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Design and Synthesis of Novel Arylpiperazine Derivatives Containing the Imidazole Core Targeting 5-HT$_{2A}$ Receptor and 5-HT Transporter

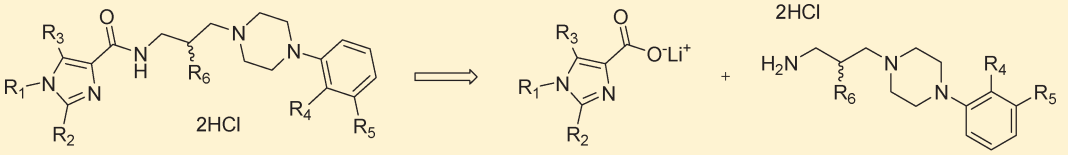
Hee Jeong Seo,[†] Eun-Jung Park,[†] Min Ju Kim,[†] Suk Youn Kang,[†] Suk Ho Lee,[†] Hyun Jung Kim,[†] Ki Nam Lee,[†] Myung Eun Jung,[†] MinWoo Lee,[†] Mi-Soon Kim,[†] Eun-Jung Son,[†] Woo-Kyu Park,[‡] Jeongmin Kim,[†] and Jinhwa Lee*[,†]

[†]Research Center, Green Cross Corporation, 303 Bojeong-dong, Giheung-gu, Yongin 446-770, Korea

[‡]Pharmacology Research Center, Korea Research Institute of Chemical Technology, 100 Jang-Dong, Yuseong-Gu, Daejeon 305-343, Korea

Supporting Information

ABSTRACT:



Serotonin antagonist reuptake inhibitor (SARI) drugs that block both 5-HT$_2$ receptors and the serotonin transporters have been developed. The human 5-HT$_{2A/2C}$ receptor has been implicated in several neurological conditions, and potent selective 5-HT$_{2A/2C}$ ligands may have therapeutic potential for treatment of CNS diseases such as depression. An imidazole moiety usually provides good pharmacokinetic properties as a drug substance, and thus considerable efforts have been devoted to develop imidazole derivatives into drug candidates. The imidazole series of compounds was evaluated against 5-HT$_{2A/2C}$ and serotonin reuptake inhibition. A few of the compounds in the series showed promising IC$_{50}$ values and antidepressant-like effect in in vivo forced swimming test (FST). On the basis of these results, further lead optimization studies resulted in identifying promising compounds potentially for therapeutic use.

INTRODUCTION

Depression, especially major depression, is an extremely serious disease affecting about 121 million people and is one of the leading causes of disability worldwide. And unipolar depression is predicted to be the second main cause of disability in 2020 by the World Health Organization.[1,2] Over the past several decades, the synaptic actions of monoamine neurotransmitters such as norepinephrine (NE) and serotonin (SER, 5-HT) were considered as important indications to psychiatric disease, including anxiety and depression.[3,4] Various psychotropic drugs which are associated with these neurotransmitters have been individually developed. Selective serotonin reuptake inhibitors (SSRIs) are a relatively newer class of antidepressants for treating depression. SSRIs such as fluoxetine, sertraline, paroxetine, and citalopram have been the most widely prescribed antidepressants since 1980s.[5] All SSRIs strongly and selectively inhibit 5-HT reuptake by the presynaptic neuron, thus increasing 5-HT concentration at the synapse. Although SSRIs offer a more favorable profile, they also have some side adverse effects including anxiety, sedation, headache, tremor, insomnia, and sexual dysfunction. More importantly, there are troublesome facts that SSRIs are generally effective only for less than two-third patients and have undesirably long therapeutic onset time.[6] Recently, there has been the achievement of an important development of an antidepressant which interacts with dual or multiple targets.[7]

5-HT$_1$ autoreceptors are widely distributed in the brain, and also serotonin transporters (SERT) are known to have a major role in the control of synaptic 5-HT levels.[8] Blockade of 5-HT$_{1A/B/D}$ autoreceptors, with or without concomitant SERT inhibition, rapidly increases brain 5-HT levels and consequently might be able to provide a fast onset of antidepressant/anxiolytic action relative to current therapies.[9] Arylpiperazine is a core fragment of a good number of active compounds displaying various pharmacological effects. The most frequently studied of arylpiperazine derivatives, so-called long-chain arylpiperazines, have been found as serotonin receptor ligands, especially 5-HT$_{1A}$ and 5-HT$_{2A}$ ones. Their general chemical structure contains an alkyl chain attached to the N4 atom of the piperazine moiety and a terminal amide or an imide fragment.[10]

Because numerous side effects are associated with nonselective binding at postsynaptic 5-HT receptors, addition of 5-HT receptor antagonistic component to 5-HT reuptake inhibitor has

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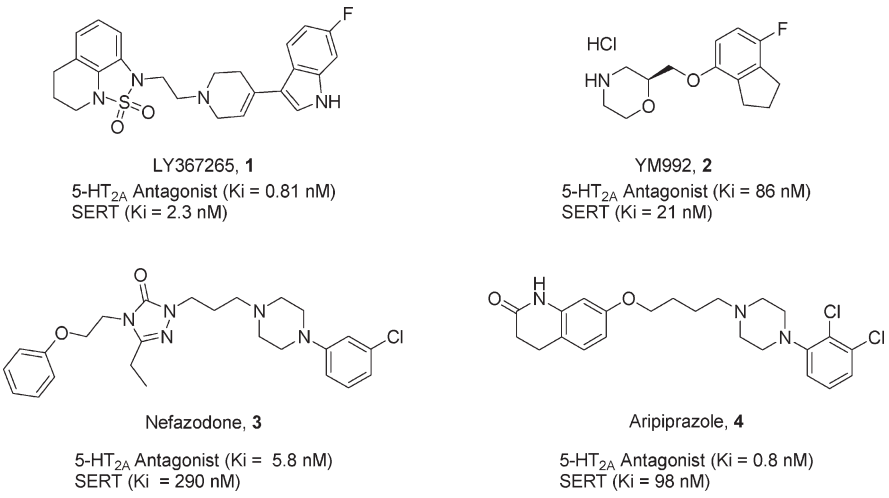


Figure 1. Chemical structures of representative antidepressant compounds.

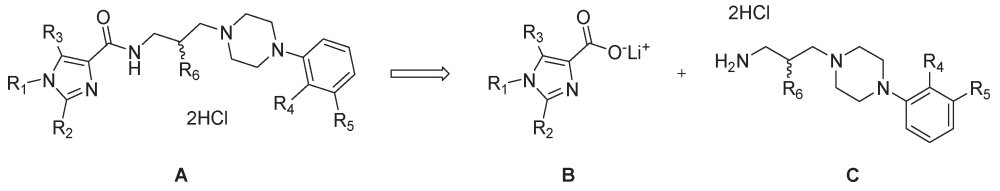


Figure 2. Preparation of target compounds using peptide bond formation.

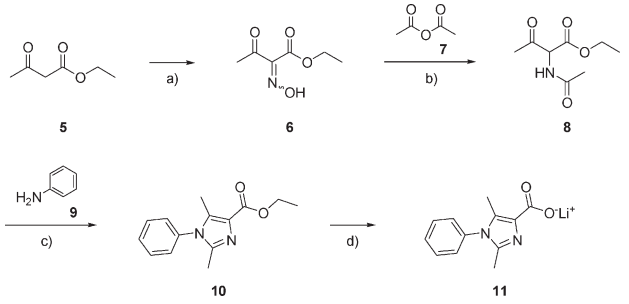
been proposed.[11−13] The level of synaptic 5-HT was expected to increase with this approach and thus eventually achieve rapid onset time. Along the line, various compounds have been proposed and developed as potential antidepressant with dual activity (Figure 1). For instance, Eli Lilly's LY367265 (1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-1(2*H*)-pyridinyl]ethyl]-5,6-dihydro-1*H*,4*H*-[1,2,5]-thiadiazolo[4.3.2-ij]quinoline-2,2,-dioxide, **1**) shows excellent binding affinities (K_i = 2.3 nM for SERT; K_i = 0.81 nM for 5-HT_{2A}).[14] Yamanouchi has also discovered YM992 ((*S*)-2-((7-fluoro-2,3-dihydro-1*H*-inden-4-yloxy)methyl)morpholine monohydrochloride, **2**) as an antidepressant with moderate affinities for SERT/5-HT_{2A} (K_i = 21 and 86 nM, respectively).[15] Bristol-Myers Squibb's nefazodone (1-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)-3-ethyl-4-(2-phenoxyethyl)-*1H*-1,2,4-triazol-5(4*H*)-one, **3**) has been described as having a similar mode of action with an improved side effect profile.[16] **3** had advantages over other antidepressants, including reduced possibility to disturb sleep or sexual dysfunction and ability to treat patients who did not respond properly to other antidepressant drugs. However, Bristol-Myers Squibb discontinued the sale in 2004 with adverse hepatic events including liver failure.[16c,d] More recently, aripiprazole (7-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one, **4**),[17,18] which had been approved for the treatment of atypical antipsychotics, was also approved by the FDA as an adjunct to treat major depressive disorder in adults. Unlike other FDA-approved atypical antipsychotics antagonizing the D_2 receptor, **4** appears as a 5-HT_{1A} partial agonist, 5-HT_{2A} antagonist, and 5-HT_{2C} partial agonist. In addition, it has moderate affinity for the serotonin transporter. However, it also has a number of side effects including headache, nausea, constipation, anxiety, restlessness, insomnia, nervousness, and so on. In this regard, there are still urgent unmet medical needs on the development of novel drugs with better developability characteristics, i.e. improved pharmacologic properties and reduced side effects.

