Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 719–730

BIOORGANIC & MEDICINAL CHEMISTRY

The New Generation Dihydropyridine Type Calcium Blockers, Bearing 4-Phenyl Oxypropanolamine, Display α-/β-Adrenoceptor Antagonist and Long-Acting Antihypertensive Activities

Jhy-Chong Liang, Jwu-Lai Yeh, Chia-Sui Wang, Shwu-Fen Liou, Chieh-Ho Tsai and Ing-Jun Chen*

Department of Pharmacology, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC

Received 11 May 2001; accepted 10 September 2001

Abstract—A new series of dihydropyridine derivatives, bearing oxypropanolamine moiety on phenyl ring at the 4-position of the dihydropyridine base, were prepared. Oxypropanolamine was synthesized by replacing the phenolic OH of vanillin or other compounds, having a phenyl aldehyde group, with epichlorohydrin, followed by cleavaging the obtained epoxide compounds with *tert*-butylamine, *n*-butylamine or 2-methoxy-1-oxyethylamino benzene (guaiacoxyethylamine), respectively. Obtained various oxypropanolamine compounds, still remaining a phenyl aldehyde moiety, were then performed by Hantzsch condensation reaction with methylacetoacetate or ethylacetoacetate, respectively, to give our new series of dihydropyridine linked with the 4-phenyl ring. These compounds were evaluated for inotropic, chronotropic, and aorta contractility that associated with calcium channel and adrenoceptor antagonist activities. Dihydropyridine derivatives that with oxypropanolamine side chain on their 4-phenyl ring associated α-/β-adrenoceptor blocking activities created a new family of calcium entry and the third generation β-adrenoceptor blockers. Optimizing this research to obtain more potent α-/β-adrenoceptor blocking and long-acting antihypertensive oxypropanolamine on the 4-phenyl ring of dihydropyridine series compounds was thus accomplished and classified as third generation dihydropyridine type calcium channel blockers, in comparison with previous short-acting type nifedipine and long-acting type amlodipine. We concluded that compounds **1a**, **1b** and **1g** showed not only markedly high calcium-antagonistic activity but also the highest antihypertensive effect; compounds **1b**, **1c**, **1f**, **1g**, **1i** and **1j** induced sustained antihypertensive effects are major and attributed to their calcium entry and α-adrenoceptor blocking activities in the blood vessel due to their introduction of 2-methoxy, 1-oxyethylamino benzene moiety in the side chain on the 4-phenyl ring of dihydropyridine. Bradycardiac effects of all the compounds **1a**–**1j** resulted from calcium entry and β-adrenoceptor blocking, which attenuate the sympathetic activation-associated reflex tachycardia in the heart. We selected compound **1b** as candidate compound for further pharmacological and pre-clinical evaluation studies.

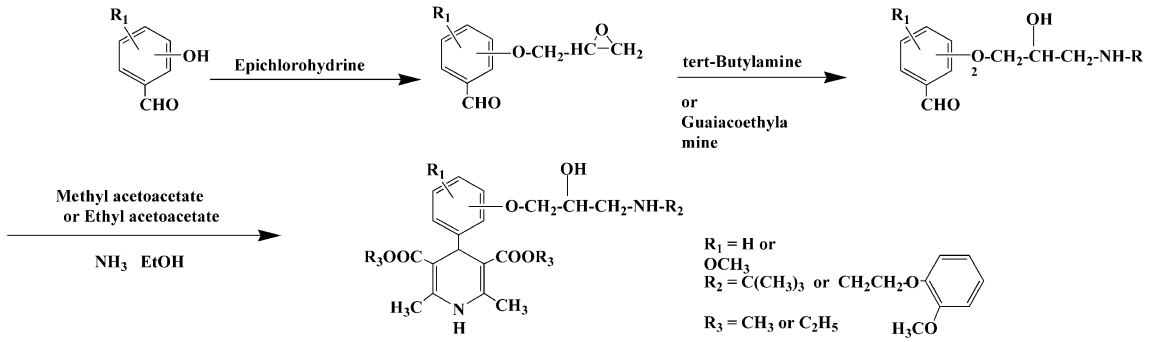
Introduction

Dihydropyridine drugs, such as nifedipine, nicardipine, amlodipine and others, are clinically effective cardiovascular agents for the treatment of hypertension and have been intensively studied to elucidate the molecular and conformational requirements for their attractive calcium antagonist activities.[1,2]

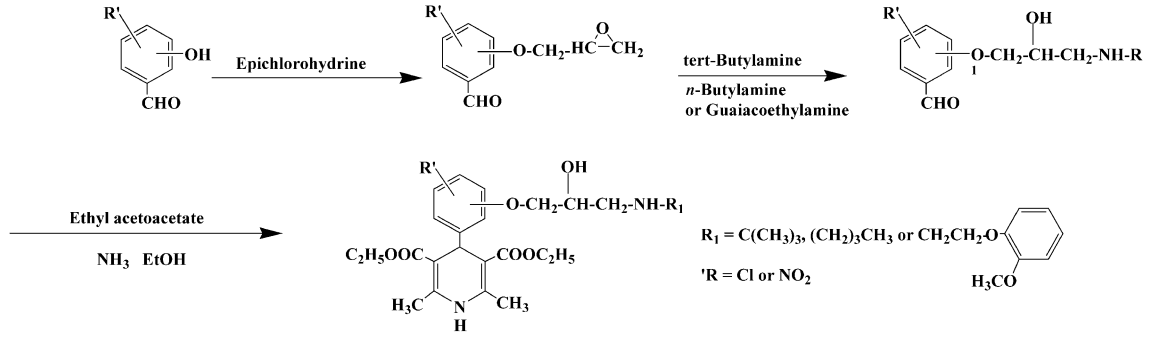
Short-acting dihydropyridine drugs must be used with caution in patients with coronary heart disease because of the increased mortality observed in patients treated with these drugs.[3–6] One of the most discussed undesirable effects is the reflex tachycardia produced by dihydropyridines.[7] This trouble is also found in the use of an α-adrenoceptor blocker for enhancing calcium channel blocking activity.[8] Obviously, a dihydropyridine type calcium channel and α-adrenoceptor blockers without revealing reflex tachycardia activity is an important target in the research of new drugs in this field. To resolve this problem, β-adrenoceptor blockade is suggested to combine with calcium channel or α-adrenoceptor blockade to reduce their vasodilatory effects associated tachycardia reflex.[9–12] Chemical incorporation of a dihydropyridine type calcium channel blocker and α-/β-adrenoceptor blocker molecule to reveal these three blockades is suggested to be reasonable in this study. Our series of compounds in this study are the hybrid products of these three blockades.

*Corresponding author. Tel.: +886-7-323-4686; fax: +886-7-323-4686; e-mail: ingjun@kmu.edu.tw

0968-0896/02/$ - see front matter
PII: S0968-0896(01)00318-2



Scheme 1.



Scheme 2.

To date, the structure–activity relationship of the dihydropyridines indicated that desired structural characteristic of the substituents at the 4-position of the dihydropyridine nucleus had been thought to be the phenyl ring. Modification of the phenyl ring of dihydropyridine compounds demonstrated that the introduction of electron donating substituents, such as the 3-hydroxy and 2-methoxy group, results in a loss of coronary blood flow and electron-withdrawing substitutents appear to contribute to increasing or maintaining the activities.[13] An electron-withdrawing *meta* substituent in the 4-phenyl ring of dihydropyridine enhanced relaxing activity but lengthy (not bulky) *meta* substituent and any *para* substituent was described to reduce this activity.[14] In order to obtain additional β- or α-adrenoceptor blocking activity in one molecule, we intended to discover appropriate substituents on the phenyl ring at the 4-position of the dihydropyridines nucleus. In the beginning of this study, we speculated that substitution of the hydroxy group on phenyl ring could be one of the candidates to introduce any moiety with adrenergic blocking function. However, by replacing the phenolic hydroxyl group of vanilloids with oxypropanolamine, we have recently succeeded in synthesizing a series of vanilloid based β-adrenoceptor blockers that with distinguish properties.[15–18] Previous success prompted us to extend the scope in this research so we decided to synthesize a new family of dihydropyridines by introducing electron-withdrawing substituents with a basic property on their 4-phenyl ring, given that a third generation β-adrenoceptor blocker carvedilol with additional vasodilatory activity, derived from α-adrenoceptor and calcium channel entry blocking activities, was reported to be useful in the treatment of hypertension and heart failure.[19–21] In the present study, we tried to introduce an oxypropanolamine moiety that associated with β-adrenoceptor blocking activity, on the phenyl ring of dihydropyridines, expecting to have extra adrenergic antagonist activities. In addition, new type long-acting antihypertensive dihydropyridines with adrenoceptor blocking activities were also expected to resist hydrolysis or metabolism in vivo. Taking into consideration that nearly all the dihydropyridine drugs were essentially neutral ester molecule with low aqueous solubility and marked first-pass effect in the liver,[14] we decided to incorporate oxypropanolamine on the 4-position with the phenyl ring that is linked with dihydropyridine. Our compounds are of basic property, however, which was not the case as alkoxymethy amine, substituted on the 2-position of the dihydropyridine base,[22] such as amlodipine. Actually, this decision of synthesis was also adapted to a previous claim about basic substitutents, such as found in amlodipine. Our basic 4-phenyl oxypropanolamine, linked with dihydropyridine, was thus also supposed to display long-acting antihypertensive activity.[23,24]

Since guaiacoxyethylamino moiety has been taken as α_1-adrenoceptor blockade-associated functional group previously,[15–18] in the present study, as shown in Schemes 1 and 2, an α-blockade-associated guaiacoxyethylamino moiety was also designed to be involved in our dihydropyridine series to enhance original dihydropyridine-associated calcium channel blocking activity in vascular smooth muscle. Propanolamine moiety (related to β-blockade) located, by chemical reaction, between the phenyl ring and guaiacoxyethylamino group (related to α-blockade), shown in Schemes 1 and 2, was suggested to have a proper α/β-adrenoceptor blockade.[25–33] The aim of this study in developing long-

acting antihypertensive dihydropyridines was to combine the advantages of both α- and β-adrenoceptor blockades, calcium channel blocking effect, without the presence of a tachycardia reflex, and thus to compensate for the untoward circulatory effect in classical short-acting dihydropyridine derivatives and α-adrenoceptor blockers.

