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The Design and Synthesis of 1,4-Substituted Piperazine Derivatives as Triple Reuptake Inhibitors

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Novel 1,4-substituted piperazine derivatives **5**, Series A and B were designed by fragment analysis and molecular modification of 4 selected piperazine-containing compounds which possess antidepressant activity. We synthesized new 39 analogues of Series A and 10 compounds of Series B, respectively. The antidepressant screening against DA, NE, and serotonin neurotransmitter uptake inhibition was carried out using the Neurotransmitter Transporter Uptake Assay Kit. The compounds in Series B showed relatively higher reuptake inhibitory activity for SERT, NET, and DAT than those in Series A. The length of spacer between the central piperazine core and the terminal phenyl ring substituted at the piperazine ring in Series B seems to exert an important role in the activity.

Key Words : Major depressive disorder, Antidepressants, Triple reuptake inhibitor, Piperazine, CNS drug

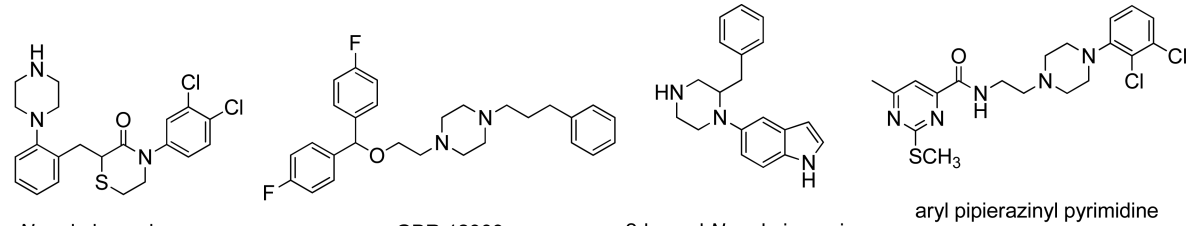
Introduction

Major depressive disorder (MDD) is a common and serious illness with the potential to become the leading cause of disability worldwide. Pathophysiologically, the cause of depression is commonly associated with a deficiency of monoamine neurotransmitters (serotonin (5-HT), norepinephrine (NE) and dopamine (DA)) in the brain, and a number of antidepressants aim to increase the levels of these neurotransmitters in the synapses. Among various monoaminergic strategies for maintaining the concentration of neurotransmitters, the blocking of pre-synaptic 5-HT, NE and DA transporters (SERT, NET and DAT) to reuptake inhibition of neurotransmitters into nerve terminals has been an important strategy in modern antidepressant therapy.[1] Although many kinds of reuptake inhibitor (RI) such as selective serotonin RIs (SSRIs) and serotonin norepinephrine RIs (SNRIs) are commercially available for the treatment of major depression, they may take several weeks of treatment to affect any improvement in symptoms and some inhibitors reveal a few side effects such as insomnia and sexual dysfunction.[2] One strategy to improve efficacy and/or reduce the delay in the onset of their action is the addition of a DA component to SSRIs or SNRIs. This is the concept of triple reuptake inhibition which blocks synaptic reuptake to all of 5-HT, NE, and DA. Recent study results supported the effect of DA in depression. For example, D_3-preferring DA receptor agonists such as pramipexole showed therapeutic efficacy in major depression.[1(a),3] DARI such as bupropion enhanced the antidepressant actions of SSRIs and SNRIs in humans.[4] A few classes of triple RI (TRI), including DOV 216,303[5] and GSK 372,475,[6] showed positive Phase II clinical effect. However, there is as yet no TRI available in the market, and combination and multiple dugs therapy to inhibit the reuptake of 5-HT, NA and DA may raise some problems of pharmacokinetics. Therefore, TRIs as a single molecule are expected to be the next generation of antidepressant and remain desirable.

