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Research Paper

Lipophilic 4-imidazolyl-1,4-dihydropyridines: synthesis, calcium channel antagonist activity and docking study

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Abstract: A new series of nifedipine analogues was synthesized and evaluated as calcium channel antagonist using the high K^+ contraction of guinea-pig ileal longitudinal smooth muscle. The *ortho*-nitrophenyl group of nifedipine is replaced by a lipophilic 2-butyl-5-chloro-1*H*-imidazol-4-yl substituent. The symmetrical analogues (**3a-f**) were prepared by condensing 2 moles of alkyl acetoacetate (**2**), ammonium acetate and 2-butyl-5-chloro-1*H*-imidazole-4-carboxaldehyde (**1**), in a classical Hantzsch synthesis, whereas the asymmetrical analogues (**6a-e**) were synthesized by a modified Hantzsch reaction, that involved, condensation of **1**, **2** and alkyl 3-aminocrotonate (**5**). The results of IC_{50} demonstrate that all compounds are similar to or more active than the reference drug nifedipine. In order to explain the differences in calcium channel antagonist activities, a docking study of **3a**, **3d** and (*S*)- and (*R*)-enantiomers of **6c** with the DHP receptor model, was performed. Bulkiness of C_3 and C_5 diester play a crucial role in binding with the DHP receptor model.

Introduction

The influx of extracellular Ca^{2+} through L-type potential dependent calcium channel is responsible for the regulation of many physiological functions, including smooth and cardiac muscle contraction [1–5]. The discovery that the substituted 4-aryl-1,4-dihydropyridine (DHP) class of calcium channel blockers such as, nifedipine inhibits Ca^{2+} influx represented a major therapeutic advance in treatment of cardiovascular diseases such as

hypertension, angina pectoris and other spastic smooth muscle disorders [6–12]. DHPs may lead to other beneficial effects such as regression of left ventricular pressure and vascular hypertrophy, renal protection, weak anti-platelet, anti-ischemic and anti-atherogenic activities [13, 14]. Recently, nifedipine was used topically to treat chronic anal fissure [15].

QSAR studies indicated that the potency of nifedipine analogues was dependent on lipophilicity, an electronic term and separated terms for each position on the aromatic ring [16]. Changes in the substitution pattern at the C_3 , C_4 and C_5

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positions of the DHP ring alter potency [17], tissue selectivity [18] and the conformation of the DHP ring [19].

Natale et al. found that lipophilic 4-isoxazolyl-1,4-dihydropyridines, that have an aryl group on isoxazole ring, exhibited high binding affinity [20]. They proposed a model for DHP binding and found a highly lipophilic pocket in the receptor's active site. Examination of their model indicated that aryl substituent could be properly oriented to interact with the lipophilic pocket of their hypothesized channel. Other studies suggested that C₄ heterocyclic substituents gave compounds with calcium channel antagonist activity [21–22].

Many previous studies have shown that bioisosteric replacement of the 4-aryl moiety with a substituted imidazole ring gave 4-imidazolyl-1,4-dihydropyridines, which retained potent calcium channel antagonist activity [23–26].

In this paper we provide synthesis, calcium channel antagonist activity and docking study of new 1,4- DHPs with the lipophilic 2-butyl-5-chloro-1*H*-imidazol-4-yl substituent at 4 position of the DHP ring as potential calcium channel blockers.

Materials and Methods

Chemistry

Melting points were determined on a Stuart SMP10 capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Micro Analytical Center, Faculty of Science, Cairo University, Cairo, Egypt and were within $\pm 0.4\%$ of the calculated values. NMR spectra were recorded on a JEOL-ECA500 MHz spectrometer.

All organic reagents were obtained from Aldrich Co. and were used without further purification. Silica gel (200–400 mesh, 60 Å) used for column chromatography was obtained from Aldrich and silica gel chromatographic sheets with a fluorescent indicator used for thin layer chromatography (TLC) were obtained from Eastman Kodak Co., Rochester, NY.

*General procedure for the synthesis of symmetrical esters of dialkyl 4-(2-butyl-5-chloro-1*H*-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (3a-f)*

To 50 ml round-bottomed flask, ammonium acetate (0.123 g, 1.59 mmol) was added to a stirring solution of 2-butyl-5-chloro-1*H*-imidazole-4-carboxaldehyde (0.25 g, 1.34 mmol) and alkyl acetoacetate (2.68 mmol) in 10 ml methanol or 2-propanol. The reaction mixture was protected from light and heated under reflux for 5-12 hr. After cooling, the precipitate was filtered and purified by crystallization from methanol or 2-propanol to afford the corresponding compound.

*Dimethyl 4-(2-butyl-5-chloro-1*H*-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (3a)*

Anal. C₁₈H₂₄ClN₃O₄, % calcd (found) C: 56.62 (56.28), H: 6.34 (6.22), N: 11.00 (10.93). ¹H-NMR (CDCl₃, δ): 0.87-0.90 (t, *J* = 7.65, 3H, -CH₂CH₃); 1.30-1.34 (m, *J* = 7.65, 2H, -CH₂CH₃); 1.60-1.64 (m, *J* = 7.65, 2H, -CH₂CH₂CH₂-); 2.28-2.35 (s, 6H, Ar-CH₃); 2.57-2.61 (t, *J* = 7.65, 2H, imidazole -CH₂CH₂-); 3.65-3.77 (s, 6H, -COOCH₃); 5.01-5.04 (s, 1H, 4-position of DHP); 6.36 (s, 1H, imidazole NH).

