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Synthesis and Evaluation of Pharmacological Activities of 3,5-Dialkyl 1,4-Dihydro-2,6-Dimethyl-4-Nitroimidazole-3,5-Pyridine Dicarboxylates

New analogues of nifedipine, in which the 2-nitrophenyl group at position 4 is replaced by a 1-methyl-5-nitro-2-imidazolyl substituent, were synthesized. The symmetrical dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates were prepared by a classical Hantzsch condensation. The asymmetrical analogues were synthesized using a procedure reported by Iwanami that involved the condensation of alkylacetoacetate with methyl-, ethyl- or isopropyl-3-aminocrotonate and 1-methyl-5-nitroimidazole-2-carboxaldehyde. Calcium channel antagonist activities were determined *in vitro* using a guinea pig ileum longitudinal smooth muscle (GPILSM) assay. Many compounds exhibited superior, or equipotent, calcium antagonist activity ($IC_{50} = 10^{-10}$ to 10^{-13} M range) relative to the reference drug nifedipine ($IC_{50} = 1.09 \pm 0.12 \times 10^{-11}$ M). Antinociceptive effects of some compounds were evaluated by the mouse tail-flick assay *in vivo*. Results demonstrate that some of the compounds were active as an antinociceptive.

Keywords: Dihydropyridine; Ca^{+2} channel antagonist; Antinociceptive; Nitroimidazole

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Introduction

The influx of extracellular Ca^{2+} through potential dependent calcium channels is responsible for the regulation of many physiological functions, including smooth and cardiac muscle contraction [1–3]. The discovery that the 1,4-dihydropyridine (Nifedipine) class of calcium channel antagonists inhibits this Ca^{2+} influx represented a major therapeutic advance in treatment of cardiovascular diseases such as hypertension, angina pectoris, and other spastic smooth muscle disorders [4–7]. In addition, the antinociceptive activities of some calcium channel blockers such as, nifedipine, nimodipine, verapamil, and diltiazem had been reported previously. These finding suggest a pharmacological role of Ca^{2+} channel blockers in the modulation of antinociception under acute condition [8–10].

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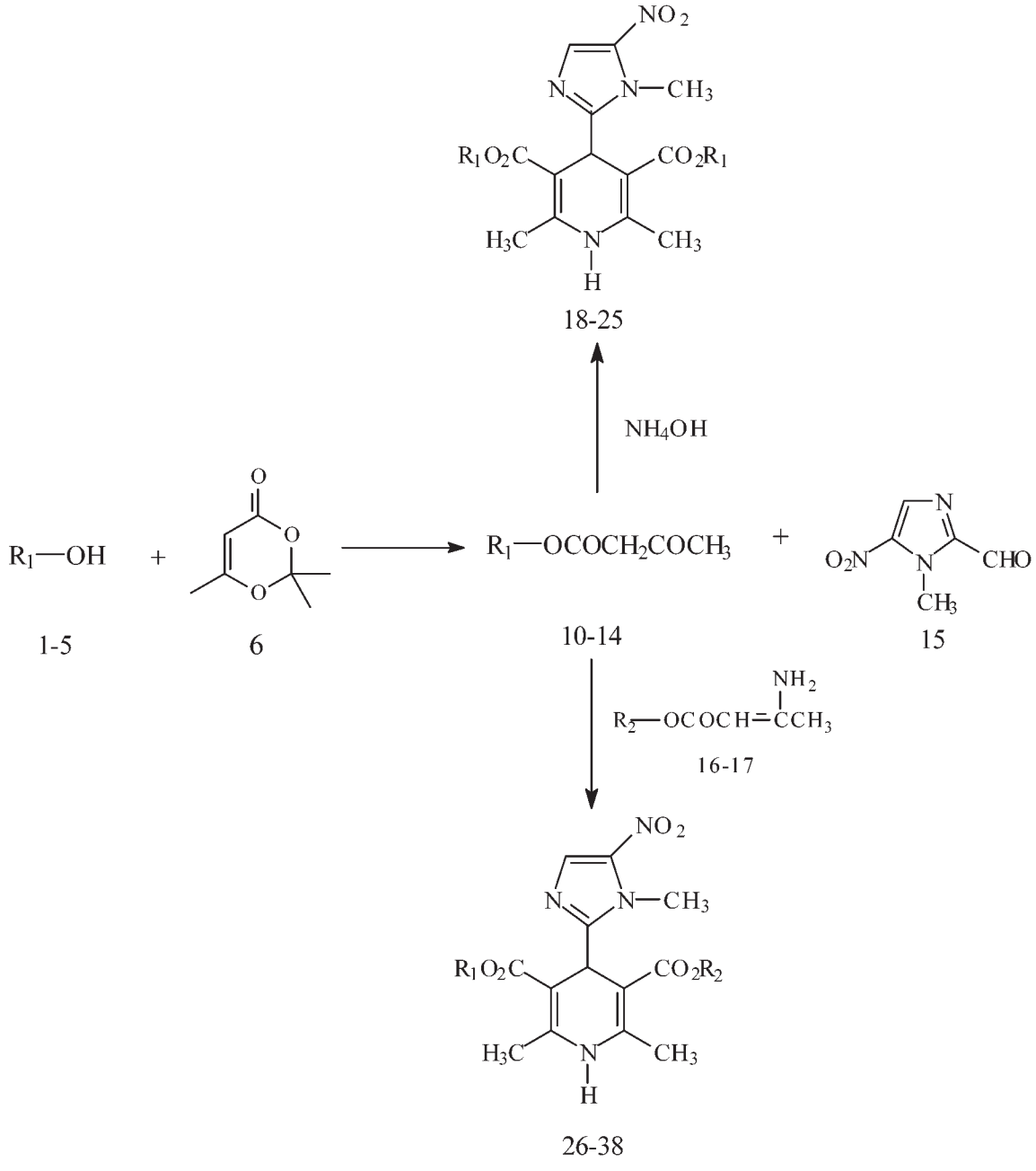
Changes in substitution pattern at the C-3, C-4, and C-5 positions of nifedipine alter activity and tissue selectivity [11–13]. It was our interest to determine the effects of different C-3 alkyl substituents, in conjugation with C-4 nitroimidazolyl substituents, on the calcium channel antagonist activity. We now report the synthesis, calcium channel antagonist and antinociceptive activities of dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicacboxylate.

