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Antioxidative activities of novel diphenylalkyl piperazine derivatives with high affinities for the dopamine transporter

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Abstract—A new series of diphenylalkyl piperazine derivatives with high affinities for the dopamine transporter (DAT), which were modified at both the diphenylalkyl moiety and the phenyl ring in the phenylamino moiety of 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine **1**, was evaluated for their inhibitory activities against auto-oxidative lipid peroxidation in canine brain homogenates. Some of these were approximately equivalent in activity to α-tocopherol as a potent antioxidant with IC_{50} values of low micromolar order, and the 4-hydroxyphenyl derivative **11** showed the most potent antioxidative activity with an IC_{50} value of 0.32 μM, exhibiting approximately 5-fold more potent activity than α-tocopherol. The structure–activity relationship (SAR) studies of the antioxidative activity of these derivatives are presented.

Although much research has been focused on the etiology and pathogenesis of the progressive neurodegenerative disorders[1–4] such as Alzheimer's disease,[5] Huntington's disease,[6] Parkinson's disease,[7–10] and amyotrophic lateral sclerosis,[11] these diseases have not been elucidated clearly. In the last two decades, it has been confirmed that oxidative stress in the central nervous system (CNS) plays an important role in the pathogenesis and progression of these neurodegenerative disorders, and also increases other risk factors for these diseases.[1–4] The brain is considered to be vulnerable to oxidative stress, because a high concentration of polyunsaturated lipids in the brain can serve as substrates for lipid peroxidation.[3] Thus, the generation and subsequent chain reaction of reactive oxygen species such as the hydroxy radical, super oxide radical, and nitric oxide, induce irreversible damage to neuronal cell membranes through extensive lipid peroxidation, leading to the death of neuronal cells.[1,2]

Parkinson's disease (PD), characterized by motor dysfunction with tremor, akinesia, rigidity, and postural instability, is one of the best-known neurodegenerative disorders. This disease is believed to be caused by the depletion of dopamine (DA) due to neurodegeneration in nigrostriatal dopaminergic neurons.[12,13] Recently there has been a focus of interest on the use of antioxidants such as α-tocopherol (Fig. 1) for the suppression of neurodegeneration in nigrostriatal dopaminergic neurons, and the clinical trials of α-tocopherol for the treatment of PD have been performed.[14] Moreover, the antioxidative activities of conventional medications for PD, such as bromocriptine[15] and lazabemide[16] (Fig. 1), have been widely studied for an additional action, which may prevent or delay the neuronal death in dopaminergic nerves.

Keywords: Diphenylalkyl piperazine derivative; Antioxidative activity; Dopamine transporter affinity; Structure–activity relationship.
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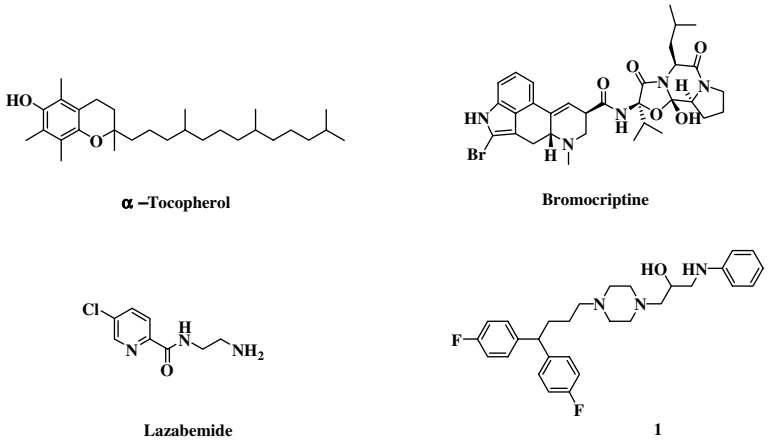
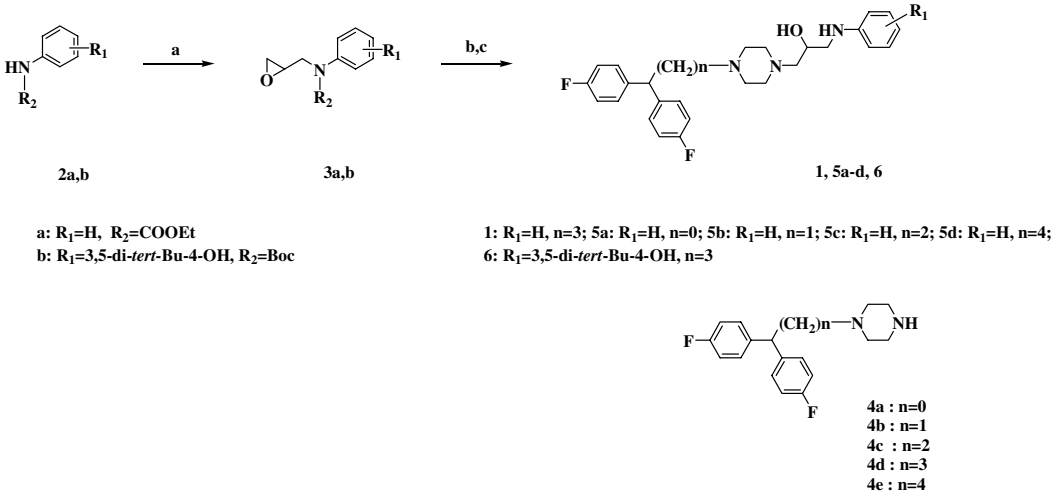