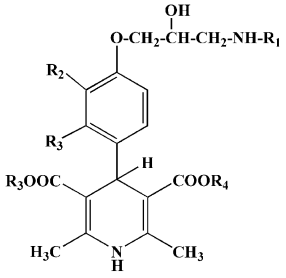
Results

Chemistry

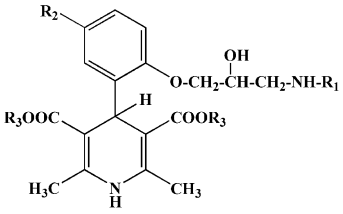
Obtained compounds were represented by the general Scheme 1 for that with *para*-substituted oxypropanolamine moiety, and Scheme 2 for that with *ortho*-substituted oxypropanolamine moiety on the 4-phenyl ring. The synthesis of compound **1** represents a typical example of the general synthesis of *para*-oxypropanolamine derivatives of vanillin (Scheme 1) and compound **1f** represents an example of the general synthesis of *ortho*-substituted oxypropanolamine derivatives of hydroxy benzaldehyde (Scheme 2), respectively. *Para*-substituted oxypropanolamines were synthesized by replacing the phenolic OH of vanillin with epichlorohydrin, followed by cleaving the obtained epoxide compound with 2-methoxy, 1-oxyethylamino benzene (guaiacoxyethylamine), *tert*-butylamine or *n*-butylamine, respectively, to yield middle products. *Ortho*-substituted oxypropanolamine derivatives were synthesized by replacing the phenolic OH of hydroxy benzaldhyde and then repeated as above (**2a**–**2j**) (Fig. 1). All obtained compounds were then reacted with methylacetoacetate or ethylacetoacetate, followed by Hantzsch condensation reaction, to obtain various dihydropyridines (compounds **1b**–**1j**) (Fig. 2). In contrast, vanillin was also directly followed by Hantzsch condensation reaction, without oxypropanolamine moiety, to present the possible calcium channel blocking activity as control, in comparison with compounds with oxypropanolamine moiety.[2]

Pharmacology

Intravenous compounds **1a**–**1j** (1.0 mg/kg) produced blood pressure lowering and heart rate decreasing effects in pentobarbital-anesthetized normotensive Wistar rats. Blood pressure lowering effects of compounds

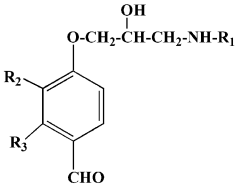


compound	R_1	R_2	R_3	R_4
1a	*t*-Butyl	OCH_3	H	CH_3
1b	guaiacoxyethyl	OCH_3	H	CH_3
1c	guaiacoxyethyl	OCH_3	H	C_2H_5
1g	guaiacoxyethyl	H	Cl	C_2H_5
1j	guaiacoxyethyl	H	H	C_2H_5

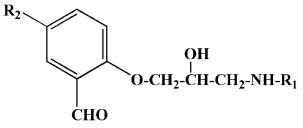


compound	R_1	R_2	R_3
1d	*t*-Butyl	Cl	C_2H_5
1e	*n*-Butyl	Cl	C_2H_5
1f	guaiacoxyethyl	Cl	C_2H_5
1h	*t*-Butyl	NO_2	C_2H_5
1i	guaiacoxyethyl	NO_2	C_2H_5

Figure 1. Structures of compounds **1a**–**1j**.



compound	R_1	R_2	R_3
2a	*t*-Butyl	OCH_3	H
2b	guaiacoxyethyl	OCH_3	H
2c	guaiacoxyethyl	OCH_3	H
2g	guaiacoxyethyl	H	Cl
2j	guaiacoxyethyl	H	H



compound	R_1	R_2
2d	*t*-Butyl	Cl
2e	*n*-Butyl	Cl
2f	guaiacoxyethyl	Cl
2h	*t*-Butyl	NO_2
2i	guaiacoxyethyl	NO_2

Figure 2. Structures of compounds **2a**–**2j**.

1a–**1j** reached their maximum about 1 min after dosing and only compounds **1a**, and **1b** lasted for above 1 h (Figs 3 and 4). Nifedipine at a dose of 1.0 mg/kg iv produced a temporary fall in blood pressure with a marked increase in heart rate (Figs 3 and 4).

Two of the most effective compounds **1a** and **1b** were selected and their antihypertensive activities were examined in pentobarbital-anesthetized normotensive Wistar rats and unanesthetized spontaneous hypertensive rats (SHR). Compound **1a** is reported as "vanidipinedilol' and compound **1b** is reported as "labedipinedilol-A'.[23,24] These two compounds showed high activity and longer-lasting actions under these experimental conditions. Oral administration of compounds **1a** and **1b** (10–50 mg/kg) in SHR reduced the blood pressure and heart rate for 24 h. More promisingly, heart rate remained relatively constant in comparison with blood pressure changes after administration of compound **1b** and returned to basal value at 24 h. The hypotensive effects of compounds **1a** and **1b** (25–50 mg/kg) reached maximum about 1 h after dosing and lasted over 10 h. Compound **1b** was shown to have more potent vasodilatory effect due to calcium antagonism with the $pK_{Ca^{-1}}$ value of 8.46 (nifedipine: $pK_{Ca^{-1}}$ 9.02). Despite its weaker vasodilatory action than that of nifedipine in vitro, the oral administration of compound **1b** produced a potent and long-lasting antihypertensive effect. Compound **1b**-induced sustained depressor effect in SHR might be major and attributed to its calcium entry and α-adrenoceptor blocking activities in the blood vessel and partly attributed to β-adrenoceptor/calcium channel blockade-associated decreased cardiac output in the heart. Compound **1a** is devoid of α-adrenoceptor blocking and is less potent than compound **1b** in anti-hypertensive activity and α/β ratio, but is more potent than compound **1b** in decreasing of mechanical cardiac function.

In the isolated Wistar rat atrium, compounds **1a**–**1j** (10^{-6} M) competitively antagonized (−)isoprenaline (10^{-10}–10^{-5} M)-induced positive chronotropic and inotropic effects in a concentration-dependent manner. Compounds **1a**–**1j** (10^{-6} M) also caused a shift to the right of the (−)isoprenaline concentration–response curve. The following order of activity was found for these compounds: **1a** > **1b** > **1g** > **1i** > **1h** > **1j** > **1f**≧**1d** > **1e** > **1c**. In electrically driven rat left atrial strips, compounds **1a**–**1j** also antagonized (−)isoprenaline-induced positive inotropic responses and produced rightward shifts of the cumulative concentration–response curves to (−)isoprenaline. The following order of activity was found for these compounds: **1b** > **1a** > **1j**≧**1g** > **1h** > **1i** > **1e** > **1c** > **1d** > **1f**. The pA_2 values and slope of regression lines were indicated in Table 1.

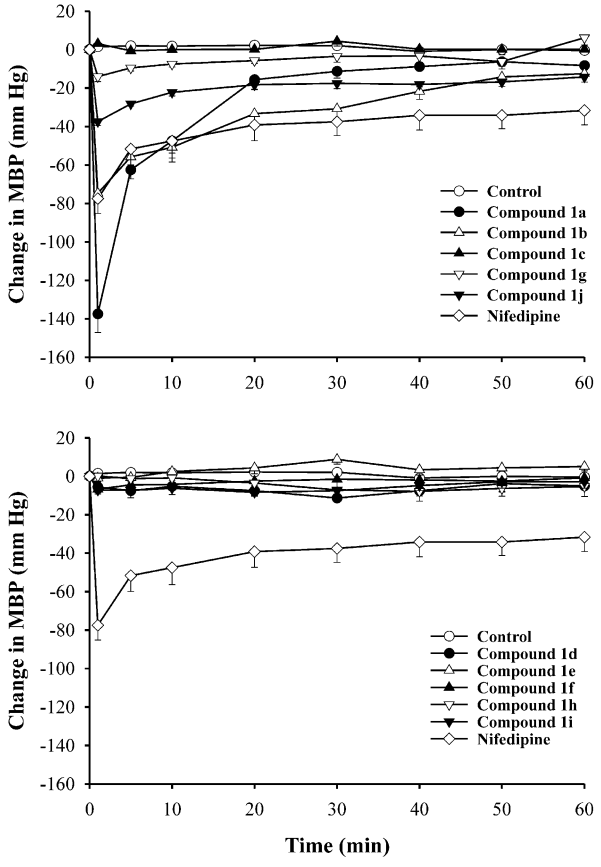


Figure 3. Effects of intravenous injection of compounds **1a**–**1j** and nifedipine (1.0 mg/kg) on blood pressure in normotensive Wistar rats, anesthetized with pentobarbital. Saline was used as a control. Vertical bars represent SE change from basal line value, which was 114±5 mm Hg for mean arterial blood pressure (MBP). Each value represents mean±SEM; $n=6$.

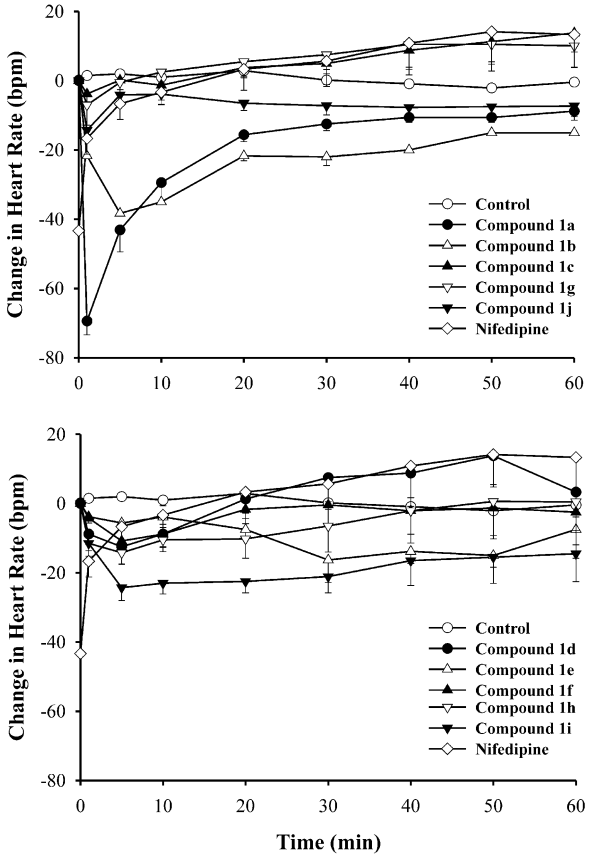


Figure 4. Effects of intravenous injection of compounds **1a**–**1j** and nifedipine (1.0 mg/kg) on heart rate in normotensive Wistar rats, anesthetized with pentobarbital. Saline was used as a control. Vertical bars represent SE change from basal line value, which was 381±25 beats/min for heart rate. Each value represents mean±SEM; $n=6$.