Among the broad range of templates, heterocyclic scaffolds represent the most promising molecules as leading structures for the discovery of novel synthetic drugs. In particular, the piperazine core is found in numerous drugs and clinical and preclinical candidates that address a broad spectrum of targets.[7] Recently, a number of compounds, including piperazine, were reported as a new class of small molecules with antidepressant activity in depressive disorders. For instance, aryl piperazine and piperidine ethers showed NE reuptake inhibitory and 5-HT_{1A} partial agonistic activities, and *N*-(1,2-diphenylethyl)piperazines exhibited dual 5-HT and NE reuptake inhibitory activities.[8] And 2-substituted *N*-aryl piperazines have received attention as potent and novel TRIs.[9] The selective DARI, GBR 12909, including a piperazine core, has already been used in the clinical treatment of depression[10] and vilazodone, which combines the effects of dual 5-HT_{1A} receptor agonist and 5-HT reuptake inhibitor, is currently undergoing evaluation in Phase III clinical studies of MDD.[11]

Results and Discussions

Design. We first selected four piperazine-containing compounds and analyzed their structures. Our strategy of exploration of target compounds for the development of a novel triple reuptake inhibitory antidepressant included a design of the structures through fragment analysis of compounds **1-4** which possess antidepressant activity (see Figure 1).[8-10,12] The characteristic common points of the structures were: a) they are basic due to 2° or 3° amino moiety, b) two or three aromatic groups are present, c) they have one or more hydrogen bonding acceptors or donors, d)



Compounds	Targets (action)	Constants	Antidepressant activity IC_{50} (nM), K_i (nM) or pK_i						ref.
			SERT	NET	DAT	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	
1	NET and 5-HT_{1A} partial agonists	K_i	-	5	-	9	-	-	8
2	DARI	K_i	-	-	12	-	-	-	10
3	TRI	pK_i	7.9	8.1	8.2	-	-	-	9
4	SERT, 5-HT_{2A} and 5-HT_{2C}	IC_{50}	881	-	-	-	55	81	12

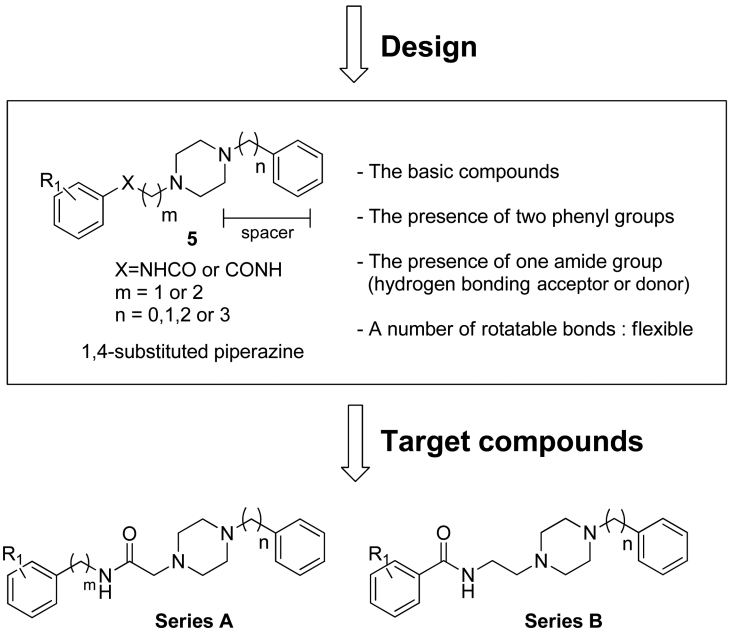
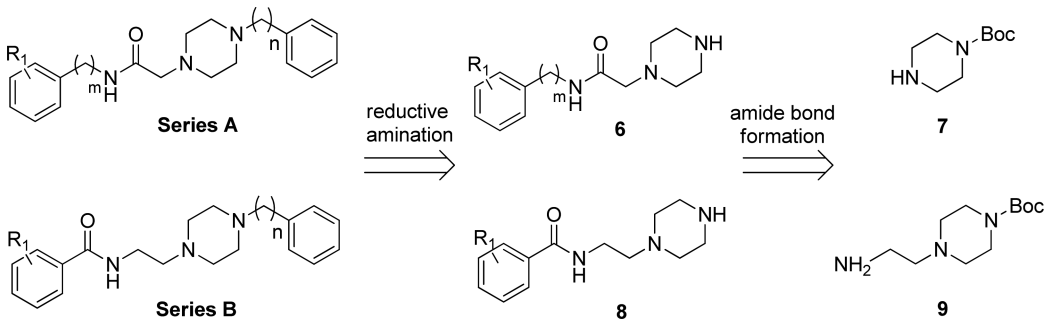
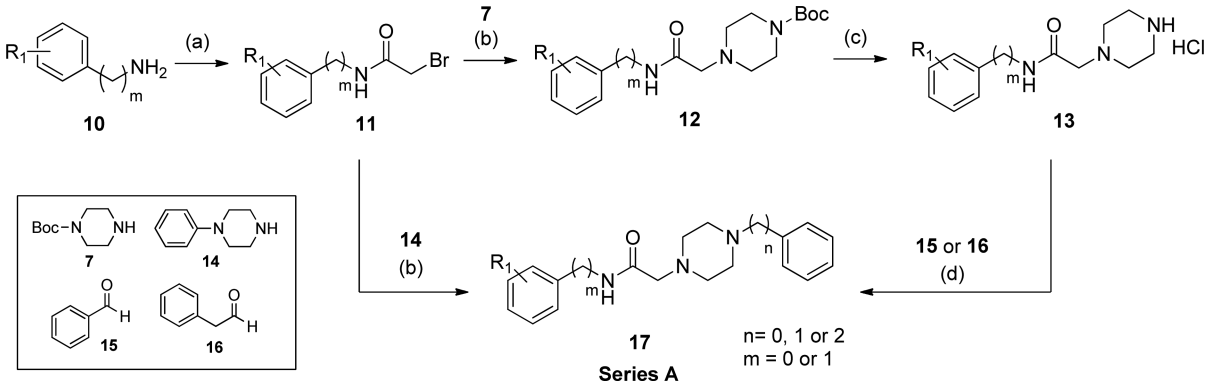