*Diethyl 4-(2-butyl-5-chloro-1*H*-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (3b)*

Anal. C₂₀H₂₈ClN₃O₄, % calcd (found) C: 58.6 (58.6), H: 6.89 (6.76), N: 10.25 (10.34).

$^1\text{H-NMR}$ (CDCl_3 , δ): 0.86-0.89 (t, $J = 7.65$, 3H, $-\text{CH}_2-\text{CH}_3$); 1.23-1.26 (t, $J = 6.90$, 6H, $\text{COOCH}_2\text{CH}_3$); 1.32-1.34 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.61-1.65 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.32 (s, 6H, Ar- CH_3); 2.67-2.69 (t, $J = 7.65$, 2H, imidazole $-\text{CH}_2\text{CH}_2-$); 4.13-4.30 (m, $J = 6.1$, 4H, $-\text{COOCH}_2\text{CH}_3$); 5.02 (s, 1H, 4-position of DHP); 6.38-6.40 (s, 1H, imidazole NH).

Diisopropyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (3c)

Anal. $\text{C}_{22}\text{H}_{32}\text{ClN}_3\text{O}_4$, % calcd (found) C: 60.33 (60.58), H: 7.36 (7.07), N: 9.59 (9.88). $^1\text{H-NMR}$ (CDCl_3 , δ): 0.85-0.89 (t, $J = 7.75$, 3H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.16-1.19 (d, $J = 6.1$, 6H, $-\text{CH}(\text{CH}_3)_2$); 1.22-1.25 (d, $J = 6.1$, 6H, $-\text{CH}(\text{CH}_3)_2$); 1.27-1.32 (m, $J = 7.6$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.52-1.69 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.22-2.25 (s, 6H, Ar- CH_3); 2.54-2.58 (t, $J = 7.6$, 2H, imidazole $-\text{CH}_2\text{CH}_2-$); 4.96 (s, 1H, 4-position of DHP); 4.98-5.01 (m, $J = 6.15$, 2H, $\text{COOCH}(\text{CH}_3)_2$); 6.58 (s, 1H, imidazole NH).

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

Anal. $\text{C}_{24}\text{H}_{36}\text{ClN}_3\text{O}_4$, % calcd (found) C: 61.86 (61.91), H: 7.79 (8.02), N: 9.02 (9.21). $^1\text{H-NMR}$ (CDCl_3 , δ): 0.88-0.91 (m, 15H, $-\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{CH}(\text{CH}_3)_2$); 1.31-1.34 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.61-1.63 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 1.90-1.93 (m, $J = 6.85$, 2H, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$); 2.30-2.32 (s, 6H, Ar- CH_3); 2.55-2.70 (t, $J = 7.6$, 2H, imidazole $-\text{CH}_2\text{CH}_2-$); 3.80-3.88 (d, $J = 6.9$, 2H, $-\text{COOCH}_2\text{CH}_2-$); 3.90-4.10 (d, $J = 6.9$, 2H, $-\text{COOCH}_2\text{CH}_2-$); 5.04 (s, 1H, 4-position of DHP); 6.38 (s, 1H, imidazole NH).

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

Anal. $\text{C}_{24}\text{H}_{36}\text{ClN}_3\text{O}_4$, % calcd (found) C: 61.86 (62.02), H: 7.79 (7.90), N: 9.02 (9.19). $^1\text{H-NMR}$ (CDCl_3 , δ): 0.89-0.93 (t, $J = 7.6$, 3H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.33-1.36 (m, $J = 7.6$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.36-1.45 (s, 18H, $-\text{C}(\text{CH}_3)_3$); 1.56-1.60 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.27-2.30 (s, 6H, Ar- CH_3); 2.67-2.87 (t, $J = 7.6$, 2H, imidazole $-\text{CH}_2\text{CH}_2-$); 4.94 (s, 1H, 4-position of DHP); 7.25 (s, 1H, imidazole NH).

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

Anal. $\text{C}_{22}\text{H}_{32}\text{ClN}_3\text{O}_6$, % calcd (found) C: 56.23 (56.58), H: 6.86 (6.99), N: 8.94 (8.63). $^1\text{H-NMR}$ (CDCl_3 , δ): 0.87-0.90 (t, $J = 7.65$, 3H, $-\text{CH}_2\text{CH}_3$); 1.30-1.34 (m, $J = 7.65$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_3$); 1.63-1.65 (m, $J = 6.9$, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$); 2.30-2.35 (s, 6H, Ar- CH_3); 2.53-2.56 (t, $J = 7.65$, 2H, imidazole $-\text{CH}_2\text{CH}_2-$); 3.57-3.59 (s, 6H, $-\text{OCH}_3$); 3.52-3.62 (t, $J = 7.1$, 2H, $-\text{CH}_2\text{CH}_2\text{OCH}_3$); 3.65-3.73 (t, $J = 7.1$, 2H, $-\text{CH}_2\text{CH}_2\text{OCH}_3$); 4.17-4.20 (t, $J = 6.9$, 4H, $-\text{COOCH}_2$); 5.01 (s, 1H, 4-position of DHP); 6.60-6.80 (s, 1H, imidazole NH).

General procedure for the synthesis of asymmetrical esters of dialkyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6a-e)

To a 50 ml round-bottomed flask were added a mixture of 2-butyl-5-chloro-1H-imidazole-4-carboxaldehyde (0.25 g, 1.34 mmol), alkyl acetoacetate (1.34 mmol) and alkyl 3-aminocrotonate (1.34 mmol) in 10 ml methanol or 2-propanol. The reaction mixture was protected from light and heated under reflux for 5-12 hr. After cooling, the precipitate was filtered and purified by crystallization from methanol or 2-propanol to afford the corresponding compound.