Compounds **1a**–**1j** competitively antagonized the (−)isoprenaline-induced relaxation from the spontaneous tone of guinea pig tracheal strips. Likewise, compounds **1a**–**1j** produced parallel shifted to the right of the agonist concentration–response curves. The following order of activity was found for these compounds: **1a** > **1g** > **1h**≧**1b** > **1f**≧**1i** > **1j**≧**1e** > **1d** > **1c**. The apparent pA_2 values for compounds **1a**–**1j** on tracheal strips were indicated in Table 1. Compounds **1a**, **1b** and **1g** with *para*-substituted *tert*-butylamino or 2-methoxy, 1-oxyethylamino benzene (guaiacoxyethylamino) terminal moiety in the side chain on 4-phenyl ring showed the greater activity in the isolated Wistar rat atrium and guinea pig tracheal strips than others in these experiments so they had more potent competitive β_1- and β_2-blockade activities.

In isolated Wistar rat aorta rings, compounds **1a**–**1j** (10^{-6} M) competitively inhibited norepinephrine-induced contraction effects, causing a shift to the right of the norepinephrine concentration–response curves. The following order of activity was found for these compounds: **1b** > **1g**≧**1j** > **1i** > **1f** > **1h** = **1e** = **1c** > **1d** > **1a**. The pA_2 values and slope of regression lines were shown in Table 1. Compounds **1b**, **1f**, **1g**, **1i** and **1j** with substitutive guaiacoxyethylamino terminal moiety showed the greater activity in the isolated Wistar rat aorta rings than others in these experiments confirming that the substitution with guaiacoxyethylamino terminal moiety was found to produce α-adrenoceptor blocking activity.[15–18]

In isolated rat thoracic aortic rings, compounds **1a**–**1j** produced inhibition or calcium channel blocking of $CaCl_2$-induced contraction with a p$K_{Ca^{-1}}$ value showed in Table 1. The slopes for compounds **1a**–**1j** on the experiments were not significantly different from unity. The following order of activity was found for these compounds: **1i** > **1h** > **1b** > **1j** > **1d** > **1g** > **1f** > **1a** > **1e** > **1c**. Compounds **1h** and **1i** with substitutive 5-NO_2 group on phenyl ring and compounds **1b** and **1j** with substitutive guaiacoxyethylamino terminal moiety in the side chain on 4-phenyl ring showed the greater activity in the isolated Wistar rat aorta rings than others. On the other hand, compounds **1a**–**1j** also inhibited high K^+-induced voltage-dependent calcium influx-associated contraction. The maximum percent relaxation and IC_{50} values of compounds **1a**–**1j** on KCl (75 mM)-contracted thoracic aortic rings are shown in Table 2. The following order of activity was found for these compounds: **1g** > **1b** > **1h** > **1i** > **1a** > **1e** > **1j** > **1f** > **1d** > **1c**. Substitution with guaiacoxyethylamino terminal or with NO_2 group on 4-phenyl ring seemed to potentiate the voltage-dependent relaxing activities in this experiment.

Discussion

Our present series of compounds, bearing oxypropanolamine on the 4-phenyl ring, belong to a new family of dihydropyridine type calcium channel blockers with additional adrenoceptor blocking properties. The contribution of the channel and receptor blockades of these

Table 1. pA_2 and p$K_{Ca^{-1}}$ values and slopes of Schild plots for new 1,4-dihydropyridine compounds in in vitro studies

Compd	β_1		β_2	α_1	Calcium	β_1/β_2[b] ratio	α/β[b] ratio	Calcium/β_1[b] ratio	Calcium/α[b] ratio
	pA_2 right atrium (Slope)	pA_2 value[a] left atrium (Slope)	pA_2 value[a] trachea (Slope)	pA_2 value[a] aorta (Slope)	pKCa^{-1} value aorta (Slope)				
1a	7.21 ± 0.12 (1.05 ± 0.08)	7.13 ± 0.09 (0.99 ± 0.07)	7.19 ± 0.09 (0.98 ± 0.07)	6.13 ± 0.24 (0.90 ± 0.07)	6.58 ± 0.87 (0.97 ± 0.05)	1.1	0.1	0.2	2.8
1b	7.18 ± 0.09 (0.98 ± 0.04)	7.43 ± 0.15 (0.88 ± 0.06)	6.83 ± 0.06 (0.73 ± 0.04)	8.28 ± 0.03 (0.90 ± 0.07)	8.46 ± 0.42 (0.87 ± 0.15)	2.2	12.6	19.1	1.5
1c	6.24 ± 0.24 (1.10 ± 0.06)	6.20 ± 0.13 (1.12 ± 0.04)	6.01 ± 0.09 (1.11 ± 0.07)	6.67 ± 0.17 (1.08 ± 0.04)	6.37 ± 0.45 (1.11 ± 0.15)	1.7	2.7	1.3	0.5
1d	6.41 ± 0.32 (1.13 ± 0.08)	6.17 ± 0.25 (1.02 ± 0.09)	6.16 ± 0.21 (0.89 ± 0.08)	6.54 ± 0.15 (0.82 ± 0.05)	7.77 ± 0.75 (0.98 ± 0.12)	1.8	1.3	22.9	17.0
1e	6.32 ± 0.17 (0.89 ± 0.02)	6.32 ± 0.52 (0.89 ± 0.08)	6.42 ± 0.19 (0.83 ± 0.02)	6.67 ± 0.13 (0.91 ± 0.07)	6.49 ± 0.19 (0.84 ± 0.09)	0.8	2.2	1.5	0.7
1f	6.46 ± 0.14 (0.91 ± 0.06)	6.07 ± 0.18 (0.81 ± 0.03)	6.69 ± 0.08 (0.93 ± 0.16)	6.89 ± 0.35 (1.12 ± 0.02)	6.66 ± 0.33 (0.87 ± 0.03)	0.6	2.7	1.6	0.6
1g	7.03 ± 0.12 (1.09 ± 0.02)	6.76 ± 0.15 (0.76 ± 0.14)	7.00 ± 0.23 (1.01 ± 0.12)	7.56 ± 0.66 (1.02 ± 0.05)	6.72 ± 0.94 (0.93 ± 0.17)	1.1	3.4	0.5	0.1
1h	6.69 ± 0.16 (1.01 ± 0.09)	6.52 ± 0.23 (1.12 ± 0.06)	6.85 ± 0.34 (0.98 ± 0.08)	6.67 ± 0.21 (1.04 ± 0.09)	8.49 ± 0.64 (1.12 ± 0.17)	0.7	1.0	63.1	66.1
1i	6.73 ± 0.03 (0.89 ± 0.04)	6.43 ± 0.06 (0.98 ± 0.04)	6.61 ± 0.28 (0.93 ± 0.08)	7.03 ± 0.12 (0.87 ± 0.14)	8.54 ± 0.22 (0.85 ± 0.08)	1.3	2.1	64.6	32.4
1j	6.61 ± 0.11 (0.97 ± 0.10)	6.81 ± 0.08 (0.83 ± 0.06)	6.48 ± 0.07 (0.85 ± 0.03)	7.55 ± 0.05 (1.01 ± 0.06)	8.02 ± 0.04 (0.87 ± 0.07)	1.4	7.9	25.7	3.2

[a]pA_2 values were obtained from the formula pA_2 = [log (DR-1)-log molar concentration antagonist] and the slope values were calculated from individual Schild plot by regression analysis. Each value was the mean ± SEM. of six to eight experimental results.
[b]The ratio values were obtained from the antilogarithm of the difference between the mean pA_2 values and p$K_{Ca^{-1}}$ values from in vitro studies.

compounds were suggested to provide a novel balance among them. In this study, we have investigated the effects of structural modifications, bearing oxypropanolamine on 4-phenyl ring of dihydropyridine. Although several previous studies suggested that the hydrogen atom was preferable at the *para* position of the 4-phenyl ring of dihydropyridine,[14] however, on which, we first test to introduce 2,5-, 2,4- or 3,4-disubstitutents on 4-phenyl ring of dihydropyridine. Substituents on the 4-phenyl ring of dihydropyridine were selected from NO_2, chloro, methoxyl, hydroxy and oxypropanolamine moieties. On the 4-phenyl ring of dihydropyridine, the replacement of its 4-hydroxyl, 3-hydroxyl and 2-hydroxyl group with a oxy- propanolamine moiety might be added with α- and β- adrenoceptor blocking activities to enhance calcium channel blocking activity that derived from their dihydropyridine base. Because of the bulkiness, basic characteristics, and hydrophobicity of this branched oxypropanolamine side chain, these compounds were expected to resist hydrolysis and had more potent and longer antihypertensive activity.

The results, expressed as blood pressure decreasing activity, calcium blocking activity and pA_2 values of the negative inotropic, chronotropic and calcium antagonist potencies, together with the respective percentage decreases of the maximum effects, are shown in Figures 3 and 4, Tables 1 and 2. These data show that all the synthetic manipulations were detrimental for calcium channel modulation and added with adrenoceptor blocking activities as far as vascular smooth muscle and heart were concerned.

