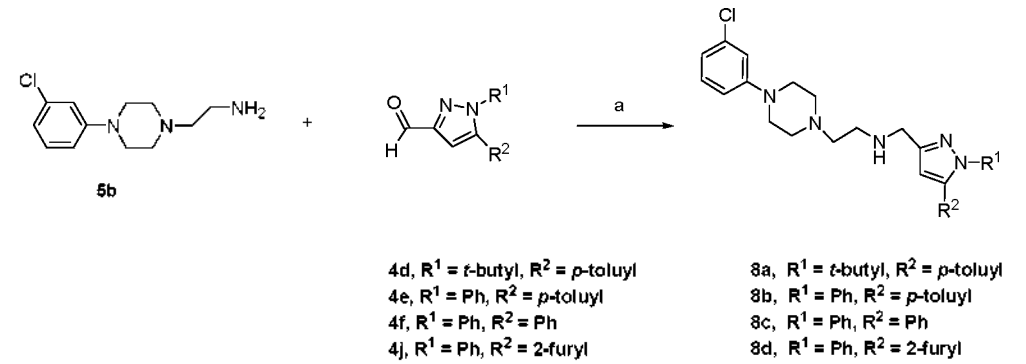


Figure 2. The designed target molecule.

The desired compounds **6-9** were synthesized by reductive amination of pyrazole aldehyde (**4**) with corresponding arylpiperazylethylamine (**5**) in moderate yields. The pyrazole aldehydes having various R^1 and R^2 substituents were prepared by previously developed process in our group.[15] Aryl piperazinylethylamine (**5**) was obtained by Gabriel amine synthetic method from the commercially available arylpiperazine. To introduce chlorophenyl substituent in core target molecule, *p*-chlorophenylpiperazine, *m*-chlorophenylpiperazine and *p*-chlorophenyl (phenyl)methylpiperazine were selected and reacted with several pyrazole aldehydes. In detail synthetic methods were noted as follow. Claisen-condensation of commercially available various ketones (**1**) with diethyldiketone gave 2,4-dioxo-4-alkyl-butyric acid ethyl esters (**2**). These β-ketoester compounds undergo cycloaddition reaction with phenylhydrazine or *t*-butylhydrazine, yielding a pyrazole 3-carboxy acid ethyl ester (**3**), which was then oxidized by DIBAL-H from ester to aldehyde (**4**). Gabriel-

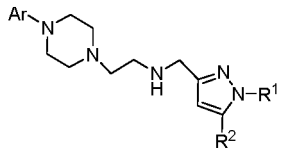


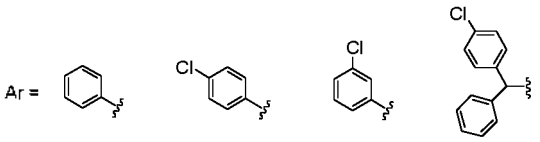
Scheme 1. Regent and condition: (a) NaOEt/EtOH, diethyloxalate, (b) $R^1NH_2NH_2$·HCl, EtOH, (c) DIBAL-H, CH_2Cl_2, (d) $NaBH(OAc)_3$, CH_2Cl_2.



Scheme 2. Reagent and condition: (a) $NaBH(OAc)_3$, CH_2Cl_2.

Table 1. *In vitro* 5-HT$_{3A}$ receptor blocking effects of 5-(*N*-arylpiperazinylethyl)aminomethylpyrazoles





