Original Article

Synthesis and evaluation of calcium channel antagonist activity of new 1, 4-dihydropyridines containing phenylamineimidazolyl substitute in guinea-pig ileal smooth muscle

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ABSTRACT

Background: 1,4-dihydropyridines are a class of drugs which are used in the treatment of some cardiovascular disorders. The prototype, Nifedipine, does not have optimal pharmacokinetic and pharmacodynamic properties. Several new derivatives of 1, 4-dihydropyridine have been produced and pharmacologically evaluated in order to find drugs with better pharmacological properties. Among them, those with a substituted heteroaromatic ring in the C_4 position of the 1, 4-dihydropyridine ring, instead of the phenyl ring in Nifedipine, are most considered. In this study, eight novel derivatives of this class with "2-methylthio-1-(phenylamino)imidazole-5-yl" in the C_4, C_3 and C_5 positions were prepared and evaluated as calcium channel antagonist agents.

Methods: To prepare these compounds, Hantzsch method for the synthesis of 1, 4-dihydropyridine derivatives was deployed. An aldehyde was reacted with appropriate acetoacetate ester and ammonium acetate. This aldehyde was prepared in three steps. Cumulative doses were applied to determine the relaxing effect of the compounds on the longitudinal smooth muscle of male albino guinea pigs.

Results: Chemical structures of the compounds were characterized by ^1H nuclear magnetic resonance, infrared and mass spectroscopy. The IC_{50} of each compound was graphically determined from the concentration-response curves.

Conclusions: Two compounds were more active than Nifedipine. Both had lipophilic ester groups with low steric hindrance that met the merits of a better receptor binding of 1, 4-dihydropyridines. These derivatives have high potential for further study.

Key words: 1, 4-dihydropyridine, Calcium channel antagonist, Phenylamineimidazolyl, Cardiovascular disorder

1, 4-Dihydropyridine calcium channel antagonists form an important class of drugs which induce relaxation of vascular smooth muscles, preferentially in arteries and display a negative inotropic effect on isolated cardiac muscle [1]. They exert these effects via binding to a high affinity binding site in L-type voltage-dependent Ca^{2+} channels[2]. In practice, this class of drugs is effective in the treatment of hypertension, angina pectoris and other cardiovascular disorders. Changes in the substitution pattern at the C_3, C_4, C_5 positions of Nifedipine, the prototype of

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Sponsored by : Isfahan University of Medical Sciences and Health Services, grant number 79219.

1, 4-dihydropyridines, alter activity and tissue selectivity[3,4]. In some studies heteroaromatic groups substituted with a phenyl group, attached directly to the heteroaryl ring or indirectly, by a methylene linkage, were used as the C_4 substitute of Ca^{2+} channel antagonist 1, 4-dihydropyri- dines [5, 8]. It was therefore of interest to determine the effect of selected C_3 and C_5 substitutes, in conjugation with 2-methylthio-1-(phenylamino) 1*H*-imidazole-5-yl as C_4 substitute on calcium channel antagonist activity. We now report the synthesis and calcium antagonist activities of new dialkyl (aryl alkyl) 1, 4-dihydro-2, 6-dimethyl-4- [2-methylthio-1-(phenylamino) 1H-imidazole-5-yl]-3,5-pyridine dicarboxy- lates.

Materials and Methods

Chemistry: Melting points were determined using a Mettler FP61 capillary apparatus. Infrared spectra acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-80 instrument was used to acquire ^{1}HNMR spectra. Chloroform-D was used as solvent. Mass spectra were acquired with a Finnigan TSQ-70 mass spectrometer. Electron-impact ionization was performed at an ionizing energy of 70 eV; the source temperature was 250 °C. The synthesis of the 1, 4-dihydropyridine derivatives 5a-h (Table 1) was achieved following the steps outlined in Figure 1. 5-Hydroxymethyl-1-phenylamino-2-mercapto-imidazole 2 was prepared according to the procedure described by Denner et al [9]. Reaction of 2 with methyl iodide afforded corresponding substituted methylthio imidazole 3. Oxidation of 3 with manganese dioxide in chloroform gave the corresponding aldehyde 4. The symmetrical 1, 4-dihydropyridine deriveatives 5a-h were prepared (23-56 % yield) by the classical Hantzsch condensation[10] in which the aldehyde 4 was reacted with the acetoacetate ester and ammonium acetate. The compounds were characterized by ^{1}H nuclear magnetic resonance, infrared and mass spectroscopy. The purity of all products was determined by thin layer chromatography using several solvent systems of different polarities. The physical properties of final compounds are summarized in Table 1. All final products were pure and stable compounds. Similar to other analogues of Nifedipine, they are also lipophilic compounds with very slight solubility in water.