Significant differences in the structure–activity relationship exist between these compounds. Intravenous administration of these compounds produced both decrease in mean blood pressure and heart beat. Of the compounds, shown in Figure 1, with a 2-chloro substituent (R_3), guaiacoxyethyl terminal (R_1) (**1g**) and 3-methoxy substituent (R_3), *t*-butyl terminal (R_1) (**1a**) on nitrogen in the side chain on the 4-phenyl ring were strongly active, and of the compound with a 3-methoxy substituent (R_3), guaiacoxyethyl terminal (R_1) on nitrogen (**1b**) in the side chain on the 4-phenyl ring showed the most potent antihypertensive activities in this series. Compound **1b**, with less tachycardiac reflex effect, was the most potent for the hypotensive effect in normotensive rats, and with long-acting antihypertensive action in SHR. Compound **1a** was the most potent for the bradycardia effect both in normotensive rats and SHR.

Table 2. Maximum relaxation (%) and IC_{50} values (M) of new dihydropyridine calcium antagonists in isolated rat thoracic aorta precontracted with 75 mM $[K^+]_0$

Compd	Maximum relaxation (%) at 10^{-4} M	IC_{50} values (M)
1a	78.5±5.2	1.26×10^{-5}
1b	89.8±2.4	3.69×10^{-6}
1c	56.4±4.4	9.24×10^{-5}
1d	70.5±4.8	2.94×10^{-5}
1e	72.8±6.3	1.66×10^{-5}
1f	75.3±2.5	2.07×10^{-5}
1g	96.2±3.7	2.99×10^{-6}
1h	97.6±4.8	4.43×10^{-6}
1i	100.6±5.1	5.02×10^{-6}
1j	90.6±3.9	1.75×10^{-5}

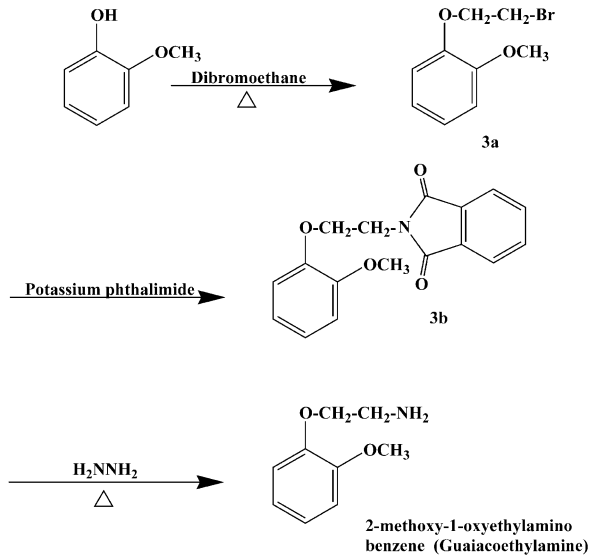
It was shown that all the compounds could either strongly or weakly inhibit Ca^{2+} dependent chronotropic, inotropic and aortic contractile effects; therefore, some of the compounds (**1a**, **1b** and **1g**) appeared to be potent and competitive calcium entry blocking agents. The IC_{50} values indicated that the relaxation of aorta rings by compound **1g** was more effective than other compounds. Compounds **1a**, **1b** and **1g** showed not only markedly high calcium-antagonistic activity but also the highest antihypertensive effect. In all cases, the 3,5-dicarboethoxy substituent derivatives were less active than those of the 3,5-dicarbomethoxy substituent on dihydropyridine base. Ester substituents at the 3,5-position of the dihydropyridine derivatives weakened the activity with increasing substituent size. From the results of the compounds, we concluded that compounds **1b**, **1c**, **1f**, **1g**, **1i** and **1j** induced sustained antihypertensive effect is major and attributed to their calcium entry and α-adrenoceptor blocking activities in the blood vessel due to their introduction of guaiacoethyl terminal moiety in the side chain on the 4-phenyl ring of dihydropyridine. Bradycardiac effects of all the compounds **1a**–**1j** are resulted from calcium entry and β-adrenoceptor blocking, which attenuate the sympathetic activation-associated reflex tachycardia in the heart.

A favorable substituent on the 4-phenyl ring of dihydropyridine derivatives was suggested to be an electron-withdrawing group such as a nitro group, a cyano group, a trifluoromethyl group, or a chlorine atom.[34] Dihydropyridine derivatives with nitroso moiety, an electron-withdrawing substitutent on the phenyl ring, was described as having coronary blood flow increasing activity.[8] Introduction of electron-donating substituents on the phenyl ring such as the hydroxy and methoxy groups found in dihydropyridines resulted in a loss of hypotensive effect and coronary blood flow,[35] however, the modification of the hydroxy group on the phenyl ring of the dihydropyridine derivative with the methoxy group and oxypropanolamine such as compound **1a** or compound **1b** still revealed potent vasorelaxing activity, in contrast to previous results.[8] In addition, 2-chloro, 5-chloro and 5-NO_2 phenyl derivatives exhibit almost decreased activities for voltage-dependent calcium channel blocking and for the hypertensive effects; this was an interesting result that had never been seen in the case of 4-substituted phenyl derivatives. Conformational and electronic change caused by the substitution at different sites of the phenyl ring might affect interactions of the concerned molecules with drug receptors and thereby influence their potencies.[36] The introduction of electron withdrawing substituents on the phenyl ring, such as found in 4-hydroxy derivative,[24] results in a loss of these activities in comparison with compounds **1a** and **1b**. The finding implied that the substitution on the *para*-hydroxy group of the 4-phenyl ring with oxypropanolamine may extend to have an additional adrenoceptor blockade and may be more important than the

chloro or NO_2 groups on the phenyl ring for their calcium channel blocking and their hypotensive activities. In contrast, the oxypropanolamine derivative (**1j**) without an electron donating 3-methoxy group decreases the hypotensive and adrenoceptor/calcium channel blocking effects[37] in comparison with compound **1b**. Our 5-nitro group substituted compounds did not show any better influence upon the antihypertensive action, but showed the most potent calcium antagonist activity (shown in compounds **1h** and **1i**, in comparison with compounds **1a** and **1b**). The 5-chloro-substituted compounds **1d**, **1e** and **1f** lost their antihypertensive activities, though the 2-chloro-substituted compound **1g** was a little less potent than the two most potent compounds, **1a** and **1b**. Compounds with 3-methoxy substituent (**1a** and **1b**) in our series seems significantly to increase the hypotensive and adrenoceptor/calcium channel blocking activities, in comparison with that without the 3-methoxy group (**1j**).[37] For the substitutions on the 3,5-position, compounds with dicarbomethyl substitutent reveal more antihypertensive activity than those with dicarboethyl substituent. This fact indicates that it is not necessary to increase the bulk of the ester substituent in our series of compounds. So far, the 3-methoxy group, *para* substituted oxypropanolamine on the phenyl ring and the 3,5-dicarcomethyl group of dihydropyridine have been found to be the most favorable for our series of compounds.

Compounds **1h** and **1i**, with nitro substituent, showed more Ca/β_1 selectivity (Table 1). This fact indicated that they have more potent calcium channel blocking activity on the vascular smooth muscle than β_1-adrenoceptor blocking activity on the cardiac muscle. However, tissue selectivity is also dependent on other structural components. Compounds **1b**, **1c**, **1f**, **1g** and **1j**, containing guaiacoxyethylamino terminal moiety that associated with α-adrenoceptor blocking activity, are suggested to be more highly tissue-selective ($\alpha > \beta_1$) on the vascular smooth muscle (Table 1). Our compounds with structurally different substituents on the 4-phenyl ring of dihydropyridine type compounds exhibit selectivity for vascular over cardiac tissue, except compound **1a** with more β_1-adrenoceptor blocking activity in comparison with its own α-adrenoceptor and calcium blocking activities.

In conclusion, this result suggested that our newly synthetic compounds belong to hypotensive and antihypertensive agents. Most of these compounds have higher vascular calcium entry and then α-adrenoceptor blocking activities. Some of them have predominantly with β-adrenoceptor blocking activity. Since these compounds have higher molecular weight and ester substituents, the lipophilities of theses compounds may be easily partitioned into the cell membrane of vascular smooth muscle where the binding events occur. From these profiles, compound **1b** was suggested to be a useful compound for the treatment of hypertension and associated cardiac hypertrophy.[23] This compound is now selected as candidate for further preclinical investigations. Dihydropyridine type calcium channel blockers have been functionally divided into three classes for their effects in the cardiovascular system: (1) short-acting calcium channel blockers, such as nifedipine; (2) long-acting calcium channel blockers, such as amlodipine; and (3) long-acting calcium channel blockers with adrenoceptor blocking activity.[37] Our new compounds and YM-430 and YM-15430-1 with β- or α-/β-adrenoceptor blocking activities,[12,38] are suggested to belong to the third generation dihydropyridine type calcium channel blockers.



Scheme 3.

Experimental

General information

All melting points were measured with a Yanaco MP-J3 micromelting point apparatus and are uncorrected. Infrared spectra were recorded through a KBr disk (ν in cm^{-1}) on a Hitachi 270-30 IR spectrophotometer. ^1H nuclear magnetic resonance spectra were recorded on a Varian Gemini-200 FT-NMR spectrometer, using DMSO-d_6 as solvent and TMS as internal standard (chemical shift in δ, ppm). Mass spectra were recorded with a JEOR-D100 GC-mass spectrometer. Column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh).

Vanillin and phenyl aldehyde such as 5-chlorosalicylaldehyde, 2-chloro-4-hydroxy-benzaldehyde, 5-nitrosalicylaldehyde were all obtained from Tokyo Chemical Industry Co. (TCI, Tokyo, Japan). (−)Isoprenaline bitartrate and norepinephrine HCl were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents used in this study were EP-grade products of E. Merck (Darmstadt, Germany). Synthetic compounds were prepared in stock solution in isotonic saline, with the addition of 10% DMSO if necessary. Animals were obtained from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan.

