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Original Article

Antihypertensive effects of new dihydropyridine derivatives on phenylephrine-raised blood pressure in rats

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Abstract

Changes in the substitutions at C-3 and C-5 positions of 4-(1-methyl-5-nitro-2-imidazolyl) dihydropyridine analogs of nifedipine have led to changes in potency of the compounds. The objective of the present study was to examine the hypotensive effects of 5 newly synthesized dihydropyridine derivatives of nifedipine in rats with phenylephrine-raised blood pressure. Anesthetized Sprague-Dawley rats were randomly assigned to 19 groups of 7 animals each. Control group received the vehicle dimethylsulfoxide (0.05 mL), 3 groups were given nifedipine at 100, 300, or 1000 μg/kg, and 5 other groups each composed of 3 subgroups administered one of the 5 new dihydropyridine compound at 100, 300, or 1000 μg/kg. All animals were initially infused with 20 μg/kg/min phenylephrine for 45 min, and were then given a bolus of either dimethylsulfoxide, nifedipine, or new dihydropyridine compounds 20 min after the commencement of phenylephrine infusion. Blood pressure and heart rate (HR) of the animals were measured before and at the end of phenylephrine infusion, or 25 min after injection of vehicle or compounds. Compared to dimethylsulfoxide, nifedipine, and new 1, 4-dihydropyridine derivatives caused significant reductions in MBP. Moreover, cyclohexyl propyl, phenyl butyl, and cyclohexyl methyl analogs of 1, 4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-3,5-pyridinedicarboxylase at 100 μg/kg, phenyl butyl, and cyclohexyl methyl analogs at 300 μg/kg, and cyclohexyl methyl analogs at 1000 μg/kg reduced MBP similar to nifedipine. There was no significant difference between HR of all groups before and after administration of the compounds. The findings indicated that changes in substitution at C-3 and C-5 positions of 2-(1-methyl-5-nitro-2-imidazolyl) dihydropyridine analogs of nifedipine were associated with changes in hypotensive activity of the compounds.

Keywords: 1, 4-dihydropyridine; Nifedipine; Phenylephrine; Arterial pressure

INTRODUCTION

Calcium channel blockers are one of the widely used drugs for the management of hypertension, ischemic heart diseases, and cardiac arrhythmias. Structurally, they are divided into three main categories including benzothiazepinone, phenylalkylamine and dihydropyridines.

Dihydropyridine calcium channel blockers are known to have more vasodilatory and less cardiodepressant effects than other calcium channel blockers. This property has made them more suitable for use in the treatment of hypertension and angina (1-3). Their useful effects in management of cardiovascular disorders are due to their ability to relax vascular smooth and cardiac muscles via inhibiting the influx of calcium ion through L-type calcium channels.

Nifedipine was the first dihydropyridine calcium channel blocker to be widely prescribed for the treatment of hypertension (4-6). After the introduction of nifedipine, a number of dihydropyridine derivatives, which are more potent and have less side effects such as reflex tachycardia, have been synthesized (7). Although a fairly good number of calcium channel blockers are available, the search for drugs with more potency, less toxicity, and better pharmacokinetic and pharmacodynamic profiles have continued.

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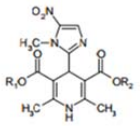
Table 1. Chemical structure and IUPAC names of new dihydropyridine derivatives.

Compound	R1=R2	Chemical structure
A	$(CH_2)_3C_6H_5$	
B	$(CH2)_3C_6H_{11}$	
C	$(CH2)_4C_6H_5$	
D	$CH_2C_6H_{11}$	
E	$CH_2C_6H_5$	

(Compound A) Bis (3- phenylpropyl) 2, 6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-1, 4-dihydropyridine-3, 5-dicarboxylate, (Compound B) Bis (3-cyclohexylpropyl) 2, 6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-1, 4-dihydropyridine-3, 5-dicarboxylate. (Compound C) Bis(4-phenylbutyl) 2, 6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-1, 4-dihydropyridine-3, 5-dicarboxylate. (Compound D) Bis (cyclohyxylmethyl) 2, 6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-1, 4-dihydropyridine-3, 5-dicarboxylate. (Compound E) Dibenzyl 2, 6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyl)-1, 4-dihydropyridine-3, 5-dicarboxylate.

