Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1947–1950

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Diphenylalkyl Piperazine Derivatives with Dual Calcium Antagonistic and Antioxidative Activities

Makoto Kimura,* Tomoko Masuda, Koji Yamada, Nobuo Kubota, Nobuyuki Kawakatsu, Masaki Mitani, Kenichi Kishii, Masato Inazu† and Takayuki Namiki*

Pharmaceutical R & D Laboratories, POLA Chemical Industries Inc., 560, Kashio-cho, Totsuka-ku, Yokohama, Kanagawa, 244-0812, Japan

Received 30 January 2002; accepted 6 May 2002

Abstract—Two types of novel diphenylalkyl piperazine derivatives containing the thio or aminopropanol moiety substituted by phenyl or benzyl group were synthesized, and evaluated for their calcium antagonistic and antioxidative activities. These compounds showed apparent inhibitions against KCl-induced contractions in isolated rat aorta. Among them, phenylamino compound **9** and benzylamino compound **13** also possessed potent inhibitory activities against auto-oxidative lipid peroxidations in canine brain homogenates. Two representative compounds **3a** and **9** were evaluated for their inhibitory activities against KCl-induced contractions in isolated canine arteries (basilar, coronary, mesenteric, and renal). Both compounds showed the most potent inhibitions to basilar artery.

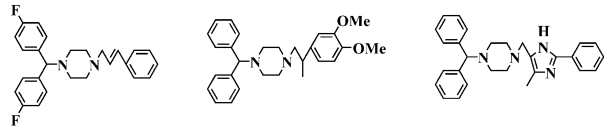
In spite of the therapeutic progress for cerebrovascular disease, stroke is one of the major causes of death in the developed countries, and has presented both health and economic problems. Until now, thrombolytic reagents[1] and neuroprotectants such as calcium antagonists,[2,3] NMDA receptor antagonists,[4] AMPA receptor antagonists,[5] 5-HT_{1A} agonists,[6] and antioxidants[7] have been used or developed in clinical trials for the treatment of patients with cerebral ischemia and traumatic injury, but their therapeutic estimations have not been established as yet.[8] The priority for the treatment of cerebral ischemia and traumatic injury is an improvement of lowered blood flow in ischemic and penumbra regions. However, after reperfusion of them, the destruction of the cell membrane and the death of nerve cells occur.[9] These are caused by a pathological increase of intracellular Ca^{2+} concentration due to a significant diminution of cellular energy reserves, following the failure of intracellular energy-dependent ion homeostasis.[10] The calcium overloading activates a large number of intracellular enzymes, which induce a marked increase in the generation of reactive oxygen species to cause irreversible damage to cell membrane through extensive lipid peroxidation.[11] The consequences of these enzyme perturbations and the production of reactive oxygen species induce widespread dysfunctions to lead to the death of nerve cells, including disruptions of neuronal membrane and cytoskeletal integrity, and damage to mitochondrial function.

Recently, it has been demonstrated that the use of a neuroprotectant acting on multiple target molecules at the same time or a combination therapy with neuroprotectant having different mechanisms of action for preventing cell death exhibits an obvious therapeutic effect in ischemic animal models.[12,13] For these findings, the neuroprotectant with multiple mechanisms of action is expected to provide a possibility of exhibiting the clinically significant efficacy. In pathological events of ischemic cascade described above, we took notice of calcium antagonistic and antioxidative activities to find a neuroprotectant having dual actions for the treatment of cerebral ischemia and traumatic injury. In this paper, we will describe the synthesis of two types of novel diphenylalkyl piperazine derivatives containing the thio and aminopropanol moiety substituted by phenyl or benzyl group, and their evaluation for calcium antagonistic and antioxidative activities.

*Corresponding authors. Tel.: +81-45-826-7264; fax: +81-45-826-7249; e-mail: m-kimura@pola.co.jp (M. Kimura); t-namiki@pola.co.jp (T. Namiki).

†Present address: Department of Pharmacology, and Intractable Disease Research Center, Tokyo Medical University, 6-1-1 Shinjyuku, Shinjyuku-ku, Tokyo, 160-8402, Japan.