Figure 1. Design of the target compounds of novel 1,4-substituted piperazines, Series A and B, based on the piperazine derivatives **1**-**4** possessing antidepressant activity.



Scheme 1. Retrosynthesis of 1,4-substituted piperazine Series A and B.



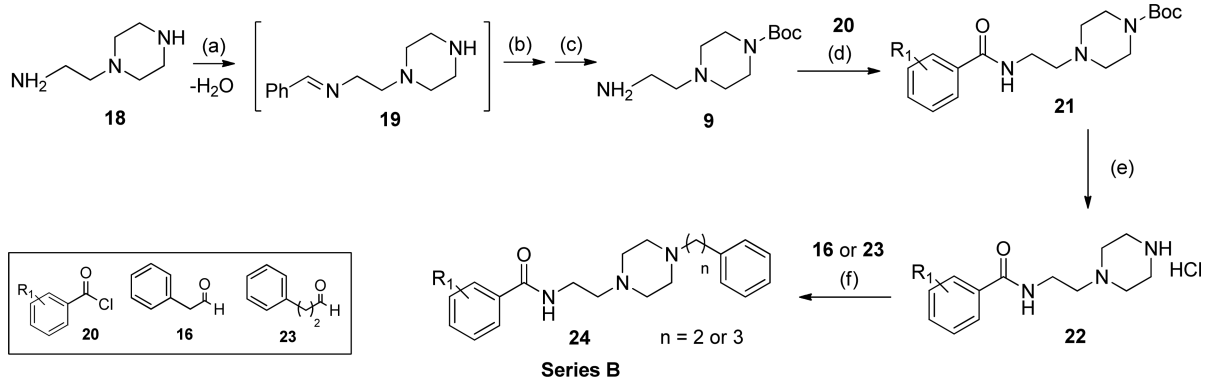
Scheme 2. Reagents and conditions: (a) bromoacetyl bromide, Et_3N, CH_2Cl_2, 0 °C to rt; (b) K_2CO_3, CH_3CN, reflux; (c) 1 N HCl, MeOH, 60 °C; and (d) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt.

the molecules are flexible; and e) they have a narrow clogP value (3.50-5.30).[6] Additionally, it is well known that the structural properties for discovery of CNS drugs are fewer hydrogen bond donors (< 2), hydrogen bond acceptors (< 6), optimal Log P (1.5-2.7), lower polar surface area (60-70 Å^2), fewer rotatable bonds (< 5) *et al.*[13]

Keeping the structures and the structural properties of CNS drugs in mind we have designed novel 1,4-substituted piperazine **5**, Series A and B. This series was assumed to be readily prepared by reductive amination of aldehyde with the secondary amines **6** and **8**, which were prepared from commercially available piperazines **7** and **9** (Scheme 1).

