Structure–Activity Relationships of Receptor Binding of 1,4-Dihydropyridine Derivatives

Daiki TAKAHASHI, Luvsandorj OYUNZUL,[a] Satomi ONOUE,[a] Yoshihiko ITO,[a] Shinya UCHIDA,[a] Rahime SIMSEK,[b] Miyase Gozde GUNDUZ,[b] Chiat SAFAK,[b] and Shizuo YAMADA*,[a]

[a] *Department of Pharmacokinetics and Pharmacodynamics and Global COE Program, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan: and* [b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University; Ankara 06532, Turkey.*
Received November 2, 2007; accepted December 18, 2007; published online December 20, 2007

The present study was undertaken to investigate binding activity of synthesized 1,4-dihydropyridine (1,4-DHP) derivatives (Compounds 1—124) to 1,4-DHP calcium channel antagonist receptors in rat brain. Sixteen 1,4-DHP derivatives inhibited specific (+)-[^{3}H]PN 200–110 binding in rat brain in a concentration-dependent manner with IC_{50} value of 0.43 to 3.49 μM. Scatchard analysis revealed that compounds 54, 69, 85, like nifedipine, caused a significant increase in apparent dissociation constant (K_d) for (+)-[^{3}H]PN 200–110, while compounds 68, 69 and 80 caused a significant decrease in maximal number of bindings sites (B_{max}). These data suggest that compounds 68, 69 and 80 exert longer-acting antagonistic effects of 1,4-DHP receptors than compounds 54, 69 and 85. The structure–activity relationship study has revealed that 1) ester groups in 3- and 5-positions are the most effective, 2) the aryl group in the 4-position of 1,4-DHP ring is the basic requirement for optimal activity, 3) position and type of electron-withdrawing groups on phenyl group at position 4 would affect the receptor-binding activity. Furthermore, compound 58 exerted α1 receptor binding activity, being 1.6 times greater than 1,4-DHP receptors. Compounds 81, 84, 91, 94, 106, 108 and 109 showed significant binding of ATP-sensitive potassium (K_{ATP}) channel, and the binding activities of compounds 81, 84, 108 and 109 were 1.6—3.8 times greater than the binding activity for 1,4-DHP receptors. Compounds 91 and 106 had similar binding activity for K_{ATP} channel and 1,4-DHP receptors. In conclusion, the present study has shown that novel 1,4-DHP derivatives exert relatively high binding affinity to 1,4-DHP receptors and has revealed new aspect of structure–activity relationships of 1,4-DHP derivatives, especially hexahydroquinoline derivatives.

Key words 1,4-dihydropyridine; hexahydroquinoline derivative; structure–activity relationship; L-type calcium channel antagonist receptor

Calcium channel antagonists inhibit muscle contraction by blocking the influx of Ca^{2+} through the L-type calcium channels and they have been used in the treatment of angina pectoris and systemic hypertension.[1—4] Although several types of compounds act as calcium channel blockers, 1,4-dihydropyridines (1,4-DHPs) are used as the most popular drug in this area. Nifedipine is the prototype drug of this group. A number of 1,4-DHP compounds were synthesized by modifying the nifedipine molecule. Some of 1,4-DHPs clinically used has exhibited the blocking activity not only at L-type calcium channels but also at T- and N-type calcium channels.[5—8] These drugs may reduce the side effect of reflex tachycardia. Similarly, 1,4-DHP derivatives have reported to show the activity at ion channels and G-protein-coupled receptors.[9]

Most of 1,4-DHPs clinically used have an aromatic ring in the 4-position and ester groups in the 3- and 5-positions of the 1,4-DHP ring. However, many active compounds have also been obtained by the introduction of the 1,4-DHP moiety to the condensed ring systems such as hexahydroquinoline and furoquinoline.[10—14] In these compounds, the orientation of the carbonyl group of the ester substituent at the 5-position in the 1,4-DHP ring of nifedipine has been fixed. The pharmacologically active antagonists have an aromatic ring in the 4-position of the 1,4-DHP ring restricting the aromatic ring to the 1,4-DHP vertical plane and flattening the 1,4-DHP ring. Previously we have synthesized hexahydroquinoline derivatives and evaluated their antagonistic effects on KCl- or $BaCl_2$-induced contraction of rat and guinea-pig smooth muscles.[10—15] Radioreceptor binding assay is a very reliable method to evaluate directly the receptor binding activity which reflects the pharmacological activity.[16—18] The aim of the current study is to investigate the structure–activity relationships of newly sunthesized 1,4-DHP derivatives in binding activities of 1,4-DHP calcium channel antagonist receptors in the rat brain. In addition, we investigated the binding activities of these derivatives to other receptors such as muscarinic receptors, (1-adrenoceptors, purinergic receptors, ATP-sensitive potassium (K_{ATP}) channels and nicotinic receptors.

MATERIALS AND METHODS

Materials [2,8-^{3}H]α,β-Methylene ATP tetrasodium salt ([^{3}H]α,β-MeATP, 0.581 TBq/mmol), [*N*-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS, 3.03 TBq/mmol), [7-methoxy-^{3}H]prazosin ([^{3}H]prazosin, 3.21 TBq/mmol), [cyclohexyl-2,3-^{3}H(*N*)]glibenclamide ([^{3}H]glibenclamide, 1.56 TBq/mmol), (+)-[5-methyl-^{3}H]PN 200–110 ((+)-[^{3}H]PN 200–110, 2.71—3.03 TBq/mmol) and [5,6-bicycloheptyl-^{3}H](±)-epibatidine ([^{3}H]epibatidine, 2.05 TBq/mmol) were purchased from Perkin-Elmer Life Sciences, Inc. (Boston, MA, U.S.A.). Some 1,4-DHP derivatives (Compounds **1**—**124**, Table 1) were synthesized by the modified before.[10—15,19] All other chemicals were purchased from Merck and Aldrich.