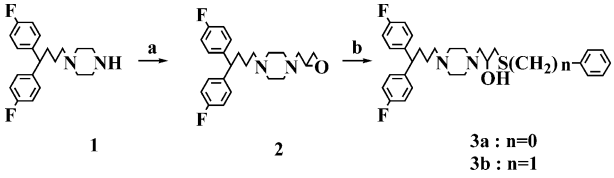
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00322-0



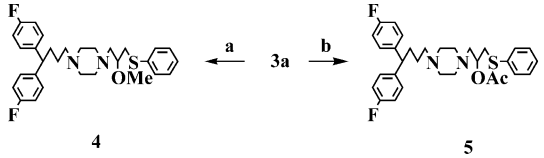
Flunarizine Tamolarizine Lifarizine

The diphenylmethyl piperazine derivatives including flunarizine,[14] tamolarizine,[15] and lifarizine[16] have been well-known as calcium antagonists with calcium overload inhibitory activity, and flunarizine has been reported to show an antioxidative activity in addition to them.[17] However, flunarizine has also been known to have a clinical risk of extrapyramidal side effects caused through the binding to D_2 receptor.[18] In order to increase the calcium antagonistic and antioxidative activities and avoid the side effect of flunarizine, we attempted to find new compounds having the diphenylalkyl piperazine moiety. And we used commercially available 1-[4,4-bis(4-fluorophenyl)butyl]piperazine in place of the diphenylmethyl piperazine moiety of flunarizine.

The syntheses of phenylthiopropanol derivatives **3a**, **4**, and **5** and benzylthiopropanol derivative **3b** are shown in Schemes 1 and 2. Alkylation of 1-[4,4-bis(4-fluorophenyl)butyl]piperazine **1** with epibromohydrin using tetrabutylammonium bromide in CH_2Cl_2 under alkaline conditions, followed by ring opening of epoxide **2** with the corresponding mercaptans in EtOH, afforded **3a** and **3b** (Scheme 1). Alkylation of **3a** with iodomethane in the presence of sodium hydride in *N,N*-dimethylformamide (DMF) gave a methoxide **4** (Scheme 2). Acetylation of **3a** with acetic anhydride in pyridine gave an acetate **5** (Scheme 2).

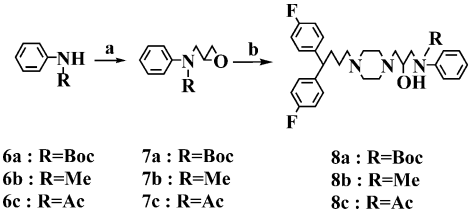


Scheme 1. Synthesis of **3a** and **3b**: (a) Bu_4NBr, NaOH (aq), epibromohydrin, CH_2Cl_2, **3a**: 65%, **3b**: 54%; (b) mercaptans, EtOH, 62%.

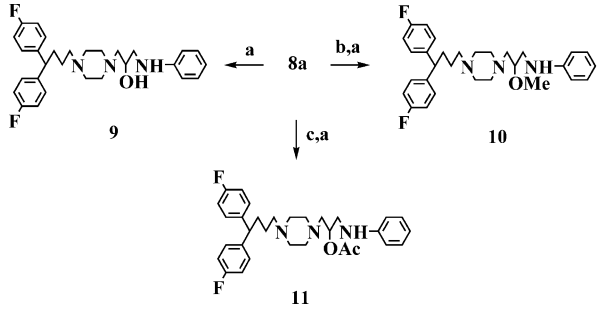


Scheme 2. Synthesis of **4** and **5**: (a) NaH, MeI, DMF, 38%; (b) Ac_2O, pyridine, 77%.

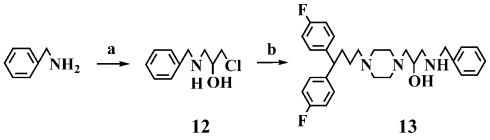
The syntheses of phenylaminopropanol derivatives **8b**, **8c**, **9**–**11**, and benzylaminopropanol derivative **13** are exemplified in Schemes 3–5. Alkylation of *N*-substituted aniline **6a**–**c** with epibromohydrin using sodium hydride as a base in DMF, followed by ring opening of the resulting epoxides **7a**–**c** with **1**, afforded *N*-substituted phenylamino compounds **8a**–**c** (Scheme 3). Deprotection of the *tert*-butoxycarbonyl (Boc) group in **8a** with HCl in EtOH gave **9** (Scheme 4). Alkylation of **8a** with iodomethane using sodium hydride in DMF, followed by deprotection of the Boc group with HCl in EtOH, afforded a methoxide **10** (Scheme 4). Acetylation of **8a** with acetic anhydride in pyridine, followed by deprotection of the Boc group, afforded an acetate **11** (Scheme 4). Ring opening of epichlorohydrin with benzylamine provided a chlorohydrin derivative **12**. Subsequently, alkylation of **1** with **12** using potassium carbonate and potassium iodide in EtOH gave the benzylamino derivative **13** (Scheme 5).