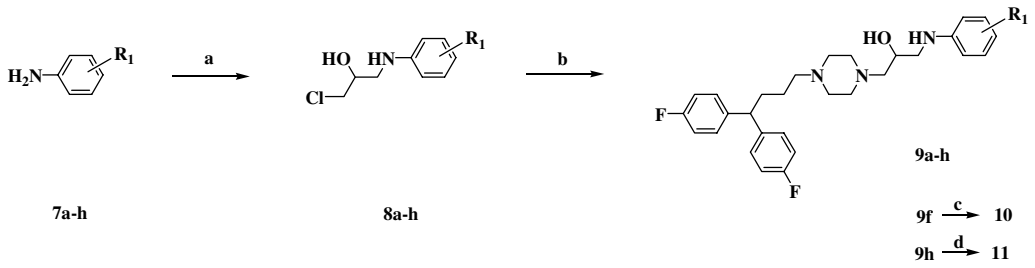
Figure 1.

In our previous study of novel diphenyl piperazine derivatives, we have found that a series of compounds represented by 1-[4,4-bis(4-fluorophenyl)butyl]-4-[2-hydroxy-3-(phenylamino)propyl]piperazine **1** shows potent DAT binding affinities on rat striatal membranes.[17,18] Moreover, from a pharmacological study using in vivo brain microdialysis, compound **1** has been found to display significant and dose-dependent increases of rat striatal extracellular DA levels.[17] These findings have suggested that these novel diphenyl piperazine derivatives are potent DA uptake inhibitors in the CNS, and may prove effective for the treatment of CNS disorders such as PD, which are caused by the depletion of DA or related to the DAT. On the other hand, compound **1** and its analogues, which were modified at the β-amino-2-propanol moiety in **1** have also been found to possess antioxidative activities against auto-oxidative lipid peroxidation in canine brain homogenates.[19] Therefore, to study further antioxidative activity of these novel diphenylalkyl piperazine derivatives as an additional action for more effective treatment of PD, we evaluated them modified at the diphenylalkyl moiety and the phenyl ring in the phenylamino moiety of **1** and examined their SARs.