3-Ethyl 5-methyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6a)

Anal. C₁₉H₂₆ClN₃O₄, % calcd (found) C: 57.64 (57.59), H: 6.62 (6.79), N: 10.61 (10.38). ¹H-NMR (CDCl₃, δ): 0.86-0.89 (t, *J* = 7.6, 3H, -CH₂CH₂CH₃); 1.23-1.26 (t, *J* = 6.9, 3H, COOCH₂CH₃); 1.31-1.33 (m, *J* = 7.6, 2H, -CH₂CH₂CH₃); 1.53-1.74 (m, *J* = 6.9, 2H, -CH₂CH₂CH₂); 2.30-2.40 (s, 6H, Ar-CH₃); 2.55-2.74 (t, *J* = 7.6, 2H, imidazole -CH₂CH₂-); 3.68 (s, 3H, -COOCH₃); 4.10-4.24 (t, *J* = 6.1, 2H, COOCH₂CH₃); 5.02 (s, 1H, 4-position of DHP); 6.27-6.50 (s, 1H, imidazole NH).

3-Isopropyl 5-methyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6b)

Anal. C₂₀H₂₈ClN₃O₄, % calcd (found) C: 58.60 (58.30), H: 6.89 (7.00), N: 10.25 (10.38). ¹H-NMR (CDCl₃, δ): 0.87-0.91 (t, *J* = 7.6, 3H, -CH₂CH₂CH₃); 1.16-1.18 (d, *J* = 6.9, 6H, -CH(CH₃)₂); 1.19-1.25 (m, *J* = 6.9, 2H, -CH₂CH₂CH₃); 1.51-1.73 (m, *J* = 6.9, 2H, -CH₂CH₂CH₂-); 2.20-2.30 (s, 6H, Ar-CH₃); 2.60-2.64 (t, *J* = 7.6, 2H, imidazole -CH₂CH₂-); 3.60-3.67 (s, 3H, COOCH₃); 4.98-5.01 (m, 2H, 4-position of DHP, -CH(CH₃)₂); 6.25-6.33 (s, 1H, imidazole NH).

3-Ethyl 5-isopropyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6c)

Anal. C₂₁H₃₀ClN₃O₄, % calcd (found) C: 59.50(59.22), H: 7.13 (7.05), N: 9.91 (9.68). ¹H-NMR (CDCl₃, δ): 0.86-0.89 (t, *J* = 7.6, 3H, -CH₂CH₂CH₃); 1.17-1.19 (d, *J* = 6.9, 6H, -CH(CH₃)₂); 1.22-1.25 (t, *J* = 6.9, 3H, COOCH₂CH₃); 1.30-1.42 (m, *J* = 6.9, 2H, -CH₂CH₂CH₃); 1.49-1.63 (m, *J* = 6.9, 2H, -CH₂CH₂CH₂-); 2.23-2.30 (s, 6H, Ar-CH₃); 2.59-2.63 (t, *J* = 7.6, 2H, imidazole-CH₂CH₂-); 4.05-4.23 (m, *J* = 6.1, 2H, COOCH₂CH₃); 4.99-5.10 (m, 2H, 4-

position of DHP, -CH(CH₃)₂); 6.27-6.33 (s, 1H, imidazole NH).

3-Isobutyl 5-methyl 4-(2-butyl-5-chloro-1H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6d)

Anal. C₂₁H₃₀ClN₃O₄, % calcd (found) C: 59.50 (59.27), H: 7.13 (7.06), N: 9.91 (9.58). ¹H-NMR (CDCl₃, δ): 0.82-0.85 (t, *J* = 7.6, 3H, -CH₂CH₂CH₃); 0.86-0.92 (d, *J* = 6.9, 6H, -CH(CH₃)₂); 1.26-1.36 (m, *J* = 7.6, 2H, -CH₂CH₂CH₃); 1.53-1.65 (m, *J* = 6.9, 2H, -CH₂CH₂CH₂-); 1.87-1.95 (m, *J* = 6.7, 1H, -CH(CH₃)₂); 2.15-2.32 (s, 6H, Ar-CH₃); 2.54-2.64 (t, *J* = 7.6, 2H, imidazole-CH₂CH₂-); 3.56-3.64 (s, 3H, COOCH₃); 3.79-3.94 (d, *J* = 6.1, 6.7, 2H, -OCH₂CH-); 4.95-5.10 (s, 1H, 4-position of DHP); 6.65-6.71 (s, 1H, imidazole NH).

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

Anal. C₂₁H₃₀ClN₃O₅, % calcd (found) C: 57.33 (57.50), H: 6.87 (7.01), N: 9.55 (9.29). ¹H-NMR (CDCl₃, δ): 0.83-0.91 (t, *J* = 7.65, 3H, -CH₂CH₂CH₃); 1.13-1.22 (t, *J* = 6.9, 3H, -COOCH₂CH₃); 1.29-1.36 (m, *J* = 7.6, 2H, -CH₂CH₂CH₃); 1.59-1.68 (m, *J* = 6.9, 2H, -CH₂CH₂CH₂-); 2.24-2.31 (2s, 6H, Ar-CH₃); 2.48-2.60 (t, *J* = 7.6, 2H, imidazole-CH₂CH₂-); 3.45-3.54 (s, 3H, -OCH₃); 3.63-3.67 (s, 1H, imidazole NH); 3.65-3.78 (t, *J* = 7.1, 2H, -OCH₂CH₂O-); 4.06-4.16 (m, *J* = 6.1, 2H, COOCH₂CH₃); 4.17-4.24 (t, *J* = 6.9, 2H, COOCH₂CH₂OCH₃); 4.89-5.12 (s, 1H, 4-position of DHP).