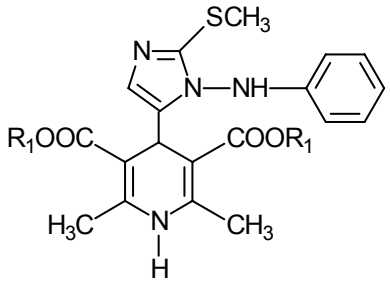

Figure 1. General reaction scheme for the preparation of symmetric 1, 4-dihydropyridines.

Table 1. Physical properties of synthesized symmetrical derivatives 5a-h.

Compound	R_1	Mp (°C)	Yield (%)
5a	CH_3	198	50
5b	C_2H_5	143	35
5c	C_3H_7	143	56
5d	$CH(CH_3)_2$	186	35
5e	$C(CH_3)_3$	227	23
5f	$CH_2C_6H_5$	177	52
5g	$CH_2CH_2C_6H_5$	133	23
5h	$CH_2CH_2CH_2C_6H_5$	133	44

5-Hydroxymethyl-2-mercapto-1-phenyl-amino imidazole 2: To a mixture of dihydroxyacetone (46 mmole, 4.26 g), potassium thiocyanate (70 mmole, 6.9 g) and phenylhydrazine (60 mmole, 8.66 g) in 34 mL of methanol, 5.4 mL of glacial acetic acid was added. The reaction mixture was stirred at room temperature for 6 hours until a creamy mixture was formed. It was filtered and the solvent was evaporated under reduced pressure. The product then was extracted from the residue and the solid mixture remained on the filter by methyl-ethyl ketone in a protonated form. After evaporation of the solvent under reduced pressure the residue was washed twice with ether in order to give the pure salt form of the desired compound.

5-Hydroxymethyl-2-methylthio-1-phenylamino imidazole 3: A mixture of 5-hydroxymethyl -2- mercapto-1-phenylamino imidazole (2) acetate (10 mmole, 2.81g) and potassium hydroxide (25 mmole, 1.4 g) in 5 mL of methanol was placed in a two- necked flask equipped with a condenser. A solution of methyl iodide (15 mmole, 2.13 g) in 2.5 mL of methanol was added drop-wise to this solution by means of a dropping funnel, while it was being stirred at room temperature. After two more hours stirring, the mixture was poured into water and the solid precipitate was collected, washed with water, and dried. The residue was recrystallized in a mixture of acetone and petroleum ether. White needle-form crystals were separated from the crystallizing solvent.

1-Phenylamino-2-methylthio imidazole-5-carboxaldehyde 4: A mixture of 5-hydroxymethyl -2- methylthio -1- phenylamino imidazole (3) (21.3 mmole, 5 g) and activated manganese dioxide (85.2 mmole, 7.4 g) in 35 mL of acetonitrile was refluxed in a round-bottom 50 mL flask for 9 hours. The reaction mixture was filtered while it was boiling on a sintered glass funnel. The residue of the filtration was washed twice with boiling chloroform. Filtered solution was evaporated with rotator evaporator under reduced pressure and the residue was crystallized in a mixture of acetone and petroleum ether to yield white needle-form crystals.

General Procedure for the synthesis of dihydropyridine derivatives 5a-h: A mixture of 1- phenylamino-2-methylthio imidazole-5-carboxaldehyde (4) (0.429 mmole, 100 mg), appropriate acetoacetate ester (0.858 mmole) and ammonium acetate (0.429 mmole, 33 mg) in 3 mL of methanol was refluxed for 24 hours. After the mixture was cooled to room temperature, the solvent was evaporated and the residue was purified by preparative thin-layer chromatography. Silicagel GF254 was used as an adsorbent and chloroform was used as the mobile phase.