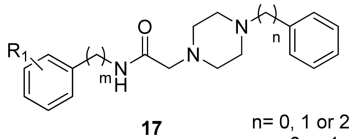
Syntheses. A synthetic route to 1,4-substituted piperazine Series A is shown in Scheme 2. The reaction of bromoacetyl bromide with either anilines or benzylamines **10** in the presence of triethylamine in methylene chloride at 0 °C afforded the corresponding α-bromo acetamide **11** in high yield. The reaction of the α-bromo acetamide **11** with either piperazine protected by *tert*-butylcarbamate (Boc) group **7** or *N*-phenyl piperazine **14** in the presence of potassium carbonate in boiling acetonitrile gave the corresponding piperazine **12** or **17** (n=0). Deprotection of Boc in **12** by the treatment of 1 N HCl in boiling methanol followed by the treatment of aldehyde **15** or **16** in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) gave the corresponding piperazine **17** (n=1 or 2). The desired products **17** could be isolated by flash chromatography from the reaction mixture, with isolated yields ranging from 52 to 96%. The structures were confirmed as a single compound by TLC and ^{1}H NMR spectroscopy. We synthesized 39 analogues of Series A in this manner.

A synthetic route to 1,4-substituted piperazine Series B is illustrated in Scheme 3. Protection of the amino group of commercially available 2-aminoethylpiperazine **18** was attained by the treatment of **18** with benzaldehyde in refluxing toluene with Dean-Stark water trap to give an intermediate benzylidene derivative **19**. Without isolation of **19**, the reaction mixture was reacted with di-*tert*-butyldicarbonate (Boc_2O) followed by deprotection of benzylidene moiety through the treatment of aqueous potassium bisulfate ($KHSO_4$) solution to afford Boc-protected 2-aminoethylpiperazine **9** (yield 97%).[14] The reaction of **9** and acyl chloride **20** in the presence of triethylamine in methylene chloride at room temperature gave the corresponding benzamidoethylpiperazine **21**. Deprotection of Boc group by the usual manner (treatment of 1 N HCl in boiling methanol) resulted in **22**. The desired products **24** could be obtained by the reductive amination of **22** either with aldehyde **16** or **23** in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) at room temperature. The reaction mixture was purified by flash chromatography to obtain **24**, with isolated yields ranging from 50 to 85% and the structures were confirmed by ^{1}H NMR spectroscopy. We synthesized 10 compounds of Series B in this manner.



Scheme 3. Reagents and conditions: (a) benzaldehyde, toluene, reflux; (b) $(Boc)_2O$, toluene, reflux; (c) $KHSO_4$, H_2O, rt; (d) Et_3N, M.C, rt; (e) 1 N HCl, MeOH, 60 °C; and (f) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt.

Table 1. Percentage inhibition of activities of 1,4-substituted piperazine derivatives **17**, series A at HEK-hSERT, HEK-hNET and HEK-hDAT