As described in our previous studies of these novel diphenyl piperazine derivatives, we have synthesized a series of diphenylalkyl piperazine derivatives modified at the diphenylalkyl moiety and the phenyl ring in the phenylamino moiety of **1**. Herein, we outline their synthetic routes. The synthetic method of these novel diphenylalkyl piperazine derivatives is divided into two routes, as shown in Schemes 1 and 2. Alkylation of the *N*-protected aniline derivatives **2a** and **2b** with epibromohydrin using sodium hydride in *N*,*N*-dimethylformamide gave the epoxides **3a** and **3b**. The ring opening of the epoxides **3a** and **3b** with the diphenylalkyl piperazines **4a**–**e** in ethanol, followed by deprotection of the *N*-protective group in the resultant intermediates, gave the diphenylalkyl piperazine derivatives **1**, **5a**–**d**, and **6** (Scheme 1). Alkylation of the diphenylbutyl piperazine **4d** with the chlorohydrin derivatives **8a**–**h** prepared from the corresponding aniline derivatives **7a**–**h** and epichlorohydrin gave the



Scheme 1. Reagents and conditions: (a) NaH, epibromohydrin, DMF, room temperature, 81% for **3a**,**3b** was used for the next reaction without purification; (b) diphenylalkyl piperazine (**4a**–**e**), EtOH, room temperature, 28–100%; (c) NaOH(aq), EtOH, reflux, for **1** and **5a**–**d**, 53–95%, and HCl, AcOEt, room temperature, for **6**, **6** was converted to its trimaleate without purification.



a:R_1=3,4-diCl, b:R_1=4-Cl, c:R_1=4-Me, d:R_1=4-OMe, e:R_1=4-N(CH_3)$_2$, f:R_1=4-NO_2, g:R_1=3,4,5-triOMe, h:R_1=4-OBn, 10:R_1=4-NH_2, 11:R_1=4-OH

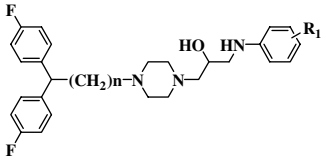
Scheme 2. Reagents and conditions: (a) epichlorohydrin, EtOH, reflux, 42–72%; (b) **4d**, K_2CO_3, KI, EtOH, reflux, 31–78%; (c) $SnCl_2$, EtOH, heat, 81%; (d) 10% Pd-black, HCOOH, MeOH, room temperature, 95%.

compounds **9a**–**h** with various substituents on the phenyl ring in the phenylamino moiety of **1** (Scheme 2). Reduction of the 4-nitro derivative **9f** with tin(II) chloride gave a 4-amino derivative **10** (Scheme 2). Deprotection of the benzyl group in the 4-benzyloxy derivative **9h** using palladium catalyst gave a 4-hydroxy derivative **11** (Scheme 2).

All of the final compounds synthesized as described above were used as their corresponding salts listed in Table 1 for their inhibitory activities against auto-oxidative lipid peroxidation in canine brain homogenates.[20] The results are shown in Table 1.

At first, the compounds **5a**–**d**, which were modified at the connective between the diphenylmethyl and piperazine moieties in compound **1**, were evaluated for their antioxidative activities, as well as **1**. All of them showed apparent antioxidative activities with IC_{50} values of approximately micromolar order, showing that the most potent compound **1** among them displayed slightly less inhibitory activity than α-tocopherol. The difference in the antioxidative activity between these five compounds was less than 2-fold, and these results suggest that the modification at the connective between the diphenylmethyl and piperazine moieties has little influence on the antioxidative activity.

Table 1. Antioxidative activities of diphenylalkyl piperazine derivatives



Compounds	*n*	R_1	Salt[a]	IC_{50} (μM)[b]
5a	0	H	A	6.9
5b	1	H	A	11.0
5c	2	H	A	11.0
1	3	H	A	6.5
5d	4	H	A	8.3
9a	3	3,4-diCl	C	23.0
9b	3	4-Cl	B	8.5
9c	3	4-Me	A	6.1
9d	3	4-OMe	A	1.6
9e	3	4-N(CH_3)$_2$	B	2.2
9f	3	4-NO_2	C	182.0
9g	3	3,4,5-triOMe	B	1.4
10	3	4-NH_2	B	2.7
11	3	4-OH	B	0.32
6	3	3,5-di-*tert*-Bu-4-OH	B	2.8
α-Tocopherol				1.5

[a] A: trihydrochloride; B: trimaleate; C: dihydrochloride.
[b] See Ref. 21.