Scheme 3. Synthesis of **8a**–**c**: (a) NaH, epibromohydrin, DMF, **7a**–**c**, 90–100%; (b) **1**, EtOH, **8a**–**c**, 18–74%.



Scheme 4. Synthesis of **9**–**11**: (a) HCl/EtOH, **9**, 98%; (b) NaH, MeI, DMF, **10**, 29%; (c) Ac_2O, pyridine, **11**, 89%.



Scheme 5. Synthesis of **13**: (a) epichlorohydrin, EtOH, 46%; (b) **1**, K_2CO_3, KI, EtOH, 62%.

Two types of novel diphenylalkyl piperazine derivatives synthesized as described above were evaluated for the inhibitory activities against KCl-induced contractile responses in isolated rat aorta.[19] The contractile response by KCl has been considered to be associated with an influx of Ca^{2+} into vascular smooth muscle through voltage-dependent calcium channels. The results are shown in Tables 1 and 2. Both types of diphenylalkyl piperazine derivatives showed apparent inhibitions against KCl-induced contractions. A typical phenylthiopropanol derivative **3a** showed a more potent inhibition than flunarizine with PD'_2 value of 6.79. The

insertion of methylene group between sulfur atom and phenyl group **3b** showed similar potency to **3a**, indicating a 10-fold potency in relation to that of flunarizine. Since hydroxy group has often been important for the molecular recognition through the hydrophilic or hydrogen bonding interaction, the modifications of the hydroxy group were attempted. The modification of the hydroxy group with methoxy group **4** had equal efficacy and that with acetoxy group **5** showed a slightly decreased activity, as compared with **3a**. Concerning aminopropanol derivatives, phenylamino compound **9**, showed an obvious calcium antagonistic activity with PD'_2 value of 6.70, exhibiting efficacy similar to that of the phenylthio compound **3a**. The insertion of methylene group between nitrogen atom and phenyl group **13** considerably decreased the activity as compared with **9**, in contrast with benzylthio derivative **3b**, but it still maintained potent efficacy comparable to flunarizine. The compound **11** modified at the hydroxy group with acetoxy group was somewhat less potent than compound **9**. The introductions of methyl group **8b** and acetyl group **8c** into nitrogen atom in compound **9** apparently decreased the activities, but they also kept similar potency to flunarizine.

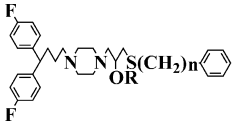


Table 1. Biological activities of phenylthio and benzylthiopropanol derivatives

Compd	*n*	R	Ca antagonistic activity[a] PD'_2	Antioxidative activity[b] IC_{50} (μM)
3a	0	H	6.79	15
3b	1	H	6.93	28
4	0	Me	6.69	20
5	0	Ac	6.55	20
Flunarizine			5.98	15
α-Tocopherol			—	1.5

[a]See ref 20.
[b]See ref 21.

Next, two types of diphenylalkyl piperazine derivatives were evaluated for the inhibitory activities against auto-oxidative lipid peroxidations in canine brain homogenates.[22] The results are shown in Tables 1 and 2. The phenylthio compound **3a** showed an apparent inhibition with IC_{50} value of 15 μM and it was equivalent in activity to flunarizine, but it was 10-fold less potent than α-tocopherol as a well-known antioxidant. The insertion of methylene group between sulfur atom and phenyl group **3b** resulted in a decrease of activity as compared with **3a** or flunarizine. The modifications of the hydroxy group with methoxy group **4** and acetoxy group **5** also showed slightly decreased potencies.