We have previously reported a series of arylpiperazine-containing heteroaryl derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2c}, and the serotonin transporter as a potential antidepressant.[19] The pyrrole series showed outstanding in vitro activity profiles and in vivo antidepressant-like activity on a forced swimming test, but it also suffered from a relatively strong *h*ERG channel inhibition and false positive effects measured via spontaneous locomotor activity.[19a,b] On the other hand, the pyrimidine series showed the favorable spontaneous locomotor activity as well as improved *h*ERG channel inhibition profile but tended to exhibit diminished binding affinity against 5-HT_{2A}, 5-HT_{2c}, and the serotonin transporter.[19c] On the basis of these earlier data, we embarked on exploring imidazole series, hoping that we would be able to collect only positive aspects out of the above pyrrole and pyrimidine series. With this in mind, the general structure of target compounds **A** can be readily prepared by typical amide coupling of imidazole 4-carboxylic acid **B** with arylpiperazinyl alkyl amine **C** at the final stage as shown in Figure 2. As a continuation of our investigation, we wish to describe the design, synthesis, and biological evaluation of novel arylpiperazine-containing imidazole 4-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C}, and the serotonin transporter as a potential antidepressant.

CHEMISTRY

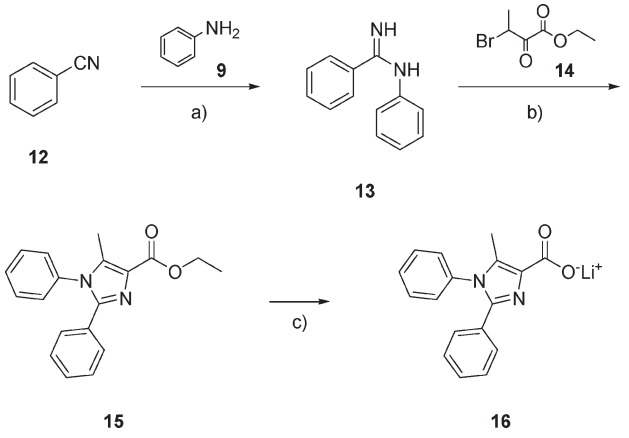
The first approach toward a key imidazole intermediate is described in Scheme 1. Thus, ethyl acetoacetate (**5**) was subjected to a reaction with sodium nitrite in acetic acid to give ethyl

Scheme 1[a]



[a] Reagents: (a) $NaNO_2$, AcOH, −10 °C to rt, 2 h, 70%; (b) **7**, Pd/C, H_2, EtOH, rt, 12 h, 86%; (c) **9**, TFA or AcOH, butyronitrile, microwave, 140 °C, 40 min, 76%; (d) $LiOH·H_2O$, $THF/H_2O(1/1)$, heat, 12 h, 90%.

Scheme 2[a]



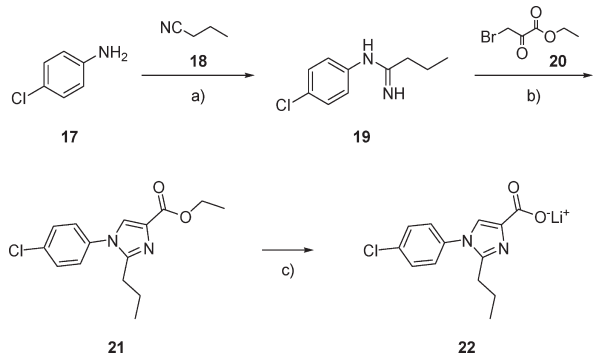
[a] Reagents: (a) **9**, NaHMDS, THF, rt; (b) **14**, $NaHCO_3$, *i*-PrOH, reflux, 55%; (c) $LiOH·H_2O$, $THF/H_2O(1/1)$, heat, 12 h.

2-(hydroxyimino)-3-oxobutanoate (**6**) in 70% yield. The compound **6** was reacted with an anhydride such as acetic anhydride (**7**) in the presence of a reducing agent such as hydrogen and a catalyst such as Pd on carbon (Pd/C) in EtOH to give an amide **8**.[20] The amide **8** was reacted with aniline **9** in acetonitrile under microwave irradiation to produce an imidazole derivative **10** in 76% yields. Hydrolysis of ester **10** with lithium hydroxide produced compound **11** as shown in Scheme 1.

The imidazole acid derivative **16** was synthesized by adopting a method reported by Solvay.[21] Thus, benzonitrile (**12**) was reacted with aniline (**9**) in the presence of sodium bis-(trimethylsilyl)amide (NaHMDS) to produce a corresponding arylbenzamidine **13**. Subsequent reaction of the resulting arylbenzamidine **13** with α-bromoketone **14** generated an intermediate ethyl 5-methyl-1,2-diphenyl-1*H*-imidazole-4-carboxylate (**15**) in 55% yield. Hydrolysis of ester **15** with lithium hydroxide produced the corresponding lithium carboxylate **16** as shown in Scheme 2.

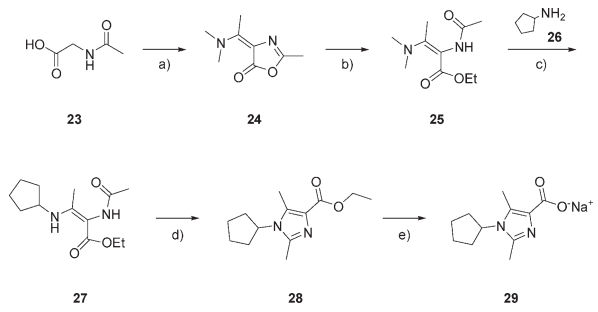
The imidazole derivative **22** was prepared by reaction of a conventional method,[22] for example, by reacting a nitrile **18** with an aniline derivative **17** using aluminum chloride to produce *N*-(4-chlorophenyl)-butyrimidamide (**19**). Subsequent reaction of the resulting compound **19** with ethyl 3-bromo-2-oxopropanoate (**20**) provided an intermediate ethyl 1-(4-chlorophenyl)-2-propyl-1*H*-imidazole-4-carboxylate (**21**). An acid

Scheme 3[a]



[a] Reagents: (a) **18**, $AlCl_3$, toluene, reflux, 5 h, 60%; (b) **20**, Na_2CO_3, toluene, reflux, 4 h, 37%; (c) $LiOH·H_2O$, $THF/H_2O(1/1)$, heat, 12 h, 86%.

Scheme 4[a]



[a] Reagents: (a) $POCl_3$, DMA, 45 °C, 50%; (b) NaH, EtOH, rt, then reflux, 1 h; (c) **26**, AcOH, rt, 12 h; (d) $(NH_4)_2SO_4$, AcOH, HMDS, reflux; (e) NaOH, aq MeOH, reflux.

form **22** was prepared from the ester **21** using lithium hydroxide, followed by acidification, as shown in Scheme 3.

Another approach toward imidazole intermediate is described in Scheme 4. The original reaction sequence was published by F. Hoffmann-La Roche.[23] *N*-Acetylglycine (**23**) and phosphorus oxychloride are mixed, to which dimethylacetamide was added dropwise slowly at low temperature (exothermic). The reaction was then stirred and warmed at 45 °C. In this way, (*E*)-4-(1-(dimethylamino)ethylidene)-2-methyloxazol-5(4*H*)-one (**24**) was generated in 50% yield. Oxazolone **24** was cleaved with in situ generated ethoxide to produce (*E*)-ethyl 2-acetamido-3-(dimethylamino)but-2-enoate (**25**). Next, an amine **26** was reacted with **25** in acetic acid at room temperature overnight to give a Michael adduct **27**. The crude intermediate **27** was then refluxed together with fine powdered ammonium sulfate in hexamethyldisilazane at 145 °C to provide the corresponding imidazoles of structure **28** in 55−73% yields for the three steps from the oxazolone **24**. The ester **28** was hydrolyzed with sodium hydroxide to produce **29** uneventfully.

As shown in Scheme 5, the synthesis of 3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-1-amine (**33**) and the like, wherein the alkyl chain between the piperazine and the terminal amine corresponds to two carbons through four carbons, commenced with *N*-(2-bromoethyl)phthalimide, *N*-(3-bromopropyl)phthalimide (**30**) and *N*-(4-bromobutyl)phthalimide by adopting a reported procedure.[24] For example, *N*-(3-bromopropyl)phthalimide (**30**)

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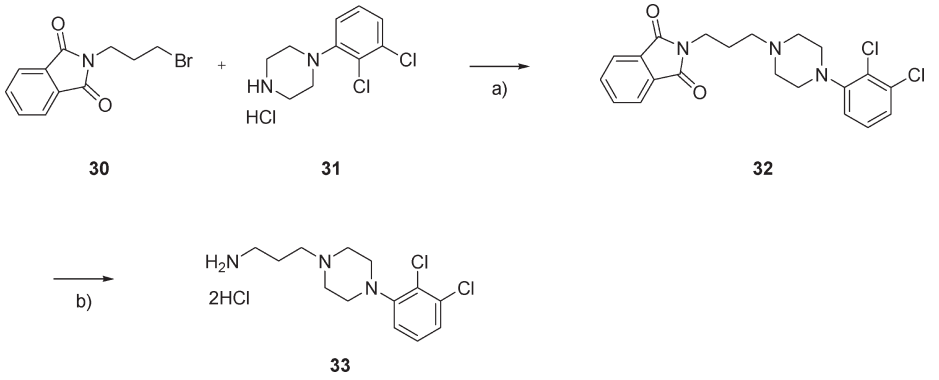
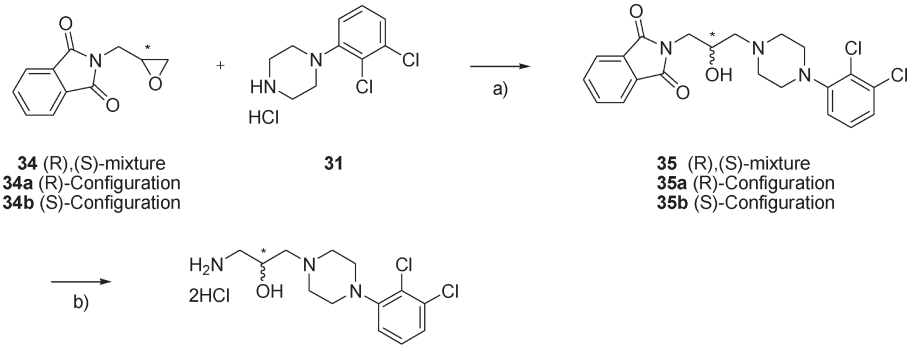
Scheme 5[a]



30 31 32 33

[a] Reagents: (a) K_2CO_3, DMF, rt, 8 h, 80%; (b) (i) $H_2NNH_2 \cdot H_2O$, EtOH, rt, 6 h, (ii) 2 M HCl solution in diethyl ether, 76%.