Dihydropyridine derivatives have been assessed in quantitative structure-activity relationship studies to develop drugs with greater tissue selectivity, longer duration of action, and slower rate of absorption than the older ones (8-12). Such studies have shown changes in the substitution pattern at C-3 and C-5 positions of nifedipine were associated with changes in potency, tissue selectivity and conformation of dihydropyridine ring (12-15). Moreover, an *in vivo* study (16) showed that substitution of R1 at C-3 with propyl or ethyl and R2 at C-5 with nitroxy-containing groups was associated with a significant reduction in mean blood pressure (MBP). Also, it was shown that some of C-3 and C-5 substituents in conjunction with C-4 l-methyl-5-nitro-2-imidazolyl substituents had better calcium channel blocking activity than nifedipine, as indicated by their *in vitro* vasorelaxing properties (17).

Considering the lack of a study examining the *in vivo* calcium channel blocking activity of C-3 and C-5 substituent in conjunction with C-4 substitution (1-methyl-5-nitro-2-imidazolyl), the present study was designed to compare blood pressure lowering effects of a number of new 1, 4-dihydropyridine derivatives (Table 1) with that of nifedipine in rats, which their blood pressure had been raised by intravenous infusion of phenylephrine.

MATERIALS AND METHODS

Materials

Sodium thiopental was obtained from Biochem GmbH (Kundl, Austria). Phenylephrine and nifedipine were purchased from Sigma-Aldrich Company (Sigma-Aldrich Chemical Co., Steinheim, Germany). Heparin **(**4500 IU/4.5 mL) was obtained from Leo (Ballerup, Denmark). Dihydropyridine derivatives (compounds A, B, C, D, and E) (Table 1) were synthesized in the laboratory of Department of Medicinal Chemistry, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The purity of the compounds was determined by thin layer chromatography using several solvent systems of different polarity (17).

Animals

Male Sprague-Dawley rats weighing 200-250 g were obtained from Laboratory Animal Breeding Center of Shiraz University of Medical Science (Shiraz, Iran). The animals were kept under standard conditions (light/dark cycle, 12 h; humidity, 25-35%; and temperature, 22-24 °C) with standard rat food and water *ad libitum*. The study protocol was approved by the Institutional Committee for Care and Use of Laboratory Animals of Shiraz University of Medical Sciences (Approval ID: 291).

Experimental design and protocol

Rats were anesthetized with intraperitoneal injections of sodium thiopental (80 mg/kg). A cervical midline incision was made and a polyethylene catheter (PE50) was placed in the right jugular vein.

A catheter was also placed in the right carotid artery used to measure the blood pressure. The catheters were filled with

heparinized saline (25 IU/mL) to prevent likely blood coagulation. Animals' body temperature was maintained at 37 ± 0.5 °C using a thermal pad and a rectal probe connected to a TCAT-LLV controller (Physitemp Instruments INC, Cliffon NJ, USA).

The animals were allowed to recover for 30 min from the surgical stress. Then, they were allocated to 19 groups of 7 animals each. One group received an intravenous injection of 0.05 mL dimethylsulfoxide (DMSO) which was used as the vehicle for nifedipine or new dihydropyridine derivatives. Six groups received an intravenous injection of nifedipine, or compounds A, B, C, D, or E at 100 μg/kg, six groups were injected an intravenous of nifedipine, or compounds A, B, C, D, or E at 300 μg/kg, and six groups received an injection of nifedipine, or compounds A, B, C, D, or E at 1000 μg/kg. Nifedipine doses were selected as directed in previous report (16).

After baseline measurement of blood pressure and HR, all animals were infused with phenylephrine at 20 μg/kg/min for 45 min through a femoral vein catheter using an infusion pump.

The total dose of phenylephrine infused over 45 min was 900 μg/kg. Twenty min after commencement of phenylephrine infusion, animals were given a bolus injection of DMSO, nifedipine or new 1,4-dihydropyridine derivatives (compounds A, B, C, D, or E). After 45 min of phenylephrine infusion or 25 min after injection of vehicle or calcium channel blocker compounds a second measurement of blood pressure and HR was performed. Animals were sacrificed at the end of the experiments.

