Arch Pharm Res Vol 34, No 9, 1417-1426, 2011
DOI 10.1007/s12272-011-0902-9

Archives of Pharmacal Research
www.springer.com/12272

Design and Synthesis of New 1,4-Dihydropyridines Containing 4(5)-chloro-5(4)-imidazolyl Substituent as a Novel Calcium Channel Blocker

Maryam Iman[1], Asghar Davood[2], Ali Reza Nematollahi[2], Ahmad Rerza Dehpoor[3], and Abbas Shafiee[4]

[1]Nanotechnology Research Center, Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 91775, Iran, [2]Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran 19419, Iran, [3]Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 14174, Iran, [4]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran

(Received November 3, 2010/Revised February 16, 2011/March 16, 2011)

New analogues of nifedipine, in which the ortho-nitro phenyl group at position 4 has been replaced by 4(5)-chloro-5(4)-imidazolyl substituent and which are able to interact with the receptor by hydrogen binding were designed, synthesized, and evaluated as calcium channel antagonists. The designed dihydropyridines were synthesized using the Hantzsch condensation and evaluated as calcium channel antagonists using the high K+ contraction of guinea-pig ileal longitudinal smooth muscle. A docking study was performed using the AutoDock4 program, and QSAR equations were obtained using multilinear regression. Our computational studies indicated that the oxygen of the ester (O10) and the N3' of the imidazole ring form a hydrogen bonding interaction with the NH of HIS 363 and NH of LYS354, respectively, and that the sum of the BEHp5 and RDF075p are the most significant descriptors. The results of calcium channel antagonist evaluation demonstrated that increasing the chain length in C3 and C5 ester substituents increased activity. The most potent compound was the bis-phenylpropyl ester (**5l**) derivative, in that it was more active than the reference drug nifedipine and that the bis-phenylethyl ester (**5k**) derivative had comparable activity with nifedipine. The present research revealed that the 4(5)-chloro-5(4)-imidazolyl moiety is a bioisoster of o-nitrophenyl in nifedipine and provided novel dihydropyridines with more activity as calcium channel antagonists.

Key words: 1,4-dihydropyridines, Calcium channels antagonist activity, Quantitative structure-activity relationship, Imidazole

Selected by Editors

INTRODUCTION

The influx of extracellular Ca^{+2} through L-type voltage dependent calcium channels regulates a number of important physiological functions, including smooth cardiac muscle contraction (Godfraind, 1986). The discovery that the 1,4-dihydropyridine (DHP) class of calcium channel antagonists inhibited the Ca^{+2} influx represented a major therapeutic advance in the treatment of cardiovascular disease such as hypertension, angina pectoris, and other spastic smooth muscle disorders (Fleckenstein, 1977). The DHP class of drugs, for which nifedipine is the prototype, has been the subject of many structure activity relationship studies (Janis and Triggle, 1983). Changes in the substitution pattern at the C_3, C_4, and C_5 positions of DHPs alter potency (Janis and Triggle, 1983; Coburn et al., 1988), tissue selectivity (Schramm et al., 1983), and the conformation of the 1,4-DHP ring. One of the structural

Correspondence to: Asghar Davood, Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, No 99, Yakhchal ave. Shariatie Street, Tehran 19419, Iran
Tel: 98-21-2260-9043, Fax: 98-21-2260-2059
E-mail: adavood@iaups.ac.ir, adavood2001@yahoo.com

requirements is that the substituted aromatic ring occupies an axial position perpendicularly bisecting the boat-like DHP ring with the substituent in a syn-periplanar orientation (Davood et al., 2001, 2006, 2010). In previous studies, we reported that a C_4 imidazole substituent created active compounds as calcium channel antagonist (Shafiee et al., 1996, 1998; Pourmorad et al., 1997; Davood et al., 2001). Herein, we describe the synthesis and calcium channel antagonist activity of new 1,4-dihydropyridines containing 4(5)-chloro-5(4)-imidazolyl substituent. For selection of this heterocycle substituent in the 4 position, the followings were considered:

1) Some heterocycle rings (imidazolyl moiety) were effective as 4-aryl in dihydropyridine structure (Shafiee et al., 1996, 1998; Davood et al., 2001).

2) Substitution of chlorine instead of NO_2 in the 4-aryl ring produced active compounds (Arrowsmith et al., 1986).

3) Considering the tatumeric forms (Fig. 1) of the 4(5)-chloro-5(4)-imidazolyl moiety, both of nitrogen atoms probably can interact with the receptor by hydrogen binding (donor, acceptor) and so both of tatumeric forms should be pharmacologically active. Our molecular modeling studies in the gas and aqueous phases indicated that the 4-chloro tautomer had very good compatibility with the reference drug, nifedipine, and was the main form and more stable than the 5-chloro tautomer.

To rationalize these findings, we docked the compounds into the active site of an L-type calcium channel. Furthermore, to obtain a quantitative understanding of the structure activity relationships (SAR) of these antagonists, a quantitative structure activity relationships (QSAR) analysis was performed.

MATERIALS AND METHODS

Molecular modeling and docking studies

The chemical structure of desired compounds was determined using HYPERCHEM software (version7, Hypercube Inc.). Conformational analysis of the designed compounds in gas phase (Fig. 1A and B) and aqueous solution (Fig. 1C and D), was performed using HYPERCHEM through semi-empirical molecular orbital (PM3) and molecular mechanic (MM+) calculation methods, respectively. The DHPs were placed in the center of 48 water molecule periodic box with dimensions of 12.0 by 10.0 by 12.0 and 2.3 Ångstroms distance between the water and DHPs, to simulate behavior in aqueous solution.