Scheme 6[a]



34 (R),(S)-mixture
34a (R)-Configuration
34b (S)-Configuration

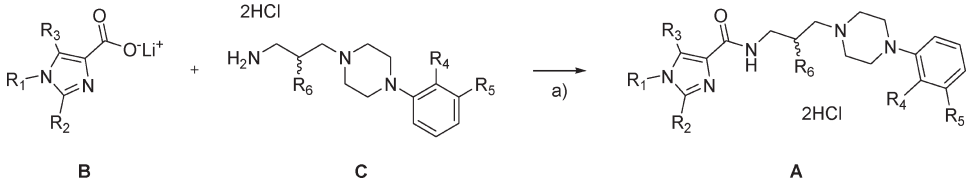
31

35 (R),(S)-mixture
35a (R)-Configuration
35b (S)-Configuration

36 (R),(S)-mixture
36a (R)-Configuration
36b (S)-Configuration

[a] Reagents: (a) TEA, THF, 80 °C, 92%; (b) (i) $H_2NNH_2 \cdot H_2O$, EtOH, rt, 6 h, (ii) 2 M HCl solution in diethyl ether, 95%.

Scheme 7[a]



B C A

[a] Reagents: (a) (i) EDCI, HOBt, NMM, DMF, rt, 12 h, (ii) 2 M HCl solution in diethyl ether.

was reacted with 1-(2,3-dichlorophenyl)piperazine (**31**) in the presence of potassium carbonate in a suitable solvent such as DMF at rt afforded the corresponding alkylated product **32** in 80% yield. Hydrazinolysis of **32**, followed by treatment of HCl solution, generated 3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-1-amine as a HCl salt form (**33**) in 76% yield.

To increase hydrophilicity for compounds such as 3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-1-amine (**33**), a compound such as 1-amino-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-2-ol (**36**) was prepared as shown in Scheme 6. Thus, commercially available *N*-(2,3-epoxypropyl)phthalimide (**34**) was treated with 1-(2,3-dichlorophenyl)piperazine (**31**) in the presence of base such as triethylamine in a suitable solvent such as THF at 80 °C produced the alcohol **35** in about 91% yield. Subsequently, hydrazinolysis of **35** generated 1-amino-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-2-ol (**36**) as a white solid in 95% yield.

Generally, a target compound was prepared by amide bond formation of acid **B** and amine **C** by use of EDCI, HOBt, and NMM in a suitable solvent such as DMF to generate **A** as shown in Scheme 7.

RESULTS AND DISCUSSION

The binding affinity of current compounds against 5-HT_{2A}, 5-HT_{2C} receptor, and serotonin transporter, stably expressed in CHO-K1 cells, was evaluated by displacement binding using [^{3}H]ketanserin, [^{3}H]mesulergine, and [^{3}H]imipramine, respectively, as radioligands.[25] The initial work was focused on exploration of substitution on the central imidazole moiety and the size

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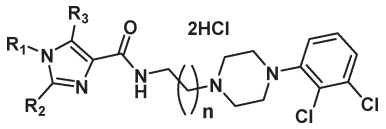
of the linker connecting the central imidazole ring to a 2,3-dichlorophenylpiperazine moiety. We decided to use 2,3-dichlorophenylpiperazine as an initial arylpiperazine, adopting a structural motif shown in **4**. The binding affinity against 5-$HT_{2A/2C}$ and inhibition against SERT are shown in Table 1. Most compounds tested displayed IC_{50} < 1 μM, implying that this series of arylpiperazinyl imidazole 4-carboxamide might hold promise as a potentially viable antidepressant. Especially, 2,5-dimethyl-1-phenyl-1*H*-imidazole (**40**) or 1-(2,3-dihydrobenzo-[*b*][1,4]dioxin-6-yl)-2,5-dimethyl-1*H*-imidazole (**57**) shows potent and balanced activity profiles against 5-$HT_{2A/2C}$ and SERT (IC_{50} = 18.6, 12.1, and 10.2 nM for **40** vs IC_{50} = 8.63, 14.6, and 9.17 nM for **57**, respectively). As the linker size reduces by one carbon, the activity profiles appear to become deteriorated as shown in **37** (IC_{50} = 31, 66, and 129 nM, respectively). Most of phenyl derivatives on the 1-position of the imidazole ring appear to be tolerated. Thus, physicochemical properties can be modulated by modifying this moiety later. Also, different groups such as methyl, ethyl, *n*-propyl, or phenyl can be introduced at 2-position of the imidazole ring without hampering overall in vitro activity profiles. A methyl (**40**) or *n*-propyl group (**42**) usually appears to be more favorable than an ethyl group (**41**), but in some cases (**47**, **55**, and **58**), a *n*-propyl group on C-2 imidazole ring was observed to diminish in vitro 5-$HT_{2A/2C}$ and SERT, implying that increased lipophilicity at this position might disrupt binding to 5-$HT_{2A/2C}$ receptors.

Some of the compounds were further evaluated with other subtypes that constitute the serotonin receptor family and dopamine receptors. The results are shown in Table 2. Interestingly, both compounds **37** and **40** proved to be highly bound to either 5-HT_{1A} or 5-HT_7 as well. In particular, compound **40** showed good activity against 5-HT receptors and SERT across the board (IC_{50} = 4.9−19 nM) except 5-HT_6 (IC_{50} = 136 nM). Such compounds might have the synergistic antidepressant properties because the blockade of 5-HT_7 receptors has been recently proposed as an alternative therapy for depression. On the contrary, binding affinity against dopamine receptors was only marginal, thereby providing good selectivity over dopamine $D_{2/3/4}$ receptors while maintaining the key target interactions. Meanwhile, a radioligand binding assay for the *h*ERG potassium channel was adopted to see if our compounds may have inhibitory activity and potential cardiotoxicity.[26] Compound **37** turned out not to be a significant inhibitor of *h*ERG channel (IC_{50} > 10 μM). But as the linker size becomes elongated (**38**), the *h*ERG profile gets slightly worse (IC_{50} = 8.11 μM) although these data are not too much problematic as shown in Table 2.

Before we move on to the in vivo efficacy study, we screened our compounds particularly against spontaneous locomotor activity to identify false positives. Although several compounds in Table 1 looked very good for in vitro activity, they appeared to have hyperactivity. For example, compounds **37** or **49** showed a high spontaneous locomotor activity (SLA) value (14802 ± 3275 at 50 mg/kg for **37**; 13040 ± 4261 at 50 mg/kg for **49**, respectively). Thus, to reduce SLA values, we decided to introduce 2,3-dimethylphenylpiperazine or 2-methyl-3-chlorophenylpiperazine which is less hydrophobic while maintain the structural motif as an approach and we also decided to keep the three-carbon distance between imidazole-amide and phenylpiperazine because they appear to provide better activity than the corresponding two-carbon linker. Also four-carbon linker was conceivable at this point, but we hesitated to explore its possibility because it looked so lengthy and too flexible that they would not be desirable from the development perspective even if they would be potent. All compounds thus designed and prepared were evaluated. The binding affinity against 5-$HT_{2A/2C}$ and inhibition against SERT are shown in Table 3. Notably, this

Table 1. Binding Affinity to Serotonin Receptor 5-HT_{2A}, 5-HT_{2C} and Serotonin Transporter (SERT) (IC_{50}, nM)



Compound	R_1	R_2	R_3	n	*in vitro* 5-HT_{2A}	5-HT_{2C}	SERT
37	Ph	Me	Me	1	31	66	129
38	Ph	Et	Me	1	27	62	245
39	Ph	Pr	Me	1	39	109	82
40	Ph	Me	Me	2	18.6	12.1	10.2
41	Ph	Et	Me	2	48	140	28
42	Ph	Pr	Me	2	21	189	13
43	Ph	Ph	Me	2	14.2	109	106
44	Pr	Me	Me	2	26	198	18
45	*c*-Pentyl	Me	Me	2	55	139	35
46	2-OMe-Ph	Me	Me	2	10.3	25.5	15.8
47	2-OMe-Ph	Pr	Me	2	139	383	84
48	4-OMe-Ph	Me	Me	2	20	88	33
49	4-OMe-Ph	Pr	Me	2	5.17	146	84
50	4-OMe-Ph	Ph	Me	2	13	64	76
51	2-F-Ph	Me	Me	2	16.8	50.8	13
52	2-F-Ph	Pr	Me	2	21	189	37
53	2-F-Ph	Ph	Me	2	19.5	185	200
54	2-Cl-Ph	Me	Me	2	30	127	82
55	4-Cl-Ph	Pr	H	2	70.9	34.6	715
56	2-CF_3-Ph	Me	Me	2	58	155	42
57	Y	Me	Me	2	8.63	14.6	9.17
58	Y	Pr	Me	2	106	297	49

Table 2. Profiles of Interesting Compounds via Competition Binding Assay at Serotonin Receptors, SERT, Dopamine Receptors (IC_{50}, nM), and Properties of *h*ERG Channels (IC_{50}, μM)

	serotonin (5-HT) receptor						dopamine receptor			
compd	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_6	5-HT_7	serotonin transpoter	D_2	D_3	D_4	*h*ERG IC_{50} (μM)
37	3.9	31	66	1682	6.8	129	908	2189	3942	>10
40	4.9	19	12	136	11	10	1369	493	>10000	8.11

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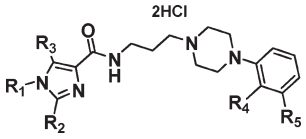
Table 3. Binding Affinity to Serotonin Receptor 5-HT$_{2A}$, 5-HT$_{2C}$ and Serotonin Transporter (SERT) (IC$_{50}$, nM)