Synthesis

2-methoxy-1-oxyethylamino benzene (guaiacoethylamine) (Scheme 3). Guaiacol (0.2 mol) and 1,2-dibromoethane (0.4 mol) were heated to 100 °C with vigorous stirring, and 125 mL of 1.6 N NaOH was added to the mixture during 30 min. Vigorous stirring and heating were continued until the pH approached 7. Then the cold reaction mixture, consisting of two layers, was taken up in ether or chloroform, the aqueous layer separated (up to 80% of unreacted quaicol can be removed from the aqueous phase), and the organic phase was washed thoroughly with 2 N NaOH (NaOH solution was necessary to remove unreacted guaiacol) and once with saturated NaCl and dried ($MgSO_4$), and the solvent was evaporated. The **3a** (2-methoxy-1-oxyethylbromide benzene) was obtained by distillation.

3a (2-methoxy-1-oxyethylbromide benzene). ^{1}H NMR ($CDCl_3$) δ 3.62–3.69 (m, 2H, C$\underline{H_2}$–Br), 3.87 (s, 3H, Ar–O–C$\underline{H_3}$), 4.30–4.37 (m, 2H, Ar–O–C$\underline{H_2}$), 6.89–6.99 (m, 4H, Ar–$\underline{H}$); MS m/s: 230 (Scan FAB+). Anal. ($C_9H_{11}O_2Br$) C, H, N.

Potassium phthalimide (0.2 mol) was added to a solution of **3a** (0.2 mol) in 200 mL of dimethylformamide. The reaction was slightly exothermic, the temperature rising to 55 °C in 5 min. Stirring was continued for 30 min, and the temperature dropped slowly to 25 °C. After the addition of 300 mL of chloroform, the mixture was poured into 500 mL of water. The aqueous phase was separated and extracted with two 100 mL portions of chloroform. The combined chloroform extract was washed with 200 mL of 0.2 N NaOH (to remove unreacted phthalimide) and 200 mL of water. After drying ($MgSO_4$) the chloroform was removed. The crystalline residue was triturated with 40 mL of ether, and then **3b** (2-methoxy-1-oxyethylphthalimide benzene) was collected by filtration.

3b (2 - methoxy - 1 - oxyethylphthalimide benzene). ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H, Ar–O–C$\underline{H_3}$), 4.10–4.15 (t, 2H, C$\underline{H_2}$–phthalimide), 4.25–4.32 (t, 2H, Ar–O–C$\underline{H_2}$), 6.83–7.88 (m, 8H, Ar–$\underline{H}$); MS m/s: 297 (Scan FAB+). Anal. ($C_{17}H_{15}O_4N$) C, H, N.

Product 2 (0.07 mol) and hydrazine hydrate (0.07 mol) in 70 mL of absolute ethanol were heated on the steam bath for 45 min. A thick white precipitate appeared, to which 20 mL of 18% HCl was added. Heating was continued for 1 h and solid material was filtered off and washed with ethanol. The solvent was evaporated from filtrate, and the residue was basified with a 20% NaOH solution. The mixture was extracted with chloroform and dried ($MgSO_4$), and the solvent was evaporated. The residual oil was distilled to obtain 2-methoxy-1-oxyethylamino benzene (guaiacoethylamine).

2 - methoxy - 1 - oxyethylamino benzene (guaiacoethylamine). ^{1}H NMR ($CDCl_3$) δ 3.08–3.13 (t, 2H, C$\underline{H_2}$–NH_2), 3.87 (s, 3H, Ar–O–C$\underline{H_3}$), 4.02–4.08 (t, 2H, Ar–O–C$\underline{H_2}$), 6.88–6.95 (m, 4H, Ar-$\underline{H}$); MS m/s: 167 (Scan FAB+). Anal. ($C_9H_{13}O_2N$) C, H, N.

4-*N*{4-[2-Hydroxy-3-(*tert*-butylamino)propoxy]-3-methoxy}benzyl-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine (1a). Epichlorohydrin (5 mol) was added to a solution of 4-hydroxy-3-methoxy-1-benzaldehyde (vanillin) (1 mol) in an aqueous ethanol solution (100 mL) containing 8 g of sodium hydroxide. The resulting solution was heated at 70 °C for 3 h with stirring. The hot reaction mixture was filtered to remove the white precipitate, and the filtrate was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with an eluent (*n*-hexane: EtOAc = 1:9). The objective fragments were combined and evaporated and the residue triturated with *n*-hexane. The resulting solid was collected, washed with *n*-hexane and dried to afford *N*-[4-(2,3-epoxypropoxy)-3-methoxy] benzaldehyde (**4a**) as a pale yellow powder. ^{1}H NMR ($CDCl_3$) δ 2.77–2.97 (m, 2H,–CH(O)C$\underline{H_2}$), 3.39–3.47 (m, 1H,–C$\underline{H}$(O)CH_2), 3.94 (s, 3H, Ar–O–C$\underline{H_3}$), 4.06–4.43 (m, 2H, Ar–O–C$\underline{H_2}$), 7.01–7.47 (m, 3H, Ar-$\underline{H}$), 9.87 (s, 1H, C$\underline{H}$O); MS m/s: 208 (Scan FAB+). Anal. ($C_{11}H_{12}O_4$) C, H, N.

To 1 mol of *tert*-butylamine were added 1 mol of compound **4a** and 100 mL of absolute ethanol. The mixture was heated at 50–55 °C for 4 h, with stirring. The reaction mixture was clarified by filtration and the filtrate concentrated under reduced pressure. The product was filtered and washed with absolute ethanol and dried, yielding a pale yellow powder. Recrystallization from EtOH afforded *N*-{4-[2-hydroxy-3-(*tert*-butylamino) propoxy]-3-methoxy}benzaldehyde (**2a**).^{1}H NMR ($CDCl_3$) δ 1.51 (s, 9H, 2×C$\underline{H_3}$), 3.17–3.51 (m, 2H, C$\underline{H_2}$–N), 3.90 (s, 3H, Ar–O–C$\underline{H_3}$), 4.17–4.25 (m, 2H, Ar–O–C$\underline{H_2}$), 4.60–4.80 (s, 1H, C$\underline{H}$–OH), 7.02–7.43 (m, 3H, Ar-$\underline{H}$), 9.86 (s, 1H, C$\underline{H}$O); MS m/s: 282 (Scan FAB+). Anal. ($C_{15}H_{23}O_4N$) C, H, N.

A solution of *N*-{4-[2-hydroxy-3-(*tert*-butylamino)propoxy]-3-methoxy} benzaldehyde (0.01 mol), methyl acetoacetate (2.4 mL, 0.02 mol) and ammonia (10 mL) in EtOH (15 mL) was refluxed for 15 h. The mixture was evaporated and treated with 50 mL aqueous Na_2CO_3 solution. The solution was extracted with $CHCl_3$ and the extract was concentrated, followed by acidification with 2 N EtOH–HCl. The residue was purified by column chromatography on silica gel with an eluent (MeOH/EtOAc = 3:7). The objective fragments were combined and evaporated and the residue triturated with EtOAc. The resulting solid was collected, washed with EtOAc and recrystallization from a mixture of MeOH and EtOAc (1:9). Compound **1a** was obtained as a yellow powder. UV (MeOH) λ_{max} nm (log ε) 229.0 (3.89); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, 3×C$\underline{H_3}$), 2.33 (s, 6H, 2×C$\underline{H_3}$), 3.00–3.45 (m, 2H, C$\underline{H_2}$–NH), 3.65 (s, 6H, 2 ×CO–OC$\underline{H_3}$ ×2), 3.77 (s, 3H, Ar–OC$\underline{H_3}$), 3.93–4.14 (m, 2H, Ar–OC$\underline{H_2}$), 4.53 (m, 1H, C$\underline{H}$–OH), 4.94 (s, 1H, Ar–C$\underline{H}$<), 5.45 (br s, 1H, exchangeable,–N$\underline{H}$–), 6.33–6.87 (m, 3H, Ar–$\underline{H}$), 8.33 (br s, 1H, exchangeable, CH_2–N$\underline{H}$–C), 9.28 (br s, 1H, exchangeable, O$\underline{H}$); IR (KBr): 3500, 3350, 3000 and 2850, 1710, 800 cm^{-1}; MS m/s: 476 (Scan FAB+). Anal. ($C_{25}H_{36}O_7N_2$) C, H, N.

The other compounds, including epoxides (compounds **4a**–**4j**, data not shown), compounds **2b**–**2j** and **1b**–**1j**,

were synthesized in a similar manner to that of compound **1a**, during the course of synthesis. The general method for the amination using 3–10 mL of each amine such as *n*-butylamine, *tert*-butylamine, and 2-methoxy-1-oxyethylamino benzene (guaiacoxyethylamine) was added with 5 g (14.3 mol) of each epoxide and 50 mL of methanol, respectively. Each mixture was heated under nitrogen at 50–55 °C for 4 h, with stirring. The reaction mixture was evaporated to dryness under reduced pressure. The residual product was passed through SiO_2 gel column chromatography and eluted with EtOAc and *n*-hexane mixture to produce various oxypropanolamines (**2b–2j**).

The synthesis of various dihydropyridine derivatives were prepared by 1 mol of aldehyde groups of oxypropanolamines and 2 mols of acetoacetic acid ester (methylacetoacetate or ethylacetoacetate) and 1 mol of ammonia according to the Hantzsch condensation reaction (**1b–1j**).

***N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy] - 3 - methoxy}benzaldehyde (2b).** ^{1}H NMR ($CDCl_3$) δ 2.50–2.89 (m, 4H, C$\underline{H}_2$–N–C$\underline{H}_2$), 3.71–3.79 (d, 6H, 2×Ar–O–C$\underline{H}_3$), 3.86–4.03 (m, 4H, 2×Ar–O–C$\underline{H}_2$), 4.19–4.24 (t, 1H, C$\underline{H}$–OH), 6.84–7.37 (m, 7H, Ar–$\underline{H}$), 8.31 (s, 1H, C$\underline{H}$O); MS m/s: 375 (Scan FAB+). Anal. ($C_{20}H_{25}O_6N$) C, H, N.