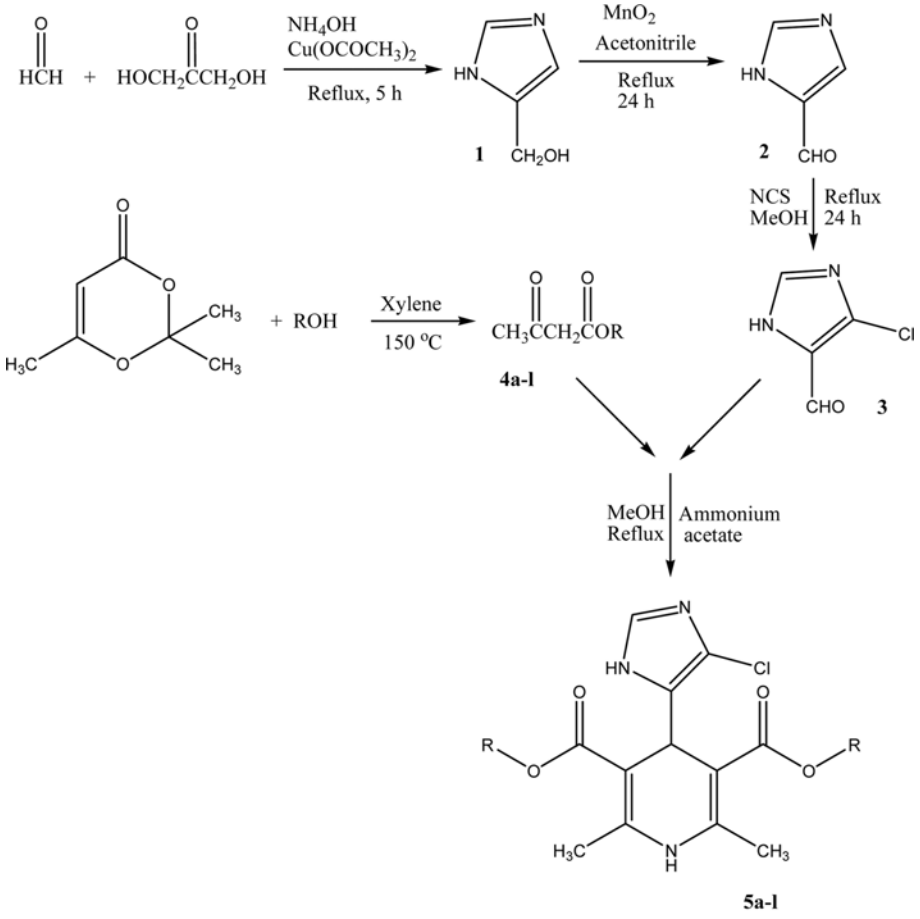
The molecular structures were optimized using the Polak-Ribiere (conjugate gradient) algorithm until the root mean square gradient was 0.01 kcal/mol. Among all energy minima conformers, the global minimum of two favored tautomeric forms of DHPs were compared with the global minimum of nifedipine as a reference drug. Thus, the superimposition of these conformers were performed to confirm whether the tautomeric form could mimic the major conformation of the reference drug to be used in the docking calculations. The resultant geometry was transferred into the Autodock (version 4) program package, and docking calculations were performed using Autodock tools. An L-type calcium channel, generated by resorting to multibody molecular dynamics simulations, was downloaded from the PDB bank server (PDB entry 1T0 J).

Autodock 4 started with a ligand molecule in an arbitrary conformation, orientation, and position and found favorable dockings in a protein-binding site using both simulating annealing and genetic algorithms. AutoDockTools (ADT), which has been released as an extension suite to the Python Molecular Viewer, was used to prepare the protein and the ligand. For the crystal structure of L-type calcium channel, polar hydrogens were added, and then Kollman United Atom charges and atomic solvation parameters were assigned. The grid maps of docking studies were computed using AutoGrid 4 included in the Autodock4 distribution. Grid center was centered on the active site and $60 \times 60 \times 60$ points with grid spacing of 0.375 were calculated. The GA-LS method was adopted to perform the molecular docking. The parameters for GA were defined as follows: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; mutation and crossover rates of 0.02 and 0.8, respectively. Pseudo-Solis & Wets parameters were used for local search and 300 iterations of Solis & Wets local search were imposed. The number of docking runs was set to 50.

Both Autogrid and Autodock computations were performed on Cygwin. After docking, all the generated structures were assigned to clusters based on a tolerance of 1 Å all-atom RMSD from the lowest-energy structure. Hydrogen bonding and hydrophobic interactions between docked potent agents and macromolecules were analyzed using ADT (Version 1.5.2). The conformation with the lowest (docked) energy was considered to be the best docking result.

Computation of structural descriptors and descriptive QSAR equation

QSAR have been widely used in the drug design process whenever detailed structural information on the ligand-receptor interactions have not been experimentally available (Hansch and Fujita, 1964; Hansch,



Scheme 1. Synthesis of new 1,4-dihydropyridines containing 4-(5)-chloro-5(4)-imidazolyl substituent.

1969; Gaudio et al., 1994).

Two-dimensional structures of molecules were drawn using the Hyperchem 7.0 software. The final geometries were obtained with the semi-empirical PM3 method in the Hyperchem program. The molecular structures were optimized using the Polak-Ribiere (conjugate gradient) algorithm until the root mean square gradient was 0.01 kcal/mol. The resulting geometry was transferred into the EDRAGON programs package (http://www.vcclab.org/lab/edragon). SPSS software (version15) was used for the MLR regression analysis.

A large number of molecular descriptors was calculated using Hyperchem, Dragon package and Autodock tools. Some chemical parameters including molecular volume (V), molecular surface area (SA), hydrophobicity (LogP), hydration energy (HE), refractivity (Rf), partial charge (PC), molecular polarizability (MP), and different quantum chemical descriptors including dipole moment (DM), local charges, HOMO, and LOMO energies were calculated using Hyperchem. Dragon software was used to calculate different functional groups, topological, geometrical, and constitutional descriptors for each molecule. Results based on the docked conformations included the intermolecular energy, Vdw-hb-desolv energy, electrostatic energy, total internal energy, torsional energy, unbound energy, predicted binding energy, and inhibition_constant (Ki) all of which we usedas descriptors in QSAR studies.

The calculated descriptors were first analysed for the existance of constant or near-constant variables, and those detected were removed. In addition, to decrease the redundancy existing in the descriptor data matrix, the correlation of descriptors with each other and with the activity (pIC_{50}) of the molecules was examined and collinear descriptors (i.e. $r > 0.9$) were detected. Among the collinear descriptors, the one that had the highest correction with activity was retained and the others were removed from the data matrix. The calculated descriptors were collected in a data matrix whose number of rows and columns were the number of molecules and descriptors, respectively.

MLR with factor analysis as the data pre-processing step for variable selection (FA-MLR) and principal component regression analysis (PCRA) methods were used to derive the QSAR equations.

Chemistry

DHP's agents (Table I) were synthesized according to Scheme 1 by using classical Hantzsch condensation (Hantzsch, 1882; Goldmann and Stoltefuss, 1991) in which 4(5)-chloro-imidazole-5(4)-carboxaldehyde 3 was reacted with 3-oxobutanoic acid ester **4a-l** and ammonium acetate. Compound **3** was prepared in three steps from formaldehyde, dihydroxyacetone, and ammonia (Davood et al., 2006). Some of the 3-oxobuthanoic acid esters (**4c-l**) were prepared according to the literature (Li et al., 1998).

