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Syntheses of Novel Diphenyl Piperazine Derivatives and Their Activities as Inhibitors of Dopamine Uptake in the Central Nervous System

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Abstract—A new series of diphenyl piperazine derivatives containing the phenyl substituted aminopropanol moiety, which were modified at sites between the diphenyl and piperazine moieties, was prepared and evaluated for dopamine transporter binding affinity with [^{3}H]GBR12935 in rat striatal membranes. These synthesized compounds showed apparent dopamine transporter binding affinities ($IC_{50} < 30$ nM) and some of them were approximately equivalent in activity to GBR12909 known as a potent dopamine uptake inhibitor, showing the activities with IC_{50} values of nanomolar range. Among them, 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine **2** was evaluated for extracellular dopamine levels in rat striatum using in vivo brain microdialysis. The intraperitoneal administration of **2** (0.01, 0.03, or 0.1 mmol/kg) induced dose-dependent increases of dopamine levels in rat striatal dialysates. The maximum increases in dopamine levels induced by **2** were greater than those by GBR12909. The pharmacological data of these novel diphenyl piperazine derivatives show that the compounds have potent dopamine uptake inhibitory activities in the central nervous system.

Introduction

The neurotransmission by biologically active molecules such as hormones and the catechol amines: dopamine (DA), noradrenaline, serotonin plays an important role in many biological processes in the central nervous system (CNS). The dopamine transporter (DAT) is located on DA nerve terminals and completes dopaminergic neurotransmission by the reuptake of DA into presynaptic neurons.[1] Dopaminergic neurotransmission is closely associated with the CNS disorders such as Parkinson's disease,[2] depression,[3] and cocaine abuse,[4,5] so the DAT is one of targets for research into new treatments of these CNS disorders. Parkinson's disease, characterized by motor dysfunction such as tremor, akinesia, rigidity, and postual instability, is one of the most well-known neurodegeneration disorders and this disease has been thought to be caused by the depletion of DA due to neurodegeneration in nigrostriatal dopaminergic neurons.[6] Levo-DOPA (L-DOPA), a frequently used treatment for Parkinson's disease, has serious disadvantages such as wearning-off, dyskinesia, motor dysfunction, and psychosis[7] which has led to the investigation of potentially better types of medicines as DA receptor agonists,[8–10] including monoamine oxidase B inhibitors[11–13] and catechol-*O*-methyl-transferase inhibitors[14] amongst others. DA uptake inhibitors, which work through the inhibition of the DAT, have been reported to increase the reduced DA levels in nigrostriatum and improve the symptoms in the animal

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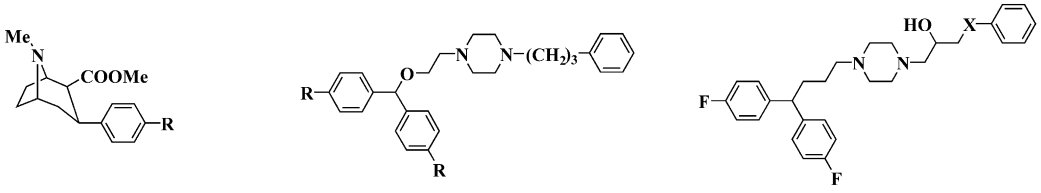
models of Parkinson's disease,[15,16] which suggests that may also provide a benefit in Parkinson's disease, possibly at early onset or at moderately advanced stages as sole therapies or concomitantly with L-DOPA. Furthermore, recent studies on the mechanism of action with cocaine in the CNS[4,17] have indicated that cocaine abuse is closely related to the DAT through cocaine binding, which has focused investigations into new treatments for cocaine abuse on this site of action. Thus, in theory DA uptake inhibitors may also provide a new approach to the improvement of the symptoms of cocaine abuse.

In last two decades, great interest and effort have been focused on the structure–activity relationships (SARs) of DA uptake inhibitors and several different classes of them have been identified.[18–24] Of these compounds, 3β-aryltropane-2β-carboxylic acid ester analogues have led to the development of potent and selective DA uptake inhibitors such as Win35,428[19] and RTI-55[23,24] shown in Figure 1, and they have helped to characterize biological and pharmacological profile of the DAT.[25] In 1980's, Van der Zee et al.[20] reported the high affinity for the DAT exhibited by diphenyl piperazine derivatives, in which the tropane moiety of benztropine was replaced by substituted piperazine. They had affinities for the DAT in the low-nanomolar range and two representative DA uptake inhibitors, GBR12909 and GBR12935 which are shown in Figure 1, were developed. Furthermore, comprehensive SAR study of the compounds of GBR series in which the piperazine,[26–28] diphenylalkyl[29–31] and phenylalkyl[32–35] moiety were modified, resulted in the discovery of notably potent and selective DA uptake inhibitors, many of which have been in pre-clinical development and some in clinical trials for the treatment of Parkinson's disease, cocaine addiction and so forth.

In the course of our study to find a calcium antagonist with an antioxidative activity,[36] we synthesized novel diphenylalkyl piperazine derivatives including compound **1** and **2** shown in Figure 1. In the preliminary biological evaluations of these compounds, to our surprise, we found that 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine **2** produced a notable stimulation of locomotor activity without a marked decrease of blood pressure. We took notice of this remarkable phenomenon of compound **2**, because DA uptake inhibitors had been reported to stimulate locomotor activities.[37] Provided that the compound **2** and its analogues show DAT binding affinities and DA uptake inhibitory activities, these compounds could serve as new structural molecules as DA uptake inhibitors different from GBR analogues having the diphenylmethoxyalkyl and phenylalkyl moieties at the 1- and 4-positions of the piperazine or piperizine ring and their bioisosteric moieties. Therefore, we synthesized its analogues and evaluated them for DAT binding affinity with [^{3}H]GBR12935 in rat striatal membranes. Furthermore, as an in vivo evaluation, we also examined the effect of compound **2** on DA levels in rat striatal dialysates.

Chemistry

The diphenylalkyl piperazine derivatives **2** and **7a–d**, which had the different length of methylene connectives between the diphenylmethyl and piperazine moieties, were synthesized as shown in Scheme 1. Alkylation of ethyl *N*-phenylcarbamate with epibromohydrin in *N,N*-dimethylformamide (DMF) using sodium hydride (NaH) gave an epoxide **3**. Epoxide **3** was allowed to react with the diphenylalkyl piperazines **4a–e**[39–42] to obtain compounds **5a–e** and the ethoxycarbonyl groups of **5a–e** were subsequently deprotected under basic conditions to provide the desirable diphenylalkyl piperazine derivatives **2** and **7a–d**. In an attempt to deprotect the ethoxycarbonyl group, the treatment of **5a** with aqueous sodium hydroxide in methanol at room temperature gave a mixture of the oxazolidone derivative **6a** and the desirable aminopropanol derivative **7a** (Route A), while the sole aminopropanol derivative **7a** was obtained by heating under reflux in alkaline ethanol (Route B). The mixture of **6a** and **7a** was separable by silica gel column chromatography. The ^{1}H NMR spectra of **6a** and **7a** exhibited multiplet signals corresponding to the oxazolidone methine proton and aminopropanol methine proton at 4.63–4.74 and 3.81–3.92 ppm, respectively. The IR spectrum of **6a** showed a strong absorption assigned to the oxazolidone C=O absorption at 1754 cm^{-1}, while that of **7a** showed an absorption assigned to the OH absorption at 3420 cm^{-1}. High resolution mass spectra of **6a** and **7a** exhibited the parent peaks at *m/z* 464.2138 and 438.2389 $[M+H]^+$, respectively. These spectral data confirmed the structure of each compound. The treatment of oxazolidone **6a** with aqueous sodium hydroxide in ethanol under reflux afforded **7a** quantitatively. Other diphenylalkyl piperazine derivatives **2** and **7b–d** were directly synthesized from the ethoxycarbonyl derivatives **5b–e** (Route B).