Compound	R_1	R_2	R_3	R_4	R_5	*in vitro* 5HT$_{2A}$	5HT$_{2C}$	SERT
59	Ph	Me	Me	Me	Me	91	170	6
60	Ph	Pr	Me	Me	Cl	154	604	3.9
61	Ph	Pr	Me	Me	Me	75	286	5
62	Ph	*i*-Pr	Me	Me	Me	105	290	16
63	Ph	Pr	H	Me	Me	133	103	16.8
64	*i*-Bu	Me	Me	Me	Cl	45.5	2221	447.2
65	*i*-Bu	Me	Me	Me	Me	216.8	10140	436.2
66	*c*-Pentyl	Me	Me	Me	Cl	80.6	4375	9.53
67	Ph	Ph	Me	Me	Me	29.4	193.4	20.5
68	Ph	Ph	Me	Me	Cl	13.9	1506	17.8
69	Ph	Ph	H	Me	Me	13.2	116	50.5
70	Ph	Ph		Me	Cl	12.3	1368	181.7
71	2-OMe-Ph	Me	Me	Me	Cl	12.8	37.4	16.8
72	2-OMe-Ph	Me	Me	Me	Me	144	146	9
73	2-OMe-Ph	Pr	Me	Me	Cl	171	473	55
74	2-OMe-Ph	Pr	Me	Me	Me	253	283	28
75	4-OMe-Ph	Me	Me	Me	Cl	23.1	93.6	12.4
76	4-OMe-Ph	Me	Me	Me	Me	62.7	190.9	31.1
77	4-OMe-Ph	Pr	Me	Me	Cl	15.2	204	10.2
78	4-OMe-Ph	Pr	Me	Me	Me	70.8	130.0	13.0
79	4-OMe-Ph	Ph	Me	Me	Cl	21	77	25
80	4-OMe-Ph	Ph	Me	Me	Me	24	96	12
81	4-OMe-Ph	Ph	H	Me	Me	19	104	42.6
82	2-F-Ph	Me	Me	Me	Cl	40	71	6.17
83	2-F-Ph	Me	Me	Me	Me	64.8	124	6.9
84	4-F-Ph	Me	Me	Me	Cl	38	113	39
85	4-F-Ph	Me	Me	Me	Me	117.9	323.7	21.6
86	4-F-Ph	Pr	Me	Me	Cl	54	229	28
87	4-F-Ph	Pr	Me	Me	Me	31	68	6.6
88	4-F-Ph	Pr	H	Me	Me	141	238	188
89	4-F-Ph	Ph	Me	Me	Cl	12.2	2239	281.9
90	4-F-Ph	Ph	Me	Me	Me	27.8	64.1	153.2
91	2-Cl-Ph	Me	Me	Me	Cl	29	39	18
92	2-Cl-Ph	Me	Me	Me	Me	80	200	17
93	2-Cl-Ph	Pr	Me	Me	Me	90	235	7.3
94	3-Cl-Ph	Me	Me	Me	Me	96.8	110.6	4.88
95	4-Cl-Ph	Me	Me	Me	Me	67	233	47
96	4-Cl-Ph	Pr	H	Me	Cl	20	367	66
97	4-Cl-Ph	Pr	H	Me	Me	32.4	37.9	67.2
98	3.5-diOMe-Ph	Me	Me	Me	Me	32	159	10
99	Y	Me	Me	Me	Cl	28	106	2.7
100	Y	Me	Me	Me	Me	40.6	3996	6.08
101	Y	Pr	Me	Me	Cl	47	61	14
102	Y	Pr	Me	Me	Me	241	237	5
103	Y	Ph	Me	Me	Me	19	68	185

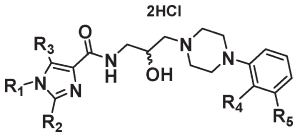
modification significantly reduced intrinsic activity against 5-HT$_{2A/2C}$ in an order of magnitude. This phenomenon is exemplified in dimethylphenylpiperazine analogue **59** (IC$_{50}$ = 91 nM for 5-HT$_{2A}$, IC$_{50}$ = 170 nM for 5-HT$_{2C}$) compared to the dichlorophenylpiperazine **40** (IC$_{50}$ = 18.6 nM for 5-HT$_{2A}$, IC$_{50}$ = 12.1 nM for 5-HT$_{2C}$). However, its inhibition activity against SERT is not decreased but rather maintained or even increased as exemplified in **40** (IC$_{50}$ = 6 nM). Among the compounds demonstrating IC$_{50}$ < 20 nM in Table 3, compound **75** appears to have hypoactivity (SLA value =5409 ± 3319 cm at 50 mg/kg). Replacement of the 2,3-dimethylpiperazine in **92** with 2-methyl-3-chloro-piperazine provided the compound **91** with an encouraging overall activity profile (IC$_{50}$ = 29 nM for 5-HT$_{2A}$, IC$_{50}$ = 39 nM for 5-HT$_{2C}$, IC$_{50}$ = 18 nM for SERT). Disappointingly, further investigation of the compound with SLA revealed its hyperactivity (SLA value =10189 ± 628 cm at 50 mg/kg). Among 45 compounds in Table 3, 13 compounds show IC$_{50}$ < 10 nM against SERT, and 8 compounds show IC$_{50}$ < 20 nM against 5-HT$_{2A}$, while none of the compounds show IC$_{50}$ < 30 nM against 5-HT$_{2C}$. It is notable that replacement of 2,3-dichloropiperazine with 2,3-dimethylpiperazine boosted in vitro inhibitory activity against the serotonin transporter while maintaining binding affinity against 5-HT$_{2A}$. Thus, this series appears to become more like SSRI compounds that happen to additionally block 5-HT$_{2A}$ at this point. There are reported studies indicating that the antidepressants which occupy 5-HT$_2$ receptors in the brain at clinical doses and block mainly 5-HT$_{2A}$ responses augment the clinical response to SSRIs in treatment-resistant patients.[27,28]

To decrease clogP and increase polarity and thus possibly improve solubility of the compounds in the series, we decided to introduce a hydroxyl group on the linker chain. Because introduction of a hydroxyl group might affect the overall efficacy and false positives profiles, the corresponding 2,3-dichlorophenylpiperazine analogues were also synthesized and evaluated.

Introduction of hydroxyl group into the linker chain also revealed a range of interesting profiles and subtle effects on intrinsic activity as shown in Table 4. Hydroxyl compound **106** afforded about 2-fold drop in 5-HT$_{2A}$ and 5-fold drop in 5-HT$_{2C}$ but with slightly increased intrinsic activity at SERT compared to the corresponding deoxy analogue **43**. A 2-F-Phenyl analogue **122** afforded about a 6-fold drop in 5-HT$_{2A}$ and a 9-fold drop in 5-HT$_{2C}$ but maintained SERT affinity. 2,3-Dihydrobenzo[*b*][1,4]dioxinyl moiety on C-1 on the imidazole ring (**138**) gave the most desirable intrinsic activity profiles yet observed, maintaining high affinities at 5-HT$_{2A}$ and SERT but again accompanied by decreased 5-HT$_{2C}$ intrinsic activity. Overall, introduction of hydroxyl group into the linker led to the discovery of compounds with good SERT inhibitory activity with additional moderate 5-HT$_{2A}$ receptor antagonism.

Interesting compounds were further evaluated in vivo with immobility in forced swimming test (FST) on mice.[29] Fluoxetine (*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine), venlafaxine ((*RS*)-1-[2-dimethylamino-1-(4-methoxyphenyl)-ethyl]cyclohexanol), paroxetine ((3*S*,4*R*)-3-[(2*H*-1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine), and sertraline ((1*S*,4*S*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine) were used as reference compounds for comparison. The results are shown in Figure 3. At 50 mg/kg, des-methyl at C-5 of imidazole (**81**) showed virtually no appreciable immobility effect. This phenomenon was also observed in another des-methyl analogue **69** which is devoid of any antidepressant-like effect at 25 mg/kg. Thus, a substituent on C-5 imidazole ring appears to be minimal requirement for in vivo efficacy against FST model on mice. Compared to sertraline, which demonstrated the most impressive immobility performance among the reference compounds tested at 50 mg/kg, compounds **46**, **57**, **68**, **70**, and **131** showed superior in vivo immobility data. Among them, compounds **68**, **70**, and **131** also displayed nice dose-dependency. As oral dose reduced down from 25 to 10 mg/kg, virtually all the compounds tested showed insufficient antidepressant activity. Either **70** or **131** demonstrated merely moderate in vivo efficacy (immobility ~80%) (see Figure 3).