Melting points were determined using a Thomas-Hoover capillary apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Bruker FT-500 spectrometer TMS was used as an internal standard. Infrared spectra were acquired on a Nicolet 550-FT spectometer. Mass spectra were measured with a Finnigan TSQ-70 spectrometer at 70 eV. Elemental analysis was carried out with a Perkin-Elmer model 240-C apparatus. The results of elemental analysis (C, H, N) were within ± 0.4% of the calculated amounts.

4 (5)-hydroxymethylimidazole (1)

This compound was prepared according to the literature (Davood et al., 2006). mp 87-88°C; IR (KBr): ν 3100 cm^{-1} (OH). ^{1}H-NMR (DMSO-d_6): δ 4.43 (s, 2H, CH_2OH), 6.90 (s, 1H, H_4 or H_5-imidazole), 7.57 (s, 1H, H_2-imidazole). Anal. Calcd. for $C_4H_6N_2O$: C, 48.97; H, 6.16; N, 28.56. Found: C, 48.81; H, 6.07; N, 28.30.

Imidazole-4(5)-carboxaldehyde (2)

This compound was prepared according to the literature (Davood et al., 2006). mp 146-147°C; IR (KBr): ν 1665 cm^{-1} (CO). ^{1}H-NMR (DMSO-d_6): δ 7.93 (s, 1H, H_4 or H_5-imidazole), 7.99 (s, 1H, H_2, imidazole), 9.74 (s, 1H, CHO), 12.98 (bs, 1H, NH). Anal. Calcd. for $C_4H_4N_2O$: C, 50.00; H, 4.20; N, 29.15. Found: C, 50.28; H, 4.43; N, 29.39.

5(4)-chloroimidazole-4(5)-carboxaldehyde (3)

This compound was prepared according to the literature (Davood et al., 2006). mp 199-201°C; IR (KBr): ν 1675 cm^{-1} (CO). ^{1}H-NMR (DMSO-d_6): δ 7.98 (s, 1H, H_2-imidazole), 9.75 (s, 1H, CHO), 12.99 (bs, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ 185.98 (CO), 142.53 (C_2, imidazole), 139.59 (C_5, imidazole), 126.68 (C_4, imidazole). Anal. Calcd. for $C_4H_3ClN_2O$: C, 36.80; H, 2.31; N, 21.46. Found: C, 36.64; H; 2.49; N, 21.29.

General procedure for the prepartation of 3-oxobutanoic acid esters (4c-l)

A solution of an alcohol and dioxin in xylene was placed in an Erlenmeyer flask. The flask was immersed in an oil bath that had been preheated to 150°C, and the solution was vigorously stirred. The evolution of acetone became apparent within several minutes and heating was continued from 1 to 3 h. The xylene was then removed, and the product was distilled.

Propyl 3-oxobutanoate (4c)

Using the general procedure and propyl alcohol provided the title compound after 2 h of reflux: Yield 69%, bp 60-62°C/10 mmHg. IR (NaCl): ν 1747 (CO ester), 1719 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.10 (t, J = 6.5 Hz, 2H, OCH_2), 3.45 (s, 2H, $COCH_2CO$), 2.27 (s, 3H, CH_3CO), 1.63 (m, 2H, CH_2CH_3), 0.88 (t, J = 6.5 Hz, 3H, CH_2CH_3).

Isopropyl 3-oxobutanoate (4d)

Using the general procedure and isopropyl alcohol provided the title compound after 3 h of reflux: Yield 55%. IR (NaCl): ν 1745 (CO ester), 1715 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.05 (m, 1H, C*H*), 3.42 (s, 2H, $COCH_2CO$), 2.2 (s, 3H, CH_3CO), 1.25 (d, 6H, CH_3CHCH_3).

Isobutyl 3-oxobutanoate (4e)

Using the general procedure and isobutyl alcohol provided the title compound after 2 h of reflux: Yield 80%, bp 70-72°C/10 mmHg. IR (NaCl): ν 1749 (CO ester), 1725 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.48 (d, J = 6.6 Hz, 2H, OCH_2), 3.46 (s, 2H, $COCH_2CO$), 2.16 (s, 3H, CH_3CO), 2.48 (m, 1H, CH), 1.48 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$).

Cyclopentyl 3-oxobutanoate (4g)

Using the general procedure and cyclopentyl alcohol provided the title compound after 1 h of reflux: Yield 69%, bp 125-127°C/20 mmHg. IR (NaCl): ν 1754 (CO ester), 1727 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.30 (m, 1H, OCH), 3.41 (s, 2H, OCH_2CO), 2.26 (s, 3H, CH_3 CO), 1.70 (m, 8H, Cyclopentyl).

Cyclohexyl 3-oxobutanoate (4h)

Using the general procedure and cyclohextyl alcohol provided the title compound after 2 h of reflux: Yield 76%, bp 84-86°C/10 mmHg. IR (NaCl): ν 1748 (CO ester), 1727 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.65 (m, 1H, OCH), 3.41 (s, 2H, OCH_2CO), 2.26 (s, 3H, CH_3 CO), 1.61 (m, 10H, Cyclohexyl).

Cyclohexylmethyl 3-oxobutanoate (4i)
Using the general procedure and cyclohexylmethyl alcohol provided the title compound after 2 h of reflux: Yield 79%, bp 81-83°C/50 mmHg. IR (NaCl): ν 1740 (CO ester), 1718 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.95 (d, J = 6.2 Hz, 2H, OCH_2), 3.44 (s, 2H, OCH_2CO), 2.27 (s, 3H, CH_3CO), 1.46 (m, 11H, Cyclohexyl).

Benzyl 3-oxobutanoate (4j)
Using the general procedure and benzyl alcohol provided the title compound after 2 h of reflux: Yield 84%. IR (NaCl): ν 1755 (CO ester), 1732 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.45 (s, 5H, Phenyl), 5.30 (s, 2H, OCH_2), 3.6 (s, 2H, OCH_2CO), 2.2 (s, 3H,CH_3CO).

Phenethyl 3-oxobutanoate (4k)
Using the general procedure and phenethyl alcohol provided the title compound after 2 h of reflux: Yeild 80%, bp 148-151°C/10 mmHg. IR (NaCl): ν 1762 (CO ester), 1721 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.32 (s, 5H, Phenyl), 4.35 (t, J = 6.5 Hz, 2H, OCH_2), 3.40 (s, 2H, OCH_2CO), 2.95 (t, J = 6.5 Hz, 2H, CH_2Ph), 2.20 (s, 3H, CH_3CO).

