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Synthesis, study of 3D structures, and pharmacological activities of lipophilic nitroimidazolyl-1,4-dihydropyridines as calcium channel antagonist

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Abstract—QSAR studies indicated that the potency of nifedipine analogues was dependent upon lipophilicity, an electronic term and separated terms for each position on the DHP ring. Changes in the substitution pattern at the C_3, C_4, and C_5 positions of DHPs alter potency, tissue selectivity, and the conformation of the 1,4-DHP ring. In this project a group of alkyl ester analogues of new derivatives of nifedipine, in which the ortho-nitrophenyl group at position 4 is replaced by a 1-methyl-5-nitro-2-imidazolyl substituent, and the methyl group at position 6 is replaced by a phenyl substituent, were synthesized and evaluated as calcium channel antagonist using the high K^+ contraction of guinea-pig ileal longitudinal smooth muscle. The results for asymmetrical esters showed that lengthening of the substituent in C_3 ester substituent increased activity. When increasing of the length is accompanied by increasing the hindrance, the activity decreased. The results demonstrate that all compounds were more active or similar in effect to that of the reference drug nifedipine.

1. Introduction

The discovery that the 4-aryl-1,4-dihydropyridine (DHP) (Nifedipine, Nimodipine) class of calcium channel blockers (CCBs) inhibits Ca^{2+} influx represented a major therapeutic advance in treatment of cardiovascular diseases such as hypertension, vasospastic angina, and other spastic smooth muscle disorders.[1–3] Recently, controversy concerning side effects has emerged. The first was an increased incidence of myocardial infarction among patients on a regimen of 'nifedipine'[4,5] which led the National Institute of Health to issue a warning to the use of time release formulations. The second was a retrospective correlation of increased incidence of cancer among older patients taking calcium channel blockers.[6,7] The current evidence on CCBs largely precludes the adverse effects of the magnitude suggested by the scare in the mid-1990 but attests to their safety and efficacy. It is also consistent with the recommendation in the Sixth Report of the Joint National Committee on Prevention, Evaluation, and Treatment of High Blood Pressure that puts β-blockers and diuretics as first-line agents for the treatment of uncomplicated hypertension, with DHPs and ACE inhibitors second.[8] Despite a rocky stretch, CCBs retain an important role in hypertension therapy and their improvement remains an important endeavor. Consequently, the search for finding analogues with novel binding properties has attracted renewed interest. Understanding the structure and function of membrane bound ion channels remains a challenge to chemists, and the design of molecules as ligands to selectively modulate ion channels holds significant therapeutic promise.

QSAR studies indicated that the potency of nifedipine analogues was dependent upon lipophilicity, an electronic term and separated terms for each position on the aromatic ring.[9] Changes in the substitution pattern at the C_3, C_4, and C_5 positions of DHPs alter potency,[10] tissue selectivity,[11] and the conformation of the 1,4-DHP ring.[12] Natale et al.[13] found that lipophilic

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4-isoxazolyl-1,4-dihydropyridines that have a aryl group on isoxazole ring exhibit high binding affinity. 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. Natale also employed Striessnig's model suggesting that potassium channel is a usable working mimic of the L-type calcium channel and observed that lipophilic substituent on isoxazolyl ring would easily be accommodated within the lipophilic channel as oriented and could be formed as a result of a π–π interaction between Tyr-1463 and the aryl moiety.[14] Other studies suggested that C_4 heterocycle substituents gave active compounds as calcium channel antagonists.[15–17] Parallel to this finding, we were able to prove that the bioisosteric replacement of the 4-aryl moiety with a 4-imidazolyl group yields 4-imidazolyl-1,4-dihydropyridines which retain potent calcium antagonist activity.[18–23]

It was therefore of interest to determine the Ca^{2+} channel antagonist activity of the hitherto unknown 1,4-dihydropyridine compounds that have 1-methyl-5-nitro-2-imidazolyl and a phenyl substituents at position 4 and 6 of DHP ring, respectively.

2. Chemistry

The synthesis of the 1,4-dihydropyridine derivatives **8a**–**f** was achieved following the steps outlined in Figure 1. Reaction of alcohol **1** with 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **2** afforded the corresponding acetoacetic esters **3c**–**f** (84–91% yield).[23] Reaction of acetoacetic esters **3c**–**f** with ammonium acetate afforded the corresponding alkyl 3-aminocrotonate **4a**–**f**. Also, reaction of 1-methyl-5-nitroimidazol-2-carboxaldehyde **5** with ethylbenzoylacetate **6** afforded the corresponding ethyl-2-benzoyl-3-(1-methyl-5-nitroimidazolyl)-2-propionate **7** (52% yield).[24,25] The asymmetrical analogues **8a**–**f** were synthesized by a modified Hantzsch reaction, using a procedure reported by Meyer et al. (8–52% yield).[26–29] The symmetrical analogue **9** was prepared (5% yield) by the classical Hantzsch condensation.[30] The yield and melting point of final compounds are summarized in Table 1.