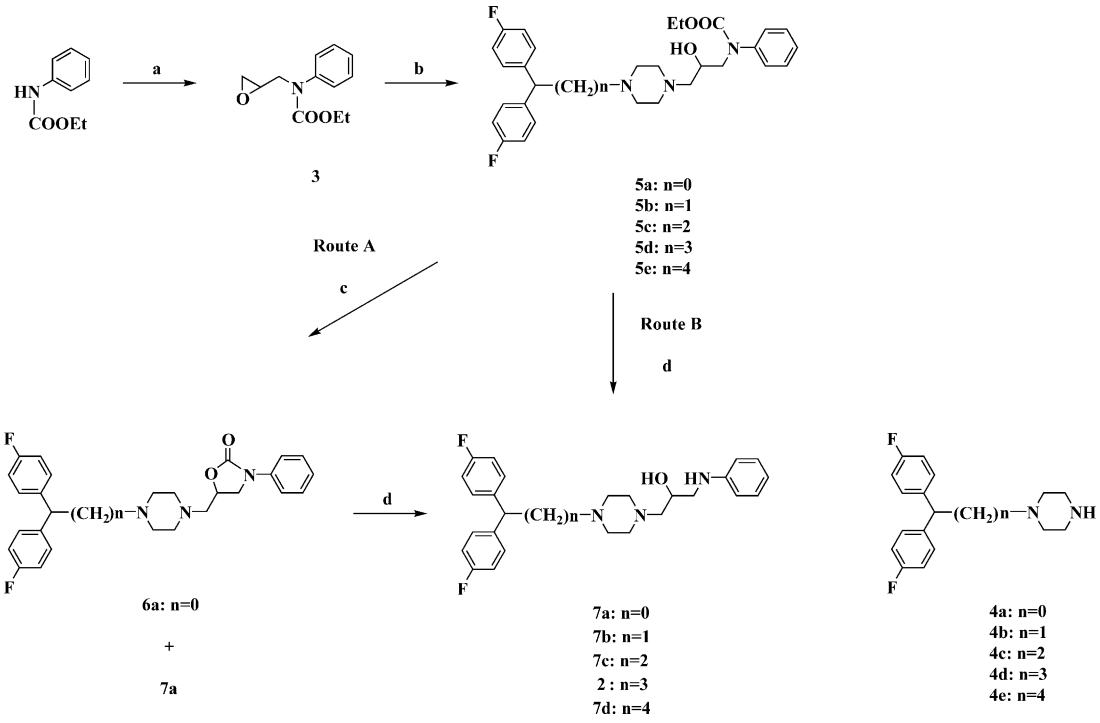


Win 35,428: R=F
RTI-55: R=I

GBR12909: R=F
GBR12935: R=H

1: X=S
2: X=NH

Figure 1.



Scheme 1. Reagents and conditions: (a) NaH, epibromohydrin, DMF, 81%; (b) diphenylalkyl piperazine (**4a–e**), EtOH, 60–100%; (c) NaOH(aq), MeOH, 51% for **6a**, 48% for **7a**; (d) NaOH(aq), EtOH, reflux, 53–95%.

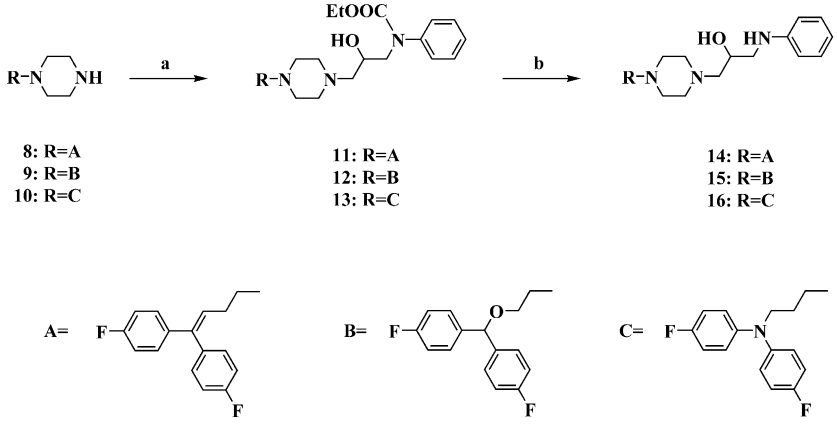
The diphenyl piperazine derivatives **14–16** and **21**, which contained the heteroatoms or functional groups in the diphenylbutyl moiety of **2**, were synthesized as shown in Schemes 2 and 3. The synthetic method for diphenylalkyl piperazine derivatives described in Scheme 1 was applied to the preparations of diphenylbutenyl **14**, diphenylmethoxyethyl **15**, and diphenylaminopropyl **16** derivatives. Ring openings of **3** with the corresponding diphenyl piperazines **8**,[43] **9**,[20] and **10**,[44] followed by deprotections of the ethoxycarbonyl groups of the resultant compounds **11–13** under basic conditions, afforded the desirable compounds **14–16**, respectively (Scheme 2). Alkylation of 1-triphenylmethylpiperazine **17** with 4-chloro-4′-fluorobutyrophenone gave the piperazine derivative **18**. Deprotection of the triphenylmethyl group of **18** with hydrogen chloride in ethanol, followed by treatment with **3**, afforded the ethoxycarbonyl derivative **19**. Deprotection of the ethoxycarbonyl group of **19** with alkaline ethanol under reflux gave the intermediate **20** and the subsequent treatment of **20** with 4-fluorophenylmagnesium bromide provided the diphenylbutyl derivative **21** containing a hydroxy group (Scheme 3).

All of the final compounds synthesized as described above were used for the pharmacological evaluations as their corresponding salts listed in Table 1.