Phenpropyl 3-oxobutanoate (4l)
Using the general procedure and phenpropyl alcohol provided the title compound after 2 h of reflux: Yield 87%, bp 169-172°C/10 mmHg. IR (NaCl): ν 1755 (CO ester), 1726 (CO, Ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.26 (s, 5H, Phenyl), 4.16 (t, J = 6.5 Hz, 2H, OCH_2), 3.43 (s, 2H, OCH_2CO), 2.71 (t, J = 6.5 Hz, 2H, CH_2Ph), 2.33 (s, 3H, CH_3CO), 1.91 (m, 2H, $\underline{CH_2}CH_2Ph$).

General procedure for preparation of dialkyl (cycloalkyl, aryl) 4-(4(5)-chloro-1H-imidazol-5 (4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5a-l)

A solution of compound 3 (0.2 g, 1.5 mmol), ammonium acetate (0.12 g, 1.5 mmol), and alkyl (cycloalkyl, aryl) 3-oxobutanote (3 mmol) in methanol (3 mL) was refluxed. The solvent was removed under reduced pressure and the residue was crystallized from diethylether to give the title compound.

Dimethyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5a)
Using the general procedure and methyl 3-oxobutanoate provided the title compound after 48 h of reflux: White crystals, yield 44%; mp > 300°C (diethyl ether). IR (KBr): ν 3395 (NH), 1716 cm^{-1} (CO). ^{1}H-NMR (DMSO-d_6): δ 2.26 (s, 6H, C_2, C_6-CH_3), 3.64 (s, 6H, OCH_3), 5.03 (s, 1H, H_4-DHP), 7.45 (s, 1H, H_2-imidazole), 8.21 (bs, 1H, NH). MS: m/z (%) 325 (M^+, 3), 290 (100), 268 (5), 223 (12), 191 (14), 132 (12), 59 (22). Anal. Calcd. for $C_{14}H_{16}ClN_3O_4$: C, 51.62; H, 4.95; N, 12.90. Found: C, 51.44; H, 5.08; N, 12.75.

Diethyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5b)
Using the general procedure and ethyl 3-oxobutanoate provided the title compound after 48 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 20:1) and crystallized from diethyl ether to give the title compound as a white crystals, yield 11%; mp > 300°C. IR (KBr): ν 3308 (NH), 1690 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 1.22 (t, J = 7.1 Hz, 6H, CH_2CH_3), 2.29 (s, 6H, C_2, C_6-CH_3), 4.08 (q, J = 7.1 Hz, 4H, OCH_2), 5.07 (s, 1H, H_4-DHP), 7.23 (s, 1H, H_2-imidazole), 8.24 (bs, 1H, NH). MS: m/z (%) 323 (M^+, 5), 323 (10), 318 (100), 307 (15), 280 (40), 234 (50), 224 (10), 196 (15). Anal. Calcd. for $C_{16}H_{20}ClN_3O_4$: C, 54.32; H, 5.70; N, 11.88. Found: C, 54.19; H, 5.85; N, 11.66.

Dipropyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c)
Using the general procedure and propyl 3-oxobutanoate (**4c**) provided the title compound after 48 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 20:1) and crystallized from diethyl ether to give the title compound as a white crystals, yield 50%; mp 201-203°C. IR (KBr): ν 3303 (NH), 1690 cm^{-1} (CO). ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 0.80 (t, J = 6.97 Hz, 6H, $CH_2\mathit{CH_3}$), 1.25-1.85 (m, 4H, $\mathit{CH_2}CH_3$), 2.22 (s, 6H, C_2, C_6-CH_3), 3.92 (t, 4H, OCH_2), 5.01 (s, 1H, H_4-DHP), 7.16 (s, 1H, H_2-imidazole), 8.14 (s, 1H, NH-DHP), 11.01 (bs, 1H, NH-imidazole). MS: m/z (%) 381 (M^+, 6), 346 (100), 294 (42), 234 (87), 196 (42), 193 (27), 149 (12), 43 (77). Anal. Calcd. for $C_{18}H_{24}ClN_3O_4$: C, 56.62; H, 6.34; N, 11.00. Found: C, 56.85; H, 6.37; N, 11.18.

Diisopropyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5d)
Using the general procedure and isopropyl 3-oxobutanoate (**4d**) provided the title compound after 48 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 20:1) and crystallized from diethyl ether to give the title compound as a white crystals, yield 15%; mp 251-254°C. IR (KBr): ν 3411 (NH), 1690 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 1.15 and 1.23 (dd, J = 6.28 Hz, 12H, $CH(\mathit{CH3})_2$), 2.28

(s, 6H, C_2, C_6-CH_3), 4.78-5.22 (m, 3H, OCH and H_4-DHP), 7.25 (s, 1H, H_2-imidazole), 7.57 (s, 1H, NH-DHP), 10.31 (bs, 1H, NH-imidazole). MS: *m/z* (%) 381 (M^+, 2), 346 (13), 296 (60), 294 (33), 252 (17), 234 (12), 45 (72), 43 (100). Anal. Calcd. for $C_{18}H_{24}ClN_3O_4$: C, 56.62; H, 6.34; N, 11.00. Found: C, 56.80; H, 6.12; N, 10.88.

Diisobutyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e)

Using the general procedure and isobutyl 3-oxobutanoate (**4e**) provided the title compound after 48 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 18:1) and crystallized from diethyl ether-petrolum ether to give the title compound as a white crystals, yield 30%; mp 151-152°C. IR (KBr): ν 3272 (NH), 1705 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$): δ 0.89 (d, *J* = 6.6 Hz, 12H, CH(*CH*$_3$)$_2$), 1.65-2.20 (m, 2H, CH), 2.31 (s, 6H, C_2, C6-CH_3), 3.88 (d, *J* = 6.11 Hz, 4H, OCH_2), 5.12 (s, 1H, H_4-DHP), 6.36 (s, 1H, NH-DHP), 7.26 pmm (s, 1H, H_2-imidazole). MS: *m/z* (%) 409 (M^+, 1), 374 (100), 308 (35), 252 (17), 233 (75), 196 (50), 151 (15), 56 (55), 41 (70). Anal. Calcd. for $C_{20}H_{28}ClN_3O_4$: C, 58.60; H, 6.89; N, 10.25. Found: C, 58.86; H, 6.92; N, 10.49.

Ditertiarybutyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5f)

Using the general procedure and t-butyl 3-oxobutanoate (**4f**) provided the title compound after 52 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 18:1) and crystallized from diethyl ether-petrolum ether to give the title compound as a white crystals, yield 20%; mp 232-234°C. IR (KBr): ν 3419, 3406 (NH), 1698 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 1.46 (s, 18H, C(*CH*$_3$)$_3$), 2.27 (s, 6H, C_2, C6-CH_3), 4.97 (s, 1H, H_4-DHP), 7.26 (s, 1H, H_2-imidazole), 7.92 (s, 1H, NH-DHP), 10.13 (bs, 1H, NH-imidazole). Anal. Calcd. for $C_{20}H_{28}ClN_3O_4$: C, 58.60; H, 6.89; N, 10.25. Found: C, 58.76; H, 6.62; N, 10.49.

Dicyclopentyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5g)

Using the general procedure and cyclopentyl 3-oxobutanoate (**4g**) provided the title compound after 36 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 19:1) and crystallized from diethylether to give the title compound as a white crystals, yield 21%; mp 234-236°C. IR (KBr): ν 3426 (NH), 1736 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$+DMSO-d_6): δ 1.67 (br, 16H, Cyclopentyl), 2.27 (s, 6H, C_2, C_6-CH_3), 4.95-5.28 (m, 3H, H_4-DHP and OCH), 7.24 (s, 1H, H_2-imidazole), 8.10 (bs, 1H, NH-DHP). MS: *m/z* (%) 433 (M^+, 2), 399 (20), 398 (32), 348 (40), 296 (52), 252 (75), 196 (98), 178 (27), 151 (26), 106 (12), 69 (100). Anal. Calcd. for $C_{22}H_{28}ClN_3O_4$: C, 60.89; H, 6.50; N, 9.68. Found: C, 61.02; H, 6.48; N, 9.81.

Dicyclohexyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5h)

Using the general procedure and cyclohexyl 3-oxobutanoate (**4h**) provided the title compound after 30 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 18:1) and crystalized from diethyl ether-petrolum ether to give the title compound as a white crystals, yield 26%; mp: 148-151°C. IR (KBr): ν 3293 (NH), 1675 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.98-2 (m, 20H, Cyclohexyl), 2.31 (s, 6H, C_2, C_6-CH_3), 4.59-5.00 (m, 2H, OCH), 5.10 (s, 1H, H_4-DHP), 6.2 (bs, 1H, NH-DHP), 7.27 (s, 1H, H_2-imidazole). MS: *m/z* (%) 461 (M^+, 1), 426 (25), 378 (22), 334 (46), 295 (27), 196 (30), 83 (65), 55 (100). Anal. Calcd. for $C_{24}H_{32}ClN_3O_4$: C, 62.40; H, 6.98; N, 9.10. Found: C, 62.54; H, 7.12; N, 9.34.

Dicyclohexylmethyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5i)

Using the general procedure and cyclohexylmethyl 3-oxobutanoate (**4i**) provided the title compound after 30 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 20:1) and crystallized from diethyl ether to give the title compound as a white crystals, yield 27%; mp 194-196°C. IR (KBr): ν 3283, 3201 (NH), 1700 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.7-1.9 (m, 22H, Cyclohexyl), 2.30 (s, 6H, C_2, C_6-CH_3), 3.90 (d, *J* = 5.58 Hz, 4H, OC*H*$_2$), 5.11 (s, 1H, H_4-DHP), 6.6 (s, 1H, NH-DHP), 7.27 (s, 1H, H_2-imidazole). MS: *m/z* (%) 489 (M^+, 1), 455 (10), 454 (20), 348 (20), 252 (15), 234 (20), 196 (17), 67 (10), 41 (100). Anal. Calcd. for $C_{26}H_{36}ClN_3O_4$: C, 63.73; H, 7.40; N, 8.57. Found: C, 63.50; H, 7.63; N, 8.75.

Dibenzyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5j)

Using the general procedure and benzyl 3-oxobutanoate (**4j**) provided the title compound after 30 h of reflux. The solvent was removed under reduced pressure and

the residue was crystalized from diethyl ether to give the title compound as a white crystals, yield 28%; mp 81-83°C. IR (KBr): ν 3224 (NH), 1669 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.28 (s, 6H, C_2, C_6-CH_3), 5.08 (bs, 5H, H_4-DHP, and OC*H_2*Ph), 7.26 (s, 11H, H_2-imidazole and Phenyl); MS: *m/z* (%) 477 (M^+, 1), 386 (27), 342 (10), 91(100), 65 (12). Anal. Calcd. for $C_{26}H_{24}ClN_3O_4$: C, 65.34; H, 5.06; N, 8.79. Found: C, 65.58; H, 5.30; N, 8.93.

Diphenethyl4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5k)

Using the general procedure and phenethyl 3-oxobutanoate (**4k**) provided the title compound after 24 h of reflux. The solvent was removed under reduced pressure and the residue was purified by TLC (silicagel, chloroform-methanol = 20:1) and crystallized from diethyl ether to give the title compound as a white crystals, yield 21%; mp 99-102°C (diethyl ether). IR (KBr): ν 3288 (NH), 1705 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.20 (s, 6H, C_2, C_6-CH_3), 2.91 (t, *J* = 6.6 Hz, 4H, C*H_2*Ph), 4.14-4.48 (m, 4H, OC*H_2*), 4.88 (s, 1H, H_4-DHP), 6.18 (bs, 1H, NH-DHP), 6.92 (s, 1H, H_2-imidazole), 7.25 (s, 10H, Phenyl). MS: *m/z* (%) 505 (M^+, 1), 470 (50), 400 (12), 358 (12), 356 (40), 296 (5), 282 (15), 234 (37), 196 (20), 104 (100), 90 (55), 76 (25), 56 (10). Anal. Calcd. for $C_{28}H_{28}ClN_3O_4$: C, 66.46; H, 5.58; N, 8.30. Found: C, 66.60; H, 5.71; N, 8.53.