4-{{*N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy]-3-methoxy}benzyl}}-2,6-dimethyl-3,5-dicarbomethoxy - 1,4 - dihydropyridine (1b). UV (MeOH) λ_{max} nm (log ε) 219.0 (4.48); ^{1}H NMR ($CDCl_3$) δ: 2.26 (s, 6H, 2 ×C$\underline{H}_3$), 2.50–2.89 (m, 4H, C$\underline{H}_2$–NH–C$\underline{H}_2$), 3.56 (s, 6H, 2×CO–OC$\underline{H}_3$), 3.68–3.74 (d, 6H, 2 ×Ar–OC$\underline{H}_3$), 3.86–4.05 (m, 4H, 2 ×Ar–OC$\underline{H}_2$), 4.4 (s, 1H, C$\underline{H}$--OH), 4.83 (s, 1H, Ar–C$\underline{H}$<), 6.84–7.37 (m, 7H, Ar–$\underline{H}$), 8.33 (s, 1H,–N$\underline{H}$–); IR (KBr): 3535, 3276, 2778, 1651, 1127 and 1023, 810 and 748 cm^{-1}; MS m/s: 570 (Scan FAB+). Anal. ($C_{30}H_{38}O_9N_2$) C, H, N.

4-{{*N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy]-3-methoxy} benzyl}}-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine (1c). UV (MeOH) λ_{max} nm (log ε) 226.0 (4.42); ^{1}H NMR ($CDCl_3$) δ: 1.20–1.27 (t, 6H, 2 ×CO–OCH$_2$C$\underline{H}_3$), 2.33 (s, 6H, 2 ×C$\underline{H}_3$), 2.50–2.89 (m, 4H, C$\underline{H}_2$–NH–C$\underline{H}_2$), 3.45 (m, 4H, 2 ×CO–OC$\underline{H}_2$CH$_3$), 3.78–3.84 (d, 6H, 2 ×OC$\underline{H}_3$), 3.88–3.92 (m, 4H, 2 ×Ar–OC$\underline{H}_2$), 4.09–4.12 (m, 1H, C$\underline{H}$–OH), 4.95 (s, 1H, Ar–C$\underline{H}$<), 5.73 (br s, 1H, exchangeable,–N$\underline{H}$--), 6.76–6.91 (m, 7H, Ar–$\underline{H}$); IR (KBr) 3420, 3332, 2968 & 2850, 1687, 1217 and 1127, 753 cm^{-1}; MS m/s: 598 (Scan FAB+). Anal. ($C_{32}H_{42}O_9N_2$) C, H, N.

***N*-{2-[2-hydroxy-3-(*tert*-butylamino)propoxy]-5-choloro}-benzaldehyde (2d).** ^{1}H NMR ($CDCl_3$) δ 1.29–1.34 (s, 9H, 3×C$\underline{H}_3$), 2.86–2.96 (m, 2H, C$\underline{H}_2$–N–), 4.07–4.17 (m, 2H, Ar–O–C$\underline{H}_2$), 4.27–4.41 (s, 1H, C$\underline{H}$–OH), 6.81–7.89 (m, 3H, Ar–$\underline{H}$), 10.34 (s, 1H, C$\underline{H}$O); MS m/s: 285.5 (Scan FAB+). Anal. ($C_{14}H_{20}O_3NCl$) C, H, N.

4-{{*N*-{2-[2-Hydroxy-3-(*tert*-butylamino)propoxy]-5-choloro}benzyl}}-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine (1d). UV (MeOH) λ_{max} nm (log ε) 227.0 (4.33); ^{1}H NMR ($CDCl_3$) δ 1.15–1.27 (s, 9H, 3 C$\underline{H}_3$), 1.36 (m, 6H, 2 ×CO–OCH$_2$C$\underline{H}_3$), 2.29–2.30 (d, 6H, 2 ×C$\underline{H}_3$), 2.69–2.98 (m, 2H, C$\underline{H}_2$–NH), 3.46–3.76 (s, 4H, 2 ×CO–OC$\underline{H}_2$CH$_3$), 3.88–4.23 (m, 2H, Ar–OC$\underline{H}_2$), 4.27 (s, 1H, C$\underline{H}$–OH), 5.21 (s, 1H, Ar–C$\underline{H}$<), 5.74 (br s, 1H, exchangeable,–N$\underline{H}$–), 6.67–7.54 (m, 3H, Ar–$\underline{H}$); IR (KBr): 3460, 3261, 3009 and 2960, 1677, 1222 and 1127, 829 cm^{-1}; MS m/s: 508.5 (Scan FAB+). Anal. ($C_{26}H_{37}O_6N_2$ Cl) C, H, N.

***N*-{2-[2-Hydroxy-3-(*n*-butylamino)propoxy]-5-choloro}-benzaldehyde (2e).** ^{1}H NMR ($CDCl_3$): 1.13–1.24 (m, 7H,–N–CH$_2$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_3$), 2.19–2.75 (m, 2H, C$\underline{H}_2$–N–C$\underline{H}_2$), 3.97–4.17 (m, 2H, Ar–O–C$\underline{H}_2$), 4.32–4.52 (s, 1H, C$\underline{H}$–OH), 6.65–7.28 (m, 3H, Ar–$\underline{H}$), 9.25 (s, 1H, C$\underline{H}$O); MS m/s: 285.5 (Scan FAB+). Anal. ($C_{14}H_{20}O_3NCl$) C, H, N.

4-{{*N*-{2-[2-Hydroxy-3-(*n*-butylamino)propoxy]-5-choloro}benzyl}}-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine (1e). UV (MeOH) λ_{max} nm (log ε) 226.0 (4.35); ^{1}H NMR ($CDCl_3$) δ 1.11–1.22 (m, 7H, NH–CH$_2$C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_3$), 1.22–1.29 (m, 6H, 2 ×CO–OCH$_2$C$\underline{H}_3$), 2.04 (s, 6H, 2 ×C$\underline{H}_3$), 2.17–2.71 (m, 4H, C$\underline{H}_2$–NH–C$\underline{H}_2$), 3.4 (m, 4H, 2 ×CO–OC$\underline{H}_2$CH$_3$), 3.97–4.14 (m, 2H, Ar–OC$\underline{H}_2$), 4.3–4.5 (m, 1H, C$\underline{H}$–OH), 5.33 (s, 1H, Ar–C$\underline{H}$<), 6.65–7.27 (m, 3H, Ar–$\underline{H}$); IR (KBr): 3475, 3365, 2967 and 2900, 1686, 1107 and 1043, 808 cm^{-1}; MS m/s: 508.5 (Scan FAB+). Anal. ($C_{26}H_{37}O_6N_2$ Cl) C, H, N.

***N*-{2-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy] - 5 - choloro}benzaldehyde (2f).** ^{1}H NMR ($CDCl_3$) δ 2.60–3.21 (m, 4H, C$\underline{H}_2$–N–C$\underline{H}_2$), 3.73–3.84 (d, 3H, Ar–O–C$\underline{H}_3$), 4.07–4.18 (m, 4H, 2×Ar–O–C$\underline{H}_2$), 4.51–4.62 (t, 1H, C$\underline{H}$–OH), 6.79–7.27 (m, 7H, Ar–$\underline{H}$), 7.70 (s, 1H, C$\underline{H}$O); MS m/s: 379.5 (Scan FAB+). Anal. ($C_{19}H_{22}O_5NCl$) C, H, N.

4-{{*N*-{2-[2-Hydroxy-3-(2-methoxy-1-oxyethylamino benzene) propoxy]-5-choloro} benzyl}}-2,6-dimethyl-3,5-dicarboethoxy - 1,4 - dihydropyridine (1f). UV (MeOH) λ_{max} nm (log ε) 226.0 (4.26); ^{1}H NMR ($CDCl_3$) δ 1.17–1.28 (m, 6H, 2 ×CO–OCH$_2$C$\underline{H}_3$), 2.27–2.29 (m, 6H, 2 ×C$\underline{H}_3$), 2.50–2.89 (m, 4H, C$\underline{H}_2$–NH–C$\underline{H}_2$), 3.67–3.77 (m, 4H, 2 ×CO–OC$\underline{H}_2$CH$_3$), 3.84 (s, 3H, Ar–OC$\underline{H}_3$), 3.85–4.14 (m, 4H, 2 ×Ar–OC$\underline{H}_2$), 4.26–4.33 (m, 1H, C$\underline{H}$–OH), 5.3 (s, 1H, Ar–C$\underline{H}$<), 5.6 (br s, 1H, exchangeable,–N$\underline{H}$–), 6.71–7.16 (m, 7H, Ar–$\underline{H}$); IR (KBr): 3467, 3332, 2918 and 2850, 1707, 1621, 1101 and 1031, 810 & 753 cm^{-1}; MS m/s: 602.5 (Scan FAB+). Anal. ($C_{31}H_{39}O_8N_2$ Cl) C, H, N.

***N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy] - 3 - choloro}benzaldehyde (2g).** ^{1}H NMR ($CDCl_3$) δ 2.81–3.12 (m, 4H, C$\underline{H}_2$–N–C$\underline{H}_2$), 3.92–3.86 (t, 3H, Ar–O–C$\underline{H}_3$), 4.02–4.04 (m, 4H, 2×Ar–O–C$\underline{H}_2$), 4.31–4.42 (t, 1H, C$\underline{H}$–OH), 6.88–7.98 (m, 7H, Ar–$\underline{H}$), 8.71 (s, 1H, C$\underline{H}$O); MS m/s: 379.5 (Scan FAB+). Anal. ($C_{19}H_{22}O_5NCl$) C, H, N.

4-{{*N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylamino benzene) propoxy]-3-choloro} benzyl}}-2,6-dimethyl-3,5-

dicarboethoxy - 1,4 - dihydropyridine (1g). UV (MeOH) λ_{max} nm (log ε) 221.0 (4.39); ^{1}H NMR ($CDCl_3$) δ 1.21–1.29 (m, 6H, 2 ×CO–OCH_2CH_3), 2.04 (m, 6H, 2 ×CH_3), 2.29–2.50 (m, 4H, CH_2–NH–CH_2), 3.82–3.86 (m, 4H, 2 ×CO–OCH_2CH_3), 3.87–3.98 (m, 3H, Ar–OCH_3), 4.06–4.24 (m, 4H, 2 ×Ar–OCH_2), 4.6 (m, 1H, CH–OH), 5.31 (s, 1H, Ar–CH<), 5.75 (br s, 1H, exchangeable,–NH–), 6.73–6.98 (m, 7H, Ar–H); IR (KBr): 3463, 3383, 2938, 1742, 1656, 1120, 810 and 748 cm^{-1}; MS m/s: 602.5 (Scan FAB+). Anal. ($C_{31}H_{39}O_8N_2$ Cl) C, H, N.