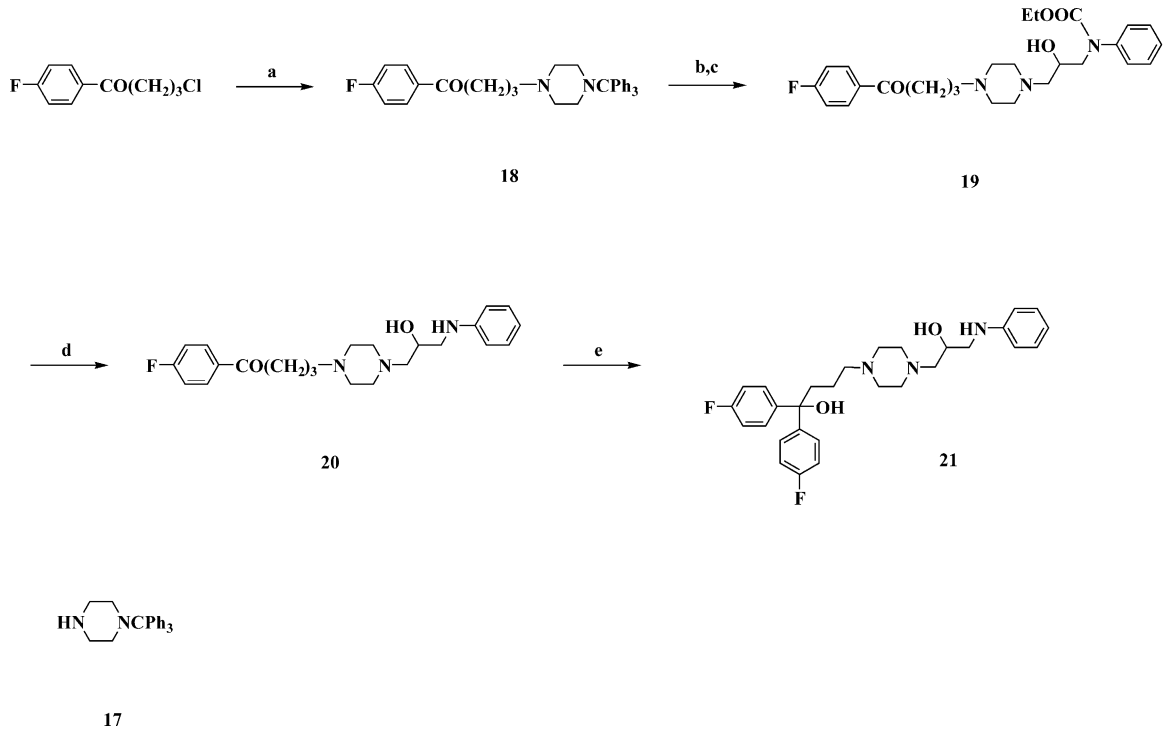
Results and Discussion

[^{3}H]GBR12935 binding studies

Diphenyl piperazine derivatives synthesized as described above along with GBR12909 were evaluated for their



Scheme 2. Reagents and conditions: (a) **3**, EtOH, 62–90%; (b) NaOH(aq), EtOH, reflux, 53–89%.



Scheme 3. Reagents and conditions: (a) **17**, K_2CO_3, KI, DMF, 72%; (b) HCl/EtOH; (c) **3**, EtOH, 51%; (d) NaOH(aq), EtOH, reflux, 83%; (e) 4-fluorophenylmagnesium bromide, THF, 68%.

competitive binding assays using [^{3}H]GBR12935 to label the DAT in rat striatal membranes.[38] The results are shown in Table 1.

At first, compound **2** ($n=3$) producing a notable stimulation of locomotor activity was evaluated for the binding affinity for the DAT. Surprisingly, compound **2** exhibited a highly potent binding affinity with IC_{50} value of 2.0 nM and was equivalent in activity to GBR12909 known as a potent dopamine uptake inhibitor. This finding also suggested strongly that the diphenyl piperazine derivatives including compound **2** containing the phenyl substituted aminopropanol moiety might be able to serve as novel DA inhibitors different from GBR analogues. Therefore, first of all, we attempted to investigate further modifications for the SAR exploration at the sites between the diphenyl and piperazine moieties of compound **2**.

Table 1. DAT binding affinities of diphenyl piperazine derivatives

Compd	n[a]	IC_{50} (nM)[b]
7a·3HCl	0	20.00±3.00
7b·3HCl	1	28.00±2.00
7c·3HCl	2	7.00±0.65
2·3HCl	3	2.00±0.29
7d·3HCl	4	6.00±0.32
14·3HCl	—	3.39±0.85
15·2$C_4H_4O_4$[c]	—	2.18±0.76
16·3HCl	—	1.75±0.59
21·2$C_4H_4O_4$[c]	—	6.41±0.71
GBR12909·2HCl		2.00±0.35

[a]The n indicates the alkyl chain length as shown in Scheme 1.
[b]The DAT was labeled with [^{3}H]GBR12935. IC_{50} values represent the concentrations inhibiting 50% of specific bindings and were calculated by non-linear regression fitting. Each value represents the mean±S.E. from three experiments conducted in duplicate.
[c]Dimaleate.

In order to examine the effect of alkyl chain length between the diphenylmethyl and piperazine moieties on the binding affinity for the DAT, compounds **7a–d** were synthesized and evaluated. These compounds showed moderate to high binding affinities ($IC_{50}<30$ nM) for the DAT. Among them, compounds **7c** ($n=2$) and **7d** ($n=4$) showed high binding affinities ($IC_{50}<10$ nM), while compounds **7a** ($n=0$) and **7b** ($n=1$) showed moderate potencies. Subsequently, compounds **14–16** and **21**, which contained various heteroatoms or functional groups in the diphenylbutyl moiety of **2**, were evaluated for DAT binding affinity. These four compounds were synthesized in the hope of having different pharmacokinetic profiles along with the expectation of increasing the binding affinity for the DAT, because they have different physico-chemical properties from **2**. As shown in Table 1, all of four compounds **14–16** and **21** showed highly potent binding affinities ($IC_{50}<10$ nM).

The results shown above indicate that the distance between the diphenylmethyl moiety and the nitrogen atom in the piperazine ring is important for DAT binding affinity, showing that two–four methylene connectives (**2**, **7c**, and **7d**) are more suitable for exhibiting the potent interaction with the DAT. On the other hand, two compounds **7a** and **7b** having shorter distances between the diphenylmethyl and piperazine moieties show almost equal moderate potencies, which may indicate the involvement of poor interactions of these compounds with the DAT as compared with **2**, **7c**, and **7d**. Namely, it is suggested that alkyl chain between the

diphenylmethyl moiety and the piperazine ring plays a critical role as a spacer. Furthermore, this consideration is confirmed by the results that the compounds **14**–**16** and **21**, which have the distances and geometric configurations similar to those of **2** between the diphenyl and piperazine moieties although they contain the heteroatoms or functional groups, also display highly potent DAT binding affinities, suggesting that the heteroatoms or functional groups of them have little influence on DAT binding affinity.

Thus, all of the tested compounds showed moderate to high binding affinities for GBR12935 binding site of the DAT. And then, in order to examine in vivo pharmacological profile of these compounds, we selected compound **2** as a representative, which was one of the compounds having the highest binding affinity.

In vivo brain microdialysis[38]

To further investigate the pharmacological activities, the changes of rat striatal extracellular DA levels were examined using in vivo brain microdialysis. The changes of DA levels in the striatal dialysates after the intraperitoneal administration of **2** (0.01, 0.03, or 0.1 mmol/kg) or GBR12909 (0.1 mmol/kg) are shown in Figure 2. The administration of GBR12909 increased extracellular DA levels significantly as compared with the basal levels. The administrations of **2** induced dose-dependent increases of extracellular DA levels and maintained high DA levels for 240 min, showing significant increases at the highest dose (0.1 mmol/kg) as compared with the basal levels. Although the in vitro DAT binding affinities of **2** and GBR12909 were equivalent, much greater increases of both initial and maintained DA levels than GBR12909 were observed after the intraperitoneal administration of 0.1 mmol/kg of **2**. This difference in the DA levels between **2** and GBR12909 seems to be owing to that in their pharmacokinetic properties such as permeability to blood–brain barrier, metabolism, or protein binding.

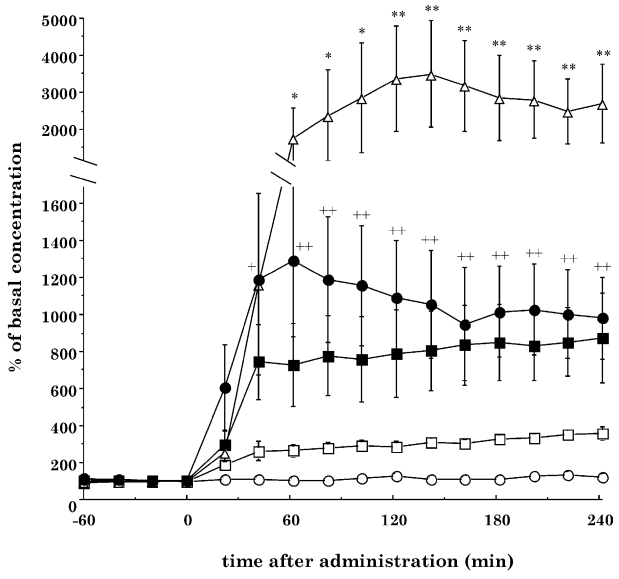


Figure 2. Effects of **2** and GBR12909 on dopamine levels in dialysates collected from the rat striatum. Saline (○ control), **2** (□ 0.01 mmol/kg, ■ 0.03 mmol/kg, or △ 0.1 mmol/kg) or GBR12909 (● 0.1 mmol/kg) was administered intraperitoneally and dialysates were collected for a further 4 h. The changes in the DA level are expressed as percentages of basal values (4.8 ± 0.5 pg/20 μL). Each point represents the mean ± S.E. for 4–6 rats. *Significantly different from control at $p < 0.05$ (Dunnett's test), **significantly different from control at $p < 0.01$ (Dunnett's test), $^{+}$Significantly different from control at $p < 0.05$ (*t*-test), $^{++}$significantly different from control at $p < 0.01$ (*t*-test).