Measurement of blood pressure and heart rate

Systolic and diastolic blood pressure was measured by a pressure transducer, which was connected to the catheters placed in the right carotid arteries.

The pressures were recorded using a Grass Polygraph. Mean blood pressure was calculated as diastolic pressure plus one-third of arterial pulse pressure. Heart rate was counted from upstrokes of arterial pulse pressure tracing on the Grass Polygraph chart.

Statistical analysis

Data, presented as mean ± SEM, were examined for normality of distribution. For between-group comparisons normally-distributed data were compared using one-way analysis of variance (ANOVA). Where a significant difference was observed with ANOVA, the source of the difference was located using Duncan's Multiple Range test. Where the data were not normally distributed, they were analyzed using Kruskal-Wallis followed by Dunn's test. A P-value of ≤ 0.05 was considered statistically significant. Data analysis was performed using Sigma stat (version 3.0) statistical software (San Jose, CA, USA). The illustrations were prepared using Sigma plot (version 8.0) software (San Jose, CA, USA).

RESULTS

The results of the effects of nifedipine and new dihydropyridine compounds at 100 μg/kg on MBP and HR are shown in Fig.1. There was no significant difference between baseline MBP of groups receiving DMSO, nifedipine or new dihydropyridine compounds (Fig. 1A).

The final MBP of groups received nifedipine, compounds B, C and D, but not compound A or E, were significantly lower than that of the group received DMSO. There was no significant difference between the final MBP of group received nifedipine, and those received compound B, C, or D. However, the MBP of groups received compound A or E was significantly higher than that of nifedipine-receiving group (Fig. 1A). There was no significant difference between baseline HRs of groups receiving DMSO, nifedipine or new dihydropyridine compounds (Fig. 1B). Moreover, there was no significant difference between the final HRs of groups received DMSO, nifedipine or dihydropyridine compounds (Fig. 1B).

The results of the effects of nifedipine and new dihydropyridine compounds at 300 μg/kg on MBP and HR are shown in Fig.2. There was no significant difference among baseline MBPs of groups received DMSO, nifedipine or new dihydropyridine (Fig. 2A).

The final MBP of group received compound A, B, C, or D, but not compound E, was significantly lower than that of the DMSO-treated group. The final MBP of groups received compounds B or C was significantly lower than that of the nifedipine-treated group, but the final MBP of group receiving compound A, D or E was significantly higher than that of nifedipine-treated group (Fig. 2A). There was no significant difference between baseline HRs of groups receiving DMSO, nifedipine or new dihydropyridine compounds (Fig. 2B). Moreover, there was no significant difference between the final HRs of groups treated with DMSO, or nifedipine or dihydropyridine compounds (Fig. 2B).

The results of the effects of nifedipine and new dihydropyridine compounds at 1000 μg/kg on MBP and HR are shown in Fig. 3. There was no significant difference among baseline MBPs of groups receiving DMSO, nifedipine, or new dihydropyridine compounds (Fig. 3A).

The final MBP of groups received nifedipine, or compounds A, B, C, D, or E, was significantly lower than that of DMSO-treated group. There was no significant difference between the final MBP of groups received compound C and nifedipine, however, the MBPs of groups receiving compounds A, B, D or E were significantly higher than that of the nifedipine group (Fig. 3A). There was no significant difference between baseline HRs of groups receiving DMSO, nifedipine or new dihydropyridine compounds (Fig. 3B). Moreover, there was no significant difference between the final HRs of groups received DMSO, nifedipine or dihydropyridine compounds (Fig. 3B).