Animals Sprague-Dawley and Wistar rats (SLC, Shizuoka, Japan) were used in this study. Rats were housed with a 12 h light–dark cycle and fed laboratory food and

* To whom correspondence should be addressed. e-mail: yamada@ys7.u-shizuoka-ken.ac.jp

water *ad libitum*.

Tissue Preparation and Radioreceptor Binding Assay The radioligand binding assays for muscarinic receptors, α_1-adrenoceptors, 1,4-DHP receptors, purinergic receptors, K_{ATP} channels and nicotinic receptors were performed using [^{3}H]NMS,[20] [^{3}H]prazosin,[17] (+)-[^{3}H]PN 200–110,[18] [^{3}H]$\alpha\beta$-MeATP,[21] [^{3}H]glibenclamide[22] and [^{3}H]epibatidine[22,23], respectively.

Rats were killed by taking the blood from the descending aorta under temporary anesthesia with diethyl ether and the brain (except for cerebellum) was excised after perfused with cold saline from the aorta. For the binding assays of [^{3}H]NMS, [^{3}H]prazosin and (+)-[^{3}H]PN 200–110, rat brain was homogenized by a Kinematica Polytron homogenizer in 19 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, (EDTA), 2 μg/ml soybean trypsin inhibitor, 10 μg/ml bacitracin. The homogenate was then centrifuged at 40000×***g*** for 20 min at 4°C, and the suspension of the pellet was centrifuged again under the similar condition. The resulting pellet was finally suspended 19 volumes of 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$ for the binding assay.

For the binding assays of [^{3}H]α,β-MeATP and [^{3}H]glibenclamide, the brain was homogenized by the homogenizer in 10 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA, 2 μg/ml soybean trypsin inhibitor, 10 μg/ml bacitracin. The homogenate was then centrifuged at 2000×***g*** for 10 min at 4°C, and the supernatant was recentrifuged at 40000×***g*** for 20 min at 4°C. The pellet was suspended 10 volumes of above buffer and centrifuged at 40000×***g*** for 20 min at 4°C. The resulting pellet was finally suspended 4 volumes of 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$ for the binding assay.

For the [^{3}H]epibatidine assay, the brain was homogenized by the homogenizer in 19 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4). The homogenate was then centrifuged at 40000×***g*** for 10 min at 4°C, and the suspension of the pellet was centrifuged again under the similar condition. The resulting pellet was finally suspended 19 volumes of 50 mM Tris–HCl buffer (pH 7.4) for the binding assay.

In the case of [^{3}H]NMS binding assay, the brain homogenate (5 mg) was incubated with 0.1 nM [^{3}H]NMS in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$. Incubation was carried out for 60 min at 25°C. In the case of [^{3}H]prazosin binding assay, the brain homogenate (5 mg) was incubated with 0.1 nM [^{3}H]prazosin in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$. Incubation was carried out for 30 min at 25°C. In the case of (+)-[^{3}H]PN 200–110 binding assay, the brain homogenate (5 mg) was incubated with 0.5 nM (+)-[^{3}H]PN 200–110 in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$. Incubation was carried out in the dark with a sodium lamp for 60 min at 25°C. In the case of [^{3}H]$\alpha\beta$-MeATP binding assay, the brain homogenate (50 mg) was incubated with 0.1 nM [^{3}H]$\alpha\beta$-MeATP in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$. Incubation was carried out for 120 min at 4°C. In the case of [^{3}H]glibenclamide binding assay, the brain homogenate (50 mg) was incubated with 0.1 nM [^{3}H]glibenclamide in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4) containing 3 mM $CaCl_2$. Incubation was carried out for 60 min at 25°C. In the case of [^{3}H]epibatidine binding assay, the brain homogenate (5 mg) was incubated with 0.5 nM [^{3}H]epibatidine in total volume of 0.5 ml in 50 mM Tris–HCl buffer (pH 7.4). Incubation was carried out for 60 min at room temperature. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD, U.S.A.) through Whatman GF/B glass fiber filters, and the filters were then rinsed three times with 3 ml of ice-cold 50 mM Na/K^+ phosphate buffer. Pretreatment of the filters with 20 mM $Na_4P_2O_7$ and 0.5% polyethylenimine served to eliminate binding of [^{3}H]$\alpha\beta$-MeATP and [^{3}H]epibatidine to the filters. Tissue-bound radioactivity was extracted from the filters by placing them overnight by immersion in scintillation fluid (2 l toluene, 1 l Triton X-100, 15 g 2,5-diphenyloxazole, 0.3 g 1,4-bis[2-(5-phenyloxazolyl)]benzene), and radioactivity was determined by a liquid scintillation counter. Specific binding of [^{3}H]NMS, [^{3}H]prazosin, (+)-[^{3}H]PN 200–110, [^{3}H]$\alpha\beta$-MeATP, [^{3}H]glibenclamide and [^{3}H]epibatidine was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine, 100 μM phentolamine, 1 μM nifedipine, 3 μM $\alpha\beta$-MeATP, 1 μM glibenclamide and 300 μM (−)-nicotine, respectively. The (+)-[^{3}H]PN 200–110 assay was performed in the dark with a sodium lamp. All assays were conducted in duplicate. Protein concentrations were measured by the method of Lowry *et al.*[25]

Data Analysis Analysis of radioligand binding data was performed as described.[16] The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for each radioligand were estimated by Rosenthal analysis of the saturation data of (+)-[^{3}H]PN 200–110 (0.025—1.0 nM). The ability of each agent to inhibit specific binding of radioligand was estimated from the IC_{50} values, namely the molar concentration of this agent necessary to displace 50% of the specific binding of radioligand (determined by log probit analysis).

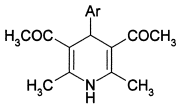
Statistical analysis of the data was performed by a one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparison. The data were expressed as mean±S.E. Statistical significance was accepted at $p<0.05$.