Pharmacology

IC₅₀ were determined at the special unit, Department of Pharmacology, National Research Centre, Dokki, 12622 Cairo, Egypt.

Determination of calcium channel antagonist activity

Male guinea-pigs weighing 300–450 g were killed by a blow to the head. The intestine was removed above the ileocecal junction and longitudinal smooth muscle segments of 2 cm length were mounted under a resting tension of 0.5 g. The segments were maintained at 37 °C in a 10 ml jacketed organ bath (automatic multi-chamber organ bath system model no. ML870B6/C, PanLab, Spain). The organ bath physiological saline solution of the following (mmol) composition: NaCl (137), CaCl₂ (1.8), KCl (2.7), MgSO₄ (1.1), NaH₂PO₄ (0.4), NaHCO₃ (12) and glucose (5), was continuously aerated with carbogen (a mixture of 95 % oxygen and 5% carbon dioxide) and its temperature was kept at 37 °C. The muscles were equilibrated for 1 hr with a solution changed every 15 min. The contractions were recorded with a force displacement transducer (model no. MLT0201, PanLab, Spain) connected to amplifier (powerlab, AD instruments, Australia). Test agents were prepared as 10⁻⁶ M stock solutions in ethanol and stored protected from light. Dilutions were made into double distilled water. The contractile response was taken as the 100% value for the tonic (slow) component of the response. The contraction was elicited with 40 mM KCl. Test compounds were cumulatively added and compound induced relaxation of contracted muscle was expressed as percent of control. The IC₅₀ values (molar concentration needed to produce 50% relaxation on contracted ileal smooth muscle) were graphically determined from the concentration-response curves.

Results and Discussion

Chemistry

The chemical structures of the prepared compounds **3a-f**, **6a-e** and reference drug nifedipine are shown in **Figure 1**. The synthesis of 1,4-dihydropyridine (DHP) derivatives **3** and **6** was achieved following the steps outlined in **Scheme 1**. Classical Hantzsch condensation in which, 2-butyl-5-chloro-1*H*-imidazole-4-carboxaldehyde (**1**) reacted with the appropriate acetoacetic ester (**2**) and ammonium acetate in methanol or 2-propanol, afforded the symmetrical DHP analogues **3**. The asymmetrical analogues **6** were synthesized by a modified Hantzsch reaction, that involved, condensation of **1** and **2** to afford alkyl 2-((2-butyl-5-chloro-1*H*-imidazol-4-yl)methylene)-3-oxobutanoate (**4**) which then underwent a cyclizing Michael addition with alkyl 3-aminocrotonate (**5**). The preparation, collection and purification of the products were carried out in the absence of oxidizing agents and in darkness.

The physical data of the prepared compounds are summarized in **Table 1**.

Pharmacology

The calcium channel antagonist activity of the prepared compounds was determined as the molar concentration needed to produce 50% inhibition of the guinea-pig ileal longitudinal smooth muscle contractility, is summarized in **Table 2**. Comparison of the activities of symmetrical and asymmetrical esters indicates that increasing of size of substituents at C₃ or C₅ position increases activity. When increasing of the size is accompanied by increasing the hindrance, the activity decreases.

The results of calcium channel antagonist activity indicate that the lipophilic 2-butyl-5-chloro-1*H*-imidazol-4-yl group is a bioisoster of *ortho*-nitrophenyl ring of nifedipine. The prepared compounds retain

calcium channel antagonist activity and are similar or slightly higher in effect than nifedipine.

When the ester substituents at the C₃ and C₅ of the DHP ring are different, the C₄ position becomes chiral and stereoselectivity of antagonism is observed. The (*S*)-lercanidipine kinetic disposition level was always higher than the (*R*)-enantiomer following administration of 20 mg racemic mixture in volunteers [27]. However, enantioselectivity is not absolute. The recently developed dihydropyridines, including amlodipine, nicardipine, isradipine and oxodipine have been subject to investigations involving their enantioselective determination and pharmacokinetics. The concentration profiles of amlodipine enantiomers have been demonstrated to be relatively similar for each enantiomer after oral administration of a racemic mixture in man, despite a reported difference in the case of nilvadipine [28]. Other study showed that *R*-(-)-nicardipine was more effective than that of *S*-(+)-nicardipine on inhibiting vasoconstriction of isolated rabbit thoracic artery [29].

Compounds **3a**, **3d** and **6c** were chosen for docking study with DHP receptor model. Compound **3a** is similar to nifedipine in ester substitution. This would allow the study of effect of bioisosteric imidazole ring. Compounds **6c** and **3d** have the highest and lowest activities, respectively.

Compound **6c** is a chiral molecule and has the highest potency. The discussion and explanation of differences among activities of **3a**, **3d**, **6c** and the reference nifedipine are given in details under docking section. Compounds **6a-e** were obtained as racemates. So, the interaction of the (*S*)- and

(*R*)-enantiomers with the DHP receptor model will also be considered.

Docking

A docking study was done to explain the variation in calcium channel antagonist activities and to correlate them to the chemical structure of compounds **3a-f** and **6a-e**.

The chemical structures were drawn by Marvin Sketch V 5.4. The most energetically favored conformer was exported as *.mol2 file format for docking using Molegro Virtual Docker V 5.5. Iterative simplex algorithm was chosen to perform docking process with 15 runs per ligand, 50 population size, 100 max iteration and 8 poses for each ligand.

Compounds **3a**, **3d**, **6c** and nifedipine were chosen for docking with DHP receptor model coordinate afforded by Dr Boris S. Zhorov, which was obtained by homology model of K⁺ channel from *Streptomyces lividans* [30].