6 7 8 9

Entry	Substituent			5-HT$_{3A}$ receptor		
	Ar	R^1	R^2	V_{max}	IC_{50} (μM)	nH
6	Ph	*t*-butyl	*p*-chloro-Ph	88.7 ± 5.8	41.9 ± 6.4	2.6 ± 0.8
7a	*p*-chloro-Ph	*t*-butyl	*p*-chloro-Ph	100.5 ± 4.3	28.3 ± 5.0	1.2 ± 0.2
7b	*p*-chloro-Ph	*t*-butyl	*n*-propyl	97.3 ± 5.6	1.3 ± 0.3	1.4 ± 0.3
7c	*p*-chloro-Ph	*t*-butyl	*i*-butyl	102.8 ± 2.1	2.1 ± 0.2	1.2 ± 0.1
7d	*p*-chloro-Ph	*t*-butyl	*p*-tolyl	102.6 ± 6.5	2.4 ± 0.4	1.4 ± 0.3
7e	*p*-chloro-Ph	Ph	*p*-tolyl	95.4 ± 3.5	2.2 ± 0.2	1.8 ± 0.3
7f	*p*-chloro-Ph	Ph	Ph	101.3 ± 2.6	3.1 ± 0.3	1.1 ± 0.1
7g	*p*-chloro-Ph	Ph	methyl	98.5 ± 1.2	2.5 ± 0.3	1.4 ± 0.1
7h	*p*-chloro-Ph	Ph	*n*-propyl	94.8 ± 4.8	1.3 ± 0.2	1.5 ± 0.3
7i	*p*-chloro-Ph	Ph	*i*-butyl	104.3 ± 3.5	3.3 ± 0.9	1.4 ± 0.4
7j	*p*-chloro-Ph	Ph	2-furyl	97.3 ± 2.0	4.3 ± 0.3	2.0 ± 0.2
7k	*p*-chloro-Ph	Ph	(4-cyclohexyl)Ph	95.0 ± 5.6	17.7 ± 2.2	1.9 ± 0.3
7l	p-chloro-Ph	Ph	(4-piperidinyl)Ph	95.9 ± 1.6	6.4 ± 0.3	1.9 ± 0.1
8a	*m*-chloro-Ph	*t*-butyl	*p*-tolyl	98.4 ± 5.5	31.0 ± 5.0	1.1 ± 0.2
8b	*m*-chloro-Ph	Ph	Ph	96.9 ± 4.3	20.3 ± 1.9	1.5 ± 0.2
8c	*m*-chloro-Ph	Ph	2-furyl	95.9 ± 2.8	9.4 ± 0.7	1.5 ± 0.2
8d	*m*-chloro-Ph	Ph	*p*-tolyl	87.6 ± 3.6	21.8 ± 1.8	1.6 ± 0.2
9a	(*p*-ClPh)CHPh	*t*-butyl	*p*-tolyl	74.2 ± 1.5	39.9 ± 0.5	1.9 ± 0.4
9b	(*p*-ClPh)CHPh	Ph	*p*-tolyl	73.1 ± 3.1	8.2 ± 0.8	2.2 ± 0.4
		MDL-72222		99.6 ± 17.7	0.77 ± 0.16	1.3 ± 0.27

synthesis of arylpiperazine gave corresponding amine (**5**). The synthetic pathway of selected compounds was presented in Scheme 1 and 2. The novel chlorophenyl substituted target compounds were synthesized and all compounds were fully characterized by spectroscopic analysis, and some of selected compounds were summarized in Table 1.

Biological activity. Next, all piperazinylethylpyrazole derivatives (**6-9**) were estimated for their *in vitro* effects on 5-HT$_{3A}$ receptor channel activities by examining of the inhibitory effects of the target compounds **6-9** on the 5-HT-mediated inward peak currents ($I_{5\text{-}HT}$) in oocytes expressing wild-type mouse 5-HT$_{3A}$ receptors. All of synthesized target compounds exhibited inhibitions on $I_{5\text{-}HT}$. The inhibitory potencies of each compound tested on $I_{5\text{-}HT}$ were estimated with IC_{50} values (Table 1).

The data indicated that several compounds showed high inhibitory effects on $I_{5\text{-}HT}$ along with low IC_{50} values. According to the structure activity relationships (SAR) studies of aromatic substituents (Ar) on piperazine, *p*-chlorophenyl group (a series of **7**) tends to exhibit better activities than those of other functional groups such as phenyl group (**6**), *m*-chlorophenyl group (**8**), and *p*-chlorophenyl(phenyl)methyl substituted ones (**9**). Among the *p*-chlorophenypiperazine substituted compounds, the compound **7b** and **7h** having one *n*-propyl group in pyrazole ring, were the most potent inhibitor that exhibited IC_{50} values of 1.3 μM, which were comparable to the well-known 5-HT$_{3A}$ receptor antagonist MDL-72222 (Table 1). Compared with a series of the compound having same R^1 and R^2 substituents such as compound **7d**, **8a**, **9a** (R^1 = *t*-butyl, R^2 = *p*-tolyl) between compound **7f**, **8b** (R^1 = R^2 = Ph), the compound **7d** (2.4 μM), **7f** (3.1 μM) having *p*-chloropnenyl as Ar group showed lower IC_{50} values than the compound **8a** (31.0 μM), **8b** (20.3 μM) and **9a** (39.9 μM) having *m*-chlorophenyl or *p*-chlorophenyl(phenyl)methyl group. And also as compared with the compound having different substituents, compound **7b** and **7h** possessing *n*-propyl group in R^2 which showed lowest IC_{50} value of 1.3 μM, these data revealed that linear alkyl-chained functionality on R^2 leads to show high inhibitory activity on $I_{5\text{-}HT}$. As shown similar activities about 2 μM of IC_{50} values of the compounds **7d** (R^1 = *t*-butyl, R^2 = *p*-tolyl) and **7e** (R^1 = Ph, R^2 = *p*-tolyl) having different R^1 substituents, R^1 substituent little affected the activity.