RESULTS

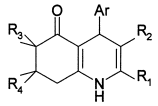
Binding Activities for 1,4-DHP Receptor, Muscarinic Receptor, α_1-Adrenoceptor, Purinergic Receptor, ATP-Sensitive Potassium Channel and Nicotinic Receptor The inhibitory effects (% of inhibition) of novel 1,4-DHP derivatives (compounds **1**—**124**, Table 1) on specific binding of (+)-[^{3}H]PN 200–110, [^{3}H]NMS, [^{3}H]prazosin, [^{3}H]$\alpha\beta$-MeATP, [^{3}H]glibenclamide and [^{3}H]epibatidine were examined. Nineteen derivatives (compounds **1**, **48**, **49**, **50**, **54**, **59**, **60**, **66**, **68**, **69**, **80**, **83**, **85**, **88**, **93**, **94**, **107**, **115**, **119**) at the concentration of 10 μM inhibited specific (+)-[^{3}H]PN 200–110 binding in the rat brain by more than 80%. In subsequent quantitative experiment, these compounds inhibited brain (+)-[^{3}H]PN 200–110 binding in a concentration-dependent manner (Fig. 1). The IC_{50} values of these 1,4-DHP derivatives were 0.43—3.49 μM which were higher than that of nifedipine (Table 2). The Hill coefficients of these compounds were not significantly different from unity.

The kinetic analysis was performed by examining the

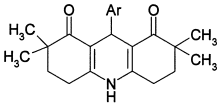
Table 1. Chemical Btructures of Synthesized 1,4-DHP Derivatives (**1**, **2**: 2,6-Dimethyl-3,5-diacetyl-4-aryl-1,4-DHP; **3**—**121**: 2,3,6,7-Substituted-5-oxo-1,4,5,6,7,8-hexahydroquinoline; **122**—**124**: 2,2,7,7-Tetramethyl-9-aryl-2,3,4,5,6,7,9,10-octahydro-1,8-acridinedione) and Their Inhibition Rates (%) (Each: 10 μM) of Specific Binding of (+)-[^{3}H]PN 200–110 (1,4-DHP), [^{3}H]NMS (Muscarinic), [^{3}H]Prazosin (α_1), [^{3}H]Glibenclamide (K_{ATP}), [^{3}H]α,β-MeATP (P2X) and [+H]Epibatidine (Nicotinic) in Rat Brain



1, 2



3 - 121



122 - 124

Compound	R_1	R_2	R_3	R_4	Ar	% inhibition of specific radioligand binding					
						1,4-DHP	Muscarinic	α_1	K_{ATP}	P2X	Nicotinic
1	–	–	–	–	2-Fluorophenyl	79.3	3.2	4.0	7.8	0.1	3.8
2	–	–	–	–	3-Pyridyl	1.5	3.3	0	2.2	0	0.6
3	CH_3	$COCH_3$	H, H	H, H	2-Chlorophenyl	26.4	1.5	3.8	10.2	4.9	4.0
4	CH_3	$COCH_3$	H, H	H, H	2,4-Dinitrophenyl	0	0	0	3.4	14.0	4.6
5	CH_3	$COCH_3$	H, H	H, H	4-Pyridyl	3.8	2.4	0	7.9	1.7	0
6	CH_3	$COCH_3$	H, H	H, H	2-Thienyl	6.5	0	1.0	4.9	2.6	0
7	CH_3	$COCH_3$	CH_3, CH_3	H, H	2-Chlorophenyl	11.7	2.3	0	2.6	3.5	0
8	CH_3	$COCH_3$	CH_3, CH_3	H, H	3-Chlorophenyl	12.5	2.4	0	2.0	8.1	0
9	CH_3	$COCH_3$	CH_3, CH_3	H, H	4-Chlorophenyl	0.1	3.3	0	9.7	2.6	0
10	CH_3	$COCH_3$	CH_3, CH_3	H, H	4-Bromophenyl	0	0	2.5	4.9	0	0
12	CH_3	$COCH_3$	CH_3, CH_3	H, H	3-Fluorophenyl	27.0	0	0	3.3	0	3.2
13	CH_3	$COCH_3$	CH_3, CH_3	H, H	4-Fluorophenyl	2.4	0	8.7	9.2	4.4	4.4
15	CH_3	$COCH_3$	CH_3, CH_3,	H, H	3-Furyl	2.0	0	1.5	0.3	1.5	0
16	CH_3	$COCH_3$	CH_3, CH_3	H, H	3-Methylphenyl	4.9	0	0	5.3	0	2.3
17	CH_3	$COCH_3$	CH_3, CH_3	H, H	4-Methylphenyl	0.5	0	1.9	4.0	0	0
18	CH_3	$COCH_3$	CH_3, CH_3	H, H	3-Methoxyphenyl	4.2	0	1.1	5.5	0	0
19	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Methylphenyl	79.2	0	1.7	1.3	0.9	0
20	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Methylphenyl	17.2	0	0	7.8	0	0
21	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Methoxyphenyl	6.8	0	5.2	0	4.8	0
22	CH_3	$COOCH_3$	CH_3, CH_3	H, H	4-Methylphenyl	0	0	0	3.4	0	1.8
23	CH_3	$COOCH_3$	CH_3, CH_3	H, H	4-Methoxyphenyl	0	0	0	0	0.7	0
24	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3,4-Methylenedioxyphenyl	0	0	0	5.9	4.9	1.2
25	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Methylphenyl	36.1	0	1.3	0	1.4	2.0
26	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Methoxyphenyl	52.0	0	0	3.2	0	0
27	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Methylphenyl	27.8	0	1.2	8.4	4.4	2.9
28	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Methoxyphenyl	62.7	0	0	9.9	10.5	6.3
29	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	4-Methylpheny	0	0	0	16.6	8.8	2.2
30	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	4-Methoxyphenyl	3.0	0	4.7	5.2	5.3	0
31	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3,4-Methylenedioxyphenyl	1.9	0	7.3	23.6	7.2	4.5
32	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	Phenyl	10.6	0	3.3	10.3	4.4	0
33	CH_2CH_3	$COOCH_3$	CH_3, CH_3,	H, H	2-Bromophenyl	42.8	0	0	3.2	8.6	0
34	CH_2CH_3	$COOCH_3$	CH_3, CH_3,	H, H	4-Methoxyphenyl	0	0	0	4.7	4.6	0
35	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Bromophenyl	32.1	0	12.8	16.3	10.3	1.6
36	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Trifluoromethylphenyl	8.7	2.4	1.1	24.5	11.1	5.3
37	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	4-Bromophenyl	4.9	0.9	16.4	21.3	8.4	2.6
38	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	4-Chlorophenyl	3.3	0.5	3.8	28.8	13.1	5.8
39	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	2,3-Dichlorophenyl	23.6	0	0	6.0	9.3	1.3
40	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	2,4-Dichlorophenyl	10.5	0	0	12.1	13.4	2.4
41	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	3,4-Dichlorophenyl	0	0	0	0.5	14.7	0
42	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Pyridyl	0	0	0	0	11.3	4.0
43	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Pyridyl	0	0	0	1.9	9.8	0.8
44	CH_2CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Chlorophenyl	16.1	0	0	11.2	8.0	1.6
45	CH_3	$COOCH_3$	CH_3, CH_3	H, H	Phenyl	37.0	0	0	9.0	4.9	0
46	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Bromophenyl	70.1	0	0	0	0	5.0
47	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Chlorophenyl	62.8	0	0	0.2	0	4.4
48	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Fluorophenyl	90.4	0.5	5.6	6.9	7.5	4.3
49	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Nitrophenyl	89.0	0	4.2	7.4	14.8	1.5
50	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Trifluoromethylphenyl	93.1	0	0.4	2.6	14.6	0.8
51	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2-Pyridyl	0.3	0.9	0.7	2.6	16.9	1.8
52	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Bromophenyl	68.2	0	7.7	17.9	21.8	0.3
53	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Fluorophenyl	66.0	0	2.0	1.1	15.0	0
54	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Nitrophenyl	100	0.9	4.7	21.1	10.7	0
55	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3-Pyridyl	2.8	0	4.0	2.8	0	0.3
56	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3,4-Dichlorophenyl	0	3.0	0	0	0	5.0
57	CH_3	$COOCH_3$	CH_3, CH_3	H, H	4-Acetylaminophenyl	0	3.3	2.9	1.4	4.5	5.2
58	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	Phenyl	39.3	0.7	64.0	1.7	8.1	3.0
59	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Bromophenyl	94.7	0	3.9	6.0	11.2	3.7