Chemistry

Alkyl acetoacetates **10**–**14** were synthesized by the reaction of 2,2,6-trimethyl-4H-1,3-dioxine-4-one **7** with alcohols **1**–**5** in 43–57 % overall yield. The symmetrical analogues **18**–**25** were prepared by the classical Hantzsch condensation [14] in which 1-methyl-5-nitroimidazole-2-carboxaldehyde 15 was reacted with the acetoacetic esters **7**–**14** and ammonium hydroxide in 49-81 % yield [15–16] (Scheme 1).



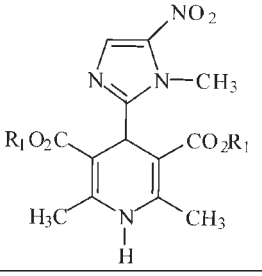
Scheme 1. Synthesis of symmetrical and asymmetrical analogues of nifedipine.

The unsymmetrical analogues **26**–**38** were synthesized by a modified Hantsch reaction using a procedure reported by Iwanami [17]. Thus, condensation of alkyl-3-aminocrotonates **16**–**17**, acetoacetic esters **7**–**14**, and 1-methyl-5-nitroimidazole-2-carboxaldehyde **15** afforded the required products in 48–76 % yield [18] (Scheme 1).

Results and discussion

The *in vitro* calcium channel antagonist activities (IC_{50}) of compounds **18**–**36** were determined as the molar concentration of the test compounds required to produce 50 % inhibition of the muscarinic receptor-mediated (carbacol, 1.67×10^{-7} M) Ca^{+2}-dependent contraction

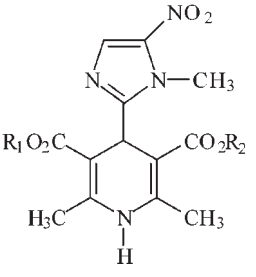
Table 1. Physical properties and calcium channel antagonist activity of symmetrical analogues **18**–**25**.



Compound	R_1	Mp (°C)	Yield (%)	Calcium channel antagonist activity IC_{50} ± SEM, (n)
18	Me	211–213	54	$9.03 \pm 0.34 \times 10^{-12}$ (6)*
19	Et	205–207	49	$1.55 \pm 0.24 \times 10^{-12}$ (4)*
20	nPro	221–223	59	$8.78 \pm 0.40 \times 10^{-11}$ (5)*
21	isoPro	229–231	81	$7.76 \pm 0.93 \times 10^{-11}$ (4)
22	nBut	196–198	53	$5.11 \pm 0.83 \times 10^{-11}$ (7)
23	isoBut	208–210	69	$4.38 \pm 1.01 \times 10^{-10}$ (4)
24	tBut	236–237	61	$8.68 \pm 0.89 \times 10^{-10}$ (4)
25	nPen	140–143	58	$9.53 \pm 0.67 \times 10^{-10}$ (5)
	Nifedipine			$1.09 \pm 0.12 \times 10^{-11}$ (3)*

* Single asterisk indicates $P < 0.05$ compared to nifedipine in that experiment using Student's t-test.