Pharmacology: Nifedipine was supplied by Sigma Quimica, Spain. All compounds were dissolved in dimethyl sulfoxide (DMSO). Solutions of dihydropyridines and the organ bath were protected from light. Other analytical grade reagents were obtained from Merck Company (Darmstadt, Germany).

Male albino guinea pigs (300-450g) were killed by a blow to the head. The intestine was removed above the ileocaecal junction and longitudinal smooth muscle segments of 2 cm in length were mounted under a resting tension of 400-500 mg. The segments were maintained at 37 ºC in a 20 ml jacketed organ bath containing oxygenated (100% O_2) physiological saline solution (PSS) of the following composition (in mM): NaCl (137), $CaCl_2$ (1.8), KCl (2.7), $MgSO_4$ (1.1), NaH_2PO_4 (0.4), $NaHCO_3$ (12) and glucose (5). The muscles were equilibrated for one hour with a solution change every 15 minutes. The concentra- tions were recorded with a force displacement transducer (F-50) on a NARCO physiograph. 0.7 mL of a 3 molar solution of KCl was used to induce contraction in the ileum. Cumulative doses of each calcium-channel antagonist were then added to the organ bath at 10 minute intervals. Each segment was treated with only one compound. The IC_{50} of each compound was graphically determined from the concentration-response curves [8].

Data were presented as mean ± SEM. Comparison between groups was made using one way analysis of variances with post-hoc test of LSD. A P-value < 0.05 was considered as statistically significant.

Results

Chemistry: 5-Hydroxymethyl -2- mercapto -1-phenylamino imidazole 2

Yield: 65%, MP 134 °C; IR (KBr): ν (cm^{1}) 3260 (*OH*); ^{1}H NMR ($CDCl_3$): δ (ppm) 8.2 (s, 1H, H_4 of imidazole), 7.9-8.1 (m, 2H, H_2 and H_6 of phenyl), 7.1-7.5 (m, 3H, H_3, H_4, H_5 of phenyl), 6.0 (s, 1H, SH), 4.6 (s, 2H, CH_2 OH), 3.7 (s, 1H, CH_2 O*H*) .

5-Hydroxymethyl-2-methylthio-1-phenylamino imidazole 3: Yield: 67%, MP: 93 °C; IR (KBr): ν (cm-1) 3260 (OH); 1H NMR (CDCl3): δ (ppm) 7.5 (m, 6H, 1H: H4 of imidazole, 5H: phenyl), 4.8 (s, 2H, CH2 OH), 3.2 (brd.s, 1H, CH2 OH), 2.7 (s, 3H, SCH3).

1-Phenylamino-2-methylthio imidazole-5-carboxaldehyde 4: Yield: 85%, MP: 85 °C; IR (KBr): ν (cm-1) 3300 (NH), 1710 (CO); 1H NMR (CDCl3): δ (ppm) 10.0 (s, 1H, CHO), 7.6 (m, 6H, 1H: H4 of imidazole, 5H: phenyl), 2.8 (s, 3H, SCH3).

Dimethyl 1, 4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole -5-yl]-3,5-pyridinedicarboxylate 5a: Yield: 50%, MP: 198 °C; IR (KBr): ν (cm^{-1}) 3348 (*NH*), 1704 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.40 (brd.s, 6H, 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.55 (brd.s, 1H, H_1 of dihydropyridine), 5.40 (s, 1H, H_4 of dihydropyridine), 3.70 (s, 6H, -$COOCH_3$), 2.65 (s, 3H, SCH_3), 2.30 (s, 6H, C_2, C_6 CH_3); Mass, *m/z* (%): 428.0 (M+), 223.9 (35), 322.9 (74), 325.0 (13), 335.0 (31), 354.8 (100), 381.9 (40), 383.0 (19), 400.0 (21), 414.0 (86).

