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Original Article

Synthesis and antihypertensive activity of novel 4-[1-(4-X-benzyl)-5-imidazolyl] dihydropyridines in rat

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Abstract

A series of 1,4-dihydropyridine calcium channel blockers bearing 1-(4-X -benzyl)-5-imidazolyl substituent at 4 position (5a-e) (X=H, F) were synthesized and tested for antihypertensive activity in desoxycorticosterone acetate (DOCA)-induced hypertension in rats. Amlodipine was used as the standard dihydropyridine. All compounds tested showed lower antihypertensive activity than that of amlodipine. The most active compound (5e) had fluorine substituent (X=F).

Keywords: Imidazole; Dihydropyridine; Antihypertension

INTRODUCTION

Very soon after the discovery of cardiovascular properties of 1,4-dihydropyridines, it was found that these substances act by inhibiting the entry of Ca^{2+} into the cardiac cells and vascular muscle through the voltage dependent calcium channels (1).

Structurally diverse group of compounds are known to be effective as calcium antagonists (2). The most potent class of antagonists comprises derivatives of 1,4-dihydropyridine of which the most widely agent used today is amlodipine (3). These classes of compounds have been the subject of many structure activity relationship studies (4-8). Previously the effects of C-4 1-(4-nitrobenzyl)-5-imidazolyl substituents in conjunction with various C-3 and C-5 diesters on blood pressure has been reported using indirect tail-cuff method in rats (9). This paper describes the effects of new 1,4-dihydro-2,6-dimethyl-4-[1-(4-X-benzyl)-5-imidazolyl]-3,5-pyridinedicarboxylates [5a-e] on blood pressure in DOCA-salt hypertensive rats.

MATERIALS AND METHODS

Chemicals

Amlodipine was purchased from Tolidarou Pharmaceuticals (Tehran, Iran). All compounds including amlodipine were dissolved in dimethyl sulfoxide (DMSO). Other analytical grade reagents were obtained from Merck Company (Darmstadt, Germany).

Chemistry

Melting points were determined using the capillary apparatus with a system of Gallenkamp. 1H-NMR spectra were run on a Bruker AC-80 spectrometer. Infrared

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spectra were recorded on a FT-IR Perkin-Elmer Paragon 1000 spectrophotometer. Compounds 1 to 4 were prepared as reported previously (10).

Preparation of dialkyl 1,4-dihydro-2,6-dimethyl-4-[1-(4-X-benzyl)-2-alkylthio-5-imidazolyl]-3,5-pyridinedicarboxylate (5e)

A solution of ammonium hydroxide (25%, 0.5 ml) was added to a stirring solution of compound 4 (1.26 mmol) and alkyl acetoacetate (2.54 mmol) in methanol (5 ml). The mixture was protected from light and refluxed overnight. The methanol was evaporated at reduced pressure to give compounds 5a-e. Spectral data of compounds (5a-e) are given in Table 1.

Induction of experimental hypertension

This study was carried out on male Sprague Dawley rats (Razi Institutes, Mashhad, Iran) weighing between 250 and 300 g. Rats were housed in temperature and humidity controlled, light-cycled quarters. Hypertension was induced by DOCA-salt injection (20 mg/kg, twice weekly, for 5 weeks, s.c., n=20) and NaCl (1%) was added to their drinking water (11).

Studies in anaesthetized rats

Five weeks after DOCA injection, animals were anaesthetized with sodium thiopental (30 mg/kg by i.p. injection). The right common carotid artery was catheterized for the measurement of blood pressure, right and left jugular veins were cannulated for the administration of anesthetic (sodium thiopental, 10 mg/kg) and different agents such as acetylcholine, test agents (5a-e) and amlodipine throughout the experiment. The trachea was cannulated and the animals were allowed to breathe spontaneously. Body temperature was recorded using a rectal thermostat probe and was maintained at 37 ± 0.5 ^{o}C using an incandescent lamp placed over the abdomen. After stabilization, arterial blood pressure (systolic, diastolic and mean) and heart rate were measured.

Measurement of antihypertensive effects

All the test agents were administered with doses of 0.6, 1.2 and 1.8 mg/kg to the hypertensive rats in a volume of 0.3 ml/kg. Equivolumetric injection of vehicle DMSO was administered to the control animals. Amlodipine was used as the standard agent with the same doses.

Statistical Analysis of Data

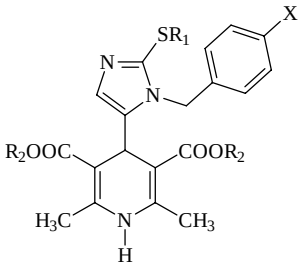
Results are expressed throughout as means ± S.E.M. and were analyzed by one way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison test (for comparison of responses to dihydropyridine with DMSO in hypertensive rats). P value of less than 0.05 was considered to be significant.