These results combined with the in vitro binding affinity for the DAT suggest to us that compound **2** increases striatal extracellular DA levels through the DA uptake inhibitory effect by binding to the DAT in rat striatum. And also, the above data of these diphenylpiperazine derivatives containing the phenyl substituted aminopropanol moiety indicate to be able to provide new DA uptake inhibitory molecules different from GBR analogues.

Conclusion

In this report, we have synthesized a new series of diphenyl piperazine derivatives containing the phenyl substituted aminopropanol moiety, which were modified between the diphenyl and piperazine moieties, and evaluated them for DAT binding affinity in rat striatal membranes. All of the tested compounds showed apparent DAT binding affinities ($IC_{50} < 30$ nM). Some of them were approximately equivalent in activity to GBR12909, showing the activities with IC_{50} values of nanomolar range. Among them, compound **2** was evaluated for extracellular DA levels in rat striatum using in vivo brain microdialysis. After the intraperitoneal administration of **2** (0.01, 0.03, or 0.1 mmol/kg), the extracellular DA levels in rat striatum increased dose-dependently. The initial increase and high maintenance of DA levels by **2** were much greater than those by GBR12909 at the dose of 0.1 mmol/kg. The above data indicate that these diphenyl piperazine derivatives are novel potent DA uptake inhibitors within the CNS. We now continue further chemical modifications and pharmacological evaluations of them.

Experimental

All melting points were determined using a Büchi micromelting point apparatus without correction. IR spectra were measured with a Nicolet FT-IR 205 spectrometer. ^{1}H NMR spectra were recorded on a JEOL GSX spectrometer (270 MHz). Chemical shifts were reported in ppm (δ) values, based on tetramethylsilane as an internal standard. The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, brs = broad singlet. MS spectra were recorded using a JEOL SX-102 mass spectrometer. Elemental analyses were performed by Yanaco CHN CORDER MT-5 (C, H, N) and Flask Combustion (Cl). Column chromatography were performed on silica gel (BW-200, Fuji Silisia Chemical, Ltd., 100–200 mesh).

***N*-(2,3-Epoxypropyl)-*N*-ethoxycarbonylaniline (3).** NaH (60% in mineral oil; 0.21 g, 5.25 mmol) was added to a solution of ethyl *N*-phenylcarbamate (0.83 g, 5.02 mmol) in DMF (30 mL) under ice bath cooling and the mixture was stirred for 1 h under a nitrogen atmosphere. A solution of epibromohydrin (0.69 g, 5.04 mmol) in DMF (5 mL) was added dropwise to it over a period of 20 min and the mixture was stirred for 12 h at room temperature. The mixture was diluted with H_2O and extracted with benzene. The extract was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$ as an eluent) to give **3** (0.90 g, 81%) as a colorless oil. IR (KBr) cm^{-1}: 1703, 1597, 1300. ^1H NMR ($CDCl_3$) δ 1.22 (3H, t, $J=6.8$ Hz), 2.50 (1H, dd, $J=2.7$, 5.1 Hz), 2.78 (1H, t, $J=4.6$ Hz), 3.21–3.33 (1H, m), 3.63 (1H, dd, $J=8.9$, 14.6 Hz), 3.95 (1H, dd, $J=3.9$, 14.6 Hz), 4.17 (2H, q, $J=6.8$ Hz), 7.27–7.54 (5H, m). HRFAB-MS calcd for $C_{12}H_{16}NO_3$ $[M+H]^+$: 222.1130. Found: 222.1173..

1-[Bis(4-fluorophenyl)methyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (5a). A mixture of 3 (1.21 g, 5.47 mmol) and 1-[bis(4-fluorophenyl)methyl]piperazine **4a**[39] (1.51 g, 5.24 mmol) in EtOH (20 mL) was stirred for 4 h at room temperature. After removal of EtOH, the residue was dissolved in AcOEt. The organic solution was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH=99{:}1$ as an eluent) to give **5a** (2.66 g, 100%) as a pale yellow oil. IR (KBr) cm^{-1}: 3482, 1690, 1601, 1222. ^1H NMR ($CDCl_3$) δ 1.18 (3H, t, $J=6.8$ Hz), 2.23–2.31 (2H, m), 2.33–2.82 (8H, m), 3.63 (1H, dd, $J=7.3$, 14.3 Hz), 3.77 (1H, dd, $J=7.3$, 14.3 Hz), 4.11–4.20 (1H, m), 4.14 (2H, q, $J=6.8$ Hz), 4.18 (1H, s), 6.95 (4H, t, $J=6.5$ Hz), 7.22–7.34 (9H, m). HRFAB-MS calcd for $C_{29}H_{34}F_2N_3O_3$ $[M+H]^+$: 510.2614. Found: 510.2568.

5-[4-[Bis(4-fluorophenyl)methyl]-1-piperazinyl]methyl-3-phenyl-2-oxazolidone (6a) and 1-[Bis(4-fluorophenyl)methyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (7a). Route A: 2.0 N NaOH (2 mL) was added dropwise to a solution of **5a** (2.00 g, 3.92 mmol) in MeOH (20 mL) and the mixture was stirred for 15 h at room temperature. After removal of MeOH, the residue was diluted with H_2O and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was separated by silica gel column chromatography ($CHCl_3/CH_3OH=99{:}1$ as an eluent) to give **6a** (0.93 g, 51%) as a pale yellow oil from the first fraction. The second fraction yielded **7a** (0.82 g, 48%) as a pale yellow oil..

Route B: 2.0 N NaOH (2 mL) was added dropwise to a solution of **5a** (2.00 g, 3.92 mmol) in EtOH (20 mL) and the mixture was heated under reflux for 15 h. After removal of EtOH, the residue was diluted with H_2O and extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH=99{:}1$ as an eluent) to give **7a** (1.64 g, 95%) as a pale yellow oil.

6a. IR (KBr) cm^{-1}: 1754, 1601, 1506, 1222. ^1H NMR ($CDCl_3$) δ 2.24–2.81 (10H, m), 3.72–3.81 (1H, m), 4.01–4.13 (1H, m), 4.23 (1H, s), 4.63–4.74 (1H, m), 6.97 (4H, t, $J=8.9$ Hz), 7.01–7.53 (9H, m). HRFAB-MS calcd for $C_{27}H_{28}F_2N_3O_2$ $[M+H]^+$: 464.2150. Found: 464.2138.

7a. IR (KBr) cm^{-1}: 3420, 1605, 1232. ^1H NMR ($CDCl_3$) δ 2.32–2.51 (8H, m), 2.63–2.71 (2H, m), 3.04 (1H, dd, $J=6.5$, 12.4 Hz), 3.25 (1H, dd, $J=4.1$, 12.4 Hz), 3.81–3.92 (1H, m), 4.22 (1H, s), 6.62 (2H, t, $J=8.4$ Hz), 7.01–7.53 (11H, m). HRFAB-MS calcd for $C_{26}H_{30}F_2N_3O$ $[M+H]^+$: 438.2357. Found: 438.2389.