Table 1. Continued

Compound	R_1	R_2	R_3	R_4	Ar	% inhibition of specific radioligand binding					
						1,4-DHP	Muscarinic	α_1	K_{ATP}	P2X	Nicotinic
60	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Chlorophenyl	92.6	2.1	7.8	7.3	17.6	3.4
61	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Fluorophenyl	78.0	0.2	6.0	4.4	8.9	2.4
62	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Nitrophenyl	56.0	0	0.7	5.0	8.0	2.9
63	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2-Pyridyl	25.1	0	2.1	1.0	0	5.9
65	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Bromophenyl	73.4	0.1	2.5	3.1	0.8	11.7
66	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Chlorophenyl	83.7	6.1	0	2.1	8.7	11.4
67	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Fluorophenyl	45.5	1.4	5.3	0	8.9	0
68	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Nitrophenyl	98.0	0.6	10.3	20.3	11.2	0
69	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Trifluoromethylphenyl	99.6	0	3.5	0	8.7	0.9
70	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3-Pyridyl	15.6	0	2.3	0	3.6	8.3
71	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,3-Dichlorophenyl	78.0	0	0	0	1.4	0
72	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,4-Dichlorophenyl	16.9	0.1	0	0	0	0
73	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,6-Dichlorophenyl	59.1	0.7	0	0	2.5	1.3
74	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	4-Acetylaminophenyl	1.6	1.2	0	0	4.0	5.9
75	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	2,4-Dichlorophenyl	0	0	0	0	11.2	7.4
76	CH_3	$COOCH_3$	CH_3, CH_3	H, H	3,4-Difluorophenyl	22.8	0	3.4	3.4	12.1	1.8
77	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2,5-Difluorophenyl	74.7	0	3.9	1.1	17.9	0
79	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2,4-Difluorophenyl	57.7	0	5.4	6.2	8.0	0
80	CH_3	$COOCH_3$	CH_3, CH_3	H, H	2.3-Difluorophenyl	85.1	0	0.1	3.0	5.9	0.8
81	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	3,4-Difluorophenyl	16.1	0	0	61.3	0	0
82	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,5-Difluorophenyl	50.0	0	0	15.4	0	0
83	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,6-Difluorophenyl	95.4	0	1.0	24.4	0	0
84	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,4-Difluorophenyl	29.7	0	0	48.5	0	0
85	CH_3	$COOC_2H_5$	CH_3, CH_3	H, H	2,3-Difluorophenyl	98.3	0	0	16.7	0	0
86	CH_3	$COOCH_3$	H, H	CH_3, CH_3	3,4-Difluorophenyl	23.7	0	0.2	0	15.3	0
87	CH_3	$COOCH_3$	H, H	CH_3, CH_3	2,5-Difluorophenyl	31.8	0	2.6	0	18.2	0
88	CH_3	$COOCH_3$	H, H	CH_3, CH_3	2,6-Difluorophenyl	96.9	0	0	0	13.3	0.7
89	CH_3	$COOCH_3$	H, H	CH_3, CH_3	2,4-Difluorophenyl	21.5	0.7	0	0	14.5	0
90	CH_3	$COOCH_3$	H, H	CH_3, CH_3	2,3-Difluorophenyl	75.9	1.4	0	16.1	0	0
91	CH_3	$COOC_2H_5$	H, H	CH_3, CH_3	3,4-Difluorophenyl	73.5	1.6	0	73.2	0	0
92	CH_3	$COOC_2H_5$	H, H	CH_3, CH_3	2,5-Difluorophenyl	49.7	0	0	13.8	0	0
93	CH_3	$COOC_2H_5$	H, H	CH_3, CH_3	2,6-Difluorophenyl	93.8	0	0	9.8	0	0
94	CH_3	$COOC_2H_5$	H, H	CH_3, CH_3	2,4-Difluorophenyl	89.3	0	0	49.8	0	0
95	CH_3	$COOC_2H_5$	H, H	CH_3, CH_3	2,3-Difluorophenyl	46.0	1.4	8.6	5.1	8.6	0