Diphenpropyl 4-(4(5)-chloro-1H-imidazol-5(4)-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5l)

Using the general procedure and phenpropyl 3-oxobutanoate (**4l**) provided the title compound after 24 h of reflux. The solvent was removed under reduced pressure and the residue was crystalized from diethyl ether to give the title compound as a white crystals, yield 15%; mp 138-140°C. IR (KBr): ν 3400 (NH), 1690 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.7-2.18 (m, 4H, C*H_2*CH_2Ph), 2.33 (s, 6H, C_2, C_6-CH_3), 2.63 (t, *J* = 7.91 Hz, 4H, C*H_2*Ph), 4.11 (t, *J* = 6.28 Hz, 4H,OC*H_2*), 5.14 (s, 1H, H_4-DHP), 6.8 (bs, 1H, NH-DHP), 7.19 (s, 11H, H_2-imidazole and Phenyl). MS: *m/z* (%) 533 (M^+, 2), 498 (50), 370 (20), 117 (25), 91 (100), 65 (17). Anal. Calcd. for $C_{30}H_{32}ClN_3O_4$: C, 67.47; H, 6.04; N, 7.87. Found: C, 67.60; H, 5.88; N, 7.99.

Pharmacology

Male albino guinea-pigs (300-450 g) were sacrificed by a blow to the head. The intestine was removed above the ileocecal junction and longitudinal smooth muscle segments 2 cm in length were mounted under a resting tension of 0.5 g. The segments were maintained at 37C in a 20 mL jacketed organ bath containing oxygenated physiological saline solution of the following (mM) composition: 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 1h with a solution change every 15 minutes, and the contraction was recorded with a force displacement transducer (F-50) on a NARCO physiograph. Test agents were prepared at 10^{-2} M stock solutions in dimethyl sulfoxide (DMSO) and stored protected from light. Dilutions were made in DMSO. The contractile response was taken as the 100% value for the tonic (slow) component of the response. The contraction was elicited with 80 mM KCl.Test compounds were cumulatively added and compound-induced relaxation of contracted muscle was expressed as percent of control. The IC_{50} values (concentration needed to produce 50% relaxation on contracted ileal smooth muscle) were graphically determined from the concentration-response curves (Bolger et al., 1983; Rovnyak et al., 1992).

The research protocol and experimental animals were approved by the ethics committee of Tehran University of Medical Sciences.

Statistical analysis

The results have been presented as mean ± S.E.M., and the statistical significance between the groups was analyzed by means of variance followed by one-way ANOVA test. *P* values less than 0.05 were considered significant.

RESULTS

Molecular modeling and docking

The conformational study of compound **5a** was done by COSMIC molecular mechanics calculations and two favored tatumers were found for this compound. They were labeled as A when the imidazole substituent at C-4 lied in a 5-chloro tautomeric form and B when the imidazole substituent lied in a 4-chloro tautomeric form (Fig. 1). In both cases, the DHP ring showed a twisted boat conformation and the imidazole ring occupied an axial position perpendicularly bisecting the boat-like DHP ring. In addition, the ab initio calculation at 298 K gave 1.54 Kcal/mol for the interconversion of tautomers B and A. Therefore, the two possible tautomers were converted into each other at room temperature.

Docking calculations were performed using Autodock software (vertion 4). L-type calcium channel that was generated by resorting to multi-body molecular

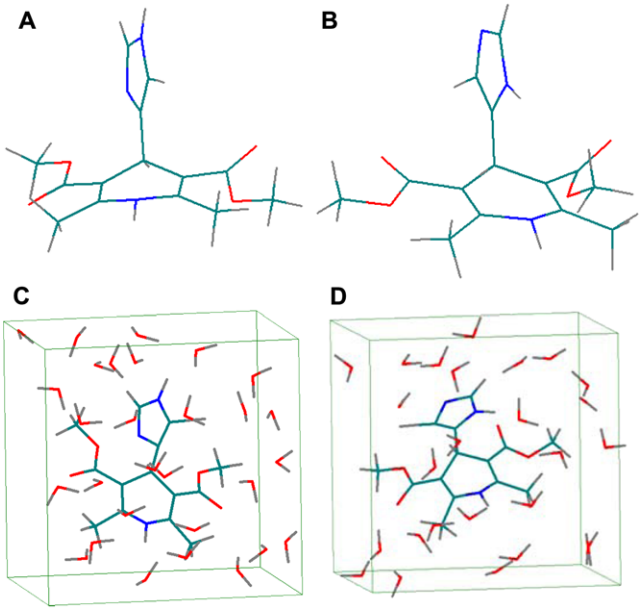


Fig. 1. Cosmic optimized geometric for lowest energy tautomeric forms in a gas phase (**A**: 5-chloro and **B**: 4-chloro) and in aqueous solution (**C**: 5-chloro and **D**: 4-chloro) for compound **5a**.

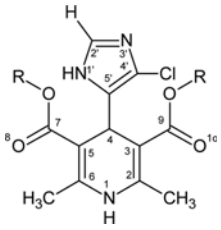
dynamics simulations, was downloaded from the PDB bank server (PDB entry 1T0J). In order to assign the perfect grid of each ligand, grid box values were obtained by trial and error and from our previous study (Davood et al., 2009, 2010). Finally, docking was performed using the implemented Lamarckin GL, and the default parameters and ten independent docking runs were performed for each DHP. Flexible docking of all data sets used for the computational study was carried out on the active site of L type calcium channel. The predicted binding energies of these inhibitors into the active site have been listed in Table I.

The orientation of the most potent calcium channel blocker, compound **5l**, in the active site of L-type calcium channel was examined through a docking experiment (Fig. 2) (Huey and Morris, 2007). This study indicated that in compound **5l**, the oxygen of ester (O10) and the N3' of imidazole ring formed a hydrogen bonding interaction with the NH of HIS 363 (distance = 2.197) and the NH of LYS354 (distance = 2.008), respectively.

QSAR Model

In this study, many descriptors were calculated for all the compounds. To select the set of descriptors that were most relevant to the IC_{50} of 1,4-DHP, multi linear regression (MLR) models were built and the QSAR equations with stepwise selection and elimination of variables was established using the MLR method. The

Table I. Calcium channel antagonist activity and docking results of new DHPs **5a-l**



DHP's	R	Binding energy[a]	pIC_{50} Exp.[b]	pIC_{50} Calc.[c]	|REP%|[d]
5a	CH_3	−4.11	5.90	6.23	0.07
5b	CH_2CH_3	−4.35	6.77	6.68	0.06
5c	$CH_2CH_2CH_3$	−4.77	8.37	7.47	0.02
5d	$CH(CH_3)_2$	−5.42	8.46	8.70	0.03
5e	$CH_2CH(CH_3)CH_3$	−5.43	8.79	8.72	0.01
5f	$C(CH_3)_3$	−5.51	8.32	8.87	0.02
5g	Cyclopentyl	−5.65	8.69	9.13	0.01
5h	Cyclohexyl	−5.71	9.04	9.24	0.03
5i	Cyclohexylmethyl	−5.77	9.11	9.36	0.01
5j	Benzyl	−5.89	9.04	9.58	0.01
5k	Phenethyl	−5.93	10.40	9.66	0.02
5l	Phenpropyl	−5.98	10.50	9.75	0.01
Nifedipine		−5.95	10.41	9.67	0.02

[a]The predicted binding energy (Kcal/mol).
[b]The pIC_{50} in guinea pig ileal smooth muscle by KCl (80 mM) was determined graphically from the dose- response curve. There were six experiments for each compounds.
[c]The pIC_{50} using multi linear regression Eq. (1).
[d]The absolute value of the percent of the relative error of prediction.