3. Pharmacology

The calcium channel antagonist activity of compounds was determined as the concentration needed to produce 50% inhibition of the guinea-pig ileal longitudinal

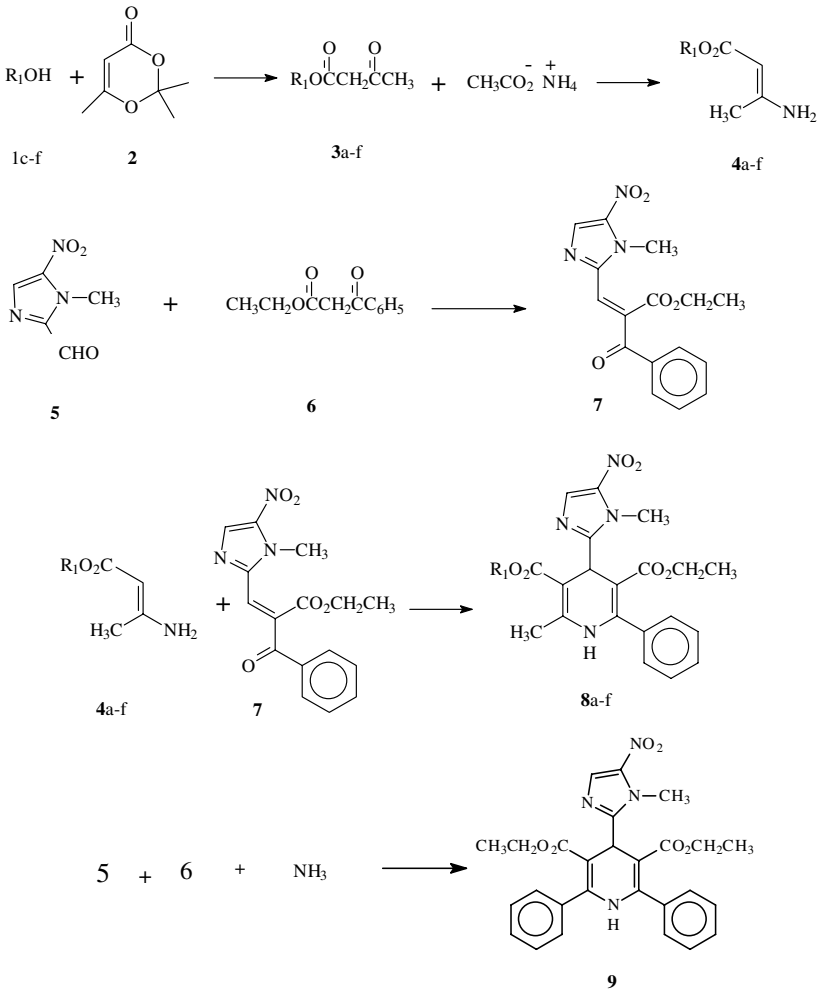
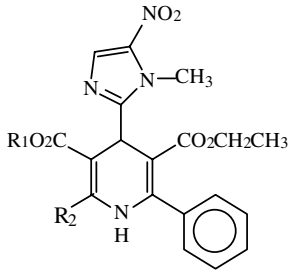


Figure 1. Synthetic pathway for the DHP derivatives used in this study.

Table 1. Physical and calcium channel modulation data for compounds **8a–f** and **9**



Compound	R_1	R_2	Mp (°C)	Yield (%)	Calcium channel antagonist activity $IC_{50} \pm SEM$[a] ($n = 5$)
$\mathbf{8_a}$	Methyl	Methyl	200–202	8	$8.24 \pm 0.41 \times 10^{-8}$
$\mathbf{8_b}$	Ethyl	Methyl	167–169	10	$1.37 \pm 0.78 \times 10^{-8}$
$\mathbf{8_c}$	*n*-Propyl	Methyl	186–188	21	$3.33 \pm 0.37 \times 10^{-9}$
$\mathbf{8_d}$	*iso*-Propyl	Methyl	157–159	29	$3.47 \pm 0.22 \times 10^{-8}$
$\mathbf{8_e}$	*n*-Butyl	Methyl	168–171	20	$2.21 \pm 0.42 \times 10^{-10}$
$\mathbf{8_f}$	*iso*-Butyl	Methyl	168–169	52	$1.54 \pm 0.25 \times 10^{-9}$
9	Ethyl	Phenyl	183–185	5	$1.14 \pm 0.73 \times 10^{-7}$
	Nifedipine				$1.12 \pm 0.36 \times 10^{-8}$