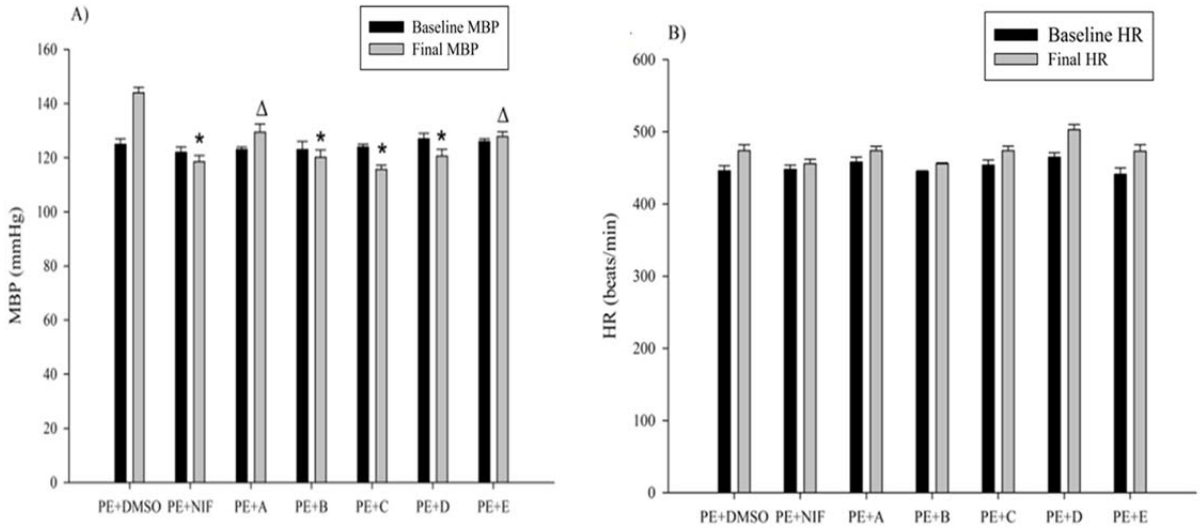


Fig. 1. Baseline and final (A) mean blood pressure (MBP) and (B) heart rate (HR) of all groups. Final measurements were performed after 45 min of phenylephrine (PE) infusion (20 μg/kg/min), or 25 min after injection (0.05 mL) of dimethylsufoxide (DMSO), or 100 μg/kg of nifedipine (NIF) or dihydropyridine derivatives (compounds A, B, C, D, or E). *Significant ($P \leq 0.05$) difference from the final MBP of PE + DMSO-treated group.$^{\Delta}$Significant ($P \leq 0.05$) difference from final MBP of PE + nifedipine-treated group.