RESULTS

Chemistry

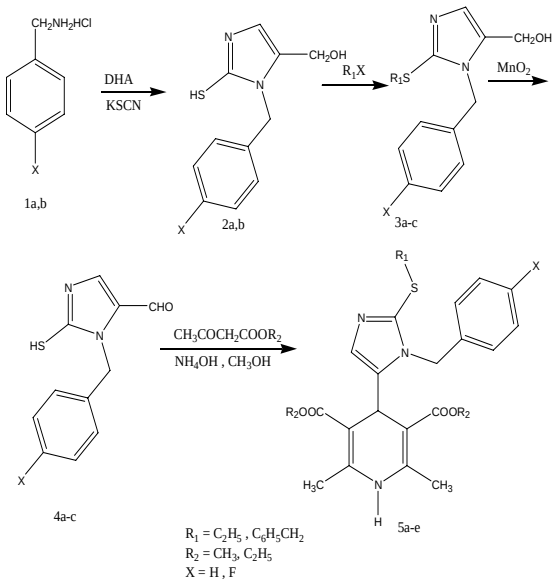
5-hydroxymethyl-1-(4-X-benzyl)-2-thoimidazole [2a,b] was prepared from 4-X-benzylamine hydrochloride [1] and dihydroxyacetone dimmer. Reaction of 2 (scheme 1) with alkyl halide afforded corresponding 2-alkylthio-1-(4-X-benzyl)-5-hydroxymethylimidazole [3a-c]. Oxidation of 3 with manganese dioxide in chloroform gave corresponding aldehyde [4a-c]. The symmetrical 1,4-dihydropyridines [5a-e] were prepared by the classical Hantzsch condensation in which the aldehyde [4a-c] were reacted with acetoacetic acid ester and ammonium hydroxide. Spectral data of compounds (5a-e) were given in Table 1.

Table 1. Characterization data of dihydropyridines (5a-e).



Compd[1]	R_1	R_2	Yield (%)	M.P. (ethyl acetate)	Mol. Formula[2] (M.W.)	IR in KBr (C=O) ν, cm^{-1}	^{1}H NMR($CDCl_3$) δ, ppm
5a	C_2H_5	CH_3	56%	157-58 °C	$C_{23}H_{27}N_3O_4S$ (441.54)	1699	7.5 -6.6 (m, 7H, arom, H-C_4 imidazole, NH), 5.4 (s, 2H, CH_2N), 5.04 (s, 1H, H-C_4 dihydropyridine), 3.4 (s, 6H, CH_3O), 2.93 (q, 2H, CH_2S, J=7.2Hz), 2.25(s, 6H, CH_3-$C_{2,6}$ dihydropyridine), 1.22(t, 3H, CH_3).
5b	C_2H_5	C_2H_5	53%	158-60 °C	$C_{25}H_{31}N_3O_4S$ (469.60)	1695	7.4 -6.6 (m, 7H, arom, H-C_4 imidazole, NH), 5.94 (s, 2H, CH_2N), 5.1 (s, 1H, H-C_4 dihydropyridine), 4.15-3.74 (m, 4H, CH_2O), 2.84 (q, 2H, CH_2S, J=8.0Hz), 2.21(s, 6H, CH_3-$C_{2,6}$ dihydropyridine), 1.33-1.00 (m, 9H, CH_3).
5c	$C_6H_5CH_2$	C_2H_5	50%	178-180 °C	$C_{30}H_{33}N_3O_4S$ (531.67)	1684	7.15-6.20 (m, 12H, arom, H-C_4 imidazole, NH), 5.17(s, 2H, CH_2N), 5.05 (s, 1H, H-C_4 dihydropyridine), 4.25-3.75(m, 6H, CH_2O, CH_2S), 2.16 (s, 6H, CH_3-$C_{2,6}$ dihydropyridine), 1.13(t, 6H, CH_3 , J=7.7Hz).
5d	$C_6H_5CH_2$	CH_3	78%	200-03 °C	$C_{28}H_{29}N_3O_4S$ (503.61)	1684	7.6-6.6 (m, 12H, arom, H-C_4 imidazole, NH), 5.14(s, 2H, CH_2N), 5.03 (s, 1H, H-C_4 dihydropyridine), 3.96 (s, 2H, CH_2S), 3.41(s, 6H, CH_3O), 2.24 (s, 6H, CH_3-$C_{2,6}$ dihydropyridine).
5e	$C_6H_5CH_2$	CH_3	40%	oily	$C_{28}H_{28}FN_3O_4S$ (521.60)	1683	7.8 -7.0 (m, 11H, arom, H-C_4 imidazole, NH), 5.24 (s, 2H, CH_2N), 4.78 (s, 1H, H-C_4 -dihydropyridine), 3.92 (s, 1H, CH_2S), 3.76 (s, 6H, CH_3O), 2.10 (s, 6H, CH_3-$C_{2,6}$ dihydropyridine).

[1] X=H in all compounds except **5e** in which X=F [2] C, H, and N analysis were within ± 0.4% of the theoretical values for the formula given.