[a] The molar concentration of antagonist test compound causing a 50% in the tonic contractile response ($IC_{50} \pm SEM$) in guinea-pig ileum smooth muscle by KCl (80 mmol/L) was determined graphically from dose–response curve. The number of experiments was 5 for all compounds.

smooth muscle (GPILSM) contractility is summarized in Table 1. Comparison of the activities of asymmetrical esters in alkyl ester series (Table 1, **8a–f**) indicates that increasing of size of substituents in C-3 ester position increases activity. When increasing of the size is accompanied by increasing the hindrance, the activity decreases. Moreover, the results show that compounds **8b** and **8d** are similar in effect to the reference drug nifedipine. The compounds **8a** and **9** represent lower calcium channel antagonist activity, while compounds **8c**, **8e** and **8f** exhibit much higher activity relative to the reference drug nifedipine. Comparison of the activities of symmetrical compound (**9**) and asymmetrical esters in alkyl ester series (**8a–f**) indicates that asymmetrical esters are more active than symmetrical compound.

The structure–activity data indicate that the 1-methyl-5-nitro-2-imidazolyl moiety is a bioisoester of ortho-nitrophenyl group. As lipophilic aromatic substituents attached to the C-6 position of DHP ring are supposed to improve penetration into organ, these compounds are more active in comparison of similar compounds that contain methyl moiety in C-6 position of DHP ring. On the other hand, the activity data listed in Table 1 indicate that one-order decrease in activity is obtained when methyl at C-2 position is replaced by a phenyl substituent (compounds **8b** and **9**, respectively). This observation that is in the reverse order of the effect of increasing lipophilicity may be due to the increase in steric hindrance by phenyl group since the binding of DHP derivatives to their receptor is sandwich-type and there is a challenge between lipophilicity and steric for the calcium channel antagonist activity of the DHP derivatives.[31] Therefore, replacement of methyl by phenyl in one site of DHP ring (i.e., C-6) increases the activity because of higher lipophilicity but replacement of both methyls at C-2 and C-6 positions of DHP ring decreasing the activity due to increase in steric hindrance.

4. Three-dimensional structure

The optimized 3D structure of the molecules obtained by DFT calculations at the level of 6–31G* is represented in Figure 2 and some geometrical properties including angles, dihedrals, and atomic distances are listed in Table 2. As one can see from Figure 2, the studied DHP derivatives adapted to relatively the same geometry similar to that we found in our previous studies for other DHP derivatives.[12] The DHP ring exhibited a semi-boat conformer and the methyl-nitroimidazolyl substituent at C-4 position of the DHP ring positioned at the axial coordinate. Interestingly, in all of the derivatives the methyl group of methyl-nitroimidazolyl oriented in the anti-preplanar with respect to the DHP ring. In addition, the nitroimidazolyl ring made a dihedral angle about 30° with the DHP ring. The phenyl ring, attached to the C-6 position of DHP ring, made a dihedral angle about 58° with the DHP ring for all derivatives except for **8e**. The dihedral angle for this compound was found to be 25°. It should be noted that the optimization was performed many times with different starting geometries and in all trials similar final geometries were found for **8e**. The major difference between the geometry of the studied molecules upon variation of the substituent pattern on the C-3 position of the DHP ring (R1) is the orientation of the CO double bond of the C3-COOEt with respect to the DHP ring. As seen, for compounds **8a** and **9** a *cis* orientation resulted and the rest adapted to the *trans* conformation.