Table 2. Physical properties and calcium channel antagonist activity of asymmetrical analogues **26**–**38**.



Compound	R_1	R_2	Mp (°C)	Yield (%)	Calcium channel antagonist activity IC_{50} ± SEM, (n)
26	Me	Et	221–224	65	$7.39 \pm 0.43 \times 10^{-12}$ (5)*
27	nPro	Me	200–202	76	$4.78 \pm 1.02 \times 10^{-12}$ (4)
28	nPro	Et	215–217	64	$1.72 \pm 0.51 \times 10^{-12}$ (6)*
29	isoPro	Me	218–220	59	$9.49 \pm 1.19 \times 10^{-13}$ (4)*
30	isoPro	Et	241–243	72	$5.16 \pm 1.05 \times 10^{-12}$ (3)*
31	nBut	Me	171–173	57	$0.33 \pm 0.22 \times 10^{-12}$ (6)*
32	nBut	Et	187–189	52	$3.25 \pm 0.61 \times 10^{-12}$ (4)*
33	isoBut	Me	232–234	63	$0.91 \pm 0.85 \times 10^{-12}$ (3)*
34	isoBut	Et	241–243	56	$1.15 \pm 0.29 \times 10^{-11}$ (5)
35	tBut	Me	241–244	73	$1.77 \pm 0.71 \times 10^{-12}$ (3)*
36	tBut	Et	238–240	58	$4.91 \pm 1.17 \times 10^{-11}$ (4)
37	nPen	Me	180–183	48	$4.66 \pm 0.42 \times 10^{-13}$ (6)*
38	nPen	Et	161–163	53	$7.34 \pm 0.04 \times 10^{-12}$ (4)*
	Nifedipine				$1.09 \pm 0.12 \times 10^{-11}$ (3)*

* Single asterisk indicates $P < 0.05$ compared to nifedipine in that experiment using Student's t-test.

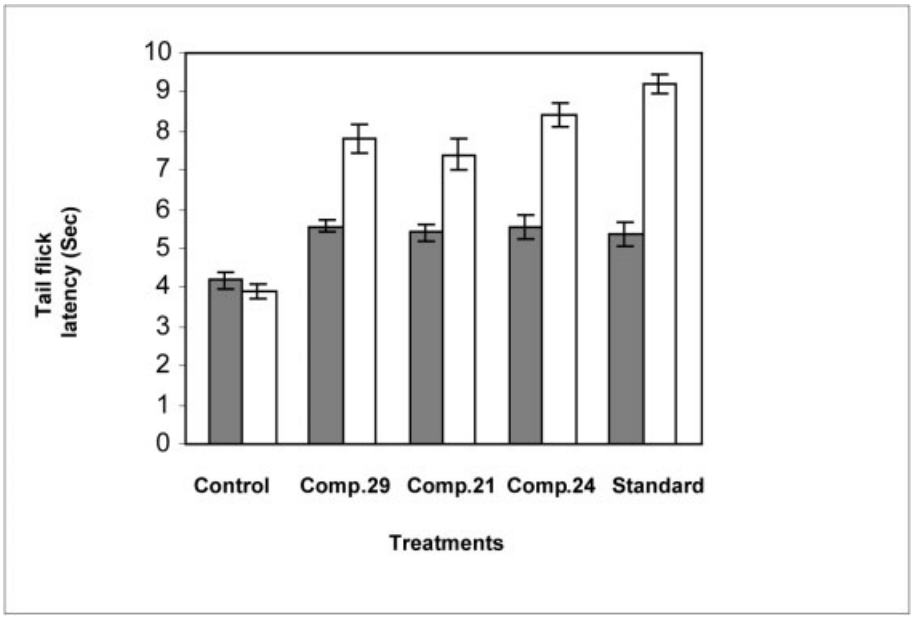


Figure 1. Comparing the effects of compounds **21**, **24**, and **29** on tail-flick latency time, Pre-drug, and post-drug latency time. n = 4, control was distilled water. Compound administrated 50 mg/kg (i.p.), standard was nifedipine.