1-[Bis(4-fluorophenyl)methyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (7a·3HCl). 4.6 N HCl in EtOH (5 mL) was added dropwise to a solution of **7a** (2.07 g, 4.73 mmol) in Et_2O (100 mL) under ice bath cooling. The resultant precipitates were collected by filtration and recrystallized from Et_2O–EtOH to give **7a**·3HCl (1.80 g, 70%) as white crystals. Mp 196–197 °C. IR (KBr) cm^{-1}: 3439, 1605, 1510, 1232. ^1H NMR (DMSO-d_6) δ 3.21–3.83 (12H, m), 4.33–4.42 (2H, m), 6.93 (2H, t, $J=7.3$ Hz), 7.02 (2H, d, $J=7.8$ Hz), 7.33–7.51 (9H, m). Anal. calcd for $C_{26}H_{29}F_2N_3O{\cdot}3HCl$: C, 57.10; H, 5.90; N, 7.68; Cl, 19.45. Found: C, 57.21; H, 5.88; N,7.81; Cl, 19.41.

1-[2,2-Bis(4-fluorophenyl)ethyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (5b). This compound was prepared as a pale yellow oil from **3** and 1-[2,2-bis(4-fluorophenyl)ethyl]piperazine (**4b**)[40] in 72% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3434, 1631, 1599, 1223. ^1H NMR ($CDCl_3$) δ 1.18 (3H, t, $J=6.8$ Hz), 2.23–2.81 (11H, m), 2.87 (2H, d, $J=7.0$ Hz), 3.75 (1H, dd, $J=4.3$, 8.9 Hz), 3.93–4.02 (1H, m), 4.13–4.21 (3H, m), 6.94–7.01 (4H, m), 7.13–7.22 (4H, m), 7.24–7.44 (5H, m). FAB-MS m/z 524 $[M+H]^+$.

1-[2,2-Bis(4-fluorophenyl)ethyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (7b). This compound was prepared as a pale yellow oil from **5b** in 81% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3436, 1698, 1599, 1223. ^1H NMR ($CDCl_3$) δ 2.21–2.63 (10H, m), 2.81 (2H, d, $J=8.1$ Hz), 2.95 (1H, dd, $J=6.2$, 12.4 Hz), 3.17 (1H, dd, $J=3.8$, 12.4 Hz), 3.82–3.93 (1H, m), 4.08 (1H, t, $J=8.1$ Hz), 6.56 (2H, d, $J=8.4$ Hz), 6.63 (1H, t, $J=7.0$ Hz), 6.89 (4H, t, $J=6.5$ Hz), 7.11–7.23 (6H, m). HRFAB-MS calcd for $C_{27}H_{32}F_2N_3O$ $[M+H]^+$: 452.2513. Found: 452.2549.

1-[2,2-Bis(4-fluorophenyl)ethyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (7b·3HCl). This compound was prepared from **7b** according to the procedure described for **7a·3HCl** and recrystallized from EtOH to give white crystals in 70% yield. Mp 232–234 °C. IR (KBr) cm^{-1}: 3359, 1602, 1226. ^1H NMR (DMSO-d_6) δ 3.12–3.23 (4H, m), 3.31–3.75 (10H, m), 3.96–4.02 (1H, m), 4.21–4.34 (1H, m), 6.76 (1H, t, $J=7.3$ Hz), 6.84 (2H, d, $J=7.3$ Hz), 7.18 (6H, t, $J=8.4$ Hz), 7.43–7.56 (4H, m). Anal. calcd for $C_{27}H_{31}F_2N_3O{\cdot}3HCl$: C, 57.81; H,6.11; N, 7.49; Cl, 18.96. Found: C, 58.09; H, 6.01; N, 7.36; Cl, 18.72.

1-[3,3-Bis(4-fluorophenyl)propyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (5c). This compound was prepared as a pale yellow oil from **3** and 1-[3,3-bis(4-fluorophenyl)propyl]piperazine (**4c**)[40] in 74% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3445, 1698, 1508, 1225. ^1H NMR ($CDCl_3$) δ 1.19 (3H, t, $J=7.0$ Hz), 2.14–2.55 (12H, m), 2.56–2.64 (2H, m), 3.65 (1H, dd, $J=6.8$, 14.3 Hz), 3.77 (1H, dd, $J=3.8$, 14.3 Hz), 3.92–4.08 (2H, m), 4.14 (2H, q, $J=7.0$ Hz), 6.95 (3H, dt, $J=1.9$, 6.2 Hz), 7.19–7.21 (3H, m), 7.35–7.53 (7H, m). HRFAB-MS calcd for $C_{31}H_{38}F_2N_3O_3$ $[M+H]^+$: 538.2881. Found: 538.2922.

1-[3,3-Bis(4-fluorophenyl)propyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (7c). This compound was prepared as a pale yellow oil from **5c** in 63% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3389, 1603, 1507, 1222. ^1H NMR ($CDCl_3$) δ 2.13–2.56 (12H, m), 2.62–2.78 (2H, m), 3.24 (1H, dd, $J=3.5$, 12.4 Hz), 3.38 (1H, dd, $J=5.9$, 12.4 Hz), 3.93–4.02 (2H, m), 6.62 (2H, d, $J=7.3$ Hz), 6.70 (1H, t, $J=7.3$ Hz), 6.95 (3H, dt, $J=2.4$, 6.5 Hz), 7.13–7.27 (7H, m). HRFAB-MS calcd for $C_{28}H_{34}F_2N_3O$ $[M+H]^+$: 466.2670. Found: 466.2713..

1-[3,3-Bis(4-fluorophenyl)propyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (7c·3HCl). This compound was prepared from **7c** according to the procedure described for **7a**·3HCl and recrystallized from EtOH to give white crystals in 82% yield. Mp 241–244 °C. IR (KBr) cm^{-1}: 3320, 1603, 1508, 1226. ^1H NMR (DMSO-d_6) δ 2.52–2.63 (2H, m), 2.92–3.81 (14H, m), 4.14 (1H, t, $J=8.1$ Hz), 4.23–4.38 (1H, m), 6.60 (2H, t, $J=6.5$ Hz), 6.73 (1H, d, $J=7.3$ Hz), 7.16–7.23 (5H, m), 7.31–7.49 (5H, m). Anal. calcd for $C_{28}H_{33}F_2N_3O·3HCl$: C, 58.49; H, 6.31; N, 7.31; Cl, 18.50. Found: C, 58.36; H, 6.52; N, 7.13; Cl, 18.22.

1-[4,4-Bis(4-fluorophenyl)butyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (5d). This compound was prepared as a pale yellow oil from **3** and 1-[4,4-bis(4-fluorophenyl)butyl]piperazine (**4d**)[41,42] in 95% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3442, 1702, 1600, 1504, 1225. ^1H NMR ($CDCl_3$) δ 1.19 (3H, t, $J=6.8$ Hz), 1.42–1.57 (2H, m), 1.91–2.06 (2H, m), 2.33–2.54 (10H, m), 2.57–2.63 (2H, m), 3.64 (1H, dd, $J=7.3$, 14.3 Hz), 3.83–3.96 (3H, m), 4.14 (2H, q, $J=6.8$ Hz), 6.88 (4H, t, $J=4.6$ Hz), 7.13–7.24 (4H, m), 7.25-7.47 (5H, m). HRFAB-MS calcd for $C_{32}H_{40}F_2N_3O_3$ $[M+H]^+$: 552.3038. Found: 552.2998.