The DHP ring position is constrained using template docking to allow a hydrogen bond formation with Tyr³³¹⁰ in all poses important for activity [31]. Compound **3a** is less potent than nifedipine. Both **3a** and nifedipine form electrostatic interaction with Ca²⁺ cofactor. The *ortho*-nitrophenyl group of nifedipine, NH group of imidazole ring of **3a** and oxygen of the carbonyl ester group of both compounds, are engaged in electrostatic interaction with Ca²⁺ cofactor.

Hydrogen bonding between NH group of the DHP ring and Tyr³³¹⁰ is important for the calcium channel blocking activity and is shown in both **3a** and nifedipine. The hydrophobic interaction of *ortho*-nitrophenyl ring of nifedipine and Tyr⁴³¹¹ is

not shown in **3a** due to improper orientation of the imidazole ring. A highly lipophilic pocket in the receptor's active site can accommodate the long butyl chain of **3a**. The *ortho*-nitrophenyl ring of nifedipine slides, while the butyl substituent is immersed in the lipophilic pocket (**Figure 2**).

Besides, as shown in **Figure 2**, the *ortho*-nitrophenyl ring of nifedipine can be engaged in a hydrophobic ring to ring interaction with Tyr⁴³¹¹. This interaction is not shown with **3a**. This explains the higher calcium antagonist activity of nifedipine compared to **3a**.

The chlorine atom of imidazole ring and the bulky isobutyl group of **3d** inhibit the proper orientation, which leads to binding with the receptor. This deprives the aromatic ring of **3d** from hydrophobic interaction with the receptor's lipophilic pocket. The bulky side chain ester leads to swing the compound away from Tyr³³¹⁰. So, **3d** does not have the proper position for hydrogen bonding with Tyr³³¹⁰. Besides, the hydrophobic ester side chain elongation in the water-lake results in a decrease in activity, c.f., **Figure 3**.

On the other hand, the optimal bulkiness of ethyl and isopropyl ester side chains of **6c** does not swing the NH group of DHP ring away from the appropriate position for hydrogen bonding with Tyr³³¹⁰ and allows the compound to make hydrophobic interaction with receptor's lipophilic pocket.

Docking of (*S*)- and (*R*)-enantiomers of **6c** with DHP receptor model was considered to retain the hydrogen bonding interaction. Both enantiomers have similar orientations regarding the DHP and imidazole rings. Both enantiomers have the ability to form a hydrogen bonding with Tyr³³¹⁰ and to

interact with the lipophilic pocket, c.f., **Figure 4**.

As shown in **Figure 5**, the only observed difference between the enantiomers of **6c** is the different orientation of the ester side chain with respect to the water-lake cavity and hydrophobic bracelet. Water-lake cavity favors lower alkyl side chain ester. Hydrophobic bracelet favors higher alkyl side chain ester. In case of the (*S*)-enantiomer of **6c**, the ethyl group projects to the water-lake cavity and the isopropyl group faces the hydrophobic bracelet. The reverse is shown in case of the (*R*)-enantiomer. So, the isopropyl ester side chain of the (*S*)-enantiomer of **6c** stabilizes the hydrophobic bracelet and the closed channel conformation [30, 31]. This observation may lead to better interaction of the (*S*)-enantiomer with the DHP receptor model.

Conclusion

As shown from the results of IC₅₀, all investigated compounds showed potent calcium channel antagonist activities. Docking demonstrates that groups of suitable bulkiness, such as ethyl and isopropyl at 3- and 5-positions of DHP ring, gave **6c**, which is more active than nifedipine. Besides, the suitable bulkiness and lipophilic characters of substituted imidazole ring, the (*S*)-enantiomer of **6c** may play a crucial role in interaction of ligands with DHP receptor model and stabilization of closed channel conformation. The future work will include the resolution and testing of the pure enantiomers.

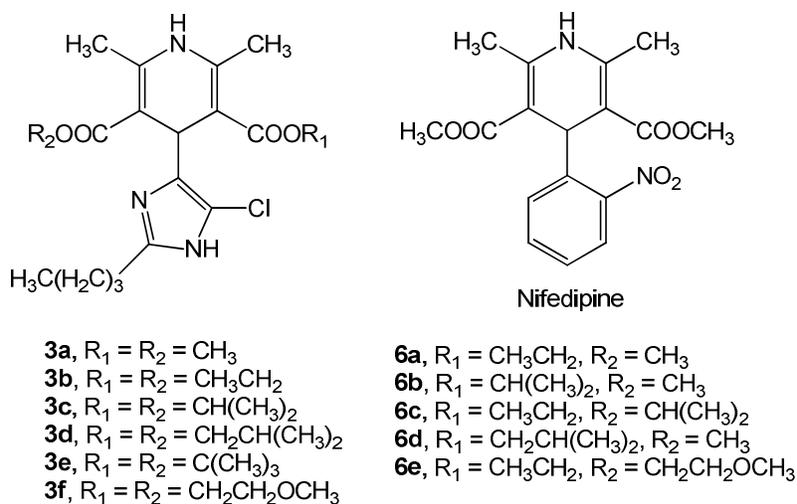
Acknowledgment

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Figure 1. Structures of **3a-f** and **6a-e** and nifedipine