Compounds of structurally bulky R^2 substituent such as 4-cychlohexylphenyl (**7k**, IC_{50} = 17.7 ± 2.2 μM), 4-piperidylphenyl (**7l**, 6.4 ± 0.3 μM), and *p*-chlorophenyl (**7a**, 28.3 ± 5.0 μM) in the pyrazole ring were showed moderate inhibitory potency

than small substituents such as methyl, propyl and phenyl group. And also, *m*-ClPh- or (*p*-ClPh)CHPh-groups in piperazine exhibited low activity in the inhibition of $I_{5\text{-}HT}$. Therefore, these results indicate that the presence of chloro group on *p*-position of phenyl play important roles in exerting the 5-HT_{3A} receptor channel current inhibitions. The Hill coefficients indicate the number of binding molecule with 5-HT_{3A} receptor. As shown in Table 1, the modification of Ar, R^1 and R^2 substituent led to no significant change in Hill coefficient. The Hill coefficients of the compound **7f** and **8a** showed 1.1 nH, and the compound **7j**, **7k** and **7l** showed 2.0 nH, 1.9 nH and 1.9 nH, respectively. It seems that one molecule of compound **7f** and **8a** might bind to 5-HT_{3A} receptors, whereas two molecules of the compounds **7k** and **7l** having comparatively large functionality such as cyclohexylphenyl or piperidinylphenyl group, might bind to 5-HT_{3A} receptor to exhibit their effects.

In summary, a new series of *N*-chlorophenyl substituted piperazinylethylamino pyrazoles were designed, synthesized and examined for the 5-HT_{3A} inhibiton potency and SAR. Among all compounds, **7b** and **7h** show the most potent for 5-HT_{3A} receptor inhibition activity. The results of structure activity relationships (SAR) indicate that the chlorophenyl group is key role in biological activity against 5-HT_{3A} receptor. The position of substituted in phenyl ring is particularly important, due to all *p*-chloro substituted compounds showed lower IC_{50} values. These findings suggest that novel chlorophenyl substituted piperazinyl-pyrazole derivatives could be a lead compound of 5-HT_{3A} inhibitor.

Experimental Section

Preparation of 3-formyl-1-(*t*-butyl)-5-(*p*-chlorophenyl)pyrazole (4a) General procedure. To a solution of ethoxycarbonylpyrazole **3** (4.34 mmol) in toluene (15 mL) was added slowly DIBAL-H (8.68 mL, 8.62 mmol) at −78 °C, and the solution was stirred for 30 min. The acquous methanol was added to the reaction mixture. The solution was filtered off by celite and extracted with ethyl acetate. The organic layer dried by $MgSO_4$, filtered, and the solvent evaporated under vacuum condition and purified by Flash column chromatography (hexane/ethyl acetate/methylene chloride = 3/1/1) to afford **4a** (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.43 (m, 2H), 7.29 (d, J = 9.6 Hz, 2H), 6.68 (s, 1H), 1.52 (s, 9H).

Synthesis of *N*-(*p*-chloropheyl)piperazinylethylamine 5a. To a solution of *p*-chloro piperazine (27.74 mmol) in DMF 30 mL was added K_2CO_3 (83.21 mmol) and *N*-(2-bromoethyl) phthalimide (33.28 mmol) and was allowed to stir for 4 h at 80 °C. The reaction mixture was extracted three times with CH_2Cl_2 and the organic layer was washed with distilled water and brine, and combined organic layers were dried over $MgSO_4$ and concentrated with under reduced pressure. The crude product was purified by flash column chromatography (Hexane : EtOAc : CH_2Cl_2 = 3 : 1 : 2). (yield; 75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 4H), 3.18 (m, 4H), 2.89 (t, J = 6.3 Hz, 4H), 2.73 (t, J = 5.7 Hz, 2H), 2.57 (m, 4H).