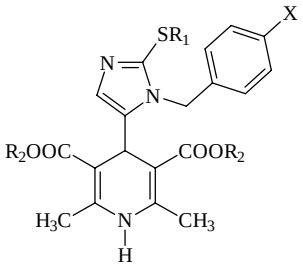
Scheme 1: General Procdure for the synthesis of dihydropyridine derivatives.

Table 2. Homodynamic effects of deoxycorticosterone acetate salt administration (Hypertensive, 20 mg/kg, twice weekly, for 5 weeks, s.c.) plus NaCl (1%, added to the rats' drinking water) in male rats. Normotensive rats received saline injection (0.5 ml/kg, twice weekly, for 5 weeks, s.c.). The values are given in mean ± S.E.M. of 15 experiments.

Groups	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Mean arterial blood pressure (mmHg)	Heart rate (beats /min)
Normotensive	111 ± 8.5	87 ± 5.9	98 ± 7.5	402 ± 22
Hypertensive	185 ± 3.4[b]	154 ± 3.8[b]	163 ± 3.5[b]	506 ± 12[a]

[a]$P < 0.05$ and [b]$P < 0.001$ vs normotensive.

Table 3. Fall in blood pressure after administration of test agents in hypertensive rats.



Compound	R_1	R_2	X	MABP fall (S.E.M)[a]		
				Dose, mg/kg, iv		
				0.6	1.2	1.8
5a	C_2H_5	CH_3	H	22.00 (1.82)	30.00 (2.44)	37.50 (1.71)
5b	C_2H_5	C_2H_5	H	21.50 (2.06)	33.00 (1.91)	42.00 (1.63)
5c	$CH_2C_6H_5$	CH_3	H	15.50 (1.70)	33.00 (1.91)	44.50 (2.21)
5d	$CH_2C_6H_5$	C_2H_5	H	19.50 (0.25)	33.00 (1.91)	40.50 (1.89)
5e	$CH_2C_6H_5$	CH_3	F	27.00 (1.29)	41.50 (3.50)	49.50 (4.22)
Amlodipine				48.00 (3.87)	71.25 (4.47)	90.00 (4.41)
DMSO				11.00 (0.57)	11.00 (0.57)	11.00 (0.57)

[a]Mean arterial blood pressure fall: standard errors of the mean (S.E.M) are indicated in parenthesis. All results were analyzed for statistically significant differences from control DMSO (0.3 ml/kg, iv) by analysis of variance and all showed significant difference ($P<0.05$).

Pharmacology

Arterial blood pressure, body and heart weight systolic, diastolic and mean arterial blood pressure was significantly increased in DOCA-salt treated rats (20 mg/kg DOCA, twice weekly, for 5 weeks, s.c., plus NaCl (1%) added to the animals' drinking water for 5 weeks) as compared to normotensive ones (0.5 ml/kg saline, twice weekly, for 5 weeks, s.c., Table 2). DOCA treatment reduced the body weight (DOCA-treated: 244 g ± 2 vs. Normotensive 379 g ± 7, $P<0.001$) and heart weight was significantly increased (DOCA-treated: 1384 mg ± 22 vs. Normotensive 1130 mg ± 37, $P<0.01$), resulting in a higher heart to body weight index.

Effects of test agents on hypertensive rats

Intravenous administration of compounds (0.6, 1.2, 1.8 mg/kg) produced blood pressure lowering effects in thiopental-anaesthetized hypertensive male Sprague Dawley rats. After stabilization, mean arterial blood pressure fall was measured (Table 3).

DISCUSSION

In the present study, administration of DOCA-salt and replacement of tap water with the NaCl (1%) for 5 weeks, increased the arterial blood pressure, which confirms the previous work (11). The increase in heart to body weight index represents a cardiac hypertrophy in DOCA-salt hypertensive rats which was similar to previous reports (12). Numerous *in vitro* studies indicate that endothelium-mediated relaxation is reduced in the DOCA-salt hypertension (13-15). It has also been reported that responses to acetylcholine are reduced in patient with essential hypertension (16). However, our *in vivo* studies showed that responses to acetylcholine are not affected by DOCA treatment. Although, the reasons for these disparate results are not clear yet, but species specific variations and the model of hypertension are important to note.

In the present study, all dihydropyridine agents (5a-e) produced a marked fall in arterial blood pressure of DOCA-treated rats. These drugs decreased both systolic and diastolic arterial blood pressure. Comparison of the activities of compounds (5a-e) with amlodipine showed that all compounds reduced the mean systolic blood pressure but were less potent than amlodipine. In the anaesthetized rats, heart rate was not significantly changed by these agents, which suggest that their effects in the heart (cardiac output) are negligible. Compound 5e with 4-fluoro substituent was found to be the most active one. So, presence of an electron-withdrawing group at the para-position of benzyl ring increases antihypertensive activity. Comparison of the activities of compound 5a and 5c with ethylthio (R1=C2H5) and benzylthio (R1=CH2C6H5) substituent respectively showed that smaller alkyl group at R1 position gave the more active compound.

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