Scheme 1. Synthesis of 3a-f and 6a-e

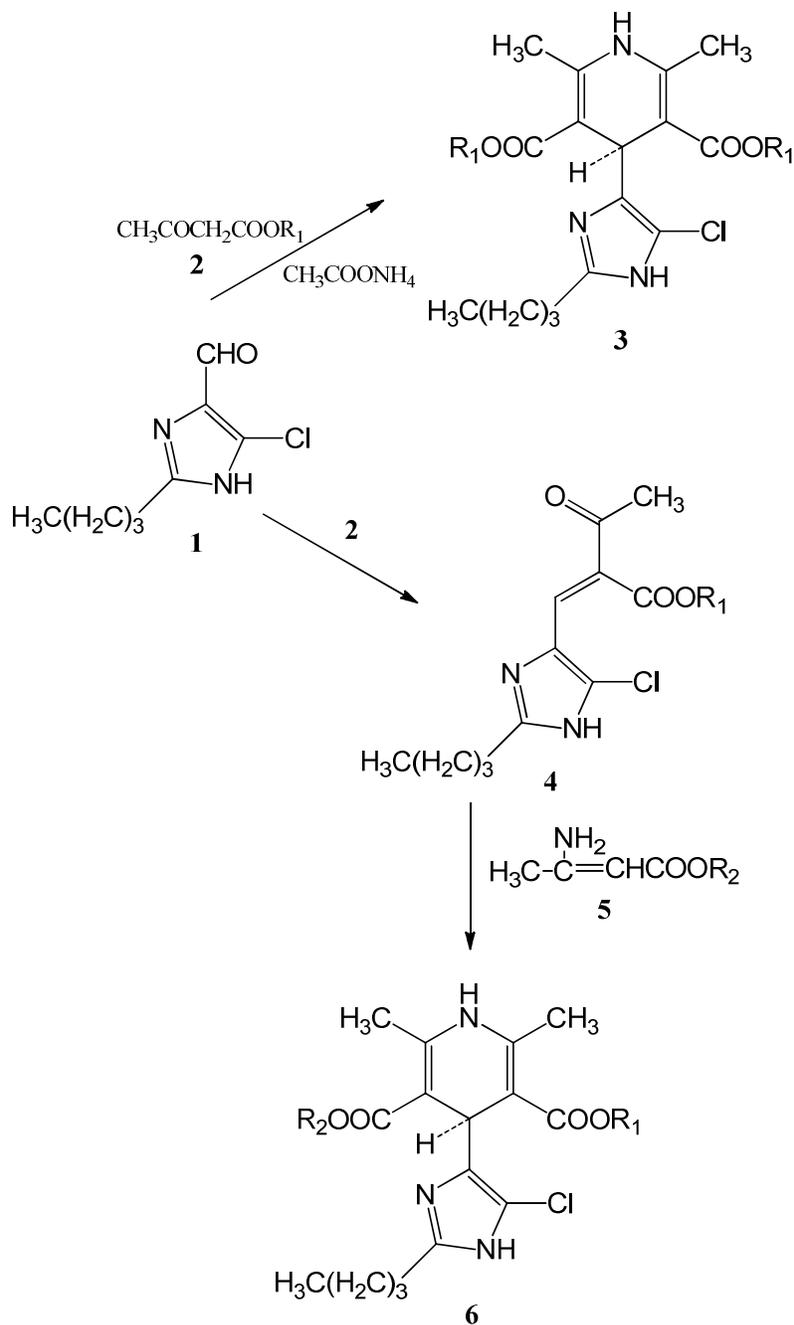
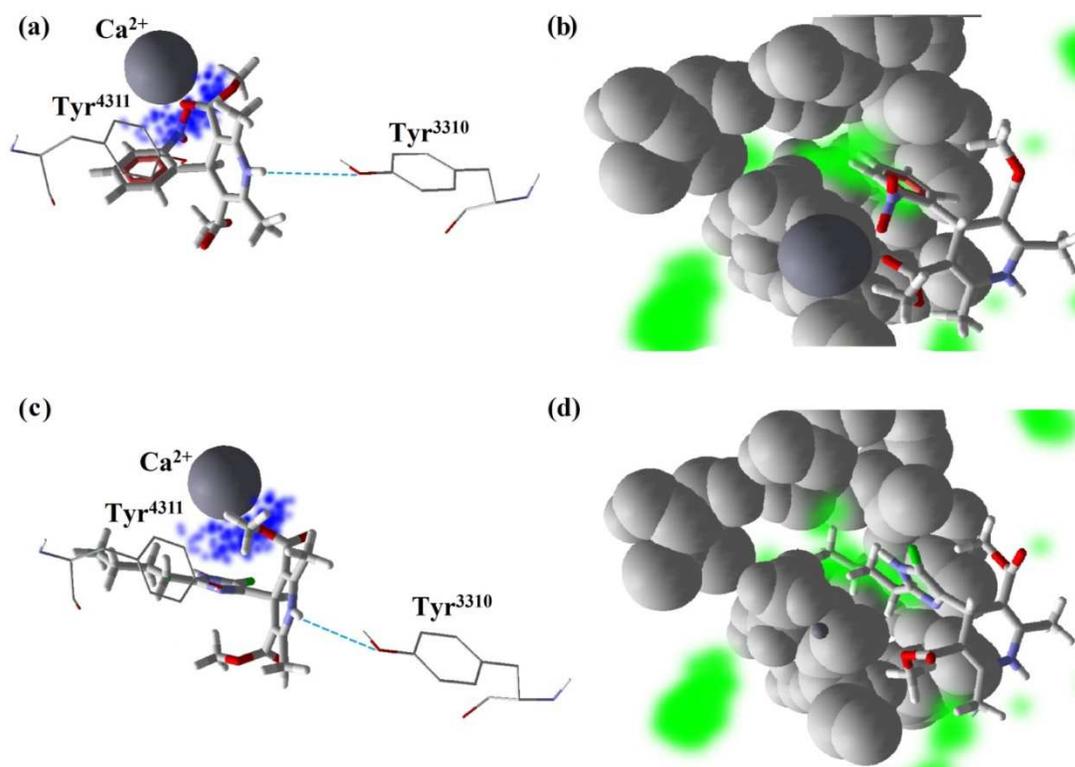
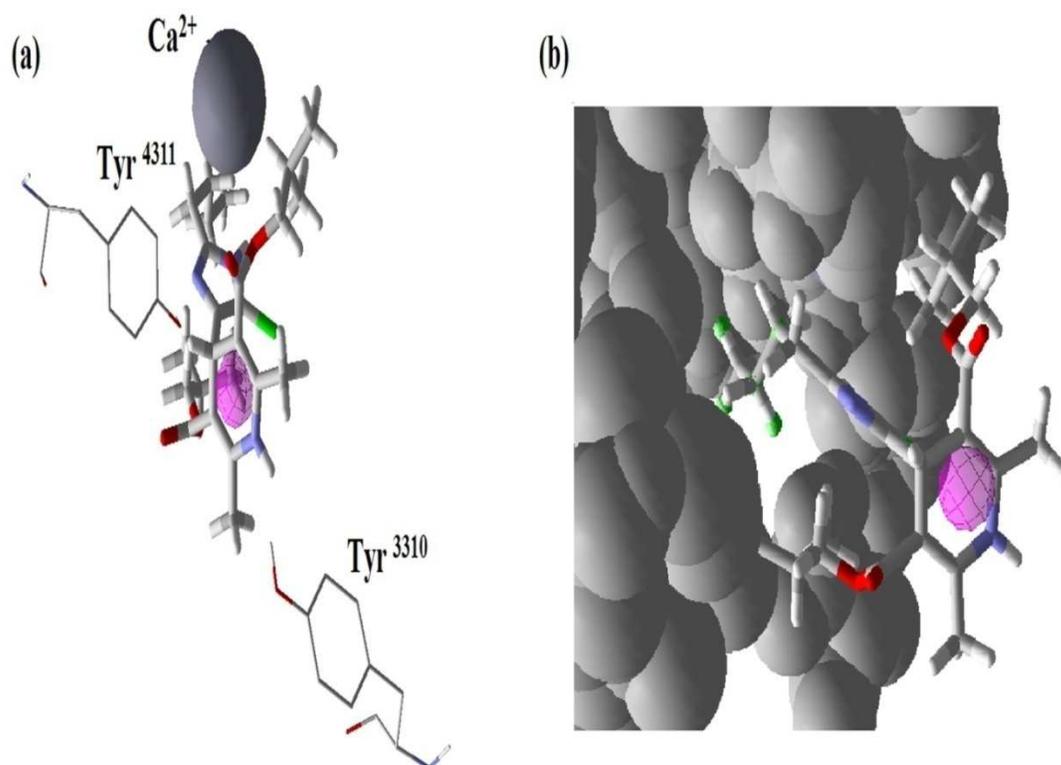