96	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	3,4-Difluorophenyl	0	0	0	0	0.2	0
97	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	2,5-Difluorophenyl	0	0	0.5	0	0	0.8
98	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	2,6-Difluorophenyl	0	0	0	0	0	1.5
99	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	2,4-Difluorophenyl	0	0	0.2	0	0	3.2
100	CH_3	$CON(C_2H_5)_2$	CH_3, CH_3	H, H	2,3-Difluorophenyl	0	0	0.2	0	0	0.1
101	CH_3	$CON(C_2H_5)_2$	H, H	CH_3, CH_3	3,4-Difluorophenyl	0	0	0.2	3.8	0	1.0
102	CH_3	$CON(C_2H_5)_2$	H, H	CH_3, CH_3	2,5-Difluorophenyl	0.4	0.5	0.2	0	0	0
103	CH_3	$CON(C_2H_5)_2$	H, H	CH_3, CH_3	2,6-Difluorophenyl	0	0	0.2	0	0	0
104	CH_3	$CON(C_2H_5)_2$	H, H	CH_3, CH_3	2,4-Difluorophenyl	0	0	3.2	10.2	6.7	5.2
105	CH_3	$CON(C_2H_5)_2$	H, H	CH_3, CH_3	2,3-Difluorophenyl	0.5	0	0	11.1	12.3	4.8
106	CH_3	$COOCH_3$	H, H	H, CH_3	2,3-Dichlorophenyl	65.1	0	0	63.6	14.7	0.8
107	CH_3	$COOC_2H_5$	H, H	H, CH_3	2,3-Dichlorophenyl	81.1	0	0	34.8	12.1	0
108	CH_3	$COOCH_3$	H, H	H, CH_3	2,4-Dichlorophenyl	31.6	0	0	56.0	15.5	0
109	CH_3	$COOC_2H_5$	H, H	H, CH_3	2,4-Dichlorophenyl	24.8	0	0	53.9	10.4	0
110	CH_3	$COOCH_3$	H, H	H, CH_3	2,5-Dichlorophenyl	47.0	0	1.8	24.8	10.0	0
111	CH_3	$COOC_2H_5$	H, H	H, CH_3	2,5-Dichlorophenyl	25.1	0	0	26.4	14.6	0
112	CH_3	$COOCH_3$	H, H	H, CH_3	2,6-Dichlorophenyl	65.4	0	0	3.0	7.5	0
113	CH_3	$COOC_2H_5$	H, H	H, CH_3	2,6-Dichlorophenyl	65.3	0	0.9	0	16.3	10.5
114	CH_3	$COOCH_3$	H, H	H, C_6H_5	2,3-Dichlorophenyl	62.7	0	41.7	22.0	18.3	7.1
115	CH_3	$COOC_2H_5$	H, H	H, C_6H_5	2,3-Dichlorophenyl	98.4	0	36.0	14.2	19.2	2.8
116	CH_3	$COOCH_3$	H, H	H, C_6H_5	2,4-Dichlorophenyl	35.8	0	13.1	12.4	19.5	0
117	CH_3	$COOC_2H_5$	H, H	H, C_6H_5	2,4-Dichlorophenyl	21.1	0	0	2.8	16.8	0
118	CH_3	$COOCH_3$	H, H	H, C_6H_5	2,5-Dichlorophenyl	61.0	0	18.6	4.3	12.8	0
119	CH_3	$COOC_2H_5$	H, H	H, C_6H_5	2,5-Dichlorophenyl	84.7	0	17.9	0	11.8	0
120	CH_3	$COOCH_3$	H, H	H, C_6H_5	2,6-Dichlorophenyl	38.1	0	6.4	0	27.0	0
121	CH_3	$COOC_2H_5$	H, H	H, C_6H_5	2,6-Dichlorophenyl	46.5	0	8.6	0.6	16.5	0
122	–	–	–	–	2-Bromophenyl	0	0.5	3.5	8.9	7.9	3.6
123	–	–	–	–	2,4-Dichlorophenyl	0	0.9	2.0	18.0	23.2	4.4
124	–	–	–	–	2-Chloro-5-nitrophenyl	0	0	0	10.9	12.9	3.4

Specific binding of (+)-[^{3}H]PN 200–110 (0.5 nM), [^{3}H]NMS (0.1 nM), [^{3}H]prazosin (0.1 nM), [^{3}H]glibencalmide (0.1 nM), [^{3}H]α,β-MeATP (0.1 nM) and [^{3}H]epibatidine (0.5 nM) in rat brain was determined in presence of different concentration of 1,4-DHP derivatives, nifedipine and nicardipine.Values are the mean of two to five determinations.

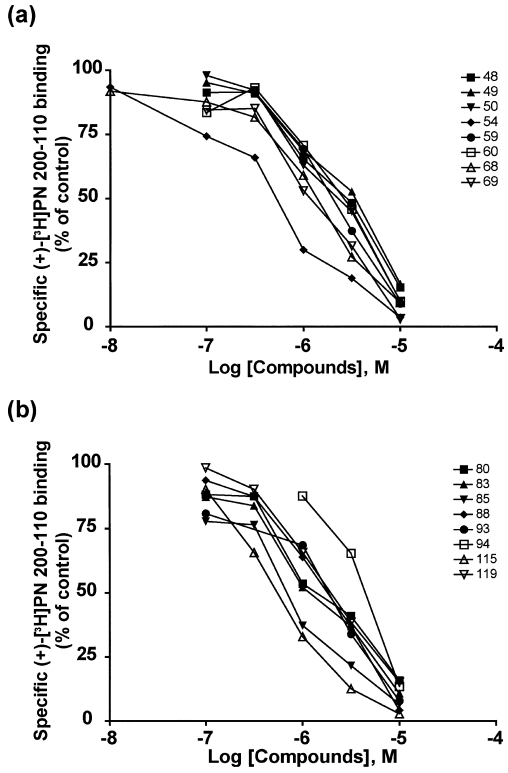


Fig. 1. Inhibition by Novel 1,4-DHP Derivatives ((a) Compounds **48**, **49**, **50**, **54**, **59**, **60**, **68** and **69**; (b) Compounds **80**, **83**, **85**, **88**, **93**, **94**, **115** and **119**, Table 1) of Specific Binding (+)-[^{3}H]PN 200–110 Binding in the Rat Brain

The ordinate is specific binding of (+)-[^{3}H]PN 200–110 (0.1 nM) (% of control). The abscissa is logarithmic molar concentration of each compound. Each point represents the average of two to four.