***N*-{2-[2-Hydroxy-3-(*n*-butylamino)propoxy]-5-nitro}benzaldehyde (2h).** ^{1}H NMR ($CDCl_3$) δ 1.16–1.30 (m, 9H, 3× CH_3), 2.71–2.95 (m, 2H, CH_2–N–), 4.03–4.07 (m, 2H, Ar–O–CH_2), 4.17–4.26 (s, 1H, CH–OH), 6.95–7.26 (m, 3H, Ar–H), 10.45 (s, 1H, CHO); MS m/s: 296 (Scan FAB+). Anal. ($C_{14}H_{20}O_5N_2$) C, H, N.

4-{{*N*-{2-[2-Hydroxy-3-(*n*-butylamino)propoxy]-5-nitro}-benzyl}}-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine (1h). UV (MeOH) λ_{max} nm (log ε) 233.0 (4.27); ^{1}H NMR ($CDCl_3$) δ 1.14–1.16 (s, 9H, 3 ×CH_3), 1.17–1.24 (m, 6H, 2 ×CO–OCH_2CH_3), 2.05 (d, 6H, 2 ×CH_3), 2.33–2.34 (m, 4H, CH_2–NH), 2.85–2.98 (m, 4H, 2 ×CO–OCH_2CH_3), 3.98–4.17 (m, 2H, Ar–OCH_2), 4.24–4.26 (s, 1H, CH–OH), 5.39 (s, 1H, Ar–CH<), 6.82–8.14 (m, 3H, Ar–H); IR (KBr): 3485, 3296, 2967 and 2880, 1681, 1197 and 1077, 848 and 768 cm^{-1}; MS m/s: 519 (Scan FAB+). Anal. ($C_{26}H_{37}O_8N_3$) C, H, N.

***N*-{2-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy] - 5 - nitro}benzaldehyde (2i).** ^{1}H NMR ($CDCl_3$) δ 2.86–3.15 (m, 4H, CH_2–N–CH_2), 3.81–3.89 (t, 3H, Ar–O–CH_3), 4.05–4.17 (m, 4H, 2×Ar–O–CH_2), 4.33–4.39 (t, 1H, CH–OH), 6.84–7.03 (m, 7H, Ar–H), 8.72 (s, 1H, CHO); MS m/s: 390 (Scan FAB+). Anal. ($C_{19}H_{22}O_7N_2$) C, H, N.

4-{{2-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy]-5-nitro}phenyl}}-2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine (1i). UV (MeOH) λ_{max} nm (log ε) 224.0 (4.28); ^{1}H NMR ($CDCl_3$) δ 1.14–1.28 (m, 6H, 2 ×CO–OCH_2CH_3), 2.04 (s, 6H, 2 ×CH_3), 2.22–2.33 (m, 4H, CH_2–NH–CH_2), 3.40–3.60 (m, 4H, 2 ×CO–OCH_2CH_3), 3.83–3.85 (s, 3H, Ar–OCH_3), 3.99–4.13 (m, 4H, 2 ×Ar–OCH_2), 4.3 (m, 1H, CH–OH), 5.41 (s, 1H, Ar–CH<), 6.5 (br s, 1H, exchangeable,–NH–), 6.82–8.12 (m, 7H, Ar–H); IR (KBr): 3483, 3342, 2989 and 2875, 1636, 1132 and 1046, 815 and 748 cm^{-1}; MS m/s: 609 (Scan FAB+). Anal. ($C_{31}H_{35}O_{10}N_3$) C, H, N.

***N*-{4-[2-hydroxy-3-(*tert*-butylamino)propoxy]}benzaldehyde (2j).** ^{1}H NMR ($CDCl_3$) δ: 2.86–3.12 (m, 4H, CH_2–N–CH_2), 3.81–3.85 (s, 3H, Ar–O–CH_3), 3.98–4.15 (m, 4H, 2×Ar–O–CH_2), 4.34 (s, 1H, CH–OH), 6.86–6.97 (m, 7H, Ar–H), 9.89 (s, 1H, CHO); MS m/s: 345 (Scan FAB+). Anal. ($C_{19}H_{23}O_5N$) C, H, N.

4-{{*N*-{4-[2-Hydroxy-3-(2-methoxy-1-oxyethylaminobenzene)propoxy]}benzyl}}-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine (1j). UV (MeOH) λ_{max} nm (log ε) 219.0 (4.48); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 6H, 2 CH_3), 2.78–3.08 (m, 4H, CH_2–NH–CH_2), 3.63 (s, 6H, 2 ×CO–OCH_3), 3.82 (s, 3H, Ar–OCH_3), 3.92 (s, 1H, CH–OH), 4.10–4.14 (m, 4H, 2 ×Ar–OCH_2), 4.93 (s, 1H, Ar–CH<), 6.73–7.17 (m, 7H, Ar–H), 8.39 (s, 1H,–NH–); IR (KBr): 3438, 3346, 3019 and 2840, 1694, 1124 and 1124, 1029 and 769 cm^{-1}; MS m/s: 540 (Scan FAB+). Anal. ($C_{29}H_{35}O_8N_2$) C, H, N.

Pharmacology

Measurement of hypotensive effect. All the compounds were examined for antihypertensive activity. The experiments were accomplished as described previously.[23,24] In brief, male Wistar rats, weighing 250–300 g were anesthetized with pentobarbital (40 mg/kg, i.p.). Following each tracheal cannulation, systemic arterial blood pressure and heart rate were taken from the femoral arterial with a pressure transducer (Model P10EZ; Spectramed, Oxnard, USA) connected to an amplifier (Model 13-4615-52, Gould, Valley View, OH, USA) and displayed on a recorder (Model 8188-4402, Gould, Valley View, OH, USA). The body temperatures were maintained at 37 °C with an electric heating pad. A femoral vein was cannulated for iv injection. All drugs were administered in a volume of 0.4 ml/kg. Equivolumetric injections of the vehicle were administered to control animals. The magnitudes of effects elicited after injections were evaluated by measuring the changes in arterial blood pressure and heart rate and comparing them to basal values.

Measurement of antihypertensive effect. Some of the compounds (**1a** and **1b**) with potent hypotensive activity in normotensive rats were tested for antihypertensive activity SHR. Male SHR of SHR/N strain weighing 200–300 g were used. Systolic blood pressure and heart rate were measured by the indirect tail cuff method using a rat tail manometer-tachometer (Natsume KN-210, Natsume Seisakusho Co. Ltd., Japan). The rats were restrained in a Plexiglas holder where temperature was maintained at 37 °C for 15–20 min to raise their body temperature, leading to dilation of the caudal artery and thereby allowing the pressure pulse to be easily detected. In all cases, at least three consecutive measurements were obtained, and the average was reported as the systolic blood pressure. Only rats with a systolic pressure of 180 mm Hg or higher were used. The measurement was performed just before and 0.5, 1, 2, 3, 4, 5, 6, 12 and 24 h even 30 h after single oral dose of compounds **1a** and **1b** (5, 10, 25, 50 mg/kg), which were dissolved in ethanol prior to diluting in acidic saline solution (pH 5.3). The solutions were protected from light and administered orally via a stomach tube in a volume of 5 mL/kg.

Measurement of β_1-, β_2-adrenergic antagonist effects. The experiments were carried out at 37 °C in a Krebs solution of the following composition (in mM): NaCl 113; KCl 4.8; $CaCl_2$ 2.2; KH_2PO_4 1.2; $MgCl_2$ 1.2; $NaHCO_3$ 25; Dextrose 11.0; bubbled with a 95% O_2/5% CO_2 mixture. The right and left atrial strips were prestretched to a baseline tension of 1.0 g. The atria were equilibrated for 90 min in aerated Krebs solution

before the experimental protocols were initiated. Isolated atria were used to evaluate the possible involvement of all compounds effects in β-adrenergic mechanism.

For the assessment of β_1-adrenergic blocking activity, a control cumulative concentration–response curve to the chronotropic effect of (−)isoprenaline was established. The right atria were allowed 60 min to restabilize, after which time various concentrations of the test agent were incubated with the atria 30 min before the cumulative concentration of the (−)isoprenaline (10^{-10}–10^{-4} M) were added. Responses were calculated as a percentage of the maximum control response to (−)isoprenaline. On the other hand, quiescent left atria were dissected free of connective tissue and mounted in organ chambers under a resting tension of 1.0 g. The tissues were bathed in an aerated Krebs solution (37 °C) and were driven at 2-s intervals via two platinum electrodes placed at either side of the atrium. β_1-Adrenergic antagonist activity was determined as follows. Cumulative concentration–response curves to the positive inotropic effects of (−)isoprenaline were obtained in the absence and presence of various concentrations of a test compound. An incubation time of 30 min was allowed for the test compound. Data were calculated as a percentage of the increase in force induced by (−)iso-)isoprenaline.

Tracheal β_2-adrenoceptor blocking activity was evaluated according to the method of our previous report.[23,24] Tracheal strips were suspended in Krebs solution at 37 °C under resting tension of 1.5 g. After the preparation were contracted by 3×10^{-6} M carbacol, cumulative concentration-relaxant response curve for (−)isoprenaline were constructed by increasing bath concentration of the agonist approximately 3-fold. The response to (−)isoprenaline was examined after 30 min incubation with the test compound. Relaxations were expressed as percentage of the maximum relaxation obtained by the first challenge of the tissues.