(tonic response) of guinea pig ileal longitudinal smooth muscle (GPILSM), and those values are presented in Tables 1 and 2.

These results indicate that compounds **18**–**36** exhibit superior or equipotent calcium channel antagonist activity (10^{-11}–10^{-13} M) relative to the reference drug nifedipine ($IC_{50} = 1.09 \pm 0.12 \times 10^{-11}$ M).Comparison of the activities of symmetrical esters **18**–**25** on GPILSM indicates that the potency order was based on the substitutions ethyl>methyl>iso-butyl>iso-propyl>n-pentyl. A comparison of the activities of the asymmetrical series of esters **26**–**38** indicates compounds possessing methyl substituent (R_2 = Me), the R_1C-3 ester substituent was a determinant of calcium channel antagonist activity where the potency order was: n-pentyl>n-butyl>t-butyl>isopropyl. Also in compounds with ethyl substituent (R_2 = Et), the R_1C-3 ester substituent was a determinant and the potency order was: n-propyl>n-butyl>n-pentyl>t-butyl.

These results show that in symmetrical esters **18**–**25** an increase in the steric size of the R_1C-3 ester substituent, decreases antagonist action ethyl>n-propyl>n-butyl>n-pentyl, and in asymmetrical series of esters **26**–**38** that compounds possessing one small R_2 substituent (R_2 = Me or Et) an increase in the steric size or lipophilic properties of R_1C-3 ester substituent increases the activity. The comparison of the activities of the compounds **18**–**36** with the compounds reported by Shafiee et al. [15] reveals that the presence of an aryl or cycloalkyl group substituted on the C-3 or C-5 position of the 1,4-dihydropyridine ring increases the smooth muscle relaxant activity. Also, comparison of the results of this study with the report of Akula et al. [19] indicated that 1,4-dihydropyridine compounds with nitroimidazole substituted on C-4 position of the ring have more activity than similar compounds with a phenyl or pyridine substitute. In addition, antinociceptive activity of the compounds **18**, **21**, **24**, **26**, and **29** have been investigated by the tail-flick method. As shown in Figure1, compounds **21**, **24**, and **29** (at 50 mg/kg) had antinociceptive effects on the tail-flick test ($P < 0.05$), but the negative control (distilled water) did not have any significant effect on the nociception.

Acknowledgement

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Experimental

Chemistry

Melting points were determined on a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H-NMR spectra were run at a Varian Unity Plus 400 MHz spectrometer (Varian, Darmstadt, Germany). Chemical shifts are reported in parts per million (δ) relative to TMS as an internal standard. The mass spectra were measured with a Finnigan TSQ-70 spectrometer (Finnigan, Bremen, Germany) at 70 eV. The IR spectra were obtained by using a Nicolet 50X-FT spectrometer (KBr disks) (Nicolet, Madison, WI, USA). Microanalyses were within ± 0.40 % of theoretical values for C, H, and N. All spectra were consistent with the assigned structures. 2,2,6-trimethyl-4H-1,3-dioxine-4-one **6**, methyl (ethyl or t-butyl) acetoacetates **7**–**9**, and methyl (ethyl) 3-aminocrotontes **16**–**17** were purchased from the Aldrich Chemical Co. (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany).

General procedure for the synthesis of acetoacetic esters **10**–**14**

A solution of alcohol **1**–**5** (50 mmol) and 2,2,6-trimethyl-4H-1,3-dioxine-4-one **6** (7.1 g, 50 mmol) in 10 mL of 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 was cooled. The xylene was removed. Distillation of the mixture afforded **10**–**14** which were used immediately in subsequent reactions.

nPropyl acetoacetate (**10**)

^{1}H NMR ($CDCl_3$): δ 4.10 (t, J = 6.5 Hz, 2 H, CO_2CH_2), 3.45 (s, 2 H, $COCH_2CO_2$), 2.27 (s, 3 H, CH_3CO), 1.63 (m, 2 H, CH_2), 0.94 (t, J = 6.4 Hz , 3 H, CH_3).

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

isoPropyl acetoacetate (**11**)

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

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

nButyl acetoacetate (**12**)

^{1}H NMR ($CDCl_3$): δ 4.15 (t, J = 6.5 Hz, 2 H, CO_2CH_2), 3.44 (s, 2 H, $COCH_2CO_2$), 2.16 (s, 3 H, CH_3CO), 1.41 (m, 4 H, CH_2CH_2), 1.01 (t, J = 6.4 Hz, 3 H, CH_3).