It is reported from the crystal structures of DHP-based derivatives that the 3D geometry of the molecules plays an important role in their activity.[12] As it is observed from Figure 2 and Table 2, the methyl of the nitroimidazolyl ring adapted to the anti-periplanar configuration with respect to the DHP ring and this is required for calcium channel antagonist activity. The syn-preplanar orientation leads to agonist activity. Another 3D

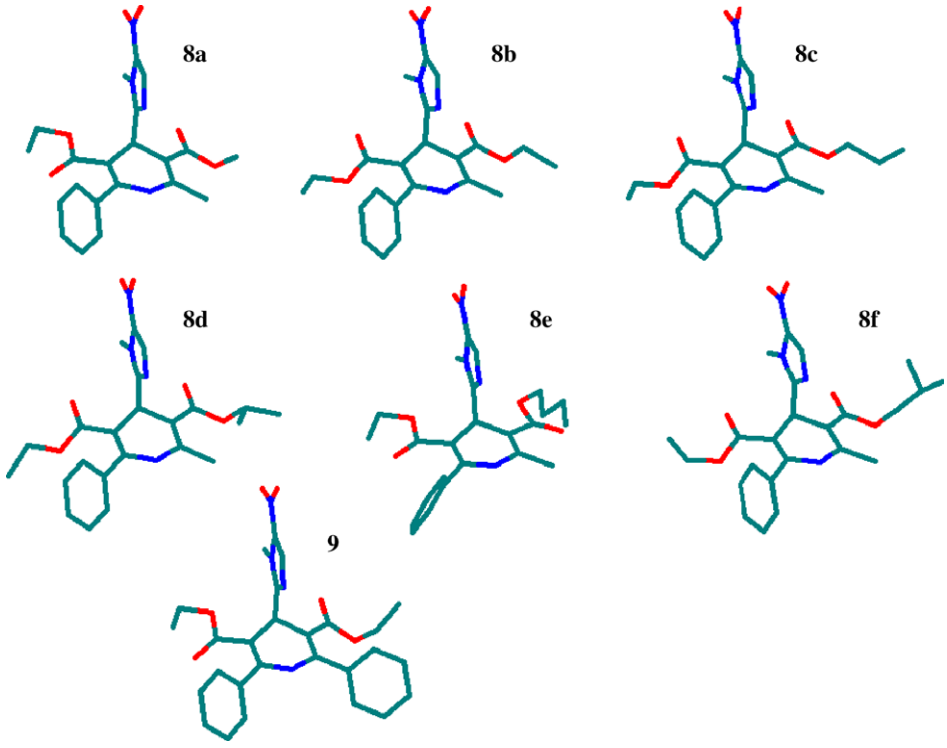


Figure 2. The optimized three-dimensional structural representation of the lipophilic DHP derivatives used in this study.

Table 2. Some geometrical features of the optimized DHP derivative

Compound	Splitting[a]	$\Phi 1$[b] (°)	$\Phi 2$[c] (°)	$\Phi 3$[d] (°)	d[e] (Å)
8a	q	58.4	32.6	*cis*	5.36
8b	m	58.8	29.1	*trans*	4.03
8c	m	57.5	28.9	*trans*	4.02
8d	m	59.1	28.9	*trans*	4.06
8e	m	−25.1	31.2	*trans*	6.23
8f	m	59.3	28.9	*trans*	4.07
9	q	56.8	32.1	*cis*	5.33

[a] q, quartet and m, multiplet.
[b] Dihedral angle between phenyl and DHP rings.
[c] Dihedral angle between nitroimidazolyl and DHP rings.
[d] Dihedral orientation between C-5-CO and DHP ring.
[e] The interatomic distance between methylene carbon and C-2′ carbon of phenyl.

geometry–activity relationship is observed for the *cis*/*trans* orientation of the CO double bond of the C3-COOEt with respect to the DHP ring. Compounds **8a** and **9** that exhibited lower activity preferred the *cis* orientation and the rest adapted to the *trans* orientation.

In the NMR investigation of the synthesized DHP, we found that the multiplicity of the ^{H}NMR of methylene's hydrogens of the C5-$COOCH_2CH_3$ is different for compounds **8a** and **9** with the other compounds. Ideally, these hydrogens must produce a quartet signal due to the presence of methyl's protons. However, all compounds exhibited multiplet signal except for **8a** and **9** that produced quartet signal. This can be attributed to the long-range coupling between the protons of methylene and those of phenyl ring, especially one attached to the C-2′ of the phenyl. A close insight into the 3D structures represented in Figure 2 reveals that at the atom C-5 of DHP ring the *cis* conformer of the CO makes the OCH_2CH_3 to be formed from the phenyl ring. In contrast, a decreased distance between the methylene's hydrogens and phenyl carbons is obtained for the *trans* conformer of CO. The inter-atomic distance between the carbon of methylene and C-2′ carbon of phenyl ring, listed in Table 2 (footnote d), supports this postulate. For compounds **8a** and **9** this distance is higher than 5.3 Å and for **8b**, **8c**, **8d**, and **8f** the distance is about 4.0 Å. Here also the distance for **8e** is an expectation.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker 80 MHz spectrometer (Bruker Analytische Messetechnik, Rheinstetten, Germany) in d_1-chloroform or d_6-DMSO and tetramethylsilane (TMS) was used as an internal standard. The mass spectra were measured with a Finnigan TSQ-70 spectrometer (Finnigan Mat, Bremen, Germany) at 70 eV. The IR spectra were obtained by using a Nicolet 50X-FT spectrometer (KBr disks) (Nicolet, Madison, WI, USA). The results of elemental analyses (C, H, and N) were within ±0.4% of the calculated values. Silica gel HT-254 (E.Merck, Darmstadt, Germany) was used for thin-layer chromatography. All spectra were consistent with the assigned structures. Methyl(ethyl) acetoacetate **1a**–**b**, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **2**, and methyl(ethyl) 3-aminocrotonate **4a**–**b** were