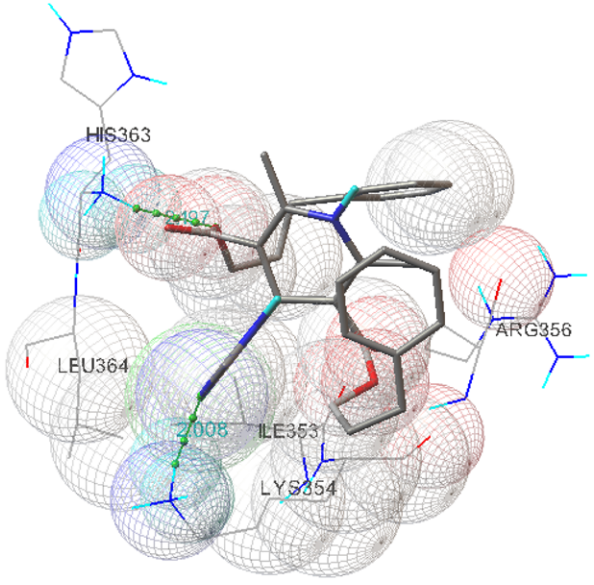


Fig. 2. Docked structures of compound **5l** in the Model of LCC. DHPs have been displayed as sticks, and hydrogen bonds (HIS 363, LYS 354) were represented with dashed lines (Docking study was performed by using ADT program and LCC model obtained from PDB server).

Table II. Correlation coefficient matrix for the descriptors of DHPs used in the MLR equation

		Correlations		
		BEHp5	RDF075p	pIc50
BEHp5	Pearson Correlation	1	.638	.925
	Sig. (2-tailed)		.026	.000
	N	12	12	12
RDF075p	Pearson Correlation	.638	1	.559
	Sig. (2-tailed)	.026		.059
	N	12	12	12
pIc50	Pearson Correlation	.925	.559	1
	Sig. (2-tailed)	.000	.059	
	N	12	12	12

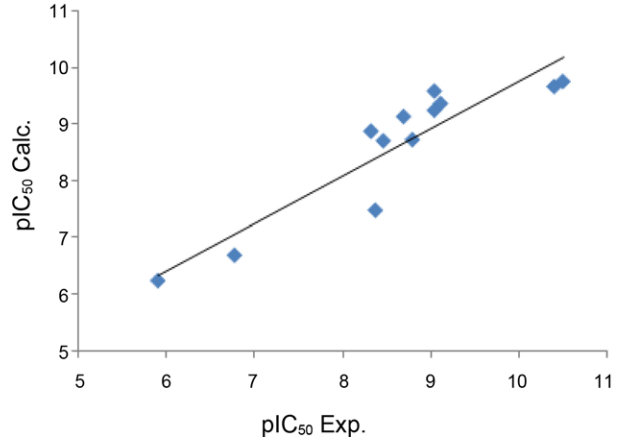


Fig. 3. Plot of cross-validated calculated activity obtained by QSAR equation for new DHP derivatives.

correlation coefficient matrix for the descriptors used in MLR equation has been provided in Table II.

$$\text{Eq. SYM } pIC_{50} = 20.397\ \text{BEHp5} - 0.062\ \text{RDF075p} - 56.659 \quad (1)$$

$n = 12$, $F = 103.59$, $R^2 = 0.95$, $S = 0.18$, $q^2 = 0.99$, $p < 0.0001$.

The calculated pIC_{50} using MLR of Eq. (1) are present in Table I and the graphical representation of cross-validated calculated activity and the experimental values using equation1 have been provided in Fig. 3.

Calcium channel antagonist evaluation

All of compounds **5a-l** were evaluated as calcium channel antagonists using the high K^+ contraction guinea-pig ileal longitudinal smooth muscle (Table I). The pIC_{50} (pIC_{50} Exp) in guinea pig ileal smooth muscle by KCl (80 mM) was determined graphically from the dose-response curve. There were six experiments for all compounds.

DISCUSSION

Based on our molecular modeling studies that revealed 4-chloro tautomeric form was the primary form and that it had good compatibility with nifedipine, 4-H was syn-perpendicular, and that in the 5-chloro tautomeric form, 4-H was anti-perpendicular, docking studies were done on the 4-chloro tautomeric form. In the docking sudies, we confirmed that the oxygen of the ester (O10) and the N3' of the imidazole ring were involved in forming a hydrogen bonding interaction with the receptor. However, due to the limitations of the scoring functions, it was often difficult to establish a quantitative correlation between the calculated and the experimentally derived activity values. However, these observations and experimental results have provided a good process for explanation of the potent and selective inhibitory activity of these compounds.

Based on the QSAR studies (Eq. (1)), the sum of the BEHp5 and RDF075p were identified as the most significant descriptors. The BEHp5 belongs to the BCUT Topological (2D) descriptors. The BCUT descriptor is a symmetrical matrix representing molecular properties and bonding information in its diagonal and off-diagonal. The RDF075p belongs to the RDF (radical distribution function) descriptors that represent the atomic polarizabilities of substituents.

Descriptors that belonged to the class of radial distribution function descriptors were based on the distance distribution in the geometrical representation of the molecule. In addition to interatomic distances in the entire molecule, the RDF also provided valuable information about bond distances, ring types, planar and non-planar systems, atom types, and other important structural motifs. By using different weighting schemes, which included atom types, electronegativity, atom mass or van der Waals radii, RDF could be adjusted to select among those atoms of the molecule, which gave rise to an important descriptor in deriving an appropriate QSAR.