Table 2. IC_{50} Values and Hill Coefficients for Inhibition by 1,4-DHP Derivatives, Nifedipine and Nicardipine of Specific (+)-[^{3}H]PN 200–110 Binding in Rat Brain

No.	IC_{50} (μM)	Hill coefficient
48	2.11±0.26	1.11±0.14
49	2.73±0.48	1.01±0.07
50	1.89±0.19	1.29±0.04
54	0.43±0.09	0.86±0.03
59	2.02±0.30	1.42±0.11
60	1.84±0.27	1.16±0.16
68	1.12±0.03	0.90±0.16
69	1.13±0.15	1.05±0.05
80	1.58±0.27	0.94±0.10
83	1.31±0.38	0.89±0.08
85	0.65±0.15	0.94±0.12
88	1.52±0.42	1.29±0.06
93	1.55±0.35	1.20±0.27
94	3.49±0.31	1.27±0.10
115	0.59±0.07	1.23±0.13
119	2.10±0.09	1.17±0.08
Nifedipine	0.004±0.00	0.89±0.09
Nicardipine	0.048±0.017	1.15±0.09

Specific (+)-[^{3}H]PN 200–110 (0.5 nM) binding in rat brain was determined in presence of different concentration of 1,4-DHP derivatives, nifedipine and nicardipine.Values are the mean±S.E. of three to five determinations.

seffects of these compounds (**50**, **54**, **68**, **69**, **80**, **85**) at concentrations around IC_{50} on binding parameters (K_d, B_{max}) of (+)-[^{3}H]PN 200–110. Scatchard analysis revealed that compounds **54** (0.5 μM), **69** (1.0 μM), **85** (0.5 μM) and nifedipine

Table 3. K_d and B_{max} for (+)-[^{3}H]PN 200–110 Binding in the Presence of Each 1,4-DHP Derivative (Compounds **50**, **54**, **68**, **69**, **80**, **85**) and Nifedipine In rat Brain

No.	K_d (nM)	B_{max} (fmol/mg protein)
Control	0.63±0.03 (1.0)	234±11 (1.0)
50 (2.0 μM)	1.18±0.13	213±10
54 (0.5 μM)	2.40±0.30 (3.81)*	292±33
68 (1.0 μM)	0.80±0.13	137±24 (0.59)*
69 (1.0 μM)	1.93±0.10 (3.06)*	115±11 (0.49)*
80 (1.5 μM)	0.80±0.11	81.0±8.4 (0.35)*
85 (0.5 μM)	1.76±0.21 (2.80)*	221±22
Nifedipine (5.0 nM)	2.34±0.50 (3.71)*	263±50

Specific (+)-[^{3}H]PN 200–110 (0.025—1.0 nM) binding in rat brain was determined in presence of concentration of IC_{50} value of each 1,4-DHP derivative and nifedipine. Values are mean±S.E. of three to five. Asterisk shows a significant difference from the values of control group, $*p<0.01$. The values in parenthesis are expressed as fold-increase (K_d) or as the value divided by control (B_{max}).

(5.0 nM) increased significantly (3.8, 3.1, 2.8, 3.7 times, respectively) K_d values for specific (+)-[^{3}H]PN 200–110 binding in the rat brain compared with the corresponding control value (Table 3). On the other hand, compounds **68** (1.0 μM), **69** (1.0 μM) and **80** (1.5 μM) decreased significantly (41, 51, 65%, respectively) B_{max} values for brain (+)-[^{3}H]PN 200–110 binding. Thus, only compound **69** decreased B_{max} with the increase of K_d.

As shown in Table 1, novel 1,4-DHP derivatives (10 μM) had little effect on specific binding of [^{3}H]NMS, [^{3}H]$\alpha\beta$-MeATP and [^{3}H]epibatidine in the rat brain. Compound **58** exhibited 64% inhibition of specific [^{3}H]prazosin binding, and compounds **81**, **84**, **91**, **94**, **106**, **108** and **109** (each 10 μM) showed 49—64% inhibition of specific [^{3}H]glibenclamide binding.

DISCUSSION

The present study was undertaken to investigate the binding activities of newly synthesized 1,4-DHP derivatives to 1,4-DHP receptors and other receptors in the rat brain with their structure–activity relationships. Although the cardiovascular tissues are clinically important targets of calcium channel antagonists, in this study, the brain tissue was used because of a similarity between brain and cardiovascular tissues of 1,4-DHP agents in the 1,4-DHP receptor binding affinity[18,26] and also because of the search for 1,4-DHP agents having a pharmacological selectivity on the central nervous system. Sixteen compounds among 124 derivatives (Tables 1, 2) inhibited significantly specific (+)-[^{3}H]PN 200–110 bindings in the rat brain with micromolar order of IC_{50} values. The kinetic analysis of radioligand binding parameters in the presence of antagonists by Scatchard analysis may provide the information about the mode of pharmacological action.[27] Scatchard analysis has revealed that compounds **54**, **69** and **85**, like nifedipine, caused a significant increase in K_d values for specific (+)-[^{3}H]PN 200–110 binding in rat brain, suggesting that these compounds bind to brain 1,4-DHP receptors in a competitive and reversible manner. On the other hand, compounds **68**, **69** and **80** caused a significant decrease of B_{max} values, possibly indicating the slowly dissociating blockade of 1,4-DHP receptor, as demonstrated previously in nonequilibrium blockade of brain nicotinic receptors by neo-

surugatoxin.[27] Inasmuch as the significant decreases in B_{max} in radioligand binding studies has been generally shown to indicate insurmountable antagonist,[27] it is anticipated that these compounds produce a sustained occupancy of 1,4-DHP receptors in the brain. In fact, our previous study has shown that preferential decrease of B_{max} value for (+)-[^{3}H]PN 200–100 binding in the rat heart after oral administration of mepirodipine may be due to the slow dissociation of this agent from Ca^{2+} antagonist receptors.[26]