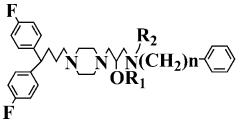


Table 2. Biological activities of phenylamino and benzylaminopropanol derivatives

Compd	*n*	R_1	R_2	Ca antagonistic activity[a] PD'_2	Antioxidative activity[b] IC_{50} (μM)
8b	0	H	Me	5.83	19
8c	0	H	Ac	5.98	390
9	0	H	H	6.70	6.5
10	0	Me	H	N.T.[c]	15
11	0	Ac	H	6.38	13
13	1	H	H	5.85	6.9
Flunarizine				5.98	15
α-Tocopherol				—	1.5

[a]See ref 20.
[b]See ref 21.
[c]N.T., not tested.

On the other hand, the phenylamino compound **9** showed a more potent inhibitory activity with IC_{50} value of 6.5 μM than flunarizine, although it was less potent than α-tocopherol. This finding prompted us to investigate further modifications at the 2-hydroxy-3-phenylaminopropyl moiety. The insertion of methylene group between nitrogen atom and phenyl group **13** showed a potent activity comparable to compound **9** on antioxidative activity. The modifications of the hydroxy group with methoxy group **10** and acetoxy group **11** decreased the activities as compared with compound **9**, but their activities were equipotent to that of flunarizine. The introduction of methyl group **8b** into nitrogen atom in compound **9** similarly decreased its activity, while that of acetyl group **8c** dramatically decreased it.

Thus, on both calcium antagonistic and antioxidative activities, the modifications of the thiopropanol moiety had a slight influence, whereas those of the aminopropanol moiety had an apparent influence.

As described above, we found that some diphenylalkyl piperazine derivatives containing the thio or aminopropanol moiety substituted by phenyl or benzyl group showed calcium antagonistic and antioxidative activities equal to or higher than flunarizine. These results suggest that the replacement of the diphenylmethyl moiety of flunarizine with the diphenylbutyl moiety and the introductions of heteroatoms such as sulfur or nitrogen atom and functional groups into the cinnamyl moiety of flunarizine can be allowed to exhibit both biological activities. In particular, it is suggested that the presence of nitrogen atom at the 3-position of the propanol moiety plays an important role in exhibiting a potent antioxidative activity.

In order to examine the selectivity to various arteries, two types of representative diphenylalkyl piperazine derivatives **3a** and **9** were evaluated for inhibitory activities against KCl-induced contractile responses in isolated canine arteries (basilar, coronary, mesenteric, and renal).[19] The results are shown in Table 3. The phenylthio compound **3a** showed potent inhibitions against all KCl-induced contractile responses in the isolated arteries. The PD'_2 values of **3a** were 7.00, 6.38,

Table 3. Vasodilatation effects of **3a** and **9**

Compd	PD'_2[a]			
	Basilar	Coronary	Mesenteric	Renal
3a	7.00	6.38	5.80	5.73
9	6.76	6.32	6.10	6.27

[a]See ref 20.

5.80, and 5.73, respectively, and the inhibitory activity to basilar artery was the most potent of those to four arteries. The phenylamino compound **9** similarly showed potent inhibitions, exhibiting the most potent inhibition to basilar artery with PD'_2 value of 6.76, but its selectivity was lower than that of **3a**.

In conclusion, we synthesized two types of novel diphenylalkyl piperazine derivatives containing the thio or aminopropanol moiety substituted by phenyl or benzyl group, and evaluated for their calcium antagonistic and antioxidative activities. Two compounds **9** and **13** possessed potent antioxidative activities along with potent calcium antagonistic activities. Two representative compounds **3a** and **9** were evaluated for inhibitory activities against KCl-induced contractile responses in isolated canine arteries (basilar, coronary, mesenteric, and renal). Both compounds **3a** and **9** showed the most potent inhibitions to basilar artery, although the selectivity of **9** was lower than that of **3a**. Further chemical modification and biological study of the diphenylalkyl piperazine derivatives containing the aminopropanol moiety have been continued to find a calcium antagonist with antioxidative activity.