Next, the compounds modified at the phenyl ring in the phenylamino moiety of **1** were evaluated for their antioxidative activities. Since the Topliss method[22] has often been applied to modification of the substituent of the phenyl ring in optimization strategies, we attempted to use this method in order to investigate the phenyl ring substituent effects. According to the Topliss method, four compounds, 3,4-dichloro **9a**, 4-chloro **9b**, 4-methyl **9c**, and 4-methoxy **9d** derivatives, excluding the unsubstituted compound **1** described above, were selected and evaluated. These four derivatives showed potent antioxidative activities. As compared with the unsubstituted compound **1**, the introduction of the methoxy group **9d** led to a 4-fold increased activity, whereas the introduction of dichlorine atoms **9a** exhibited a 3.5-fold less activity. Of the above compounds, the 4-methoxy derivative **9d** showed the most potent antioxidative activity, with an IC_{50} value of 1.6 μM, which was approximately equipotent in activity to α-tocopherol. The ranked order in the antioxidative activity of these five compounds including **1**, was : **9d** > **9c** ≧ **1** ≧ **9b** > **9a**. When these results are applied to the Topliss method, it is suggested that a contribution of the $-\sigma$ parameter, which is closely related to the electron-donating property of a substituent on the phenyl ring, is dominant in the antioxidative activity of these diphenylalkyl piperazine derivatives.

These results encouraged us to make further investigation of the phenyl ring substituent effects according to the Topliss method, and the compounds possessing a substituent with a higher $-\sigma$ effect at the *para*-position of the phenyl ring were expected to increase the antioxidative activity. Three compounds possessing the 4-dimethylamino group **9e**, 4-amino group **10**, and 4-hydroxy group **11** on the phenyl ring were evaluated. As expected, they all exhibited notably potent

antioxidative activities. Surprisingly, the 4-hydroxy derivative **11** displayed the most potent activity, showing a 4.7-fold more potent activity than α-tocopherol, with an IC_{50} value of 0.32 μM. This may be due to its higher capacity as a hydrogen donor to form a stable radical, as well as the phenols known as antioxidants,[23,24] in addition to the $-\sigma$ effect. In contrast with the results described above, the nitro derivative **9f** having a high σ effect as an electron-withdrawing substituent decreased the antioxidative activity dramatically.

Subsequently, as another approach to the enhancement of antioxidative activity by modification of the phenyl ring, the compounds replaced by an antioxidant structure known in the literature[25,26] such as 3,4,5-tri-methoxy **9g** and 3,5-di-*tert*-butyl-4-hydroxy **6** derivatives, were also evaluated. Both compounds showed potent antioxidative activities with IC_{50} values of 1.4 and 2.8 μM, respectively, and the activity of the 3,4,5-trimethoxy derivative **9g** was comparable to that of α-tocopherol. These findings suggest that the compounds containing an antioxidant structure in the phenylamino moiety also show potent antioxidative activities.

Thus, the SAR studies of compounds modified at the diphenylalkyl moiety and the phenyl ring in the phenylamino moiety of **1** suggest that the phenylamino moiety is the essential pharmacophore for the antioxidative activity.

In conclusion, a series of novel diphenyl piperazine derivatives with high affinities for the DAT, which were modified at both of the diphenylalkyl moiety and the phenyl ring in the phenylamino moiety of compound **1**, was evaluated for antioxidative activity in canine brain homogenates. Most of them showed apparent antioxidative activities with IC_{50} values of approximately micromolar order, including some compounds with activity equal to or higher than α-tocopherol. Among them, the 4-hydroxyphenyl derivative **11** exhibited the most potent activity with an IC_{50} value of 0.32 μM, which was approximately 5-fold more potent than α-tocopherol. The SAR studies suggested that the modification at the site between the diphenylmethyl and piperazine moieties has no great influence on the antioxidative activity, and that the introduction of a substituent having a $-\sigma$ effect as an electronic property into the phenyl ring of the phenylamino moiety is more likely to increase the activity. Moreover, compound **11** was suggested to display the extraordinary antioxidative activity, probably due to the formation of a stable radical in addition to the $-\sigma$ effect. These results also suggest that these novel diphenylalkyl piperazine derivatives may serve as effective treatments for PD, with dual actions of dopamine uptake inhibition and antioxidative activity.

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