purchased from the Aldrich Chemical Co. (Sigma–Aldrich Chemie GmbH, Deisenhofon, Germany).

5.1.1. General procedure for the synthesis of acetoacetic esters (3c–f). A solution of alcohol **1c–f** (50 mmol) and 2,2,6-trimethyl-4*H*-1,3-dioxine-4-one **2** (7.1 g, 50 mmol) in 10 ml xylene was placed in a 50 ml 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, heating was continued for a total 30 min. The reaction mixture was cooled. The xylene was removed. Distillation of the mixture afforded **3c–f** which were used immediately in subsequent reactions.

5.1.1.1. *n*-Propyl acetoacetate (3_c). ^{1}H NMR ($CDCl_3$): δ 4.10 (t, J = 6.5 Hz, 2H, CO_2CH_2), 3.45 (s, 2H, $COCH_2CO_2$), 2.27 (s, 3H, CH_3CO), 1.63 (m, 2H, CH_2), 0.94 (t, J = 6.5 Hz, 3H, CH_3).

IR (KBr): ν 1747 (C=O, ester), 1719 cm^{-1} (C=O, ketone).

5.1.1.2. Iso-propyl acetoacetate (3_d). ^{1}H NMR ($CDCl_3$): δ 5.06 (septet, J = 6.1 Hz, 1H, CO_2CH), 3.41 (s, 2H, $COCH_2CO_2$), 2.26 (s, 3H, CH_3CO), 1.26 (d, J = 6.1 Hz, 6H, $CH(CH_3)_2$).

IR (KBr): ν 1742 (C=O, ester), 1727 cm^{-1} (C=O, ketone).

5.1.1.3. *n*-Butyl acetoacetate (3_e). ^{1}H NMR ($CDCl_3$): δ 4.15 (t, J = 6.4 Hz, 2H, CO_2CH_2), 3.44 (s, 2H, $COCH_2CO_2$), 2.16 (s, 3H, CH_3CO), 1.41 (m, 4H, CH_2CH_2), 1.01 (t, J = 6.4 Hz, 3H,CH_3).

IR (KBr): ν 1757 (C=O, ester), 1729 cm^{-1} (C=O, ketone).

5.1.1.4. Iso-butyl acetoacetate (3_f). ^{1}H NMR ($CDCl_3$): δ 4.48 (d, J = 6.6 Hz, 2H, CO_2CH_2), 3.46 (s, 2H, $COCH_2CO_2$), 2.26 (s, 3H, CH_3CO), 2.46 (m, 1H, CH), 1.48 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$).

IR (KBr): ν 1756 (C=O, ester), 1727 cm^{-1} (C=O, ketone).

5.1.2. General procedure for the synthesis of alkyl 3-aminocrotonate (4c–f). A solution of alkyl acetoacetic esters **3a–f** (4 mmol) and ammonium acetate (6 mmol) in 5 ml ethanol was placed in a 10 ml Erlenmeyer flask. The flask was immersed in an oil bath that had been preheated to 90 °C, and the solution was vigorously stirred. Heating was continued for a total 24 h. The reaction mixture was cooled. The ethanol was removed and IR spectra of the compounds were recorded to confirm the structure of **4a–f**. Then, the compounds were immediately used in subsequent reactions.

5.1.2.1. *n*-Propyl 3-aminocrotonate (4_c). IR (KBr): ν 3449 and 3342 (NH_2), 1715 cm^{-1} (C=O, ester).

5.1.2.2. Iso-propyl 3-aminocrotonate (4_d). IR (KBr): ν 3453 and 3336 (NH_2), 1717 cm^{-1} (C=O, ester).

5.1.2.3. *n*-Butyl 3-aminocrotonate (4_e). IR (KBr): ν 3449 and 3334 (NH_2), 1713 cm^{-1} (C=O, ester).