Although compound **1** (10 μM), having 3,5-diacetyl-1,4-dihydro pyridine moiety, exhibited 79% inhibition of specific (+)-[^{3}H]PN 200–110 binding, nifedipine, having 3,5-dicarbomethoxy-1,4-dihydro pyridine moiety, showed more potent inhibitory effect on the (+)-[^{3}H]PN 200–110 binding (IC_{50}: 4.25±0.37 nM). In the previous chemical modification study on nifedipine, derivative with 3,5-diacetyl-1,4-dihydro pyridine moiety showed higher biological activities, however replacement of 3,5-dicarbmethoxy groups of nifedipine by 3,5-acetyl groups resulted in decrease of the pharmacological activity.[19,28] These observations are consistent with our presented data, and carbomethoxy groups at position 3 and/or 5 would be one of important pharmacophores for the receptor-binding activity. Compound **2** (10 μM), having pyridine moiety, had little 1,4-DHP receptor binding activity (Table 1), which agrees with the previous pharmacological data.[19] This suggested that phenyl group at the 4-position in the 1,4-DHP ring would play an important role in the receptor-binding.

In addition to monocyclic derivatives, receptor-binding activities of hexahydroquinoline derivatives (compounds **3** to **121**) were also evaluated. The substitutions of 2-pyridyl (compounds **42**, **51**, **63**) and 3-pyridyl (compounds **43**, **55**, **70**) at the 4-position exerted less binding activity to 1,4-DHP receptors compared with phenyl groups. Substitution of phenyl group also affected the binding activities. *Para*-substituted analogues had little inhibition of brain (+)-[^{3}H]PN 200–110 binding. On the contrary, ortho and/or *meta*-substituted analogues exhibited much higher activies in this study. These findings suggest that phenyl ring at position 4 with halogen or other electron-withdrawing groups would be essential for their receptor-binding activity. Further, 1,4-DHP analogues having 3-acetyl group (compounds **3**—**18**) were found to be less potent as compared to 3-carbomethoxy groups (compounds **19**—**24**, **45**—**57**, **76**—**80**), even though the phenyl groups at position 4 were halogenated. There appeared to be no significant differences in receptor-binding activities between 3-carbomethoxy derivatives (compounds **19**—**24**, **45**—**57**, **76**—**80**) and 3-carboethoxy derivatives (compounds **25**—**31**, **58**—**74**, **81**—**85**) in the 1,4-DHP ring. In addition, the compounds having diethylamide group at 3-position in 1,4-DHP group (compounds **75**, **96**—**105**) showed no binding activity, suggesting the essential role of ester groups at 3-position for the binding activities to 1,4-DHP receptors.

On the basis of the data obtained, we could provide some structure–activity relationships on 1,4-DHP derivatives as follows: 1) ester groups in 3- and 5-positions are the most effective, 2) the aryl group in the 4-position of 1,4-DHP ring is the basic requirement for optimal activity,[28—30] 3) position and type of electron-withdrawing groups on phenyl group at position 4 would affect the receptor-binding activity. Thus, previous structure–activity relationships of the 1,4-DHP derivatives having calcium antagonistic activity may adapt to that of these hexahydroquinoline derivatives.

Some arylacridine derivatives showed no or low binding activities to any receptors in this study. It was reported that compounds **123** and **124** induced concentration-dependent relaxation response of rat mesenteric arteries previously contracted with phenylephrine with pD_2 values (5.25, 5.13, respectively), the negative logarithm of the concentration at 50% of the maximum response.[15] Although some 1,4-DHP and its tricycle (acridine) analogues are also identified as potassium channel agonists, the biological activities of compounds tested in this study were not affected by glibenclamide and tetraethylammonium, which are K_{ATP} channel blocker and calcium-sensitive K^+ channel blocker, respectively. These findings indicated that the relaxant effect of these compounds might be mediated via activation of other receptors.

Although 1,4-DHP calcium antagonists inhibit the influx of Ca^{2+} through the L-type calcium channels, some 1,4-DHP derivatives were also demonstrated to exert binding activity at other ion channels and G-protein-coupled receptors. For example, ZM244085 [9-(3-cyanophenyl)hexahydro-1,8-acridinedione] is a bladder-selective agent designed as part of a search for new pathways to control urinary incontinence. This agent is an activator of K_{ATP} channels where they are more active than calcium.[31—33] SNAP 5540 is selective at the α_{1a}-adrenoceptor over α_{1b} and α_{1d} receptors and other ion channels and receptors.[34—36] In the preset study, compound **58** exhibited 64% inhibition of specific [^{3}H]prazosin binding, and compounds **81**, **84**, **91**, **94**, **106**, **108** and **109** (each 10 μM) showed 49—64% inhibition of specific [^{3}H]glibenclamide binding. Thus, the binding activity of compounds **81**, **84**, **108** and **109** for K_{ATP} channel was shown to be 1.6—3.8 times greater than that for 1,4-DHP receptors. Compounds **91** and **106** had similar binding activity for K_{ATP} channel and 1,4-DHP receptors. The binding activity of compound **58** to α_1 receptor was shown to be 1.6 times greater than that to 1,4-DHP receptors. Thus, it is plausible that the chemical modification of these 1,4-DHP derivatives leads to the development of dual receptor antagonists.