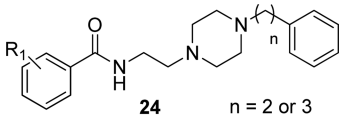


17 n= 0, 1 or 2
m = 0 or 1
series A

Entry	Compound	R_1	m	n	clogP value	(% re-uptake inhibition, 10 μM)		
						SERT	NET	DAT
1	**17a**	H	0	0	3.28	3	24	16
2	**17b**	4-CH_2CH_3	0	0	4.31	4	5	11
3	**17c**	4-F	0	0	3.68	8	23	27
4	**17d**	4-OCH_3	0	0	3.36	6	23	24
5	**17e**	4-OC_6H_5	0	0	5.38	5	8	12
6	**17f**	3,5-di Cl	0	0	5.06	8	8	6
7	**17g**	3,5-diCF_3	0	0	5.69	44	10	11
8	**17h**	4-CH_3	0	0	3.78	7	19	27
9	**17i**	4-Cl	0	0	4.25	14	26	60
10	**17j**	4-F	1	0	3.45	6	22	18
11	**17k**	4-OCH_3	1	0	3.23	12	17	19
12	**17l**	H	1	0	3.31	7	12	11
13	**17m**	4-CH_3	1	0	3.81	3	11	12
14	**17n**	3,4-di Cl	1	0	4.62	10	18	22
15	**17o**	4-Cl	1	0	4.02	3	10	18
16	**17p**	H	0	1	3.58	3	13	15
17	**17q**	4-CH_2CH_3	0	1	4.60	17	23	29
18	**17r**	4-F	0	1	3.98	5	19	22
19	**17s**	4-OCH_3	0	1	3.65	12	22	19
20	**17t**	4-OC_6H_5	0	1	5.67	22	50	67
21	**17u**	3,5-di Cl	0	1	5.35	33	88	60
22	**17v**	4-Cl	0	1	4.55	15	33	49
23	**17w**	3,5-diCF_3	0	1	5.95	10	66	54
24	**17x**	4-CH_3	0	1	4.07	11	18	24
25	**17y**	H	1	1	3.60	5	13	24
26	**17z**	4-F	1	1	3.74	5	14	26
27	**17aa**	4-CH_3	1	1	4.10	8	12	62
28	**17ab**	4-OCH_3	1	1	3.52	8	15	58
29	**17ac**	4-Cl	1	1	4.31	15	20	62
30	**17ad**	3,4-di Cl	1	1	4.91	35	44	74
31	**17ae**	H	0	2	2.46	13	27	47
32	**17af**	4-CH_2CH_3	0	2	3.49	34	55	71
33	**17ag**	4-F	0	2	2.86	13	34	60
34	**17ah**	4-OCH_3	0	2	2.54	9	33	57
35	**17ai**	4-OC_6H_5	0	2	4.56	55	90	82
36	**17aj**	3,5-di Cl	0	2	4.23	45	80	82
37	**17ak**	4-Cl	0	2	3.43	24	38	79
38	**17al**	3,5-diCF_3	0	2	4.83	14	82	76
39	**17am**	4-CH_3	0	2	2.96	12	29	52

Biological Screening. DA, NE, and serotonin neurotransmitter uptake activities were measured using the Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices, Sunnyvale, CA, USA) with the FDSS6000 96-well fluorescence plate reader, a high throughput screening device (Hamamatsu Photonics, Hamamatsu, Japan).[15] In this study, the human embryonic kidney 293 (HEK293) cells stably transfected with human DA transporter (HEK-hDAT), human NE transporter (HEK-hNET), or human serotonin transporter (HEK-hSERT) were used for the assay. Primary screening results of the synthesized compounds (Series A and B) at a concentration of 10 μM are summarized in Tables 1 and 2 respectively.

Table 2. Percentage inhibition of activities of 1,4-substituted piperazine derivatives **24**, series B at HEK-hSERT, HEK-hNET and HEK-hDAT



24 n = 2 or 3
series B

Entry	Compound	R_1	n	clogP value	(% re-uptake inhibition, 10 μM)		
					SERT	NET	DAT
1	**24a**	4-CH_3	2	2.92	40	48	59
2	**24b**	4-CH_2CH_3	2	3.45	57	65	58
3	**24c**	4-OCH_2CH_3	2	3.15	60	65	58
4	**24d**	4-F	2	2.76	27	45	44
5	**24e**	3,5-di Cl	2	4.12	49	87	70
6	**24f**	4-CH_3	3	3.30	64	62	53
7	**24g**	4-CH_2CH_3	3	3.83	77	74	58
8	**24h**	4-OCH_2CH_3	3	3.53	69	71	52
9	**24i**	4-F	3	3.15	56	50	63
10	**24j**	3,5-di Cl	3	4.50	91	91	88

Comparing the activities of the compounds recorded in Table 1 with those in Table 2, the compounds in Series B showed relatively higher reuptake inhibitory activity for SERT, NET, and DAT than those in Series A. Most of the compounds of Series A showed moderate reuptake inhibitory activity for DAT but little activity for SERT (see Table 1). In contrast, **24j** (entry 10 in Table 2) showed the most potent reuptake inhibitory activities for SERT, NET, and DAT. Interestingly, the length of spacer (n=2 or 3 in Table 2) between the central piperazine core and the terminal phenyl ring substituted at the piperazine ring seems to exert an important role in the activity. For example, when the spacer was three methylene units (n=3, see entries 6-10 in Table 2) the reuptake activities for SERT of the compounds were higher than those of the corresponding compounds when the spacer was two methylene units (n=2, see entries 1-5 in Table 2)(compare **24a** with **24f**, **24b** with **24g**, **24c** with **24h**, **24d** with **24i**, **24e** with **24j**). Further studies on expanding the library of the 1,4-substituted piperazine series A and B and on elucidating the structure activity relationship of these series are in progress.