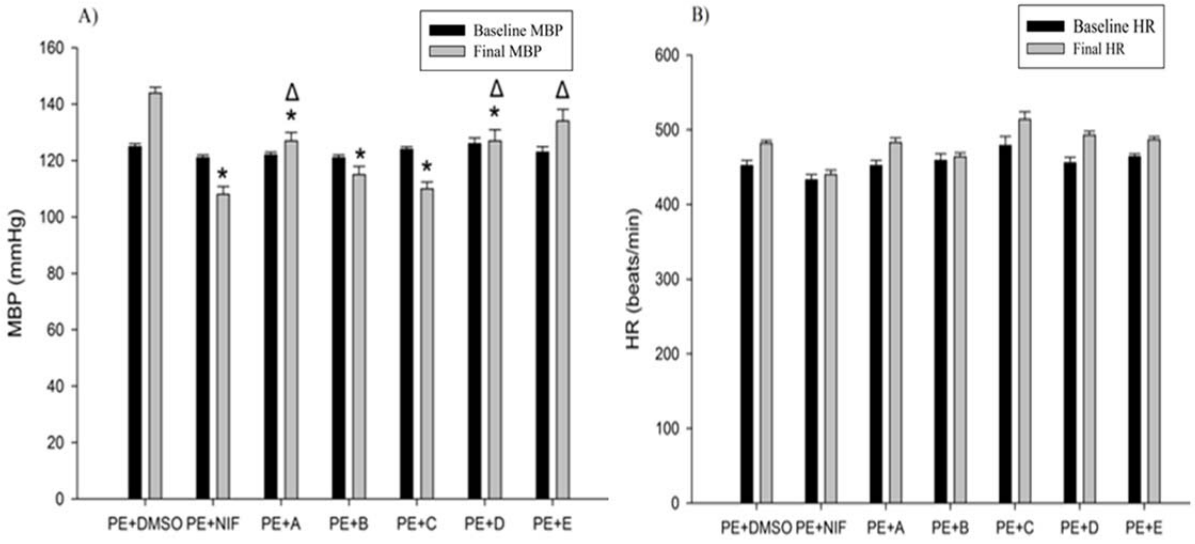


Fig. 2. Baseline and final (A) mean blood pressure (MBP) and (B) heart rate (HR) of all groups. Final measurements were performed after 45 min of phenylephrine (PE) infusion (20 µg/kg/min), or 25 min after injection of (0.05 mL) of dimethylsufoxide (DMSO), or 300 µg/kg of nifedipine (NIF) or dihydropyridine derivatives (A, B, C, D, or E). *Significant ($P \leq 0.05$) difference from the final MBP of PE + DMSO-treated group.$^{\Delta}$Significant ($P \leq 0.05$) difference from final MBP of PE + nifedipine-treated group.

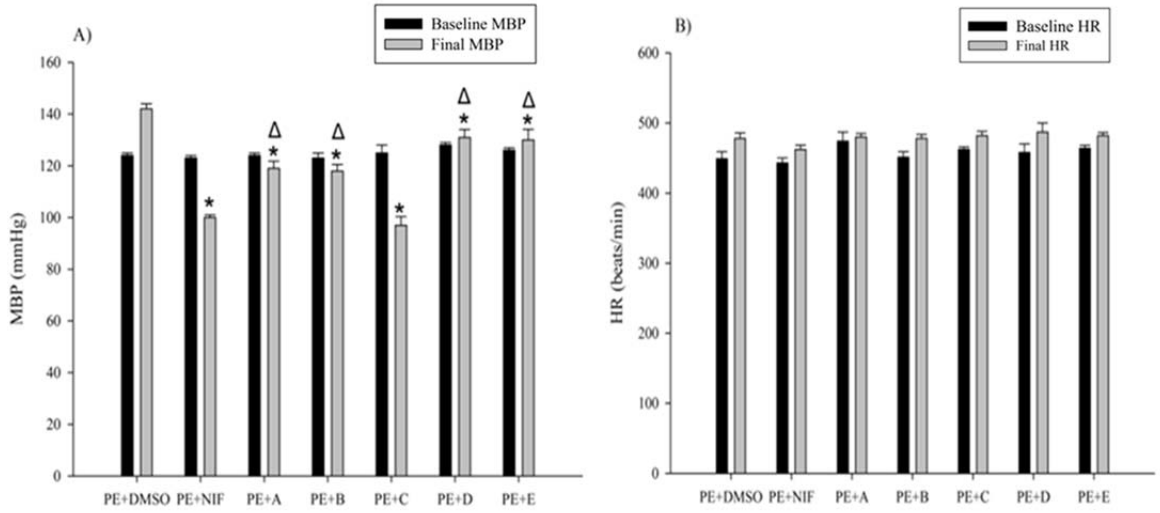


Fig. 3. Baseline and final (A) mean blood pressure (MBP) and (B) heart rate (HR) of all groups. Final measurements were performed after 45 min of phenylephrine (PE) infusion (20 µg/kg/min), or 25 min after injection of (0.05 mL) of dimethylsufoxide (DMSO), or 1000 µg/kg of nifedipine (NIF) or dihydropyridine derivatives (A, B, C, D, or E). *Significant ($P \leq 0.05$) difference from the final MBP of PE+DMSO-treated group.$^{\Delta}$Significant ($P \leq 0.05$) difference from final MBP of PE + nifedipine-treated group.

DISCUSSION

The findings of the present study show that compared to the vehicle, new 1,4-dihydropyridine compounds lowered MBP in rats with phenylephrine-raised blood pressure. They also showed that compounds B, C and D at 100 µg/kg, compounds B and C at 300 µg/kg, and compound C at 1000 µg/kg reduced MBP to an extent similar to those of nifedipine at similar doses. Moreover, they indicate that, new 1,4-dihydropyridines containing different bis-cyclohexyl or bis-phenyl alkyl ester substitutes at C-3 and C-5

and the 4-(1-methyl-4-nitro-5-imidazolyl) moiety at C-4 position of dihydropyridine core reduced MBP, the extent of which were comparable to that of nifedipine.