The results of the pharmacologycal evaluation (Table I) revealed that the test compounds showed a significant calcium channel antagonist activity in comparison to the reference drug nifedipine. The results indicated that increasing the length of the chain in C3 and C5 ester substituents increased activity (5c > 5b > 5a). When increasing of the length or lipophilicity was accompanied with increasing the hindrance, the activity decreased (5e >5f). Our results demontrated that aromatic compounds (**5j-l**) were more active than

aliphatic compounds (**5a-g**). The most potent compound was bis-phenylpropyl ester derivative (**5l**), which was more active than the reference drug nifedipine, while the bis-phenylethyl ester derivative (**5k**) had comparable activity with nifedipin.

Our pharmacological evalution revealed that all of designed compounds were active and the present study demonstrated that 4(5)-chloro-5(4)-imidazolyl moiety is a bioisoster of o-nitrophenyl in nifedipine.

ACKNOWLEDGEMENTS

This research was supported by grants (78059) from the research council of Tehran and Azad Universities of Medical Sciences, TWAS-IC (The Academy of Sciences for the Developing World- Iran Chapter), and INSF (Iran National Science Foundation).

REFERENCES

Arrowsmith, J. E., Campbell, S. F., Cross, P. E., Stubbs, J. K., Burges, R. A., Gardiner, D. G., and Blackburn, K. J., Long-acting dihydropyridine calcium antagonists. 1. 2-Alkoxymethyl derivatives incorporating basic substituents. *J. Med. Chem*., 29, 1696-1702 (1986).

Bolger, G. T., Gengo, P., Klockowski, R., Luchowski, E., Siegel, H., Janis, R. A., Triggle, A. M., and Triggle, D. J., Characterization of binding of the Ca++ channel antagonist, [3H]nitrendipine, to guinea-pig ileal smooth muscle. *J. Pharmacol. Exp. Ther*., 225, 291-309 (1983).

Coburn, R. A., Wierzba, M., Suto, M. J., Solo, A. J., Triggle, A. M., and Triggle, D. J., 1,4-Dihydropyridine antagonist activities at the calcium channel: a quantitative structure-activity relationship approach. *J. Med. Chem*., 31, 2103-2107 (1988).

Davood, A., Khodarahmi, G., Alipour, E., Dehpour, K., Amini, M., and Shafiee, A., Synthesis and calcium channel antagonist activity of nifedipine analogues containing 4(5)-chloro-2-methyl-5(4)-imidazolyl substituent. *Boll. Chim. Farm*., 140, 381-386 (2001).

Davood, A., Mansouri, N., Rerza Dehpour, A., Shafaroudi, H., Alipour, E., and Shafiee, A., Design, synthesis, and calcium channel antagonist activity of new 1,4-dihydropyridines containing 4-(5)-chloro-2-ethyl-5-(4)-imidazolyl substituent. *Arch. Pharm (Weinheim).*, 339, 299-304 (2006).

Davood, A., Nematollahi, A. R., Iman, M., and Shafiee, A., Synthesis and docking studies of new 1,4-dihydropyridines containing 4-(5)-Chloro-2-ethyl-5-(4)-imidazolyl substituent as novel calcium channel agonist. *Arch. Pharm. Res.*, 32, 481-487 (2009).

Davood, A., Nematollahi, A., Iman, M., and Shafiee, A., Computational studies of new 1,4-dihydropyridines containing 4-(5)-chloro-2-ethyl-5-(4)-imidazolyl substituent: QSAR and docking. *Med. Chem. Res*., 19, 58-70 (2010).

Fleckenstein, A., Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol*., 17, 149-166 (1977).

Gaudio, A. C., Korolkovas, A., and Takahata, Y., Quantitative structure-activity relationships for 1,4-dihydropyridine calcium channel antagonists (nifedipine analogues): a quantum chemical/classical approach. *J. Pharm. Sci*., 83, 1110-1115 (1994).

Godfraind, T., Calcium entry blockade and excitation contraction coupling in the cardiovascular system (with an attempt of pharmacological classification). *Acta Pharmacol. Toxicol (Copenh).,* 58 Suppl 2, 5-30 (1986).

Goldmann, S. and Stoltefuss, J., 1,4-Dihydropyridines: effects of chirality and conformation on the calcium antagonist and calcium agonist activities. *Angew. Chem. Int. Ed. Engl*., 30, 1559-1578 (1991).

Hansch, C., A quantitative approach to biochemical structure-activity relation-ships. *Acc. Chem. Res*., 2, 232-239 (1969).

Hansch, C. and Fujita, T., A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc*., 86, 1616-1626 (1964).

Hantzsch, A., Ueber die Synthese Pyridinartiger Verbindungen aus Acetessigather und Aldehydammoniak. *Justus Liebigs Ann. Chem*., 215, 1-82 (1882).

Huey, R. and Morris, G. M., Using AutoDock 4 with AutoDockTools, (2007).

Janis, R. A. and Triggle, D. J., New developments in Ca2+ channel antagonists. *J. Med. Chem*., 26, 775-785 (1983).

Li, A. H., Moro, S., Melman, N., Ji, X. D., and Jacobson, K. A., Structure-activity relationships and molecular modeling of 3, 5-diacyl-2,4-dialkylpyridine derivatives as selective A3 adenosine receptor antagonists. *J. Med. Chem*., 41, 3186-3201 (1998).

Pourmorad, F., Hadizadeh, F., and Shafiee, A., Synthesis and calcium channel activity of 4-imidazolyl-1,4- dihydropyridines. *Pharm. Sci*., 3, 165-168 (1997).

Rovnyak, G. C., Atwal, K. S., Hedberg, A., Kimball, S. D., Moreland, S., Gougoutas, J. Z., O'Reilly, B. C., Schwartz, J., and Malley, M. F., Dihydropyrimidine calcium channel blockers. 4. Basic 3-substituted-4-aryl-1,4-dihydropyrimidine-5-carboxylic acid esters. Potent antihypertensive agents. *J. Med. Chem*., 35, 3254-3263 (1992).

Schramm, M., Thomas, G., Towart, R., and Franckowiak, G., Novel dihydropyridines with positive inotropic action through activation of Ca2+ channels. *Nature*, 303, 535-537 (1983).

Shafiee, A., Dehpour, A. R., Hadizadeh, F., and Azimi, M., Syntheses and calcium channel antagonist activity of nifedipine analogues with methylsulfonylimidazolyl substituent. *Pharm. Acta Helv*., 73, 75-79 (1998).

Shafiee, A., Rastkary, N., and Jorjani, M., Synthesis and calcium channel antagonist activity of 1,4-dihydropyridine derivatives containing 4-nitroimidazolyl substituents. *Arzneimittelforschung*, 52, 537-542 (2002).