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

isoButyl acetoacetate (**13**)

^{1}H NMR ($CDCl_3$): δ 4.48 (d, J = 6.6 Hz, 2 H, CO_2CH_2), 3.46 (s, 2 H, $COCH_2CO_2$), 2.26 (s, 3 H, CH_3CO), 2.46 (m, 1 H, CH), 1.48 (d, J = 6.5 Hz, 6 H, $CH(CH_3)_2$).

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

nPentyl acetoacetate (**14**)

^{1}H NMR ($CDCl_3$): δ 4.13 (t, J = 6.4 Hz, 2 H, CO_2CH_2), 3.43 (s, 2 H, $COCH_2CO_2$), 2.26 (s, 3 H, CH_3CO), 1.52 (m, 6 H, $CH_2CH_2CH_2$), 0.91 (t, J = 6.1 Hz, 3 H, CH_3).

IR (KBr): ν 1752 (C=O, ester), 1721 cm^{-1} (C=O, ketone).

General procedure for the synthesis of symmetrical esters of 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates **18**–**25**

A solution of ammonium hydroxide (25 %, 0.75 mL) was added to a stirring solution of 1-methyl-5-nitro-imidazole-2-carboxaldehyde **15** (0.78 g, 5 mmol) or the respective acetoacetic esters **7**–**14** (11 mmol) in absolute ethanol (5 mL). The mixture was heated under reflux overnight. The reaction mixture was cooled and poured into ice-water (10 mL). The sticky solid which precipitated was removed by filtration and recrystallized from ethanol.

3,5-Dimethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **18**

^{1}H NMR ($CDCl_3$): δ 8.92 (br s, 1 H, NH), 7.71 (s, 1 H, imidazole H-4), 5.21 (s, 1 H, C4-H), 4.61 (s, 6 H, CO_2CH_3), 4.18 (s, 3 H, N-CH_3), 2.31 (s, 6 H, C_2-CH_3 & C_6-CH_3).

IR (KBr): ν 3374 (NH), 1747 (C=O), 1543 and 1333 cm^{-1} (NO_2).

3,5-Diethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **19**

^{1}H NMR ($CDCl_3$): δ 9.01 (br s, 1 H, NH), 7.78 (s, 1 H, imidazole H-4), 5.09 (s, 1 H, C_4-H), 4.87 (q, J = 7.1 Hz, 4 H, CO_2CH_2), 4.09 (s, 3 H, N-CH_3), 2.97 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.67 (t, J = 7.1 Hz, 6 H, CH_3).

IR (KBr): ν 3426 (NH), 1698 (C=O), 1522 and 1385 cm^{-1} (NO_2).

3,5-Di-npropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **20**

^{1}H NMR ($CDCl_3$): δ 9.16 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.17 (s, 1 H, C_4-H), 4.24 (s, 3 H, N-CH_3), 4.04 (t, J = 6.8 Hz, 4 H, CO_2CH_2), 2.21 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.61 (m, 4 H, CH_2), 0.88 (t, J = 7.1 Hz, 6 H, CH_3).

IR (KBr): ν 3426 (NH), 1698 (C=O), 1522 and 1385 cm^{-1} (NO_2).

3,5-Di-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **21**

^{1}H NMR ($CDCl_3$): δ 8.95 (br s, 1 H, NH), 7.96 (s, 1 H, imidazole H-4), 5.12 (s, 1 H, C_4-H), 5.03 (m, 2 H, $CH(CH_3)_2$), 4.26 (s, 3 H, N-CH_3), 2.23 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.24 and 1.19 (dd, J = 6.4 Hz, 6 H each, $CH(CH_3)_2$).

IR (KBr): ν 3426 (NH), 1698 (C=O), 1522 and 1385 cm^{-1} (NO_2).

3,5-Di-nbutyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **22**

^{1}H NMR ($CDCl_3$): δ 8.81 (br s, 1 H, NH), 7.94 (s, 1 H, imidazole H-4), 5.15 (s, 1 H, C_4-H), 4.28 (s, 3 H, N-CH_3), 4.08 (t, J = 6.9 Hz, 4 H, CO_2CH_2), 2.19 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.61 & 1.26 (two m, each 4 H, CH_2CH_2), 0.89 (t, J = 6.8 Hz, 6 H, CH_3).