Synthesis of 1-*tert*-butyl-5-(4-chlorophenyl)-3-[2-(4-phenylpiperazin-1-yl)-ethyl]aminomethylpyrazole 6 (General procedure). To a solution of *N*-phenylpiperazinylethyl amine (0.20 mmol) and corresponding 3-formyl-1-(*t*-butyl)-5-(*p*-chlorophenyl)pyrazole (**4a**) (0.20 mmol) in CH_2Cl_2 (5 mL) was added $NaBH(OAc)_3$ (131.8 mg, 0.622 mmol) and molecular sieves at room temperature. After stirring for 1 h, the reaction mixture was quenched with a saturated sodium bicarbonate solution and extracted with CH_2Cl_2. The organic layer washed with brine, dried by $MgSO_4$, filtered, and the solvent evaporated in vacuum. Flash column chromatography (Hexane/Ethyl acetate = 3/1) afforded product in moderate yields (69%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15-7.47 (m, 6H), 6.85 (m, 3H), 6.40 and 6.17 (s, 1H), 4.90 (brs, 1H), 4.24 and 3.99 (s, 2H), 2.51-3.38 (m, 12H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.4, 143.4, 142.3, 134.6, 132.2, 131.5, 129.1, 128.0, 120.3, 116.3, 110.2, 61.3, 57.7, 54.3, 52.5, 51.6, 49.1, 48.0, 31.1; FT-IR (KBr, cm^{-1}) 3252 (-NH), 2932, 2824, 1600, 1494, 1234, 1092; FABHRMS *m/z* calcd for $C_{26}H_{35}ClN_5$ $(M+H)^+$ 452.2603, Found 452.2581.

1-*tert*-Butyl-5-(4-chlorophenyl)-3-{2-[4-(4-chlorophenyl) piperazin-1-yl]ethyl} aminomethylpyrazole 7a. (68%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.42 (m, 4H), 7.18-7.33 (m, 9H), 6.15 (s, 1H), 3.93 (s, 2H), 3.17 (m, 2H), 2.97 (t, J = 6.0 Hz, 2H), 2.62-2.84 (m, 6H).

1-*tert*-Butyl-3-{2-[4-(4-chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-propyl pyrazole 7b. (52%): Mp = 151 ~ 152 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-7.34 (m, 4H), 6.08 (s, 1H), 3.44 (s, 2H), 3.18 (m, 2H), 2.74 (t, J = 5.4 Hz, 4H), 2.63(t, J = 5.8 Hz, 2H), 2.55 (m, 6H), 1.58 -1.43 (m, 11H), 0.89 (t, J = 7.6 Hz, 3H).

1-*tert*-Butyl-5-*iso*-butyl-3-{2-[4-(4-chlorophenyl)piperazin-1-yl]ethyl} -aminomethylpyrazole 7c. (28%): Mp = 133 ~ 134 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-7.37 (m, 4H), 6.09 (s, 1H), 3.51 (s, 2H), 3.18 (m, 4H), 2.74 (t, J = 5.6 Hz, 2H), 2.62 (t, J = 5.6 Hz, 2H), 2.45-2.59 (m, 6H), 1.80 (m, 1H), 1.44 (s, 9H), 0.90 (d, J = 7.2 Hz, 6H).

1-*tert*-Butyl-3-{2-[4-(4-chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-(4-methylphenyl)pyrazole 7d. (81%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20-7.31 (m, 5H), 7.07-7.19 (m, 8H), 5.74 (s, 1H), 3.78 (s, 2H), 3.06 (m, 2H), 2.77 (m, 4H), 2.50-2.68 (m, 6H), 2.38 (s, 3H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-1,5-diphenylpyrazol 7f. (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11-7.36 (m, 14H), 6.03 (s, 1H), 3.97 (s, 2H), 3.16 (m, 4H), 2.94 (t, J = 6.0 Hz, 2H), 2.68 (m, 4H), 2.50 (m, 2H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-methyl-1-phenylpyrazole 7g. (51%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.51 (m, 5H), 7.19-7.52 (m, 4H), 6.21 (s, 1H), 3.94 (s, 2H), 2.69 (m, 2H), 2.12-2.41 (m, 10H), 2.05 (s, 3H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-1-phenyl-5-propylpyrazole 7h. (52%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.39 (m, 6H), 7.21-7.32 (m, 3H), 6.21 (s, 1H), 3.89 (s, 2H), 2.89 (m, 2H), 2.23-2.75 (m, 12H), 1.45 (m, 2H), 0.90 (t, J = 7.0 Hz, 3H).