Diethyl 1, 4-dihydro-2, 6-dimethyl- 4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl]-3,5-pyridinedicarboxylate 5b : Yield: 35%, MP: 143 °C; IR (KBr): ν (cm^{-1}) 3343 (*NH*), 1692 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.45 (brd.s, 6H, 1H: H_4 of imidazole, 5H:-NH-C_6H_5), 6.90 (brd.s, 1H, H_1 of dihydropyridine), 5.40 (s,1H, H_4 of dihydropyridine), 4.20 (q, 4H, -CH_2CH_3), 2.65 (s, 3H, SCH_3), 2.25 (s, 6H, C_2, C_6 CH_3), 1.30 (t, 6H, -CH_2CH_3); Mass, *m/z* (%): 456.0 (M+), 251.9 (37), 322.9 (64), 349.0 (26), 368.8 (100), 395.7 (20), 442.0 (37).

Di n-propyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl]-3,5-pyridinedicarboxylate 5c : Yield: 56%, MP: 143 °C; IR (KBr): ν (cm^{-1}) 3206, 3270 (*NH*), 1694 (*CO*). ^{1}H NMR ($CDCl_3$): δ (ppm) 7.40 (brd.s, 6H, 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.90 (brd.s, 1H, H_1 of dihydropyridine), 5.40 (s, 1H, H_4 of dihydropyridine), 4.05 (t, 4H, -$CH_2CH_2CH_3$), 2.50 (s, 3H, SCH_3), 2.10 (s, 6H, C_2, C_6 CH_3), 1.65 (m, 4H, $CH_2CH_2CH_3$), 0.90 (t, 6H, -$CH_2CH_2CH_3$). Mass, *m/z* (%): 484.0 (M+), 195.8 (24), 279.9 (54), 322.9 (68), 363.0 (30), 382.8 (100), 385.0 (18), 409.8 (18), 470.0 (34).

Di iso-propyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl] -3, 5-pyridinedicarboxylate 5d: Yield: 35%, MP: 186 °C; IR (KBr): ν (cm^{-1}) 3252 (*NH*), 1688 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.50 (brd.s, 6H, 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.30 (brd.s, 1H, H_1 of dihydropyridine), 5.40 (s, 1H, H_4 of dihydropyridine), 5.05 (m, 2H, -C*H* $(CH_3)_2$), 2.60 (s, 3H, SCH_3), 2.25 (s, 6H, C_2, C_6 CH_3), 1.30 (d, 12H, -CH $(CH_3)_2$); Mass, *m/z* (%): 484.0 (M+), 42.9 (13), 195.6 (19), 279.9 (29), 296.7 (62), 341.0 (66), 364.0 (23), 382.9 (100), 409.9 (19), 411.0 (31), 470.0 (57), 473.0 (26).

Di tert-butyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl]-3,5-pyridinedicarboxylate 5e: Yield: 23%, MP: 227 °C; IR (KBr): ν (cm^{-1}) 3270 (*NH*), 1690 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.50 (brd.s, 6H, 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.70 (brd.s, 1H, H_1 of dihydropyridine), 5.40 (s, 1H, H_4 of dihydropyridine),2.65 (s, 3H, SCH_3), 2.25 (s, 6H, C_2, C_6 CH_3), 1.50 (s, 18H, -$C(CH_3)_3$);

Mass, *m/z* (%): 512.0 (M+), 424.9 (86), 429.0 (30), 441.0 (20), 499.1 (100), 502.0 (34).

Dibenzyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1- (phenylamino)1H-imidazole-5-yl]-3,5-pyridinedicarboxylate 5f : Yield: 52%, MP: 177 °C; IR (KBr): ν (cm^{-1}) 3210, 3280 (*NH*), 1698 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.00-7.65 (m, 16H, 10H: -$CH_2C_6H_5$ 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.85 (brd.s, 1H, H_1 of dihydropyridine), 5.55 (s, 1H, H_4 of dihydropyridine), 5.20 (s, 4H, -$CH_2C_6H_5$), 2.50 (s, 3H, SCH_3), 2.25 (s, 6H, C_2, C_6 CH_3); Mass, *m/z* (%): 580.0 (M+), 322.9 (23), 376.0 (42), 431.0 (100), 433.0 (22), 566.0 (25).

Diphenethyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl]-3,5-pyridinedicarboxylate 5g: Yield: 23%, MP: 133 °C; IR (KBr): ν (cm^{-1}) 3200, 3270 (*NH*), 1693 (*CO*); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.00- 7.60 (m, 16H, 10H: -$CH_2CH_2C_6H_5$ 1H: H_4 of imidazole, 5H: -NH-C_6H_5), 6.30 (brd.s, 1H, H_1 of dihydropyridine), 5.45 (s, 1H, H_4 of dihydropyridine), 4.40 (t, 4H, -$CH_2CH_2C_6H_5$), 2.95 (t, 4H, -$CH_2CH_2C_6H_5$), 2.55 (s, 3H, SCH_3), 2.20 (s, 6H, C_2, C_6 CH_3); Mass, *m/z* (%): 608.3 (M+), 323.0 (37), 404.1(48), 426.0 (28), 445.1 (100), 447.0 (19), 594.2 (18).

Diphenpropyl 1,4-dihydro-2,6-dimethyl-4-[2-methylthio-1-(phenylamino)1H-imidazole-5-yl] -3, 5-pyridinedicarboxylate 5h: Yield: 44%, MP: 133 °C; IR (KBr): ν (cm-1) 3219, 3272 (NH), 1692 (CO); 1H NMR (CDCl3): δ (ppm) 6.90-7.70 (m, 16H, 10H: -CH2CH2CH2C6H5 1H: H4 of imidazole, 5H: -NH-C6H5), 6.85 (brd.s, 1H, H1 of dihydropyridine), 5.50 (s, 1H, H4 of dihydropyridine), 4.15 (t, 4H, -CH2CH2CH2C6H5), 2.70 (t, 4H, -CH2CH2CH2C6H5), 2.50 (s, 3H, SCH3), 2.20 (s, 6H, C2, C6 CH3), 1.95 (m, 4H, -CH2CH2CH2C6H5); Mass, m/z (%): 636.0 (M+), 90.8 (53), 116.9 (21), 195.6 (13), 322.9 (76), 328.0 (19), 432.0 (40), 438.0 (36), 458.9 (100), 486.1 (26), 612.2 (28).

Pharmacology: The calcium-channel antagonist activities (IC_{50}) of 5a-h were determined as the concentration needed to produce 50% relaxation of contracted guinea pig ileal longitudinal smooth muscle. The IC_{50} values are presented in Table 2.

Table 2. Calcium channel antagonist activities (IC_{50}) of compounds **5a-h.**

Compound	IC_{50} ±SEM (n=6)
5_a	$3.54 \pm 0.85 \times 10^{-9}$
5_b	$2.29 \pm 0.79 \times 10^{-9}$
5_c	$1.69 \pm 0.43 \times 10^{-9}$
5_d	$2.16 \pm 0.76 \times 10^{-8}$
5_e	$1.21 \pm 0.27 \times 10^{-7}$
5_f	$1.49 \pm 0.39 \times 10^{-9}$
5_g	$2.79 \pm 0.65 \times 10^{-9}$
5_h	$3.15 \pm 0.71 \times 10^{-9}$
Nifedipine	$2.55 \pm 0.21 \times 10^{-9}$

Discussion

Comparison of the activities of synthesized symmetrical compounds indicated that IC_{50} of compounds $\mathbf{5_d}$ and $\mathbf{5_e}$ were significantly higher than Nifedipine. Therefore, in compounds possessing two large ester substitutes, with a branch in the first carbon atom of the substitute (*iso*-propyl and *tert*-butyl) the steric parameters cause a diminution in their activity. The same relationship between the steric effects and calcium channel antagonist activity has been reported for the 1,4-dihydropyridines with 1-(4-nitrobenzyl)-5-imidazolyl or 2-methylthio-1- (4-nitrobenzyl)-5-imidazolyl substitute in the C-4 position of the dihydropyridine ring[8].

Finally the results show that two of the compounds, $\mathbf{5_c}$ and $\mathbf{5_f}$, were more active than Nifedipine. Both of them have lipophilic ester groups with low steric hindrance, which meet the merits of a better receptor bonding of 1, 4-dihydropyridines. These derivatives have high potential for further studies.

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