IR (KBr): ν 3387 (NH), 1716 (C=O), 1519 and 1363 cm^{-1} (NO_2).

3,5-Di-isobutyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **23**

^{1}H NMR ($CDCl_3$): δ 9.32 (br s, 1 H, NH), 7.81 (s, 1 H, imidazole H-4), 5.17 (s, 1 H, C_4-H), 4.24 (s, 3 H, N-CH_3), 3.89 (d, J = 6 Hz, 4 H, CO_2CH_2), 2.16 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.19 (m, 2 H, $CH(CH_3)_2$), 1.23 and 1.19 (d, J = 6.8 Hz, 12 H, $CH(CH_3)_2$).

IR (KBr): ν 3416 (NH), 1718 (C=O), 1522 and 1371 cm^{-1} (NO_2).

3,5-Di-tbutyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **24**

^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1 H, imidazole H-4), 7.81 (br s, 1 H, NH), 5.07 (s, 1 H, C_4-H), 5.03 (m, 2 H, $CH(CH_3)_2$), 4.24 (s, 3 H, N-CH_3), 2.20 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.43 (m, 18 H each, $C(CH_3)_3$).

IR (KBr): ν 3407 (NH), 1709 (C=O), 1521 and 1378 cm^{-1} (NO_2).

3,5-Di-npentyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **25**

^{1}H NMR ($CDCl_3$): δ 9.26 (br s, 1 H, NH), 7.96 (s, 1 H, imidazole H-4), 5.16 (s, 1 H, C_4-H), 4.25 (s, 3 H, N-CH_3), 4.12 (t, J = 6 Hz, 4 H, CO_2CH_2), 2.26 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.29 (m, 12 H, $CH_2CH_2CH_2$), 0.91 (t, J = 6.1 Hz, 6 H, CH_3).

IR (KBr): ν 3411 (NH), 1706 (C=O), 1516 and 1362 cm^{-1} (NO_2).

General procedure for the synthesis of unsymmetrical esters of 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates **26**–**38**

A mixture of the respective acetoacetic esters **7**–**14** (5.0 mmol), 1-methyl-5-nitro-imidazole-2-carboxaldehyde **15** (0.78 g,

5 mmol), and the respective alkyl 3-aminocrotonates (5.0 mmol) **16**–**18** in absolute ethanol (25 mL) was refluxed for 16 h with stirring. After cooling, the precipitated product was filtered off, washed with cold ethanol, and then dried in vacuo. Recrystallization from methanol gave **26**–**36** (27–53 %) as yellow or white crystals.3-Ethyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **26**

^{1}H NMR ($CDCl_3$): δ 8.94 (br s, 1 H, NH), 7.81 (s, 1 H, imidazole H-4), 5.15 (s, 1 H, C_4-H), 4.27 (s, 3 H, N-CH_3), 4.26 (t, J = 6.8 Hz, 2 H, CO_2CH_2), 3.77 (s, 3 H, CO_2CH_3), 2.22 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.67 (t, J = 6.8 Hz, 3 H, CH_3).

IR (KBr): ν 3462 (NH), 1698 (C=O), 1597,1365 cm^{-1} (NO_2).

3-nPropyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **27**

^{1}H NMR ($CDCl_3$): δ 9.23 (br s, 1 H, NH), 7.94 (s, 1 H, imidazole H-4), 5.17 (s, 1 H, C_4-H), 4.22 (s, 3 H, N-CH_3), 4.05 (t, J = 6.7 Hz, 2 H, CO_2CH_2), 3.71 (s, 3 H, CO_2CH_3), 2.19 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.62 (m, 2 H, CH_2), 0.87 (t, J = 6.7 Hz, 3 H, CH_3).

IR (KBr): ν 3341 (NH), 1718 (C=O), 1504,1368 cm^{-1} (NO_2).

3-nPropyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **28**

^{1}H NMR ($CDCl_3$): δ 8.57 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.15 (s, 1 H, C_4-H), 4.22 (s, 3 H, N-CH_3), 4.14 (q, J = 7.2 Hz, 2 H, CO_2CH_2), 4.04 (t, J = 6.2 Hz, 2 H, CO_2CH_2), 2.24 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.62 (m, 2 H, CH_2), 1.24 (t, J = 7.2 Hz, 3 H, CO_2CH_3), 0.89 (t, J = 6.2 Hz, 3 H, CH_3).

IR (KBr): ν 3421 (NH), 1723 (C=O), 1528, 1365 cm^{-1} (NO_2).