1-[4,4-Bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (2). This compound was prepared from **5d** according to the procedure described for **7a** from **5a** by Route B and recrystallized from EtOH to give a white solid in 90% yield. Mp 99–100 °C. IR (KBr) cm^{-1}: 3329, 1603, 1222. ^1H NMR ($CDCl_3$) δ 1.53–1.68 (2H, m), 2.00–2.17 (2H, m), 2.33–2.65 (10H, m), 2.66–2.74 (2H, m), 3.06 (1H, dd, $J=5.9$, 12.9 Hz), 3.25 (1H, dd, $J=4.1$, 12.9 Hz), 3.86 (1H, t, $J=7.8$ Hz), 3.93–4.04 (1H, m), 6.63 (2H, d, $J=7.6$ Hz), 6.91–7.01 (4H, m), 7.03 (1H, t, $J=7.6$ Hz), 7.21–7.37 (6H, m). HRFAB-MS calcd for $C_{29}H_{36}F_2N_3O$ $[M+H]^+$: 480.2826. Found: 480.2839.

1-[4,4-Bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (2·3HCl). This compound was prepared from **2** according to the procedure described for **7a**·3HCl and recrystallized from EtOH to give white crystals in 92% yield. Mp 232–233 °C. IR (KBr) cm^{-1}: 3318, 1602, 1508, 1223. ^1H NMR (DMSO-d_6) δ 1.52–1.68 (2H, m), 2.03–2.09 (2H, m), 2.24–2.94 (12H, m), 3.10–3.34 (2H, m), 4.02 (1H, t, $J=7.8$ Hz), 4.21–4.33 (1H, m), 6.83 (2H, d, $J=7.3$ Hz), 7.10–7.33 (7H, m), 7.44–7.72 (4H, m). Anal. calcd for $C_{29}H_{35}F_2N_3O·3HCl$: C, 59.14; H, 6.50; N, 7.13; Cl, 18.06. Found: C, 59.32; H, 6.47; N, 7.10; Cl, 17.98.

1-[5,5-Bis(4-fluorophenyl)pentyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (5e). This compound was prepared as a pale yellow oil from **3** and 1-[5,5-bis(4-fluorophenyl)pentyl]piperazine (**4e**)[40] in 60% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3399, 1752, 1600, 1508, 1223. ^1H NMR ($CDCl_3$) δ 1.19 (3H, t, $J=7.6$ Hz), 1.23–1.34 (2H, m), 1.87–2.03 (2H, m), 2.24–2.49 (11H, m), 2.62–2.72 (2H, m), 3.63 (1H, dd, $J=7.3$, 14.3 Hz), 3.74–4.05 (4H, m), 4.17 (2H, q, $J=7.6$ Hz), 6.95 (4H, t, $J=7.3$ Hz), 7.13–7.21 (4H, m), 7.23–7.54 (5H, m). HRFAB-MS calcd for $C_{33}H_{42}F_2N_3O_3$ $[M+H]^+$: 566.3194. Found: 566.3206.

1-[5,5-Bis(4-fluorophenyl)pentyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (7d). This compound was prepared as a pale yellow oil from **5e** in 53% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3389, 1603, 1223. ^1H NMR ($CDCl_3$) δ 1.23–1.32 (2H, m), 1.42–1.49 (2H, m), 1.93–2.02 (2H, m), 2.24–2.83 (12H, m), 3.82–3.99 (1H, m), 4.04–4.15 (2H, m), 4.86–4.94 (1H, m), 6.93 (3H, t, $J=8.9$ Hz), 7.16–7.23 (4H, m), 7.41–7.47 (3H, m), 7.48–7.54 (3H, m). HRFAB-MS calcd for $C_{30}H_{38}F_2N_3O$ $[M+H]^+$: 494.2983. Found: 494.2975.

1-[5,5-Bis(4-fluorophenyl)pentyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (7d·3HCl). This compound was prepared from **7d** according to the procedure described for **7a**·3HCl and recrystallized from EtOH to give white crystals in 67% yield. Mp 236–239 °C. IR (KBr) cm^{-1}: 3402, 1603, 1223. ^1H NMR (DMSO-d_6)δ 1.13–1.24 (2H, m), 1.63–1.72 (2H, m), 2.01–2.14 (2H, m), 2.98–3.83 (14H, m), 4.00 (1H, t, $J=7.8$ Hz), 4.21–4.33 (1H, brs), 6.96 (3H, t, $J=7.8$ Hz), 7.11 (4H, t, $J=8.9$ Hz), 7.23 (3H, t, $J=7.3$ Hz), 7.33–7.44 (3H, m). Anal. calcd for $C_{30}H_{37}F_2N_3O·3HCl$: C, 59.76; H, 6.69; N, 6.97; Cl, 17.64. Found: C, 59.45; H, 6.57; N, 7.06; Cl, 17.42.

1-[4,4-Bis(4-fluorophenyl)-3-butenyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (11). This compound was prepared as a pale yellow oil from **3** and 1-[4,4-bis(4-fluorophenyl)-3-butenyl]piperazine (**8**)[43] in 74% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3380, 1698, 1599, 1508, 1222. ^1H NMR ($CDCl_3$) δ 1.19 (3H, t, $J=7.0$ Hz), 2.24–2.53 (12H, m), 2.61–2.73 (2H, m), 3.65 (1H, dd, $J=7.3$, 14.3 Hz), 3.79 (1H, dd, $J=4.6$, 14.3 Hz), 3.79–3.91 (1H, m), 4.14 (2H, q, $J=7.0$ Hz), 5.98 (1H, t, $J=7.0$ Hz), 6.94 (2H, t, $J=7.3$ Hz), 7.02–7.48 (11H, m). FAB-MS m/z 550 $[M+H]^+$.

1-[4,4-Bis(4-fluorophenyl)-3-butenyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (14). This compound was prepared as a pale yellow oil from **11** in 62% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3497, 1751, 1601, 1507, 1222. ^1H NMR ($CDCl_3$) δ 2.33–2.44 (4H, m), 2.45–2.83 (10H, m), 3.80 (1H, t, $J=8.4$ Hz), 4.06 (1H, t, $J=8.4$ Hz), 4.76–4.82 (1H, m), 5.99 (1H, t, $J=6.8$ Hz), 6.92–7.01 (2H, m), 7.04–7.23 (7H, m), 7.41–7.53 (2H, m), 7.63–7.71 (2H, m). HRFAB-MS calcd for $C_{29}H_{34}F_2N_3O$ $[M+H]^+$: 478.2670. Found: 478.2635.

1-[4,4-Bis(4-fluorophenyl)-3-butenyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (14·3HCl). This compound was prepared from **14** according to the procedure described for **7a**·3HCl and recrystallized from EtOH to give white crystals in 72% yield. Mp 215–219 °C. IR (KBr) cm^{-1}: 3449, 1602, 1509, 1227, 838. ^1H NMR (DMSO-d_6) δ 3.17 (2H, t, $J=7.3$ Hz), 3.24–4.21 (14H, m), 4.22–4.34 (1H, m), 6.20 (1H, t, $J=7.0$ Hz), 6.71 (1H, t, $J=7.3$ Hz), 6.78 (2H, d, $J=8.1$ Hz), 7.21–7.44 (10H, m). Anal. calcd for $C_{29}H_{33}F_2N_3O·3HCl$: C, 59.34; H, 6.18; N, 7.16; Cl, 18.12. Found: C, 59.44; H, 6.29; N, 7.10; Cl, 18.01.