5-*iso*-Butyl-3-{2-[4-(4-chlorophenyl)piperazin-1-yl]ethyl} amino-methyl-1-phenylpyrazole 7i. (53%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.50 (m, 6H), 7.15-7.34 (m, 3H), 6.19 (s, 1H), 3.89 (s, 2H), 3.15 (m, 2H), 2.68 (m, 2H), 2.31-2.61 (m, 10H), 1.78 (m, 1H), 0.86 (d, J = 6.9 Hz, 6H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-(2-furyl)-1-phenylpyrazole 7j. (63%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.49 (m, 6H), 7.18-7.21 (m, 4H), 6.65 (s,

1H), 6.31 (m, 1H), 5.95 (m, 1H), 3.94 (s, 2H), 3.15 (m, 2H), 2.91 (t, J = 6.0 Hz, 4H), 2.69 (t, J = 6.0 Hz, 4H), 2.50 (m, 2H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-(4-cyclohexylphenyl)-1-phenylpyrazole 7k. (55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22-7.38 (m, 7H), 7.05-7.15 (m, 5H), 7.04 (m, 1H), 6.02 (s, 1H), 4.00 (s, 2H), 3.17 (m, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.71 (m, 4H), 2.36-2.59 (m, 5H), 1.69-1.92 (m, 6H), 1.42 (m, 4H).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-1-phenyl-5-(4piperidin-1-ylphenyl)pyrazole 7l. (56%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19-7.35 (m, 8H), 6.96-7.14 (m, 6H), 6.00 (s, 1H), 3.98 (s, 2H), 3.16 (m, 4H), 3.00 (t, J = 5.9 Hz, 2H), 2.70 (m, 6H), 2.48 (m, 4H), 1.56-1.76 (m, 6H).

1-*tert*-Butyl-3-{2-[4-(3-chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-5-(4-methylphenyl)pyrazole 8a. (70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (m, 5H), 6.69-6.89 (m, 3H), 6.16 (s, 1H), 4.01(s, 2H), 3.18 (m, 4H), 3.01 (m, 2H), 2.71 (m, 2H), 2.61 (m, 2H), 2.41 (s, 3H), 1.44 (s, 9H).

1,5-Diphenyl-3-{2-[4-(3-chlorophenyl)piperazin-1-yl] ethyl}-aminomethylpyazole 8b. (89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (m, 5H), 6.69-6.89 (m, 3H), 6.16 (s, 1H), 4.01 (s, 2H), 3.18 (m, 4H), 3.01 (m, 2H), 2.71 (m, 2H), 2.61 (m, 2H), 2.41 (s, 3H), 1.44 (s, 9H).

5-{2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl} aminomethyl-3-(2-furyl)-1-phenylpyrazole 8c. (69%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.56 (m, 6H), 7.13 (t, J = 7.8 Hz, 1H), 6.71-6.90 (m, 3H), 6.67 (s, 1H), 6.33 (m, 1H), 5.97 (m, 1H), 3.99 (s, 2H), 3.13 (m, 4H), 2.90 (m, 2H), 2.48-2.71 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 151.2, 144.4, 142.6, 140.1, 135.4, 134.9, 130.0, 129.1, 128.6, 125.9, 119.2, 115.6, 113.8, 111.2, 108.9, 106.1, 105.2, 57.1, 52.9, 48.6, 46.5, 45.3.