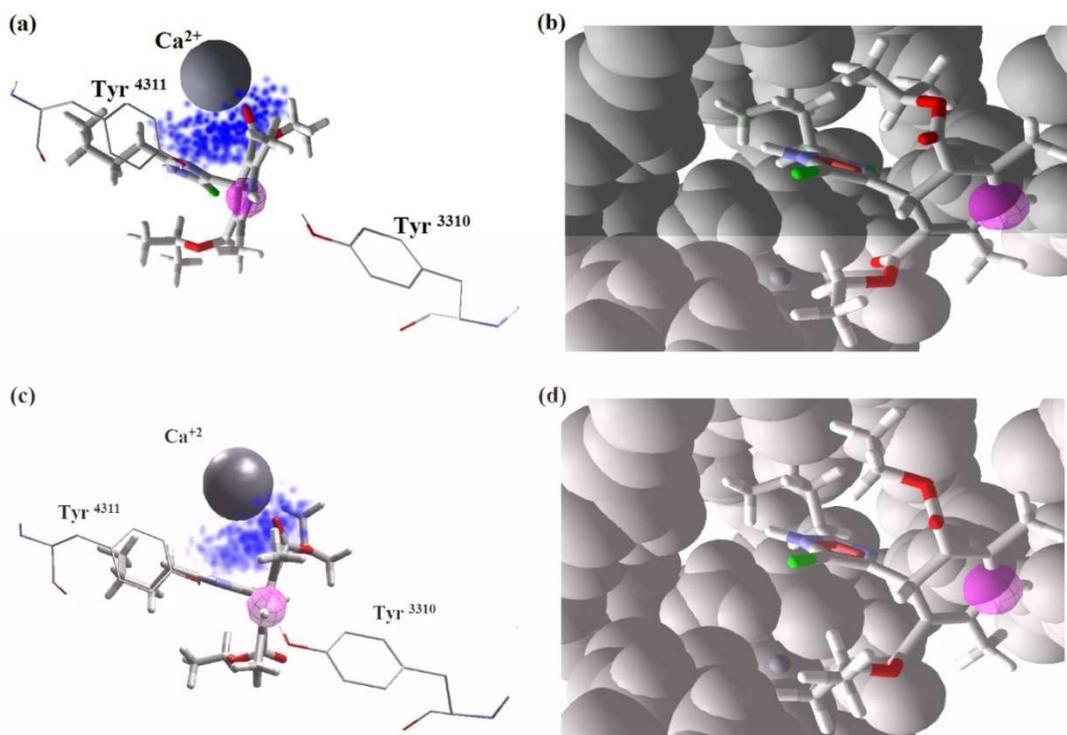
Figure 2. Interaction of nifedipine (a, b) and **3a** (c, d) with DHP receptor model

Electrostatic interaction of the aromatic ring, oxygen of the carbonyl ester group and calcium cofactor (blue), hydrogen bond between NH of the DHP and Tyr³³¹⁰ (dashed line), hydrophobic pocket (grey space-fill style), steirc favored regions (green) and ligand (stick style).