1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (12). This compound was prepared as a pale yellow oil from **3** and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-piperazine (**9**)[20] in 90% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3446, 1600, 1507, 1222. ^1H NMR ($CDCl_3$) δ 1.19 (3H, t, $J=7.6$ Hz), 2.24–2.71 (10H, m), 2.72 (2H, t, $J=7.3$ Hz), 3.54 (2H, t, $J=5.9$ Hz), 3.65 (1H, dd, $J=7.0$, 13.8 Hz), 3.77 (1H, dd, $J=4.3$, 13.8 Hz), 3.93–4.01 (1H, m), 4.15 (2H, q, $J=7.6$ Hz), 5.32 (1H, s), 7.00 (4H, dt, $J=1.9$, 8.9 Hz), 7.28–7.43 (9H, m). HRFAB-MS calcd for $C_{31}H_{38}F_2N_3O_4$ $[M+H]^+$: 554.2830. Found: 554.2866.

1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (15). This compound was prepared as a pale yellow oil from **12** in 89% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3332, 1604, 1506, 1220. ^1H NMR ($CDCl_3$) δ 2.43–2.64 (8H, m), 2.66 (4H, t, $J=5.4$ Hz), 3.04 (1H, dd, $J=6.2$, 13.0 Hz), 3.25 (1H, dd, $J=4.1$, 13.0 Hz), 3.56 (2H, t, $J=6.5$ Hz), 3.96–4.04 (1H, m), 5.33 (1H, s), 6.63 (1H, d, $J=8.1$ Hz), 6.71 (2H, t, $J=7.3$ Hz), 6.90–7.02 (4H, m), 7.17 (2H, t, $J=7.3$ Hz), 7.23–7.31 (4H, m). HRFAB-MS calcd for $C_{28}H_{34}F_2N_3O_2$ $[M+H]^+$: 482.2619. Found: 482.2588.

1-[2-[Bis(4-fluorophenyl)methoxy]ethyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine dimaleate (15·2$C_4H_4O_4$). A solution of maleic acid (0.36 g, 3.10 mmol) in Et_2O (80 mL) was added to a solution of **15** (0.45 g, 0.94 mmol) in Et_2O (50 mL) under ice bath cooling and the mixture was stirred for 30 min. The resultant precipitates were collected by filtration and recrystallized from EtOH to give **15·2$C_4H_4O_4$** (0.54 g, 81%) as white crystals. Mp 157–158 °C. IR (KBr) cm^{-1}: 3382, 1605, 1506, 1218. ^1H NMR (DMSO-d_6) δ 2.62–3.60 (14H, m), 3.61–3.74 (2H, m), 3.95–4.03 (1H, m), 5.53 (1H, s), 6.14 (4H, s), 6.55 (1H, t, $J=7.6$ Hz), 6.60 (2H, d, $J=7.6$ Hz), 7.08 (2H, t, $J=7.6$ Hz), 7.14–7.23 (4H, m), 7.31–7.39 (4H, m). Anal. calcd for $C_{28}H_{33}F_2N_3O_2·2C_4H_4O_4$: C, 60.58; H, 5.79; N, 5.89. Found: C, 60.58; H, 5.72; N, 5.87.

1-[3-[*N*,*N*-Bis(4-fluorophenyl)amino]propyl]-4-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]piperazine (13). This compound was prepared as a pale yellow oil from **3** and 1-[3-[*N*,*N*-bis(4-fluorophenyl) amino]propyl]piperazine (**10**)[44] in 62% yield according to the procedure described for **5a**. IR (KBr) cm^{-1}: 3446, 1602, 1506, 1223. ^1H NMR ($CDCl_3$) δ 1.14–1.32 (5H, m), 1.74–1.83 (2H, m), 2.33–2.59 (8H, m), 2.63–2.71 (2H, m), 3.64–3.71 (3H, m), 3.72–3.82 (1H, m), 3.84–3.91 (1H, m), 4.15 (2H, q, $J=6.8$ Hz), 6.81–7.04 (7H, m), 7.08–7.44 (6H, m). FAB-MS m/z 553 $[M+H]^+$.

1-[3-[*N*,*N*-Bis(4-fluorophenyl)amino]propyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (16). This compound was prepared as a pale yellow oil from **13** in 53% yield according to the procedure described for **7a** from **5a** by Route B. IR (KBr) cm^{-1}: 3342, 1506, 1226. ^1H NMR ($CDCl_3$) δ 1.73–1.82 (2H, m), 2.34–2.51 (10H, m), 2.62–2.75 (2H, m), 3.04 (1H, dd, $J=6.5$, 12.4 Hz), 3.25 (1H, dd, $J=4.1$, 12.4 Hz), 3.68 (2H, t, $J=7.6$ Hz), 3.91–4.01 (1H, m), 6.62–6.69 (3H, m), 6.93–7.02 (7H, m), 7.11–7.23 (3H, m). HRFAB-MS calcd for $C_{28}H_{35}F_2N_4O$ $[M+H]^+$: 481.2779. Found: 481.2786.

1-[3-[*N*,*N*-Bis(4-fluorophenyl)amino]propyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine trihydrochloride (16·3HCl). This compound was prepared from **16** according to the procedure described for **7a**·3HCl and recrystallized from EtOH to give white crystals in 53% yield. Mp 228–231 °C. IR (KBr) cm^{-1}: 3384, 1505, 1227. ^1H NMR (DMSO-d_6) δ 2.13–2.21 (2H, m), 3.23–4.01 (16H, m), 4.27–4.42 (1H, m), 6.91–7.03 (4H, m), 7.14–7.21 (4H, m), 7.22–7.34 (5H, m). Anal. calcd for $C_{28}H_{34}F_2N_4O·3HCl$: C, 57.00; H, 6.32; N, 9.50; Cl; 18.03. Found: C, 57.21; H, 6.33; N, 9.41; Cl, 17.92.

1-(4-Fluorophenyl)-4-(4-triphenylmethyl-1-piperazinyl)-1-butanone (18). A mixture of 1-triphenylmethylpiperazine **17** (6.02 g, 18.3 mmol), 4-chloro-4′-fluorobutyrophenone (10.82 g, 53.9 mmol), potassium carbonate (3.03 g, 21.9 mmol), and potassium iodide (2.99 g, 18.0 mmol) in DMF (120 mL) was heated under reflux for 2.5 h. The mixture was cooled to room temperature, diluted with H_2O, and extracted with benzene. The extract was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 95:5 as an eluent) to give 18 (6.51 g, 72%) as a colorless oil. IR (KBr) cm^{-1}: 1683, 1597, 1489, 1223. ^1H NMR ($CDCl_3$) δ 1.89 (2H, t, $J=7.0$ Hz), 2.l4 (2H, t, $J=7.3$ Hz), 2.43–2.81 (8H, m), 2.89 (2H, t, $J=7.0$ Hz), 6.94–7.29 (12H, m), 7.35–7.62 (5H, m), 7.81–7.93 (2H, m). HRFAB-MS calcd for $C_{33}H_{34}FN_2O$ $[M+H]^+$: 493.2655. Found: 493.2670.

1-[3-[(*N*-ethoxycarbonyl-*N*-phenyl)amino]-2-hydroxypropyl]-4-[(4-Fluorophenyl)-4-oxobutyl]-4-piperazine (19). 4.0 N HCl in AcOEt (22.5 mL) was added dropwise to

a solution of **18** (11.09 g, 22.5 mmol) in EtOH (100 mL) with stirring under ice bath cooling and the mixture was stirred for 5 h under the same condition to give a clear solution. The mixture was neutralized with triethylamine, diluted with benzene, dried over Na_2SO_4, and concentrated in vacuo. The residue was used for the next reaction without further purification.