References and Notes

1. Hacke, W.; Kaste, M.; Fieschi, C.; Toni, D.; Lesaffre, E.; Kummer, R. V.; Boysen, G.; Bluhmki, E.; Hoxter, G.; Mahagne, M. H.; Hennerici, M. *JAMA* **1995**, *274*, 1017.
2. Annoura, H.; Nakanishi, K.; Toba, T.; Takemoto, N.; Imajyo, S.; Miyajima, A.; Tamura-Horikawa, Y.; Tamura, S. *J. Med. Chem.* **2000**, *43*, 3372.
3. Hu, L. Y.; Ryder, T. R.; Rafferty, M. F.; Feng, M. R.; Lotarrski, S. M.; Lotarrski, D. M.; Rock, D. M.; Sinz, M.; Stoehr, S. J.; Taylor, C. P.; Weber, M. L.; Bowersox, S. S.; Miljanich, E. M.; Wang, Y. X.; Szoke, B. G. *J. Med. Chem.* **1999**, *42*, 4239.
4. Mueller, A. M.; Artman, L. D.; Balandrin, E. B.; Chien, Y. E.; Delmar, E. G.; Gerge, K.; Kierstead, A.; Marriott, T. B.; Moe, S. T.; Kewman, M. K.; Raszkiewicz, J. R.; Sanguinetti, E. L.; VanWagebnen, B. C. *Ann. N.Y. Acad. Sci.* **1999**, 450.
5. Kawasaki-Yatsugi, S.; Yatsugi, S.; Koshiya, K.; Shimizu-Sasamata, M. *Jpn. J. Pharmacol.* **1997**, *74*, 253.
6. Semkova, I.; Wolz, P.; Krieglstein, J. *Eur. J. Pharmacol.* **1998**, *359*, 251.
7. Hampson, A. J.; Grimalidi, M.; Lolic, M.; Wink, D.; Rosenthal, R.; Axelord, J. *Ann. N.Y. Acad. Sci.* **2000**, *899*, 274.
8. (a) Martinez-Vila, E.; Sieira, P. I. *Cerebrovasc. Dis.* **2001**, *11*, 60. (b) Hickenbottom, S. L.; Grotta, J. *Semin. Neuro.* **1998**, *18*, 485.
9. Farber, J. L. *Life Sci.* **1989**, *29*, 1289.
10. Kristian, T.; Siesjo, B. K. *Stroke* **1998**, *29*, 705.
11. Halliwell, B. *Drug Aging* **2001**, *18*, 685.
12. Stuiver, B. T.; Douma, B. R. K.; Bakker, R.; Nyakas, C.; Luiten, P. G. M. *Neurodegeneration* **1996**, *5*, 153.
13. Hicks, C. A.; Ward, M. A.; Swettenham, J. B.; O'Neill, M. J. *Eur. J. Pharmacol.* **1999**, *381*, 113.
14. Van Zwieeten, P. A. *Eur. Neurol.* **1986**, *25*, 57.
15. Shanklin, J.; Jonson, C.; Proakis, A. *J. Med. Chem.* **1991**, *34*, 3011.
16. Ito, C.; Im, W. B.; Takagi, H.; Takahashi, M.; Tsuzuki, K.; Liou, S.-Y.; Kunihara, M. *Eur. J. Pharmacol.* **1994**, 203.
17. Goncalves, T.; Carvalho, A. P.; Oliverira, C. R. *Eur. J. Pharmacol.* **1991**, *12*, 315.
18. Daniel, J. R.; Mauro, V. F. *Ann. Pharmacother.* **1995**, *29*, 73.
19. Sundberg, R. J.; Biswas, S.; Murthi, K. K.; Donna, R. *J. Med. Chem.* **1998**, *41*, 4317.
20. The evaluation of calcium antagonistic activity was carried out by the modification of the method described in ref 19. In brief, each smooth muscle of rat aorta cut into ring segment was mounted in organ bath filled with Krebs–Henseleit solution saturated with 95% O_2 and 5% CO_2 mixture. After the equilibration at 37 °C, the test compounds were added, subsequently KCl (10–60 mM) was added cumulatively, and the changes of isometric tension were recorded. Taking the contraction at 60 mM KCl as 100%, a concentration–response curve was drawn from 4–5 separate experiments. PD'_2 value was calculated as −log value of the concentration of the test compound required to inhibit the 60 mM KCl-induced contractions to 50%.
21. The evaluation of an antioxidative activity was carried out according to the method described in ref 22. A concentration–response curve was obtained from the three separate experiments and the IC_{50} value was calculated as the concentration of the test compound required to inhibit the auto-oxidative lipid peroxidations in canine brain homogenates by 50%.
22. Yao, K.; Ina, Y.; Nagashima, K.; Ohmori, K.; Ohno, T. *Biol. Pharm. Bull.* **2000**, *23*, 766.