5.1.2.4. Iso-butyl 3-aminocrotonate (4_f). IR (KBr): ν 3453 and 3336 (NH_2), 1716 cm^{-1} (C=O, ester).

5.1.3. Procedure for the synthesis of ethyl-2-benzoyl-3-(1-methyl-5-nitroimidazolyl)-2-propionate (7). A solution of 1-methyl-5-nitroimidazol-2-carboxaldehyde **5** (460 mg, 3 mmol), ethylbenzoylacetate **6** (580 mg, 3 mmol), glacial acetic acid (0.1 ml), piperidine (0.05 ml), and dry benzene (5 ml) was refluxed for 7 h, during which the resultant water was removed via a Dean–Stark trap. After cooling, the benzene was removed and the residue was purified by chromatography on silica gel with chloroform–methanol (20:1 v/v), to give pure compound **7** (52% yield) as solid (mp 135–137 °C).

^{1}H NMR ($CDCl_3$): δ 7.82 (s, 1H, imidazol H-4), 7.70 (s, 1H, =C–H), 7.41–7.33 (m, 5H, C_6H_5), 4.26 (q, J = 7.2 Hz, 2H, CO_2CH_2), 4.10 (s, 3H, N–CH_3), 1.18 (t, J = 7.2 Hz, 3H, CH_3).

IR (KBr): ν 1739 (C=O, ester), 1716 (C=O, ketone), 1627 (C=C), 1520, 1370 cm^{-1} (NO_2).

MS: *m*/*z* (%) 392 (M^+, 9), 300 (61), 272 (14), 242 (10), 228 (17), 192 (10), 155 (18), 125 (28), 105 (100) and 77 (37).

5.1.4. General procedure for the synthesis of alkyl ethyl 1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates (8a–f). A solution of compounds **4a–f** (1.5 mmol) and ethyl-2-benzoyl-3-(1-methyl-5-nitroimidazolyl)-2-propionate **7** (493 mg, 1.5 mmol) in ethanol (5 ml) was protected from light and refluxed for 24–30 h. After cooling, the solution was concentrated under reduced pressure and purified by thin-layer chromatography on silica gel with chloroform–methanol (50:1 v/v). The product was recrystallized from chloroform to give pure compounds **8a–f**.

5.1.4.1. 5-Ethyl-3-methyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (8a). ^{1}H NMR ($CDCl_3$): δ 8.01 (s, 1H, imidazole H-4), 7.41–7.27 (m, 5H, C_6H_5), 6.22 (br s, 1H, NH), 5.20 (s, 1H, C_4-H), 4.23 (s, 3H, N–CH_3), 3.81 (q, J = 7.2 Hz, 2H, CO_2CH_2), 3.71 (s, 3H, CO_2CH_3), 2.38 (s, 3H, C_2–CH_3), 0.75 (t, J = 7.2 Hz, 3H, CH_3).

IR (KBr): ν 3421 (NH), 1702 (C=O), 1526, 1373 cm^{-1} (NO_2).

MS: *m*/*z* (%) 426 (M^+, 51), 380 (8), 367 (15), 353 (36), 321 (100), 300 (31), 272 (13) and 252 (7).

Elemental analysis calculated for $C_{21}H_{22}N_4O_6$: C, 59.15; H, 5.20; N, 13.14. Found: C, 59.34; H, 5.18; N, 13.08.

5.1.4.2. 3,5-Diethyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (8b). ^{1}H NMR ($CDCl_3$): δ 7.99 (s, 1H, imidazole H-4), 7.40–7.31 (m, 5H, C_6H_5), 6.35 (br s, 1H, NH), 5.20 (s, 1H, C_4-H), 4.24 (s, 3H, N–CH_3), 4.17 (q, J = 7.2 Hz, 2H, C_3–CO_2CH_2), 3.82–3.78 (m, 2H, C_5–CO_2CH_2), 2.38 (s, 3H, C_2–CH_3), 1.27 (t, J = 7.2 Hz, 3H, C_3–$CO_2CH_2CH_3$), 0.75 (t, J = 7.1 Hz, 3H, C_5–$CO_2CH_2CH_3$).

IR (KBr): *v* 3193 (NH), 1673 (C=O), 1525, 1370 cm^{-1} (NO_2).

MS: *m*/*z* (%) 440 (M^+, 39), 394 (6), 367 (44), 321 (100), 314 (22), 286 (6), 258 (11) and 240 (10).