Table 4. Binding Affinity to Serotonin Receptor 5-HT$_{2A}$, 5-HT$_{2C}$, and Serotonin Transporter (SERT) (IC$_{50}$, nM)



Compound	R_1	R_2	R_3	R_4	R_5	*in vitro* 5HT$_{2A}$	5HT$_{2C}$	SERT
104	Ph	Me	Me	Me	Me	247.6	2741	5
105	Ph	Pr	Me	Me	Cl	201	398	6.2
106	Ph	Ph	Me	Cl	Cl	24	551	71.1
107	Ph	Ph	Me	Me	Cl	57	739	370
108	Ph	Ph	Me	Me	Me	58.6	525	19.1
109	*n*-Pr	Me	Me	Me	Me	839.9	>10000	10.4
110	*i*-Bu	Me	Me	Me	Me	1011	595.4	1262
111	*c*-Pentyl	Me	Me	Me	Cl	97.6	166.3	39.1
112	2-OMe-Ph	Me	Me	Cl	Cl	52.1	76.6	12.2
113	2-OMe-Ph	Me	Me	Me	Cl	370	478	19
114	4-OMe-Ph	Me	Me	Me	Cl	56	323	24
115	4-OMe-Ph	Me	Me	Me	Me	174.9	546.4	21.6
116	4-OMe-Ph	Pr	Me	Cl	Cl	63.1	544	51.8
117	4-OMe-Ph	Pr	Me	Me	Cl	582	1365	17
118	4-OMe-Ph	Pr	Me	Me	Me	126	425	6
119	4-OMe-Ph	Ph	Me	Me	Cl	170	526	443
120	4-OMe-Ph	Ph	Me	Me	Me	185	609	201
121	3.4-diOMe-Ph	Me	Me	Me	Me	204.5	1184	70.3
122	2-F-Ph	Me	Me	Cl	Cl	104	459	13
123	2-F-Ph	Me	Me	Me	Cl	197	774	36
124	2-F-Ph	Me	Me	Me	Me	579	>10000	7.59
125	4-F-Ph	Me	Me	Me	Cl	254	1454	20
126	4-F-Ph	Me	Me	Me	Me	78.3	653.7	16.6
127	4-F-Ph	Pr	Me	Cl	Cl	196	1509	65
128	4-F-Ph	Pr	Me	Me	Me	327	1001	6.3
129	4-F-Ph	Ph	Me	Me	Me	71	184.6	150.1
130	4-F-Ph	Ph	Me	Cl	Cl	94	64	195
131	4-F-Ph	Ph	Me	Me	Cl	26.7	>10000	478.4
132	2-Cl-Ph	Me	Me	Me	Me	318	311	5
133	3-Cl-Ph	Me	Me	Me	Cl	182.1	556.5	14.2
134	3-Cl-Ph	Me	Me	Me	Me	275.1	535.2	2.9
135	4-Cl-Ph	Pr	H	Cl	Cl	176.6	855.9	1276
136	4-Cl-Ph	Pr	H	Me	Cl	107	953	80
137	4-Cl-Ph	Pr	Me	Me	Me	147	661	347
138	Y	Me	Me	Cl	Cl	9.86	94.3	12
139	Y	Me	Me	Me	Cl	127	663	9.1
140	Y	Me	Me	Me	Me	231.5	609.6	12.6
141	Y	Pr	Me	Me	Cl	237	284	20
142	Y	Pr	Me	Me	Me	294	593	9.3

Y: 

The second in vivo test involves spontaneous locomotor activity test. The results are illustrated in Figure 3. Dichlorophenylpiperazine compound **106** was observed to show obvious hyperactivity. Additional dichlorophenylpiperazine analogues **43**, **46**, and **138** also appear to have hyperactivity. At this point, it was observed that as arylpiperazine was altered from dichlorophenylpiperazine into the corresponding dimethylphenylpiperazine, some degree of spontaneous locomotor activity was decreased as exemplified in compounds **69**, **100**, and **103**. Encouragingly, 3-chloro-2-methylphenylpiperazine analogues **68**, **70**, and **131**, which we had introduced as novel arylpiperazine analogues to reduce hyperactivity with maintaining antidepressant activity, did not show hyperactivity but normal activity.

In passing, an interesting observation during exploration of this series is described in Table 5. When hydroxyl group was introduced onto the linker chain in the molecule, it was observed that SERT affinity becomes improved in conjunction with a drop in 5-HT$_{2A/2C}$. Notably, significant increases in intrinsic activity at SERT were observed for compounds with (*R*)-configuration as shown in Table 5. Thus, compounds **143** and **145** showed subnanomolar SERT affinities in this series for the first time (IC$_{50}$ = 0.64 nM, IC$_{50}$ = 0.75 nM, respectively). The affinity difference between (*R*)- and (*S*)-configuration is approximately an order of magnitude. However, it is noted that racemic compounds **104** or **124** rather gave the highest activity in in vivo antidepressant activity in forced swimming test on mice as shown in Figure 4.

To further evaluate this series of compounds, some of the representative compounds have been tested for *h*ERG inhibition, cytotoxicity at HT22, genotoxicity (Ames test), and inhibition against a panel of cytochrome P450 isoforms, respectively. For instance, compound **68** showed relatively strong inhibition against *h*ERG (IC$_{50}$ = 1.3 μM). Also **68** demonstrated moderate inhibitory activity against CYP 2C9 (IC$_{50}$ = 2.9 μM) and 2D6 (IC$_{50}$ = 5.4 μM), while **68** lacks inhibitory activity against 1A2 or 3A4. **68** appeared to show neither cytotoxicity nor mutagenicity as shown in Table 6. The profile has been improved in dihydrobenzodioxin **100**. For example, **100** improved *h*ERG inhibition in approximately 7-fold, showing IC$_{50}$ = 8.7 μM. Inhibition against CYP 2D6 was reduced in greater than 3-fold. However, all of three compounds showed moderate liability against the CYP 2C9 isoform, displaying IC$_{50}$ = 2.9−4.4 μM.

Subsequently, the pharmacokinetic properties of **57** were measured in male SD rats. After oral administration of 5 mg/kg of **57** to rats, a C_{max} of 0.208 μg/mL was obtained at 15 min. The elimination half-life of **57** following oral administration was 0.37 h in rats. Compound **57** showed only moderate oral bioavailability (F = 14.92%) in rats. This profile became even worse in 2-methoxyphenylimidazole **71** as shown in Table 7, suggesting its solubility-limited absorption.

CONCLUSION

In summary, serotonin antagonist reuptake inhibitor (SARI) drugs that block both 5-HT$_2$ receptors and the serotonin transporters have been developed. We investigated a series of arylpiperazine containing imidazole 4-carboxamide derivatives for the treatment of depressive disorders. The human 5-HT$_{2A/2C}$ receptor has been implicated in several neurological conditions, and potent selective 5-HT$_{2A/2C}$ ligands may have therapeutic potential for treatment of CNS diseases such as depression. The imidazole series of compounds was evaluated against 5-HT$_{2A/2C}$ and serotonin reuptake inhibition. A few of the compounds in the series showed promising in vitro IC$_{50}$ values and in vivo antidepressant-like effect in the forced swimming test (FST). On the basis of these results, current imidazole series of compounds is being used as tool compound to identify a development candidate for promising antidepressant.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. All references to ether are to diethyl ether; brine refers to a saturated aqueous solution of NaCl. Unless otherwise indicated, all temperatures are expressed in °C. All reactions are conducted under an

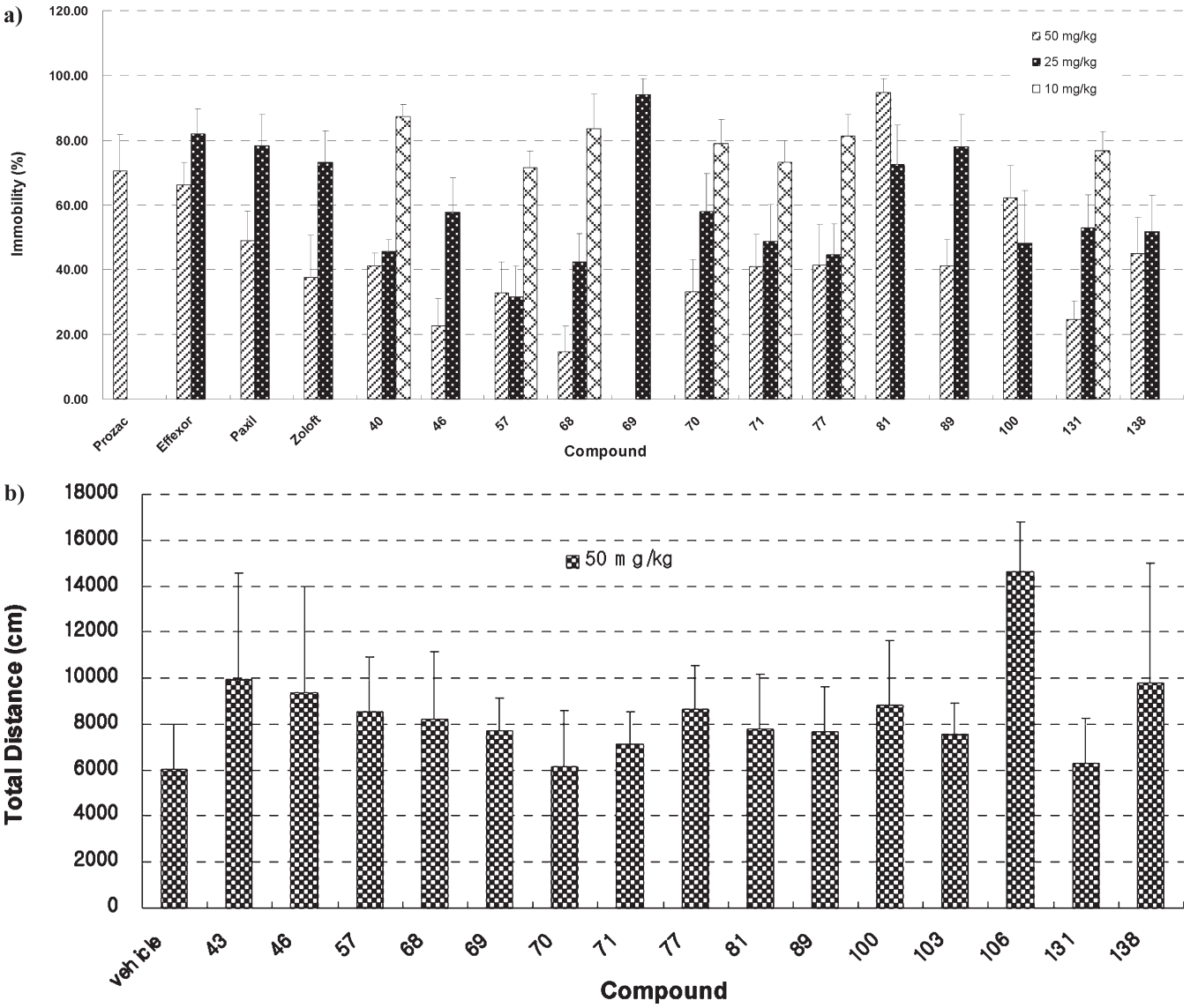


Figure 3. Antidepressants activity immobility in forced swimming test on mice (FST, %) and spontaneous locomotor activity (SLA, cm). Effects of the drugs on immobility in forced swimming test on mice. a) Drugs (50, 25, and 10 mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. (Values are means ± SEM). b) Locomotor activities of the mice treated with compounds or vehicle were counted for 30 min by Activity Analyzer. Compound (50 mg/kg) or vehicle was administered orally (po) 60 min before the test. Data were expressed as mean ± SEM of 6−7 mice.