Experimental

Preparation of the 1,4-Substituted Piperazine Series A.

Synthesis of α-Bromoacetamide 11 (General Procedure): Bromoacetyl bromide (14 mol) was added dropwise to a solution of aniline or benzylamine **10** (13 mol) and triethylamine (15 mol) dissolved in methylene chloride (100 mL) at 0 °C in an ice-bath. The cooling bath was then removed and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$ solution, and water and then dried over anhydrous $MgSO_4$. The solvent was removed by evaporation to afford the corresponding compound **11** (solid, 61-91% yields).

Synthesis of 2-Benzamino-4-Boc Piperazine 12 (General Procedure): To a solution of **11** (6.7 mol) in acetonitrile (80 mL) at room temperature was added K_2CO_3 (17 mol) and *N*-Boc piperazine **7** (6.7 mol). The mixture was refluxed for 6 h and the reaction mixture was then cooled to room temperature. The solvent was removed by evaporation and the residue was dissolved in methylene chloride, washed with water, and then dried over $MgSO_4$. The solvent was removed in vacuo, and the resulting solid was then washed with cold isopropyl ether. Drying in air gave **12** (solid, 74-93% yields).

Synthesis of 2-Benzamino Piperazine Hydrochloride 13 (General Procedure): To a solution of **12** (5.0 mol) dissolved in methanol (20 mL) was added 1 N HCl (10 mL). The reaction mixture was stirred at 60 °C for 6 h and then cooled to room temperature. The solvent was removed by evaporation and the resulting solid was washed with methylene chloride, and then dried in air to afford the corresponding compound **13** (solid, 80-98% yields).

Synthesis of 2-Benzamino-4-substituted Piperazine 17 (General Procedure):

(a) Synthesis of compound **17** (n=0) – This compound was synthesized starting from **11** and *N*-phenylpiperazine **14** by a similar procedure to that used for the synthesis of **12** described above (52-96% yields).

(b) Compound **17** (n=1 or 2) – To a solution of 13 (1.0 mmol) in methylene chloride (6 mL) at room temperature under N_2 atmosphere was added sequentially aldehyde **15** or **16** (2.0 mmol), acetic acid (1.0 mmol) and sodium triacetoxyborohydride (3.0 mmol). The reaction mixture was stirred at the same temperature for 2 h. To the resulting reaction mixture was added 1 N aqueous NaOH solution while stirring. This mixture was stirred at room temperature for 0.5 h and then extracted with methylene chloride. The organic extract was dried over anhydrous $MgSO_4$. The solvent was removed by evaporation and the crude product was then purified by flash chromatography on silica gel to obtain **17** (n=1 or 2) (53-87% yields)

Preparation of the 1,4-Substituted Piperazine Series B.

Synthesis of 1-(2-Aminoethyl)-4-Boc Piperazine 9: To a solution of 2-aminoethylpiperazine **18** (10.0 mL, 77.4 mmol) in toluene (50.0 mL) was added benzaldehyde (7.82 mL, 77.4 mmol). The mixture was refluxed for 8 h attached with a Dean-Stark water trap. To the reaction mixture cooled to 0 °C in an ice-bath was added di-*tert*-butyldicarbonate (19.5 g, 85.1 mmol) at the same temperature while stirring. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured into an aqueous 1 M $KHSO_4$ solution (154.8 mmol) and stirred at room temperature for 3 h. The aqueous layer was washed with methylene chloride and the water was evaporated. The produced residue was triturated with a mixture of methanol and methylene chloride. The filtrate organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation to afford the corresponding compound **9**.

Yield 97%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.33 (m, 4H, piperazine H), 3.64 (t, J = 6.0 Hz, 2H, NH$\underline{CH_2}$CH$_2$), 2.35-2.28 (m, 6H, piperazine H, NHCH$_2\underline{CH_2}$), 1.63 (s, 2H, NH_2), 1.36 (s, 9H, *tert*-butyl H).