α_1-Adrenoceptor antagonism. Vascular α-adrenoceptor blocking activity was evaluated in the Wistar rat aorta preparation. Isolated rat aorta preparations were mounted in a 10 mL organ bath and suspended in Krebs solution at 37 °C under a resting tension of 1.0 g. Compounds were added to the bath medium after a control concentration-response curve to norepinephrine (10^{-9}–10^{-4} M) has been obtained. The tissue was exposed to compounds for 30 min before re-challenging with norepinephrine. Inhibitions were expressed as percentage of the maximum contraction obtained by the challenge of the tissues with norepinephrine.

Calcium antagonism activity. Calcium antagonistic activity was evaluted according to the method of our previous study.[23,24] Isolated rat aorta was sectioned into 2.5-mm rings segments. An aortic ring segment was mounted between two triangular pins and was suspended in Krebs solution at 37 °C under a resting tension of 1.0 g and was equilibrated for 60 min. The segments were then incubated with or without test compounds for 10 min, using four segments per pharmacological subset. Normal Krebs solution was then exchanged for a calcium-free and depolarizing solution (containing 40 mM KCl, 0.5 mM EGTA, with or without test compounds, pH 7.5). Cumulative contraction–response curves for $CaCl_2$ were then generated by adding $CaCl_2$. Responses were recorded in the presence and absence of three concentrations of the test compounds.

Voltage-dependent vasorelaxing activity. The other method of measurement of vasorelaxing activity was a modification of one we published previously to evaluate the aorta relaxing activity of these compounds.[23] The tissues were allowed to equilibrate for 1 h in physiological solution. To induce stable contractions of thoracic aorta rings mounted in an organ bath, high potassium solution (75 mM KCl) was added to the bath. When response was considered 100%, compounds were added. The 75 mM KCl solution was prepared by substituting 70 mM NaCl for an equimolar amount of KCl.

Statistical evaluation of data. In the in vitro study, the dose ratio was obtained from the ratio of the EC_{50} values of the agonist with and without the test compound. Dissociation constant (K_B) in α-, β_1- and β_2-adrenoceptor antagonism of the test compounds were calculated from the method described by our previous study,[23,24] using the equation: K_B = [antagonist]/(dose ratio-1). The pA_2 values were then expressed as the negative logarithm of K_B and the slope of the regression line were calculated by the method described by our previous study.[23,24] Since compounds reduced the maximum responses to $CaCl_2$ in the rat aorta, double-reciprocal regression plots according to our previous report were performed.[15] In the radioligand binding assay, the K_d (the dissociation constant of radioligand), B_{max} (the maximum binding capacity) and K_i (inhibition constant) values were determined by Scatchard analysis using the non-linear curve fitting LIGAND program. All values were presented in terms of mean ± SEM. Analysis of the data and plotting of the figures were done with the aid of software (SigmaStat and SigmaPlot, Version 5.0, San Rafael, CA, USA; GraphPad PRISM™, Version 2.0, San Diego, CA, USA) run on an IBM compatible computer and a Power Macintosh.

Acknowledgements

This work in chemical synthesis was supported by research grants from the Lotus Medical Supply Inc. (Taipei, Taiwan).

References and Notes

1. Bühler, F. R.; Kiowski, W. *J. Hypertens. 5, S3* **1987**.
2. Reid, J. L.; Meredith, P. A.; Pasanisi, F. *J. Cardiovasc. Pharmacol. 7, S18* **1985**.
3. Furberg, C. D.; Psaty, B. M.; Meyers, J. V. *Circulation* **1995**, *92*, 1326.

4. Furberg, C. D.; Pahor, M.; Psaty, B. M. *Eur. Heart J.* **1996**, *17*, 1142.
5. Poole-Wilson, P. A. *Eur. Heart J.* **1996**, *17*, 1131.
6. Psaty, B. M.; Hecjbert, S. R.; Koepsell, T. D.; Siscovick, D. S.; Raghanathan, T. E.; Weiss, N. S.; Rosendaal, F. R.; Lemaitre, R. N.; Smith, N. L.; Wahl, P. W.; Furberg, C. D. *JAMA* **1995**, *274*, 620.
7. Bala, S. V. *Herz* **1982**, *7*, 211.
8. Gomes, C.; Henning, M.; Persson, B.; Trolin, G. *Clin. Exp. Hypertens* **1978**, *1*, 141.
9. Honda, K.; Takenaka, T.; Shiono, K.; Miyata-Osawa, A.; Nakagawa, C. *Japan. J. Pharmacol.* **1985**, *38*, 31.
10. Dargie, H. J. *Br. J. Pharmacol.* **1986**, *21*, 155 S.
11. Asano, M.; Uchida, W.; Shibasaki, K.; Terai, M.; Inagaki, O.; Takenaka, T.; Matsumoto, Y.; Fujikura, T. *J. Pharmacol. Exp. Ther.* **1990**, *296*, 204.
12. Shibasaki, K.; Arai, Y.; Uchida, W.; Okazaki, T.; Asano, M.; Takenaka, T. *Gen. Pharmac.* **1997**, *29*, 545.
13. Satoh, Y.; Ichihashi, M.; Okumura, K. *Chem. Pharm. Bull.* **1991**, *39*, 3189.
14. Kanno, H.; Yamaguchi, H.; Okamiya, Y.; Sunakawa, K.; Takeshita, T.; Naruchi, T. *Chem. Phar. Bull* **1992**, *40*, 2049.
15. Huang, Y. C.; Yeh, J. L.; Wu, B. N.; Lo, Y. C.; Liang, J. C.; Lin, Y. T.; Sheu, S. H.; Chen, I. *J. Drug Dev. Res.* **1999**, *47*, 77.
16. Huang, Y. C.; Wu, B. N.; Lin, Y. C.; Chen, S. J.; Chiu, C. C.; Cheng, C. J.; Chen, I. J. *J. Cardiovasc. Pharmacol.* **1999**, *34*, 10.
17. Yeh, J. L.; Yang, T. H.; Liang, J. C.; Huang, Y. C.; Lo, Y. C.; Wu, J. R.; Lin, Y. T.; Chen, I. *J. Drug Dev. Res.* **2000**, *51*, 29.
18. Chiu, C. C.; Lin, Y. T.; Tsai, C. H.; Liang, J. C.; Chiang, L. C.; Wu, J. R.; Chen, I. J.; Yeh, J. L. *Gen. Pharmacol.* **2001**, *34*, 391.
19. Moser, M.; Frishman, W. *Am. J. Hypertens.* **1998**, *11*, 15 S.
20. Bril, A.; Slivjak, M.; DiMartino, M. J.; Feuerstein, G. Z.; Linee, P.; Poyser, R. H.; Ruffolo, R. R.; Smith, E. F. III. *Cardiovasc. Res.* **1992**, *26*, 518.
21. Olsen, S. L.; Gilbert, E. M.; Renlund, D. G.; Taylor, D. O.; Yanowitz, F. D.; Bristow, M. R. *J. Am. Coll. Cardiol.* **1995**, *25*, 1225.
22. Burges, R.; Moisey, D. *Am. J. Cardiol* **1994**, *73*, 2 A.
23. Liang, J. C.; Yeh, J. L.; Chiang, L. C.; Yang, Y. C.; Sheu, S. H.; Lai, W. T.; Chen, I. *J. Drug Dev. Res.* **2000**, *49*, 94.
24. Yeh, J. L.; Liou, S. F.; Liang, J. C.; Huang, Y. C.; Chiang, L. C.; Wu, J. R.; Lin, Y. T.; Chen, I. J. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 51.
25. Wu, B. N.; Yang, C. R.; Yang, J. M.; Chen, I. *J. Gen. Pharmacol.* **1994**, *25*, 651.
26. Wu, B. N.; Hwang, T. L.; Liao, C. F.; Chen, I. *J. Biochem Pharmacol.* **1994**, *48*, 10.
27. Chen, I. J.; Yeh, J. L.; Lo, Y. C.; Sheu, S. H.; Lin, Y. T. *Br. J. Pharmacol.* **1996**, *119*, 7.
28. Chen, S. J.; Huang, Y. C.; Wu, B. N.; Chen, I. *J. Drug Dev. Res.* **1997**, *40*, 239.
29. Lin, Y. T.; Wu, B. N.; Wu, J. R.; Lo, Y. C.; Chen, L. C.; Chen, I. J. *J. Cardiovasc. Pharmacol.* **1996**, *28*, 149.
30. Wu, B. N.; Ho, W. C.; Chiang, L. C.; Chen, I. *J. Asia Pac. J. Pharmacol.* **1996**, *11*, 5.
31. Sheu, M. M.; Wu, B. N.; Ho, W. C.; Hong, S. J.; Chen, S. J.; Lin, Y. T.; Chen, I. *J. Pharmacology* **1997**, *54*, 211.
32. Wu, B. N.; Huang, Y. C.; Wu, H. M.; Hong, S. J.; Chiang, L. C.; Chen, I. J. *J. Cardiovasc. Pharmacol.* **1998**, *31*, 750.
33. Lin, Y. T.; Wu, B. N.; Horng, C. F.; Huang, Y. C.; Hong, S. J.; Lo, Y. C.; Chen, C. J.; Chen, I. *J. Jap. J. Pharmacol.* **1999**, *80*, 127.
34. Kobayashi, T.; Inoue, T.; Nishino, S.; Fujihara, Y.; Oizumi, K.; Kimura, T. *Chem. Pharm. Bull.* **1995**, *43*, 797.
35. Ashimori, A.; Ono, T.; Uchida, T.; Ohtaki, Y.; Fukaya, C.; Watanabe, M.; Yokoyama, K. *Chem. Pharm. Bull.* **1990**, *38*, 2446.
36. Alker, D.; Campbell, S. F.; Cross, P. E. *J. Med. Chem.* **1991**, *34*, 19.
37. Yeh, J. L.; Tsai, C. H.; Liang, J. C.; Wu, J. R.; Huang, Y. C.; Chen, I. *J. Drug Dev. Res.* **2001**, *52*, 462.
38. Shibasaki, K.; Uchida, W.; Shirai, Y.; Inagaki, O.; Asano, M.; Takenaka, T. *Arch. Int. Pharmacodyn.* **1994**, *328*, 213.