To examine the antihypertensive activity of nifedipine and new dihydropyridine compounds, the present study used intravenous infusion of phenylephrine to raise the blood pressure. This preparation allows rapid and inexpensive screening of compounds with blood pressure lowering activity. At the dose used (20 μg/kg/min), phenylephrine did significantly increase blood pressure. An increase in blood pressure is expected to cause reflex bradycardia by a vagally-mediated mechanism (18). However, despite increasing blood pressure, phenylephrine did not cause reflex bradycardia in the present study. Such a finding has been attributed to the phenylephrine-induced attenuation of vagally-induced bradycardia (19).

The findings show that among the novel dihydropyridine derivatives, compound C containing bis-phenyl butyl ester substitutes demonstrated significant potency comparable to that of nifedipine in reducing MBP. Moreover, as it is the case for compound D and E, substitution of cyclohexyl methyl ester or benzyl ester substitute moiety at C-3 and C-5 positions of dihydropyridine core resulted in decreased calcium channel blocking activity. From structure-activity relationship point of view, the results indicate that elongation of methylene linker of cyclohexyl or phenyl ester substitutes resulted in enhanced potency of the compounds. This finding is in agreement with the results of previous structure-activity relationship studies of dihydropyridines that substitution of C-3 and C-5 positions of nifedipine with a group larger than carboxymethyl generally maintain or increase calcium channel blocking activity, which might be associated with increased lipophilicity (13,20). However, the findings of the present *in vivo* study are not in agreement with those of an *in vitro* study (17), which showed that IC_{50} of compound D in relaxing the KCl-precontracted aortic rings was 10 times less than that of nifedipine. The mechanism of binding of dihydropyridines to calcium channel is such that the drug sandwiches between binding sites, therefore, large groups are expected to hold back such a sandwich type of binding. It has been shown that at low hydrophobicity the steric effect is more imperative and determines the activity of the compounds. However, at high hydrophobicity, binding is controlled by the hydrophobic interaction (12).

Meanwhile, the hydrophobic interaction between the drug and its binding site on calcium channels is the reason for its better bonding. Therefore, in the present study the increased hydrophobicity in compound C may explain its higher potency than those of the other compounds. Other explanations involved in the differences between the results of our study with those of the *in vitro* study (17) may stem from pharmacokinetic differences, physiological reflexes, channel-binding kinetics, frequency and voltage dependence of drug-channel interaction, and pathological state of the tissue used (21).

The findings of the present study show that there is no significant difference between the antihypertensive potency of phenyl alkyl ester derivatives and cyclohexyl ester counterparts. Benzyl ester derivative of dihydropyridine scaffold (compound E) and its cyclohexyl methyl ester counterpart (compound D) demonstrated similar antihypertensive profile at doses examined. Such a finding is also observed in phenyl propyl ester derivative (compound A) and cyclohexylpropyl ester counterpart (compound B). Moreover, the findings of the *in vitro* study (17) indicated that cyclohexyl derivatives or the phenyl substituent did not make any significant difference for their potency. This might imply that cyclohexyl or phenyl substituent in symmetric derivatives is not imperative for their attachment to their binding sites, and thereby their activities.

Traditionally, one important way to develop new drugs has been through random testing of chemicals or modification of drugs already known to have some effects (22). The present study was based on the modifications of some of dihydropyridine analogs. In order for a new molecule to reach clinical use in human it must have a higher potency than a standard drug. However, a drug with an efficacy profile equal

to that of a standard one, but with a better safety, more convenience to patient, or cost-effectiveness profiles can be a good candidate for drug development studies (23). Our findings show that some of the compounds tested had comparable hypotensive effects to that of nifedipine. Therefore, our findings can be used as a rationale for further exploration of safety, convenience to the patient, or cost-effectiveness of such compounds to reveal whether or not they have the potential of clinical use in human.

CONCLUSION

In conclusion, the present study shows that some of the new 1,4 –dihydropyridine derivatives containing different bis-cyclohexyl or bis-phenyl alkyl ester substitutes at C-3 and C-5, and 4-(1-methyl-4-nitro-5-imidazolyl) moiety at C-4 positions of dihydropyridine rings reduced MBP to a level comparable to that of nifedipine. Possible future use of these compounds in human requires further pharmacological and toxicological investigations.

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