A mixture of the residue and **3** (4.21 g, 19.0 mmol) in EtOH (40 mL) was heated under reflux for 5 h. The mixture was cooled to room temperature, poured into H_2O, and extracted with benzene. The extract was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH = 98:2$ as an eluent) to give 19 (4.58 g, 51%) as a pale yellow oil. IR (KBr) cm^{-1}: 1686, 1598, 1504, 1228. ^1H NMR ($CDCl_3$) δ 1.20 (3H, t, $J = 7.3$ Hz), 1.94 (2H, t, $J = 7.0$ Hz), 2.32–2.71 (12H, m), 2.96 (2H, t, $J = 7.0$ Hz), 3.65 (1H, dd, $J = 7.0$, 14.3 Hz), 3.76 (1H, dd, $J = 3.8$, 14.3 Hz), 3.92–4.03 (1H, m), 4.14 (2H, q, $J = 7.3$ Hz), 7.12 (2H, t, $J = 6.5$ Hz), 7.23–7.37 (5H, m), 7.92–8.04 (2H, m). HRFAB-MS calcd for $C_{26}H_{35}FN_3O_4$ $[M+H]^+$: 472.2612. Found: 472.2598.

1-[(4-Fluorophenyl)-4-oxobutyl]-4-[(2-hydroxy-3-phenylamino)propyl]piperazine (20). 2.0 N NaOH (21.2 mL) was added dropwise to a solution of **19** (5.46 g, 11.6 mmol) in EtOH (77 mL) and the mixture was heated under reflux for 8 h. After removal of EtOH, benzene was added to the residue. The organic solution was washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH = 99:1$ as an eluent) to give 20 (3.82 g, 83%) as a pale yellow oil. IR (KBr) cm^{-1}: 3359, 1686, 1602, 1505, 1227. ^1H NMR ($CDCl_3$) δ 1.94 (2H, t, $J = 7.3$ Hz), 2.33–3.83 (12H, m), 2.97 (2H, t, $J = 7.0$ Hz), 3.04 (1H, dd, $J = 6.5$, 12.4 Hz), 3.26 (1H, dd, $J = 3.2$, 12.4 Hz), 3.92–4.01 (1H, m), 6.63 (2H, d, $J = 7.6$ Hz), 6.71 (1H, t, $J = 7.0$ Hz), 7.13–7.24 (4H, m), 7.92–8.04 (2H, m). HRFAB-MS calcd for $C_{23}H_{31}FN_3O_2$ $[M+H]^+$: 400.2400. Found: 400.2438.

1-[4,4-Bis(4-fluorophenyl)-4-hydroxybutyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine (21). To tetrahydrofuran (THF, 30 mL) containing magnesium (0.022 g, 0.90 mmol) was added dropwise a solution of 1-bromo-4-fluorobenzene (0.16 g, 0.91 mmol) in THF (5 mL) with stirring under ice bath cooling and the mixture was stirred for 4 h to give a Grignard reagent. A solution of **20** (0.18 g, 0.45 mmol) in THF (15 mL) was added dropwise to a solution containing Grignard reagent in THF under ice bath cooling. The mixture was heated under reflux for 1 h and cooled to room temperature. The mixture was poured into saturated aqueous NH_4Cl and extracted with benzene. The extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH = 99:1$ as an eluent) to give **21** (0.15 g, 68%) as a colorless oil. IR (KBr) cm^{-1}: 3442, 1506, 1227. ^1H NMR ($CDCl_3$) δ 1.52-1.73 (2H, m), 2.24–2.61 (12H, m), 2.68–2.84 (2H, m), 3.05 (1H, dd, $J = 5.9$, 12.4 Hz), 3.27 (1H, dd, $J = 4.1$, 12.4 Hz), 3.92–4.04 (1H, m), 6.63 (2H, d, $J = 7.8$ Hz), 6.72 (1H, t, $J = 7.0$ Hz), 6.96 (4H, t, $J = 8.6$ Hz), 7.19–7.28 (2H, m), 7.43–7.54 (4H, m). HRFAB-MS calcd for $C_{29}H_{36}F_2N_3O_2$ $[M+H]^+$: 496.2776. Found: 496.2785.

1-[4,4-Bis(4-fluorophenyl)-4-hydroxybutyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine dimaleate (21·2C$_4$H$_4$O$_4$). This compound was prepared from **21** according to the procedure described for **15**·2C$_4$H$_4$O$_4$ and recrystallized from EtOH to give white crystals in 73% yield. Mp 151–153 °C. IR (KBr) cm^{-1}: 3440, 1579, 1507, 1226. ^1H NMR (DMSO-d_6) δ 1.52–1.63 (2H, m), 2.21–2.33 (2H, m), 2.82–3.34 (13H, m), 3.19 (1H, dd, $J = 9.7$, 15.4 Hz), 3.91–4.02 (1H, m), 6.25 (4H, s), 6.60 (1H, t, $J = 7.0$ Hz), 6.68 (3H, d, $J = 7.8$ Hz), 7.13–7.22 (5H, m), 7.47–7.62 (4H, m). Anal. calcd for $C_{29}H_{35}F_2N_3O_2 \cdot 2C_4H_4O_4$: C, 61.07; H, 5.96; N, 5.77. Found: C, 61.02; H, 5.83; N, 5.76.

Animals and materials

Male Wistar rats (6–7 weeks) from Sankyo Labo Service Co. (Tokyo, Japan) were used throughout the biological experiments. All of the tested compounds including GBR12909 were evaluated as their corresponding salts.

[^{3}H]GBR12935 binding studies

Binding assay for the DAT was determined according to the published procedure.[38] Briefly, the rat striatal membranes were incubated with [^{3}H]GBR12935 (1 nM final concentration) and test compounds (final concentration range: 10^{-11}–10^{-5} M), which were diluted with dimethyl sulfoxide solution (final dimethyl sulfoxide concentration was less than 0.1%), for 60 min at 4 °C in 50 mM Tris–citrate (pH 7.4) buffer containing 120 mM NaCl and 4 mM $MgCl_2$. [^{3}H]GBR12935 (53 Ci/mmol) was purchased from Du Pont-NEN (Boston, MA, USA). The assay was terminated by filtration through Whatman GB/F glass fiber filtermats, presoaked with 0.1% bovine serum albumin solution, with a Brandel Cell Harvester (Gaithersberg, MD, USA). Filters were assayed for radioactivity with Packard Tris-Carb Liquid Scintillation Counter (Meriden, CT, USA) in 4 mL Aquasol-2.

In vivo brain microdialysis

In vivo brain microdialysis assay was determined according to the published procedure.[38] Briefly, rats were anesthetized with pentobarbital sodium (40 mg/kg intraperitoneal administration) and placed in a stereotaxic frame. Dialysis probes (membrane length: 3 mm, diameter: 200 μm, Eicom) with guide cannula (G-5, Eicom, Kyoto, Japan) were implanted above the striatum (A: 0.5 mm, L: 2.5 mm, V: 3.0 mm from bregma) and fixed with dental cement (G-C Dental Industrial Corp., Tokyo, Japan). Following surgery, the animals were returned to the plastic cages with free access to food and water. Two or three days after surgery, the dialysis probes were connected to syringe pumps and perfused at 1 μL/min with Ringer's solutions (147 mM

NaCl, 4 mM KCl, 2.3 mM $CaCl_2$ and 1 mM sodium phosphate, pH 7.4). After equilibration period of 2 h, the perfusates were collected every 20 min. At least three control samples were taken before the administration of **2** (0.01, 0.03, or 0.1 mmol/kg) or GBR12909 (0.1 mmol/kg). The perfusate samples were assayed for dopamine using HPLC with electrochemical and coulometric detection. The data were expressed as percentages of the basal dopamine concentrations, which were the means of the dopamine concentrations in three basal dialysates.

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