Synthesis of 2-Benzamidoethyl-4-Boc Piperazine 21 (General Procedure): Acyl chloride **20** (1.3 mmol) was added to a solution of **9** (1.3 mmol) and triethylamine (1.4 mmol) dissolved in methylene chloride (20 mL) at 0 °C in an ice-bath. The cooling bath was removed and the reaction mixture was then stirred at room temperature for 3 h. The reaction mixture was washed sequentially with 0.1 N HCl, saturated aqueous $NaHCO_3$ solution, and water and then dried over anhydrous $MgSO_4$. The solvent was removed by evaporation to afford the corresponding compound **21** (solid, 78-97% yields).

Synthesis of 2-Benzamidoethyl Piperazine Hydrochloride 22 (General Procedure): To a solution of **21** (0.6 mmol) dissolved in methanol (5.0 mL) was added 1 N HCl (2.0 mL). The reaction mixture was stirred at 60 °C for 6 h. The reaction mixture was cooled to room temperature and the solvent was removed by evaporation. The produced precipitates were filtered, washed with methylene chloride and then dried in air to afford the corresponding compound **22** (solid, 92-99% yields).

Synthesis of 1-(2-Benzamidoethyl)piperazine Derivatives 24 (General Procedure): To a solution of **22** (0.9 mmol) in methylene chloride (5.0 mL) under N_2 atmosphere was added sequentially aldehyde **16** (or **23**) (1.8 mmol), acetic acid (0.9 mmol) and sodium triacetoxyborohydride (2.7 mmol). The reaction mixture was stirred at room temperature under N_2 atmosphere for 2 h. To the resulting reaction mixture was added 1 N NaOH aqueous solution and the mixture was extracted with methylene chloride. The organic extract was dried over anhydrous $MgSO_4$. The solvent was removed by evaporation and the crude product was purified by flash chromatography on silica gel to obtain **24** (50-85% yields).

Biological Screening Method.

Cell Culture: HEK293 cells stably expressing human DA transporter (HEK-hDAT), human NE transporter (HEK-hNET) and human serotonin transporter (HEK-hSERT) were kindly provided by Professor Bryan Roth (University of North Carolina at Chapel Hill). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Welgene, South Korea) supplemented with 10%(v/v) fetal bovine serum (FBS, Welgene, South Korea), penicillin (100 U/mL), and streptomycin (100 μg/mL) in the presence of Geneticin G418 (350 μg/mL, 200 μg/mL, and 500 μg/mL for HEK-hDAT, HEK-hNET, and HEK-hSERT, respectively) in a

humidified 5% CO_2 incubator at 37 °C. The cells were subcultured every 3-4 days.

Neurotransmitter Reuptake Assay and Data Analysis: Cells were seeded in 50 μg/mL, poly-L-lysine coated, 96-well, black-walled, clear-bottomed plates (Corning, NY, USA) at a density of 5×10^4 cells/well and cultured overnight. DA, NE, and serotonin neurotransmitter uptake activities were measured using the Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices, Sunnyvale, CA, USA) with the FDSS6000 96-well fluorescence plate reader (Hamamatsu Photonics, Hamamatsu, Japan). The prepared cells were washed three times with the HEPES-buffered solution (150 mM NaCl, 5 mM KCl, 10 mM Glucose, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and pH 7.4) using an ELx405 Select plate washer (BioTek Instruments, Winooski, VT, USA). The assay loading dye solution prepared according to the supplier's instruction was added to the cells and the fluorescent intensity was measured for 30 min. The cells were excited at 440 nm light and the emission was collected every 10 seconds at 520 nm light. Cells treated with test compounds were incubated for 15 min in a humidified atmosphere of 5% CO_2 at 37 °C and the assay loading dye solution was then added to the cells. The fluorescent intensity was measured for 30 min using the FDSS6000 system. GBR12909, Nisoxetine, and Fluoxetine (Tocris Bioscience, Ellisville, MO, USA) were used as a selective inhibitor for hDAT, hNET, and hSERT, respectively.

Supplementary Material. The yields, melting point, and ^{1}H NMR data for all the compounds can be found in the online version of the supplementary materials associated with this article at http://journal.kcsnet.or.kr.

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