inert atmosphere at room temperature unless otherwise noted, and all solvents are of the highest available purity unless otherwise indicated. Microwave reaction was conducted with a Biotage Initiator microwave synthesizer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz AVANCE III. Chemical shifts were expressed in parts per million (ppm, δ units). Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Mass spectra were obtained with either a Micromass, Quattro LC triple quadruple tandem mass spectometer, ESI or Agilent, 1200LC/MSD, ESI. For preparative HPLC, ca. 100 mg of a product was injected in 1 mL of DMSO onto a SunFire Prep C18 OBD 5 μm 19 mm × 100 mm column with a 10 min gradient from 10% CH_3CN to 90% CH_3CN in H_2O (purification systems from Gilson, Inc. or Waters, Inc.). The tested compounds were determined to be >95% pure by HPLC. Flash chromatography was carried using Merck Silica Gel 60 (230−400 mesh). A Biotage SP1 FLASH purification system or Biotage Isolera FLASH purification system was used for normal phase column chromatography with ethyl acetate/hexane or tetrahydrofuran/hexane. Most of the reactions were monitored by thin-layer chromatography on 0.25 mm E. Merck silica gel plates (60F-254), visualized with UV light using a 5% ethanolic phosphomolybdic acid or *p*-anisaldehyde solution. Except where otherwise noted, all reactions were run under an inert atmosphere of nitrogen gas using anhydrous solvents.

*Ethyl 2-(Hydroxyimino)-3-oxobutanoate (**6**).* To a solution of ethyl acetoacetate (**5**, 30 g, 0.23 mol) in acetic acid (35 mL) was added slowly sodium nitrite (18.0 g, 0.25 mol) in cold water (40 mL) at −10 °C. The reaction mixture was stirred for 1 h, then cold water (120 mL) was added and then stirred for 3 h. The mixture was extracted with diethyl ether (300 mL). The organic layer was washed with saturated $NaHCO_3$ (400 mL × 2), then dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The obtained product (21.46 g, white solid) was used for the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 4.21 (q, J = 7.2 Hz, 2H), 2.32 (s, 3H), 1.92 (t, J = 6.8 Hz, 3H).

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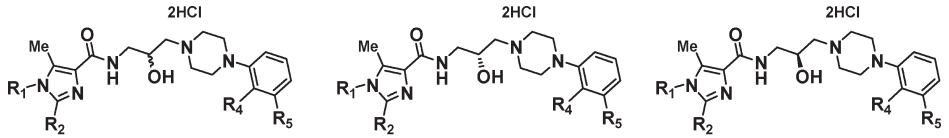
Table 5. Binding Affinity to Serotonin Receptor 5-HT$_{2A}$, 5-HT$_{2C}$ and Serotonin Transporter (SERT) (IC$_{50}$, nM)



						in vitro		
compd	R_1	R_2	R_4	R_5		5HT$_{2A}$	5HT$_{2C}$	SERT
104	Ph	Me	Me	Me	racemic	247.6	2741	5
143	Ph	Me	Me	Me	(*R*)-configuration	72.5	227	0.64
144	Ph	Me	Me	Me	(*S*)-configuration	646	1779	5.08
124	2-F-Ph	Me	Me	Me	racemic	579	>10000	7.59
145	2-F-Ph	Me	Me	Me	(*R*)-configuration	270	193	0.75
146	2-F-Ph	Me	Me	Me	(*S*)-configuration	244	1031	10.3
131	4-F-Ph	Ph	Me	Cl	racemic	26.7	>10000	478.4
147	4-F-Ph	Ph	Me	Cl	(*R*)-configuration	95	479	257
148	4-F-Ph	Ph	Me	Cl	(*S*)-configuration	20.1	534	232

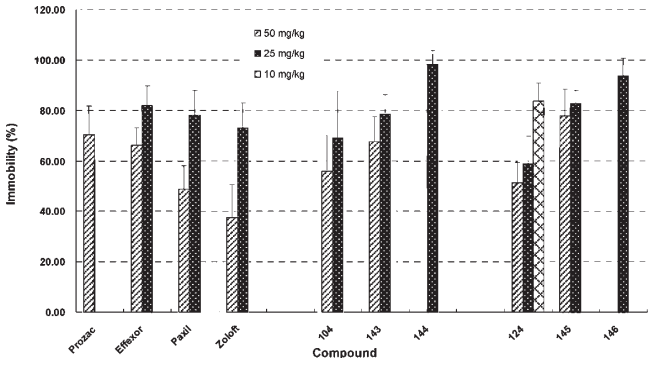


Figure 4. Antidepressants Activity immobility in forced swimming test on mice (FST, %). Effects of drugs on immobility in forced swimming test on mice. Drugs (50, 25, and 10 mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. (Values are means ± SEM).

Ethyl 2-Acetamido-3-oxobutanoate (***8***). To a mixture of ethyl 2-(hydroxyimino)-3-oxobutanoate (**6**, 9.32 g, 58.5 mmol) and Pd/C (400 mg, palladium on carbon, 10 wt %, support activated carbon, wet, Degussa type E101 NE/W) in EtOH was added acetic anhydride (**7**, 11.0 mL, 117.0 mmol) at room temperature. The reaction mixture was stirred at room temperature under H_2 for 15 h. The palladium was filtered off (Filter aid, Celite 521 AW), and then the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography (Biotage SP1 FLASH purification system) to provide the title compound (9.48 g, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (br s, 1H), 5.25 (d, J = 6.4 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.07 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H).

Ethyl 2,5-Dimethyl-1-phenyl-1H-imidazole-4-carboxylate (***10***). To a solution of ethyl 2-acetamido-3-oxobutanoate (**8**) (5.0 g, 26.7 mmol) and aniline (**9**, 7.3 mL, 80.1 mmol) in butyronitrile (10 mL) was added trifluoroacetic acid (6.2 mL, 80.1 mmol) at room temperature. The reaction mixture was irradiated in a microwave reactor (Biotage Initiator) for 40 min at 140 °C. The mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with aqueous K_2CO_3. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (Biotage SP1 FLASH purification system) to provide the title compound (4.97 g, 76%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57−7.51 (m, 3H), 7.20−7.18 (m, 2H), 4.41 (q, J = 6.8 Hz, 2H), 2.31 (s, 3H), 2.22 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H); MH^+ 245.

Lithium 2,5-Dimethyl-1-phenyl-1H-imidazole-4-carboxylate (***11***). To a solution of ethyl 2,5-dimethyl-1-phenyl-1*H*-imidazole-4-carboxylate (**10**, 4.97 g, 20.3 mmol) in THF/water (15/15 mL) was added lithium hydroxide monohydrate at room temperature. The reaction mixture was stirred at 55 °C for 12 h, and then the mixture was concentrated in vacuo. The crude solid was washed with diethyl ether to provide the title compound (4.06 g, 90%) as a white solid. The obtained product was used for the next step without purification. ^{1}H NMR (400 MHz, CD_3OD) δ 7.59−7.54 (m, 3H), 7.31−7.28 (m, 2H), 2.26 (s, 3H), 2.14 (s, 3H). MS m/z: 217 ($MH^+ - Li^+$).

N-Phenylbenzimidamide (***13***). To NaHMDS (49 mL, 48.5 mmol, 1.0 M solution in THF) was added dropwise a solution of aniline (**9**, 4.5 mL, 48.5 mmol) in anhydrous THF (10 mL) under N_2. After 20 min, a solution of benzonitrile (**12**, 5.0 mL, 48.5 mmol) in anhydrous THF (10 mL) was slowly added. The reaction mixture was stirred for 12 h, poured into cold water, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was used for the next step without purification.

Ethyl 5-Methyl-1,2-diphenyl-1H-imidazole-4-carboxylate (***15***). A mixture of *N*-phenylbenzimidamide (**13**, 1.0 g, 5.10 mmol), 3-bromo-2-oxovalerate (**14**, 1.3 g, 6.12 mmol), and $NaHCO_3$ in *i*-PrOH was stirred at 90 °C for 12 h. The reaction mixture was concentrated under reduced pressure, and then the residue was diluted with EtOAc and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (Biotage Isolera FLASH purification system) to provide the title compound (0.86 g, 55%) as a yellow solid.

Lithium 5-Methyl-1,2-diphenyl-1H-imidazole-4-carboxylate (***16***). Following the procedure for compound **11**, the desired compound was obtained as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.52−7.48 (m, 3H), 7.34−7.31 (m, 2H), 7.26−7.17 (m, 5H), 2.36 (s, 3H). MS m/z: 279 ($MH^+ - Li^+$).