3-isoPropyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **29**

^{1}H NMR ($CDCl_3$): δ 8.63 (br s, 1 H, NH), 7.96 (s, 1 H, imidazole H-4), 5.14 (s, 1 H, C_4-H), 5.02 (m, 1 H, $CO_2CH(CH_3)_2$), 4.23 (s, 3 H, N-CH_3), 3.68 (s, 3 H, CO_2CH_3), 2.22 (two s, 3 H each, C_2-CH_3 & C_6-CH_3), 1.23 and 1.16 (two d, J = 6.0 Hz, 3 H each, $CH(CH_3)_2$).

IR (KBr): ν 3383 (NH), 1737 (C=O), 1517, 1362 cm^{-1} (NO_2).

3-isoPropyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **30**

^{1}H NMR ($CDCl_3$): δ 8.25 (br s, 1 H, NH), 7.97 (s, 1 H, imidazole H-4), 5.13 (s, 1 H, C_4-H), 5.06 (m, 1 H, $CH(CH_3)_2$), 4.23 (s, 3 H, N-CH_3), 4.17 (q, J = 7.2 Hz, 2 H, CO_2CH_2), 2.19 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.58 (t, J = 7.2 Hz, 3 H, CH_3) 1.21 and 1.19 (two d, J = 6.0 Hz, 3 H each, $CH(CH_3)_2$).

IR (KBr): ν 3411 (NH), 1728 (C=O), 1542,1364 cm^{-1} (NO_2).

3-nButyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **31**

^{1}H NMR ($CDCl_3$): δ 9.01 (br s, 1 H, NH), 7.99 (s, 1 H, imidazole H-4), 5.08 (s, 1 H, C_4-H), 4.19 (s, 3 H, N-CH_3), 4.02 (t, J = 6.6 Hz, 2 H, CO_2CH_2), 3.76 (s, 3 H, CO_2CH_3), 2.21 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.51 (m, 4 H, CH_2CH_2), 0.91 (t, J = 6.7 Hz, 3 H, CH_3).

IR (KBr): ν 3317 (NH), 1728 (C=O), 1542, 1391 cm^{-1} (NO_2).

3-nButyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **32**

^{1}H NMR ($CDCl_3$): δ 8.67 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.17 (s, 1 H, C_4-H), 4.23 (s, 3 H, N-CH_3), 4.11 (m, 4 H, CO_2CH_2), 2.23 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.34 (m, 4 H, CH_2CH_2), 1.24 (t, J = 7.1 Hz, 3 H, CH_3), 0.90 (t, J = 7.1 Hz, 3 H, CH_3).

IR (KBr): ν 3418 (NH), 1728 (C=O), 1548,1369 cm^{-1} (NO_2).

3-isoButyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **33**

^{1}H NMR ($CDCl_3$): δ 9.02 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.17 (s, 1 H, C_4-H), 4.23 (s, 3 H, N-CH_3), 3.88 (t, J = 6.8 Hz, 2 H, CO_2CH_2), 3.76 (s, 3 H, CO_2CH_3), 2.23 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.90 (m, 1 H, $CH(CH_3)_2$), 0.87 (d, J = 6.6 Hz, 6 H, $CH(CH_3)_2$).

IR (KBr): ν 3401 (NH), 1715 (C=O), 1561, 1368 cm^{-1} (NO_2).

3-isoButyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **34**

^{1}H NMR ($CDCl_3$): δ 8.78 (br s, 1 H, NH), 7.94 (s, 1 H, imidazole H-4), 5.16 (s, 1 H, C_4-H), 4.23 (s, 3 H, N-CH_3), 4.14 (q, J = 6.8 Hz, 2 H, CO_2CH_2), 3.89 (d, J = 6.6 Hz, 2 H, CO_2CH_2), 2.23 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.90 (m, 1 H, $CH(CH_3)_2$), 1.23 (t, J = 6.8 Hz, 3 H, CH_3), 0.87 (d, J = 6.4 Hz, 6 H, $CH(CH_3)_2$).

IR (KBr): ν 3411 (NH), 1731 (C=O), 1547, 1381 cm^{-1} (NO_2).

3-tButyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **35**

^{1}H NMR ($CDCl_3$): δ 8.21 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.19 (s, 1 H, C_4-H), 4.23 (s, 3 H, N-CH_3), 3.68 (s, 3 H, CO_2CH_3), 2.46 & 2.19 (two s, 3 H each, C_2-CH_3 & C_6-CH_3), 1.42 (s, 9 H, $C(CH_3)_3$).