Figure 3. Docking of **3d** with DHP receptor model

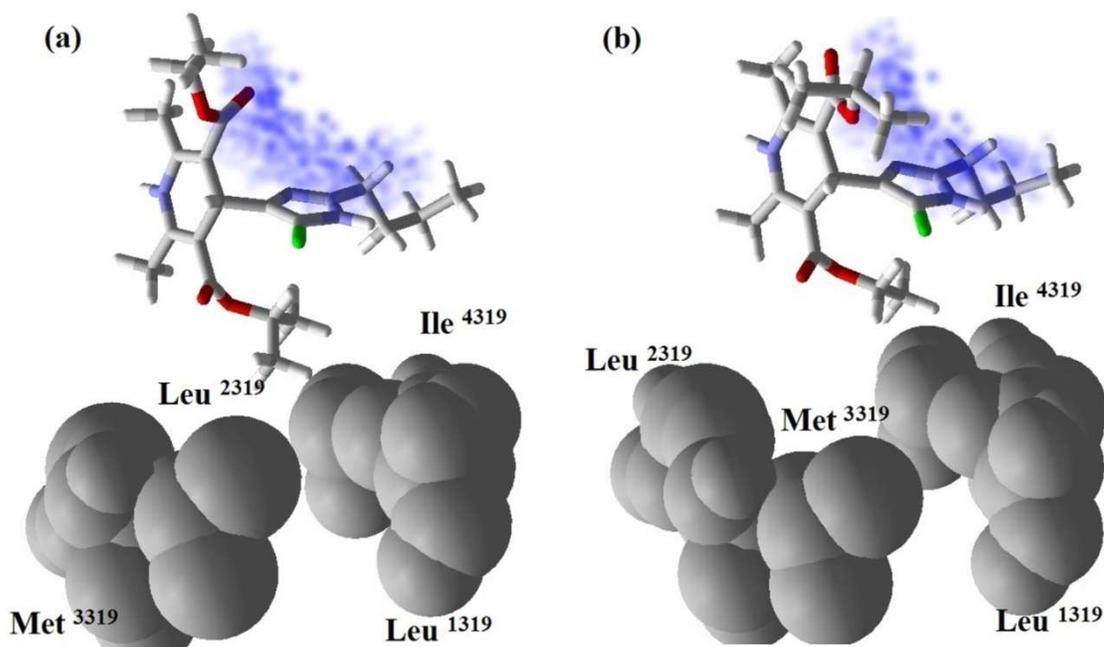
The proposed position of hydrogen bonding (magenta ball), hydrophobic pocket (grey space-fill style) and ligand (stick style).

Figure 4. Docking of **6c** (*S*)-enantiomer (a, b) and (*R*)-enantiomer (c, d) with DHP receptor model



Electrostatic interaction and water-lake (blue), the proposed position of hydrogen bonding (magenta ball), hydrophobic pocket (grey space-fill style) and ligand (stick style).

Figure 5. Interaction of (*S*)-enantiomer (a) and (*R*)-enantiomer (b) of **6c** with hydrophobic bracelet (Met³³¹⁹, Leu²³¹⁹, Leu¹³¹⁹ and Ile⁴³¹⁹)



Water-lake (blue), the proposed position of hydrogen bonding (magenta ball), hydrophobic bracelet (grey space-fill style) and ligand (stick style).

Table 1. Physical data of the prepared compounds

Compound	M.p. (°C)	Yield (%)	Reaction/crystallization solvents
3a	230-232	70	MeOH/MeOH
3b	212-214	77	MeOH/MeOH
3c	236-238	82	MeOH/MeOH
3d^a	190-192	39	iPrOH/MeOH
3e	191-193	73	iPrOH/iPrOH
3f	205-207	85	MeOH/MeOH
6a	213-215	90	MeOH/MeOH
6b	219-221	74	MeOH/MeOH
6c	236-238	73	MeOH/MeOH
6d^a	175-177	37	iPrOH/MeOH
6e	171-173	60	MeOH/MeOH

^aPurified by column chromatography; silica gel, CHCl₃: MeOH (9:1)

Table 2. Calcium channel antagonist activity data of **3a–f** and **6a–e**

Compound	Calcium channel antagonist activity (IC ₅₀ (M) ± SD ^a)
3a	2.534 ± 0.42 X 10 ⁻⁹
3b	1.377 ± 3.5 X 10 ⁻⁹
3c	3.267 ± 0.22 X 10 ⁻⁹
3d	1.798 ± 0.33 X 10 ⁻⁸
3e	2.174 ± 0.44 X 10 ⁻⁹
3f	2.355 ± 0.12 X 10 ⁻⁹
6a	5.267 ± 0.41 X 10 ⁻⁹
6b	8.618 ± 0.61 X 10 ⁻⁹
6c	9.792 ± 0.23 X 10 ⁻¹⁰
6d	2.955 ± 0.41 X 10 ⁻⁹
6e	1.178 ± 0.22 X 10 ⁻⁹
Nifedipine	1.79 ± 0.16 X 10 ⁻⁹

^aIC₅₀ ± SD was determined graphically from dose–response curve.
The number of experiments was 3 for all compounds.

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