Elemental analysis calculated for $C_{22}H_{24}N_4O_6$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.87; H, 5.51; N, 12.77.

5.1.4.3. 5-Ethyl-3-*n*-propyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-*3*,5-pyridinedicarboxylate (8c). ^1H NMR ($CDCl_3$): δ 8.01 (s, 1H, imidazole H-4), 7.40–7.26 (m, 5H, C_6H_5), 6.11 (br s, 1H, NH), 5.20 (s, 1H, C_4-H), 4.24 (s, 3H, N–CH_3), 4.09–4.06 (m, 2H, C_3–CO_2CH_2), 3.82–3.78 (m, 2H, C_5–CO_2CH_2), 2.39 (s, 3H, C_2–CH_3), 1.66 (m, 3H, C_3–$CO_2CH_2CH_2CH_3$), 0.92 (t, J = 7.2 Hz, 3H, C_3–CO_2 $CH_2CH_2CH_3$), 0.75 (t, J = 7.1 Hz, 3H, C_5–$CO_2CH_2CH_3$).

IR (KBr): *v* 3421 (NH), 1676 (C=O), 1527, 1372 cm^{-1}(NO_2).

MS: *m*/*z* (%) 454 (M^+, 68), 440 (15), 395 (6), 367 (52), 321 (100), 276 (7), 258 (9) and 240 (10).

Elemental analysis calculated for $C_{23}H_{26}N_4O_6$: C, 60.78; H, 5.77; N, 12.33. Found: C, 60.56; H, 5.79; N, 12.37.

5.1.4.4. 5-Ethyl-3-*iso*-propyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridine dicarboxylate (8d). ^1H NMR ($CDCl_3$): δ 7.99 (s, 1H, imidazole H-4), 7.39–7.27 (m, 5H, C_6H_5), 6.31 (br s, 1H, NH), 5.18 (s, 1H, C_4-H), 5.05 (m, 1H, CO_2CH), 4.25 (s, 3H, N–CH_3), 3.82–3.79 (m, 2H, CO_2CH_2), 2.37 (s, 3H, C_2–CH_3), 1.27 and 1.20 (two d, J = 6.0 Hz, 3H each, $CH(CH_3)_2$), 0.74 (t, J = 7.1 Hz, 3H, C_5–$CO_2CH_2CH_3$).

IR (KBr): *v* 3419 (NH), 1677 (C=O), 1528, 1372 cm^{-1} (NO_2).

MS: *m*/*z* (%) 454 (M^+, 27), 381 (9), 367 (47), 339 (10), 321 (100), 307 (6) and 286 (14).

Elemental analysis calculated for $C_{23}H_{26}N_4O_6$: C, 60.78; H, 5.77; N, 12.33. Found: C, 60.61; H, 5.74; N, 12.28.

5.1.4.5. 5-Ethyl-3-*n*-butyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridine dicarboxylate (8e). ^1H NMR ($CDCl_3$): δ 8.00 (s, 1H, imidazole H-4), 7.41–7.26 (m, 5H, C_6H_5), 6.09 (br s, 1H, NH), 5.20 (s, 1H, C_4-H), 4.24 (s, 3H, N–CH_3), 4.18–4.10 (m, 2H, C_3–CO_2CH_2), 3.83–3.78 (m, 2H, C_5–CO_2CH_2), 2.38 (s, 3H, C_2–CH_3), 1.64–1.58 (m, 3H, C_3–$CO_2CH_2CH_2CH_2$), 1.37–1.0 (m, 3H, C_3–$CO_2CH_2CH_2CH_2CH_3$), 0.92 (t, J = 7.0 Hz, 3H, C_3–CO_2 $CH_2CH_2CH_2CH_3$), 0.75 (t, J = 7.1 Hz, 3H, C_5–$CO_2CH_2CH_3$).

IR (KBr): *v* 3420 (NH), 1677 (C=O), 1529,1373 cm^{-1} (NO_2).

MS: *m*/*z* (%) 468 (M^+, 29), 440 (7), 395 (16), 367 (38), 342 (13), 321 (100), 276 (7), 258 (8) and 240 (9).

Elemental analysis calculated for $C_{24}H_{28}N_4O_6$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.72; H, 5.99; N, 12.01.