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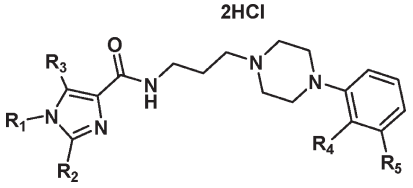
Table 6. In Vitro Toxicity of Compound 68, 70, and 100



Compound	R_1	R_2	R_3	R_4	R_5	*h*ERG (IC_{50}, μM)	HT22 (CC_{50}, μM)	AMES	CYP 1A2	CYP 2C9	CYP 2D6	CYP 3A4
68	Ph	Ph	Me	Me	Cl	1.3	23.6	Non-mutagen	> 20	2.9	5.4	> 20
70	Ph	Ph		Me	Cl	9.8	19.1	Non-mutagen	15.3	3.3	4.8	> 20
100		Me	Me	Me	Me	8.7	19.3	Non-mutagen	> 20	4.4	17.9	> 20

Table 7. Pharmacokinetic Parameters and Brain/Plasma Ratio of Compounds 57 and 71 after Oral (5 mg/kg) and iv (1 mg/kg) Administration to Rat

	compd			
	57		71	
PK parameters	iv (1 mg/kg, n = 3)	oral (5 mg/kg, n = 3)	iv (1 mg/kg, n = 3)	oral (5 mg/kg, n = 3)
C_{max} (μg/mL)	0.245 ± 0.032	0.208 ± 0.117	0.408 ± 0.056	0.065 ± 0.046
C_{last} (μg/mL)	0.001 ± 0.000	0.000 ± 0.000	0.004 ± 0.001	0.000 ± 0.000
T_{max}(h)		0.250 ± 0.000		0.250 ± 0.000
$t_{1/2}$ (h)	0.438 ± 0.064	0.372 ± 0.012	0.226 ± 0.019	0.121 ± 0.038
AUC_{inf} (min·μg/mL)	10.931 ± 2.019	10.811 ± 2.986	7.611 ± 0.488	1.312 ± 0.839
$AUC_{inf/dose}$	10.931 ± 2.019	1.631 ± 1.246	7.611 ± 0.488	0.262 ± 0.168
BA (%)	14.92		3.45	

*N-(4-Chlorophenyl)butyrimidamide (**19**).* To a solution of butyronitrile (**18**, 7.5 mL, 86.2 mmol) and $AlCl_3$ in toluene was added 4-chloroaniline (**17**, 10.0 g, 78.4 mmol). The reaction mixture was stirred at 115 °C for 5 h. The mixture was diluted with water (200 mL) and extracted with EtOAc (200 mL). The aqueous layer was neutralized with saturated $NaHCO_3$ (500 mL) and extracted with EtOAc (300 mL × 2). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The obtained product (9.2 g, pale-brown solid) was used for the next step without purification.

*Ethyl 1-(4-Chlorophenyl)-2-propyl-1H-imidazole-4-carboxylate (**21**).* To a solution of *N*-(4-chlorophenyl)butyrimidamide (**19**, 3.5 g, 17.80 mmol) and $NaHCO_3$ (3.14 g, 37.38 mmol) in *i*-PrOH was added ethyl bromopyruvate (**20**, 4.7 mL, 37.38 mmol) under N_2. The reaction mixture was stirred at 85 °C for 72 h, and then AcOH (15 mL)was added. After 4 h, the mixture was concentrated under reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ and 1N HCl solution and then dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (Biotage Isolera FLASH purification system) to provide the title compound (1.28 g, 25%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.51−7.47 (m, 2H), 7.26−7.23 (m, 2H), 4.39 (q, *J* = 7.2 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 1.71−1.61 (m, 2H), 1.39 (t, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H). MH^+ 293.

*Lithium 1-(4-Chlorophenyl)-2-propyl-1H-imidazole-4-carboxylate (**22**).* Following the procedure for compound **11**, the desired compound was obtained as a pale-brown solid (3.00 g, 86%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.58−7.56 (m, 2H), 7.49 (s, 1H), 7.43−7.40 (m, 2H), 2.63−2.59 (m, 2H), 1.70−1.61 (m, 2H), 0.86 (t, *J* = 7.6 Hz, 3H). MS *m*/*z*: 265 ($MH^+ - Li^+$).

*4-[1-Dimethylamino-eth-(Z)-ylidene]-2-methyl-4H-oxazole-5-one (**24**).* *N*-Acetylglycine (**23**, 10 g, 85.5 mmol) was dissolved in *N*,*N*′-dimethylacetamide (20 mL, 21.4 mmol), and $POCl_3$ (19.6 mL, 21.4 mmol) was added dropwise slowly at 0 °C. The reaction mixture was stirred at 50 °C for 3 h and then cooled to room temperature. CH_2Cl_2 (50 mL) was added and the mixture poured into ice−water. The resulting solution was basified with ammonium hydroxide to over than pH 8. The organic extracts were washed with 50 mL of water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by normal phase preparative column, and the desired compound was obtained as an orange solid (6.7 g, 47%). MH^+ 169.

*(E)-Ethyl 2-Acetamido-3-(dimethylamino)but-2-enoate (**25**).* 4-[1-Dimethylamino-eth-(*Z*)-ylidene]-2-methyl-4*H*-oxazole-5-one (**24**, 7.54 g, 44.8 mmol) was dissolved in ethanol (50 mL), and sodium hydride (179 mg, 4.5 mmol, 60% dispersion in mineral oil) wad added at room temperature. The solution was refluxed for 1 h. The solvent was evaporated, and the crude product was used without any further purification for the next step. MH^+ 215.

*(E)-Ethyl 2-Acetamido-3-(cyclopentylamino)but-2-enoate (**27**).* (*E*)-Ethyl 2-acetamido-3-(dimethylamino)but-2-enoate (**25**, 1 g, 4.67 mmol) and cyclopentylamine (**26**, 0.5 mL) were stirred at room temperature in AcOH (10 mL) for overnight. The reaction mixture was diluted slowly with water (10 mL) and evaporated under reduced pressure to obtain the desired product as a dark-brown oil, which

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could be used without any further purification for the next step. MH^+ 256.

*Ethyl 1-Cyclopentyl-2,5-dimethyl-1H-imidazole-4-carboxylate (**28**).* Ammonium sulfate (100 mg) was added to a solution of (*E*)-ethyl 2-acetamido-3-(cyclopentylamino)but-2-enoate (**27**, 1.5 g, 5.9 mmol) and hexamethyldisilazane (15 mL) and refluxed overnight at 150 °C. The reaction mixture was evaporated and extracted with EtOAc and water. The organic layer was evaporated, and the residue was purified with 20% methanol in CH_2Cl_2 to produce as a light-brown solid (1.0 g, 71.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (q, J = 6.5 Hz, 2H), 3.77 (m, 1H), 2.59 (s, 3H), 2.33 (s, 3H), 2.15−2.06 (m, 2H), 1.83−1.80 (m, 2H), 1.72−1.54 (m, 2H), 1.32 (t, J = 6.5 Hz, 3H). MH^+ 237.

*2-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propyl)isoindoline-1,3-dione (**32**).* The mixture of 1-(2,3-dichlorophenyl)piperazine hydrochloride (**31**, 10.0 g, 37.4 mmol), *N*-(3-bromopropyl)phthalimide (**30**, 9.09 g, 33.9 mmol), and potassium carbonate (11.7 g, 84.8 mmol) in DMF was stirred at room temperature. The mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The obtained product was washed with EtOH to provide the title compound (11.2 g, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88−7.83 (m, 2H), 7.74−7.69 (m, 2H), 7.15−7.09 (m, 2H), 6.82 (dd, J = 7.6, 2.4 Hz, 1H), 3.80 (t, J = 7.2 Hz, 2H), 2.93−2.87 (m, 4H), 2.62−2.56 (m, 4H), 2.51 (t, J = 5.6 Hz, 2H), 1.94−1.87 (m, 2H). MH^+ 418; mp 119.2 °C.

*3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propan-1-amine Dihydrochloride (**33**).* To a suspension of 2-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propyl)isoindoline-1,3-dione (**32**, 11.1 g, 26.5 mmol) in EtOH was added hydrazine monohydrate. The reaction mixture was stirred at room temperature for 6 h and then the white solid filtered off. The filtrate was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. To the crude product in diethyl ether was added 2 M HCl solution in diethyl ether (10 mL). The obtained product was washed with diethyl ether to provide the title compound (6.98 g, 73%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.22−7.18 (m, 2H), 7.08 (dd, J = 7.2, 2.4 Hz, 1H), 3.06−3.02 (m, 6H), 2.72−2.64 (m, 4H), 2.60 (t, J = 6.8 Hz, 2H), 1.90−1.84 (m, 2H). MS m/z: 288 (MH^+ − 2HCl).

*1-Amino-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-2-ol Dihydrochloride (**36**).* To a stirred solution of *N*-(2,3-epoxypropyl)phthalimide (**34**, 10 g, 0.049 mol) in THF (100 mL) was added 1-(2,3-dichlorophenyl)piperazine hydrochloride (**31**, 8.7 g, 0.033 mol) and triethylamine (4.6 mL, 0.033 mol) at room temperature, and then the resultant solution was heated at 80 °C overnight. The reaction was quenched with H_2O and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated. The solid residue was solidified with CH_2Cl_2 (20 mL)/diethyl ether (200 mL), filtered, and dried in vacuo, which was used for the following synthesis without further purification. To the prepared white solid piperazine (**35**, 13 g, 0.030 mol) in EtOH was added hydrazine monohydrate (20 mL), and the reaction solution was refluxed at 80 °C for 2 h. The reaction solution was cooled to room temperature and evaporated. The oily crude compound was extracted with EtOAc/H_2O, and organic the layer was combined and evaporated. The pale-yellow solid was tritulated with ether to afford pure targeted amine (**36**, 8.7 g, 95%) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.31−7.27 (m, 2H), 7.19−7.15 (m, 1H), 4.40−4.36 (m, 1H), 3.42−3.39 (m, 4H), 3.37−3.32 (m, 4H), 3.24−2.93 (m, 4H). MS m/z: 304 (MH^+ − 2HCl).

General Procedure (Synthesis of A). *N-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propyl)-5-methyl-1,2-diphenyl-1H-imidazole-4-carboxamide Dihydrochloride (**43**).* To a mixture of lithium 5-methyl-1,2-diphenyl-1*H*-imidazole-4-carboxylate (**16**, 300 mg, 1.06 mmol), 1-amino-3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propan-2-ol dihydrochloride (459 mg, 1.27 mmol), EDCI (305 mg, 1.59 mmol), and HOBt (286 mg, 2.12 mmol) in anhydrous DMF (5 mL) was added NMM (580 μL, 5.30 mmol). The reaction mixture was stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure. The residue was diluted with EtOAc and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by preparative HPLC (purification system, Gilson Inc.) and followed by treatment of 2 M HCl solution in diethyl ether generated to provide the title compound (267 mg, 46%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.45 (s, 1H), 7.59−7.20 (m, 13H), 6.97 (dd, J = 7.2, 2.4 Hz, 1H), 3.81−3.68 (m, 2H), 3.65−3.51 (m, 2H), 3.50−3.31 (m, 4H), 2.77−2.60 (m, 4H), 2.45 (s, 3H), 2.09−1.95 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161,4, 149.5, 144.3, 134.9, 134.8, 132.7, 130.1, 129.9, 129.8, 128.8, 128.6, 128.3, 128.0, 127.4, 127.0, 126.0, 125.2, 119.8, 53.6, 51.1, 47.6, 35.9, 23.7, 10.2. MS m/z: 548 (MH^+ − 2HCl); positive HR-FAB-MS m/z 548.1990 (MH^+ − 2HCl) (calcd for $C_{30}H_{31}Cl_2N_5O$: 548.1984).