In conclusion, the present study has shown that novel 1,4-DHP derivatives exert relatively high binding affinity to 1,4-DHP receptors and has revealed new aspect of structure–activity relationships of 1,4-DHP derivatives, especially hexahydroquinoline derivatives.

REFERENCES

1) Janis R. A., Triggle D. J., *J. Med. Chem.*, **26**, 775—785 (1983).
2) Godfraind T., Miller R., Wibo M., *Pharmacol. Rev.*, **38**, 321—416 (1986).
3) Frishman W. H., Michaelson M. D., *Am. J. Cardiol.*, **79**, 33—38 (1997).
4) Vandergoten P., Benit E., Dendale P., *Acta Cardiol.*, **54**, 71—76 (1999).
5) Fujii S., Kameyama K., Hosono M., Hayashi Y., Kitamura K., *J. Pharmacol. Exp. Ther.*, **280**, 1184—1191 (1997).
6) Furukawa T., Nukada T., Suzuki K., Fujita Y., Mori Y., Nishimura M., Tamanaka M., *Br. J. Pharmacol.*, **122**, 1136—1140 (1997).
7) Uneyama H., Takahara A., Dohmoto H., Yoshimoto R., Inoue K., Akaike N., *Br. J. Pharmacol.*, **122**, 37—42 (1997).
8) Masumiya H., Tanaka H., Shigenobu K., *Eur. J. Pharmacol.*, **335**, 15—21 (1997).
9) Triggle D. J., *Cell. Mol. Neurobiol.*, **23**, 293—303 (2003).

10) Altas Y., Safak C., Batu O. S., Erol K., *Arzneim.-Forsch.*, **49**, 824—829 (1999).
11) Kismetli E., Safak C., Erol K., Sirmagul B., Linden A., *Arzneim.-Forsch*., **54**, 371—375 (2004).
12) Simsek R., Safak C., Erol K., Sirmagul B., *Pharmazie*, **56**, 665—666 (2001).
13) Simsek R., Safak C., Erol K., Ataman S., Ulgen M., Linden A., *Arzneim.-Forsch.*, **53**, 159—166 (2003).
14) Simsek R., Gunduz M. G., Sirmagul B., Safak C., Erol K., Linden A., *Arzneim.-Forsch.*, **56**, 529—534 (2006).
15) Simsek R., Ozkan M., Kismetli E., Uma S., Safak C., *Farmaco*, **59**, 939—943 (2004).
16) Yamada S., Yamamura H. I., Roeske W. R., *J. Pharmacol. Exp. Ther.*, **215**, 176—185 (1980).
17) Yamada S., Ashizawa N., Ushijima H., Nakayama K., Hayashi E., Honda K., *J. Pharmacol. Exp. Ther.*, **242**, 326—330 (1987).
18) Yamada S., Kimura R., Harada Y., Nakayama K., *J. Pharmacol. Exp. Ther.*, **252**, 327—332 (1990).
19) Safak C., Sahin I., Sunal R., *Arzneim.-Forsch.*, **40**, 119—122 (1990).
20) Ehlert F. J., Tran L. P., *J. Pharmacol. Exp. Ther.*, **255**, 1148—1157 (1990).
21) Bo X., Fischer B., Maillard M., Jacobson K. A., Burnstock G., *Br. J. Pharmacol.*, **112**, 1151—1159 (1994).
22) Challinor-Rogers J. L., Kong D. C., Iskander M. N., McPherson G. A., *J. Pharmacol. Exp. Ther.*, **273**, 778—786 (1995).
23) Houghtling R. A., Davila-Garcia M. I., Kellar K. J., *Mol. Pharmacol.*, **48**, 280—287 (1995).
24) Ulich Y. M., Hargreaves K. M., Flores C. M., *Neuropharmacology*, **36**, 1119—1125 (1997).
25) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).
26) Yamada S., Matsuoka Y., Kato Y., Kimura R., Inagaki O., *J. Pharmacol. Exp. Ther.*, **262**, 589—594 (1992).
27) Yamada S., Isogai M., Kagawa Y., Takayanagi N., Hayashi E., Tsuji K., Kosuge T., *Mol. Pharmacol.*, **28**, 120—127 (1985).
28) Loev B., Goodman M. M., Snader M. K., Tedeschi R., Macko E., *J. Med. Chem.* **17**, 956—965 (1974).
29) Triggle D. J., Langs D. A., Janis R. A., *Med. Res. Rev.*, **9**, 123—180 (1989).
30) Safak C., Simsek R., *Mini. Rev. Med. Chem.*, **6**, 747—755 (2006).
31) Frank C., Forst J. M., Grant T., Harris R. J., Kau S. T., Li J. H., Ohmmachb C. J., Smith R. W., Trainor D. A., Trivedi S., *Med. Chem. Lett.*, **3**, 2725—2726 (1993).
32) Li J. H., *Cardiovasc. Drug Rev.*, **15**, 220—231 (1997).
33) Trivedi S., Potter-Lee L., McConville M. W., Li J. H., Ohnmacht C. J., Trainor D. A., Kau S. T., *Res. Commun. Mol. Phathol. Pharmacol.*, **88**, 137—151 (1995).
34) Hamaguchi N., True T. A., Saussy D. L., Jeffs P. W., *Biochemistry*, **35**, 14312—14317 (1996).
35) Nagarathnam D., Wetzel J. M., Miao S. W., Marzbadi M. R., Chiu G., Wong W. C., Hong X., Fang J., Forray C., Branchek T. A., Heydorn W. E., Chang R. S., Broten T., Schorn T. W., Gluchowski C., *J. Med. Chem.*, **41**, 5320—5333 (1998).
36) Wong W. C., Chiu G., Wetzel J. M., Marzabadi M. R., Nagarathnam D., Wang D., Fang J., Miao S. W., Hong X., Forray C., Vaysse P. J., Branchek T. A., Gluchowski C., Tang R., Lepor H., *J. Med. Chem.*, **41**, 2643—2650 (1998).