IR (KBr): ν 3414 (NH), 1728 (C=O), 1549, 1367 cm^{-1} (NO_2).

3-tButyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **36**

^{1}H NMR ($CDCl_3$): δ 8.31 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.12 (s, 1 H, C_4-H), 4.24 (s, 3 H, N-CH_3), 4.13 (q, J = 5.6 Hz, 2 H, CO_2CH_2), 2.25 & 2.19 (two s, 3 H each, C_2-CH_3 & C_6-CH_3), 1.98 (s, 9 H, $C(CH_3)_3$), 1.23 (t, J = 5.6 Hz, 3 H, CH_3).

IR (KBr): ν 3371 (NH), 1738 (C=O), 1561,1388 cm^{-1} (NO_2).

3-nPentyl, 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **37**

^{1}H NMR ($CDCl_3$): δ 8.87 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.15 (s, 1 H, C_4-H), 4.21 (s, 3 H, N-CH_3), 4.07 (t, J = 6.0 Hz, 2 H, CO_2CH_2), 3.76 (s, 3 H, CO_2CH_3), 2.22 & 2.17 (two s, 3 H each, C_2-CH_3 & C_6-CH_3), 1.29 (m, 6 H, $CH_2CH_2CH_2$), 0.89 (t, J = 7.2 Hz, 3 H, CH_3).

IR (KBr): ν 3361 (NH), 1717 (C=O), 1549, 1367 cm^{-1} (NO_2).

3-nPentyl, 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate **38**

^{1}H NMR ($CDCl_3$): δ 9.08 (br s, 1 H, NH), 7.95 (s, 1 H, imidazole H-4), 5.16 (s, 1 H, C_4-H), 4.23 (s, 3 H, N-CH_3), 4.12 (m, 2 H, CO_2CH_2), 2.21 (s, 6 H, C_2-CH_3 & C_6-CH_3), 1.59 (t, J = 6.8 Hz, 3 H, CH_3). 1.27 (m, 6 H, $CH_2CH_2CH_2$), 0.86 (t, J = 6.7 Hz, 3 H, CH_3).

IR (KBr): ν 3421 (NH), 1731 (C=O), 1518, 1351 cm^{-1} (NO_2).

Pharmacology

Investigations on isolated ileum of guinea pigs

Male albino guinea pig (300–450 g) was killed by a blow to the head. The intestine was removed above the ileocecal junction.

Smooth muscle segments of about 1 cm length were mounted under a resting tension of 500 mg and were maintained at 37 °C in a 20 mL jacketed organ bath containing oxygenated (95 % O_2, 5 % CO_2) physiologic saline solution of the following millimolar compositions: 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 forced displacement transducer (FTO3C) on a GRASS physiograph (GRASS Instruments, Quincy, MA, USA). All compounds were dissolved in DMSO and the same volume of solvent was used as the control. The contractile response was taken as the 100 % value for the tonic (slow) component of the response. Test compounds were added in accumulative increment after the dose response for carbacol (1.67×10^{-7} M). Test compound-induced relaxation of contracted muscle was expressed as the percent of the control [20, 21]. The IC_{50} values were graphically determined from the contraction-response curve.

Tail-flick test

All experiments were preformed on mice (albino males, in-house bred, 25–35 g). 12 animals were kept in one cage on straw bedding in an animal holding room at 24 ± 2 °C with a 12 h light-dark cycle. Balanced diet pellets and tap water were continuously available. Changes in pain perception due to drug treatments were determined in a quiet laboratory with ambient illumination and temperature close to those of the holding room. Mice were allowed to acclimate to the testing area for 1 h before the experiments began. Nociceptive response was assessed with a tail flick apparatus (HSE, March-Hugstetten, Germany) using a method initially described by D'Amour and Smith [22]. Animal responded to a focused heat-stimulus by flicking or removing their inflicted tail. The base-line latency time (reaction time, in seconds) was obtained with three measurements. The mean of these measurements was considered as the pre-drug latency time. The drug was administered intraperitoneal (50 mg/kg) immediately after the third pre-drug measure (n = 4). Another set of three measurements was taken 25 min afterwards for intraperitoneal (i.p.) administrations, and their mean was considered as post-drug latency time. A cut-off time of 10 s was used to prevent tissue damage [23, 24].

Statistical analysis

The results are presented as mean ± S.E.M. and evaluated statistically using Student's t-test. P values less than 0.05 were considered significant.

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