5.1.4.6. 5-Ethyl-3-*iso*-butyl-1,4-dihydro-2-methyl-6-phenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (8f). ^1H NMR ($CDCl_3$): δ 7.99 (s, 1H, imidazole H-4), 7.40–7.29 (m, 5H, C_6H_5), 6.41 (br s, 1H, NH), 5.20 (s, 1H, C_4-H), 4.24 (s, 3H, N–CH_3), 4.00–3.81 (m, 2H, C_3–CO_2CH_2), 3.80–3.77 (m, 2H, C_5–CO_2CH_2), 2.38 (s, 3H, C_2–CH_3), 1.97 (m, 1H, $CH_2CH(CH_3)_2$), 0.90 (d, J = 7.0 Hz, 6H, $CH(CH_3)_2$), 0.75 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$).

IR (KBr): *v* 3415 (NH), 1677 (C=O), 1532, 1376 cm^{-1} (NO_2).

MS: *m*/*z* (%) 468 (M^+, 39), 395 (22), 367 (39), 342 (16), 333 (10), 321 (100), 286 (9) and 240 (10).

Elemental analysis calculated for $C_{24}H_{28}N_4O_6$: C 61.53; H, 6.02; N, 11.96. Found: C, 61.44; H, 6.05; N, 11.91.

5.1.5. Procedure for the synthesis of 3,5-diethyl-1,4-dihydro-2,6-diphenyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9). A solution of ammonium hydroxide (25%, 0.75 ml) was added to a stirring solution of 1-methyl-5-nitro-imidazole-2-carboxaldehyde **5** (0.78 g, 5 mmol) ethylbenzoylacetate **6** (1.92 g, 10 mmol) in absolute ethanol (5 ml). The mixture was heated under reflux for 20 h. After that, the reaction mixture was cooled, solvent removed under vacuum. The residue was purified by thin-layer chromatography on silica gel using chloroform–methanol (98:2 v/v), to give pure compound **9** (5% yield) as solid (mp 183–185 °C).

^1H NMR ($CDCl_3$): δ 8.07 (s, 1H, imidazole H-4), 7.42–7.37 (m, 10H, C_6H_5), 6.19 (br s, 1H, NH), 5.29 (s, 1H, C_4-H), 4.31 (s, 3H, N–CH_3), 3.86 (q, J = 7.2 Hz, 4H, C_3 and C_5–CO_2CH_2), 0.81 (t, J = 7.2 Hz, 6H, C_3 and C_5–$CO_2CH_2CH_3$).

IR (KBr): *v* 3181 (NH), 1676 (C=O), 1532, 1381 cm^{-1} (NO_2).

MS: *m*/*z* (%) 502 (M^+, 58), 456 (16), 429 (54), 383 (100), 376 (32) and 230 (11).

Elemental analysis calculated for $C_{27}H_{26}N_4O_6$: C, 64.53; H, 5.21; N, 11.15. Found: C, 64.80; H, 5.20; N, 11.14.

5.2. Pharmacology

5.2.1. Determination of calcium channel antagonist activity. Male albino guinea pigs (300–450 g) were purchased from SUMS animal house department. They had free access to standard rodent chow and tap water at all times. The animals were housed in a room maintained at 23 ± 2 °C temperature, 55 ± 10 % humidity, and on a 12 h light/dark cycle. The feeding was disrupted 1 day before starting in vitro tests. The animals were sacrificed 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 20 ml jacketed organ bath containing oxygenated physiological saline solution of the following (mmol) 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 muscle was equilibrated for 1 h with a solution changing every 15 min. The contractions were recorded with a force displacement transducer (F-50) on a Narco Physiograph (Narco Biosystems, Houston, TX, USA). Test agents were prepared as 10–2 mol/L stock solutions in DMSO and stored protected from light. Dilutions were made with DMSO. The contractile response was taken as the 100% value for the tonic (slow) component of the response. The contraction was elicited with 80 mmol/L 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.[32,33] Nifedipine was used as reference compound.

5.2.2. Statistical analysis. Data are expressed as means ± SEM. The one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons was used to analyze the data. A value of $P < 0.05$ was considered as the significance level between the groups.

5.3. Computation details

Chemical structure of each molecule was built by Hyperchem software (Version 7, Hypercube Inc.) for the structural chemistry. Gaussian98 program[34] was operated to optimize the molecular structure. The structures were optimized by the B3LYP method of density functional theory (DFT) at the level of 6-31G*. No molecular symmetry constraint was applied; rather full optimization of all bond lengths and angles was carried out. The root mean square of 0.1 kcal mol^{-1} was used as ending criteria in geometry optimization. Then, the molecules were reloaded to Hyperchem and some angles, dihedrals, and distances were calculated in this software.

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