Biological Methods. *Measurement of Binding Affinity for Serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors.* Receptor binding affinities of the compounds for serotonin receptors were measured by the method described in the literature.[25]

For serotonin 5-HT$_{2A}$ binding, an aliquot of human recombinant serotonin 5-HT$_{2A}$ receptor (PerkinElmer Life and Analytical Sciences, USA) expressed in CHO-K1 cells (5 μg/well) and 1 nM [^{3}H]Ketanserin (Perkin-Elmer) were used in the presence of mianserin (20 μM) as nonspecific. The reaction mixture was incubated for 60 min at 27 °C using 50 mM Tris-HCl (pH 7.4) buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid and harvested through filtermate A glass fiber filter (Wallac, Finland) presoaked in 0.5% polyethyleneimine (PEI) by MicroBeta Filtermate-96 harvester (PerkinElmer) to terminate the reaction and then washed with ice-cold 50 mM Tris-HCl buffer solution (pH 7.4). The filter was then covered with MeltiLex, sealed in a sample bag, and dried in an oven. The radioactivity retained in the filter was finally counted using MicroBeta Plus (Wallac).

The binding affinity (IC_{50}) of a compound for the receptor was calculated by computerized nonlinear regression analysis (GraphPad Prism Program, San Diego, USA) using 7−8 varied concentrations of the compound run in duplicate tubes.

For serotonin 5-HT$_{2C}$ binding, frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT$_{2C}$ receptor (PerkinElmer, 4 μg/well), [^{3}H]Mesulergine (Amersham, 1.3 nM), and test compounds were added into 50 mM Tris-HCl (pH 7.4) buffer containing 0.1% ascorbic acid and 4 mM $CaCl_2$. Nonspecific binding was determined using 100 μM mianserin. The incubations were performed for 60 min at 27 °C, and these were terminated by rapid filtration through Filtermate A glass fiber filter presoaked in 0.5% PEI.

Measurement of Binding Affinity for Serotonin Transporter. For serotonin transporter binding assays, a reaction mixture with a final volume of 0.25 mL was prepared by mixing a test compound, human serotonin transporter membrane expressed in HEK-293 cells (Perkin-Elmer, 5 μg/well), [^{3}H]Imipramine (Perkin-Elmer, 2 nM), and 50 mM Tris-HCl (pH 7.4) buffer containing 120 mM NaCl and 5 mM KCl. The reaction mixture was incubated for 30 min at 27 °C and harvested through Filtermate A glass fiber filter presoaked in 0.5% PEI with ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.9% NaCl.

Measurement of Antidepressants Activity in Forced Swimming Test. To evaluate the antidepressants activity of the compounds, the inhibitory effects on immobility in forced swimming test in mice were measured according to the methods described by Porsolt et al.[29]

Each mouse was placed in a 25 cm glass cylinder (10 cm diameter) containing 15 cm of water maintained at 22 ± 1 °C and was forced to swim for 10 min. Twenty-four hours later, the mouse was replaced into

the cylinder and the total duration of immobility was recorded during the last 5 min of the 6 min testing period. Mice are judged immobile when they float in an upright position and make only small movements to keep their head above water. Test drugs were suspended in 3% Tween 80 solution and administered orally (po) 60 min before the testing.

Measurement of Spontaneous Locomotion Activity. Locomotor activity was assessed with an animal activity monitoring apparatus (EthoVision, Nolduls, Netherlands.). Fifty minutes after the administration, mice were placed individually in 32 cm × 45 cm × 32 cm plastic cages, to which they had not been previously exposed, under dim light and sound-attenuated conditions. After an initial 10 min of familiarization period, locomotor activity was monitored for 30 min. The results of the locomotor activity tests were expressed as mean ± SEM.

Pharmacokinetic Study. Male Sprague−Dawley rats (260−280 g) were purchased from Orient Bio Inc. (Gyeonggi-Do, Korea). Two days before dosing, the femoral artery and vein (intravenous (iv) only) were cannulated using polyethylene tube SP28 (Natsume, Tokyo, Japan). For intravenous administration, prepared dosing solution was injected via the femoral vein. The rats were fasted overnight before drug administration and until 6 h after dosing. For the po experiment, rats (three in each group) were given a single dose of 5 mg/kg, and heparinized samples of blood (0.3 mL) were collected at 5, 15, 30, 60, 90, 120, 240, 360, 480, 600 min, and 24 h postdose. For the iv experiment, rats (three in each group) were given a single 1 mg/kg dose, and blood samples were collected at 5, 10, 20, 40, 60, 90, 120, 240, 360, and 480 min postdose. Plasma was harvested after centrifugation and stored frozen at −20 °C until analyzed. The concentrations of compounds in plasma were determined by LC/MS/MS (API3200). The results are shown as the maximum plasma concentration (C_{max}), the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$), and the area under the plasma concentration−time curve from zero to time infinity ($AUC_{0-\infty}$).

Measurement of hERG Inhibition. HEK293 cells stably expressing the *h*ERG potassium channel are a *Homo sapien* embryonic kidney epithelial cell line transformed with the adenovirus 5 DNA. The parental HEK293 cell line was devoid of the IKr currents before transfection of the *h*ERG cDNAs. The HEK293 cells were cotransfected with the *h*ERG cDNA and G418-resistant gene incorporated into a modified pcDNA3 plasmid. The stable transfectants were maintained under constant selection pressure incorporated into the culture media. The culture media was Minimum Essential Medium (MEM, including Earle's Salts and L-glutamine) supplemented with approximately 10% fetal bovine serum, 100 U/mL of penicillin-g, 100 μg/mL streptomycin, 0.1 mM nonessential amino acids (NEAA) solution, 1 mM sodium pyruvate, and 400 μg/mL G418.

Measurement of CYP. To evaluate the CYP of the compounds, we used P450-Glo screening system. The systems include a membrane preparation containing recombinant human cytochrome P450 (CYP) enzyme, negative control membranes, a luminogenic substrate appropriate for the CYP enzyme, NADPH regeneration system, reaction buffer, Luciferin detection reagent, and Luciferin-Free water. The membranes are prepared from baculovirus-infected insect cells and contain human CYP enzyme and P450 reductase (and cytochrome b5 for CYP2C9, 2C19, and 3A4). The negative control membranes are devoid of CYP activity. The assays are ideal for testing the effects of drugs and new chemical entities on CYP enzyme activities.

Measurement of Cytotoxicity. HT22 cells (mouse hippocampal neuron, Salk Institute and KRIBB) were plated in a 96-well plates 3×10^3 cells/well for 18 h before treatment. 3-HM·HBr were treated and incubated for 48 h in growth media (DMEM with 10% FBS and 1% penicillin streptomycin). MTT (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) was treated for 4 h and detected with a plate reader at a wavelength = 450 nm.

Measurement of AMES. This test performed essentially as described by Ames et al. (1973, 1975). Media and positive control chemicals were obtained from commercial sources and were the purest grades available. Strain TA 100 was chosen as the representative tester strain because of its high spontaneous reversion rate. Spontaneous revertant numbers were counted and plotted against the dose of the test chemical to produce a survival curve for the *his*$^+$ genotype.

The mutagenicity assay was performed by mixing one of the tester strains which was cultured overnight with the test substance in the presence and in the absence of S9 mixture condition, sodium phosphate buffer added instead of S9 mixture both in negative and positive control in a test tube. Then, incubating the mixture in water bath for 30 min at 37 °C and after incubation, the mixture was mixed with top agar containing a minimal amount of histidine and then poured onto the surface of a γ-ray sterile Petri dish (Falcon, USA) containing 25 mL of solidified bottom agar. The finished plates were incubated for 48 h at 37 °C, and revertant colonies were counted later. Negative control plates containing no added test chemical, but positive control plates containing appropriate amounts of chemicals known to be active were included with each tester strain. Sodium azide (SA) and 2-aminoanthracene (2-AA) were used as positive control substances. Compounds were tested at seven concentration points in duplicate at a range of concentrations up to 2500 μg/plate. A response was considered to be positive in our criteria if there was a dose-dependent increase in revertants per plate, resulting in at least a doubling of the background reversion rate for strains TA 100.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for molecules not described in main paper text and ^{1}H NMR, ^{13}C NMR, and HPLC spectrum data for compounds **57**, **68**, **70**, **71**, **100**, **131**, **143**, and **145**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +82-31-260-9892. Fax: +82-31-260-9020. E-mail: jinhwalee@greencross.com.

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■ ABBREVIATIONS USED

SARI, serotonin antagonist and reuptake inhibitor; 5-HT, serotonin or 5-Hydroxytryptamine; $5\text{-HT}_{2A/2C}$, serotonin receptor subtype 2A/2C; CNS, central nervous system; FST, forced swimming test; SSRIs, selective serotonin reuptake inhibitors; SERT, serotonin transporter; DMF, *N*,*N*′-dimethylformamide; EDCI, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; NMM, *N*-methylmorpholine; HOBt, 1-hydroxybenzotriazole; CHO-K1 cells, Chinese hamster ovary-K1 cells; DMSO, dimethyl sulfoxide; SLA, spontaneous locomotor activity; SD rat, Sprague−Dawley rat; PK, pharmacokinetics; AUC, area under the plasma concentration time curve; IC_{50}, half-maximal inhibitory concentration; BA, bioavailability

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