1-*tert*-Butyl-3-{2-[4-(4-chlorobenzhydryl)piperazin-1-yl] ethyl} aminomethyl-5-(4-methylphenyl)pyrazole 9a. (41%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.40 (m, 4H), 7.18-7.31 (m, 5H), 7.13 (d, J = 5.8 Hz, 2H), 7.08 (d, J = 6.3 Hz, 2H), 6.15 (s, 1H), 4.18 (s, 1H), 4.06 (s, 2H), 2.72 (t, J = 5.6 Hz, 4H), 2.49 (m, 4H), 2.32-2.45 (m, 7H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9, 141.1, 138.5, 132.6, 130.4, 130.1, 129.1, 128.7, 128.6, 128.5, 128.4, 127.7, 127.2, 108.8, 61.5, 52.8, 51.6, 45.0, 43.5, 31.1, 31.1, 29.7, 21.3; FT-IR (KBr, cm^{-1}) 3356 (-NH), 2924, 2814, 1450, 1010, 912, 806; HRMS (FAB) *m/z* calcd for $C_{34}H_{44}ClN_5$ $(M+H)^+$ 556.3207. Found 556.3207.

Preparation of *Xenopus* oocytes and microinjection. *Xenopus laevis* frogs were purchased from Xenopus I (Ann Arbor, MI, USA). Their care and handling were in accordance with the highest standards of institutional guidelines. For isolation of oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester. Oocytes were surgically removed and separated by collagenase treatment followed by agitation for 2 hours in a Ca^{2+}-free medium containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM sodium pyruvate, 100 units/mL penicillin and 100 μg/mL streptomycin. Stage V-VI oocytes were collected and stored in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) supplemented with 0.5 mM theophylline and 50 μg/mL gentamicin. This oocyte-containing solution was maintained at 18 °C with continuous gentle shaking and changed daily. Electrophysiological experiments with oocytes were performed within 5 ~ 6 days of their isolation. Chemicals were bath-applied. One day after harvest, 40 nL of cRNAs were injected into the animal or vegetal pole of each oocyte using a 10 μL VWR microdispenser (VWR Scientific, San Francisco, CA, USA) fitted with a tapered glass pipette tip that was 15 - 20 μm in diameter.[15]

***In vitro* transcription of 5-HT$_{3A}$ receptor cDNAs.** For generation of wild-type cRNAs, recombinant plasmids containing the wild-type 5-HT$_{3A}$ receptor cDNA were linearized by digestion with appropriate restriction enzymes. All cRNAs were prepared using T3 RNA polymerase and the mMessage mMachine transcription kit (Ambion, Austin, TX, USA). The final cRNA products were resuspended at 1 μg/μL with RNase-free water and stored at −80 °C until use.[15] The absence of degraded RNA was confirmed by denaturing agarose gel electrophoresis followed by ethidium bromide staining.

Data recording. A custom-made Plexiglas net chamber was used for two-electrode voltage-clamp recordings. The chamber was constructed by milling two concentric wells into the chamber bottom (diameter/height: upper well, 8/3 mm; lower well, 6/5 mm) and gluing plastic mesh (~ 0.4 mm grid diameter) onto the bottom of the upper well. The perfusion inlet (~ 1 mm in diameter) was formed through the wall of the lower well, and a suction tube was placed on the edge of the upper well. The oocyte was placed on the net that separated the upper and lower wells, with the net grids serving to keep the oocyte in place during the electrophysiological recordings. Oocytes were impaled with two microelectrodes filled with 3 M KCl (0.2 ~ 0.7 MΩ). Recordings were performed in ND96 solution. The electrophysiological experiments were performed at room temperature using an Oocyte Clamp (OC-725C; Warner Instruments, Hamden, CT, USA) and stimulation and data acquisition were controlled by pClamp 8 (Axon Instruments, Union City, CA, USA).[16]

Data analysis. To obtain concentration-response curves of the effect of Compounds on $I_{5\text{-}HT}$, the peak amplitudes at different concentrations of compounds were plotted and then fitted to the following Hill equation using the Origin software (OriginLab Corp, Northampton, MA, USA): Response = $E_{max} - E_{min}/1 + (IC_{50}/[A]^{nH}) + E_{min}$, where E_{max} and E_{min} are maximal and minimal responses, respectively. [A] is concentration of compounds and nH is the Hill coefficient. IC_{50} is the concentration of compounds required to decrease the response by 50%. All values are presented as means ± S.E.M. The differences between means of control and treatment data were determined using the unpaired Student's t-test or one-way ANOVA. A value of $p < 0.05$ was considered statistically significant.

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