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Synthesis and calcium channel antagonist activities of 3-nitrooxyalkyl, 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates

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Abstract

A group of racemic 3-[(2-nitrooxyethyl), (3-nitrooxypropyl), (4-nitrooxybutyl) or (1,3-dinitrooxy-2-propyl)], 5-methyl (ethyl or propyl) 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates (**18**–**29**) were synthesized using modified Hantzsch reaction that involved the condensation of 2-nitrooxyethyl (**8**), 3-nitrooxypropyl (**9**), 4-nitrooxybutyl (**10**) or 1,3-dinitrooxy-2-propyl (**13**) acetoacetate with methyl (**14**), ethyl (**15**) or isopropyl (**16**) 3-aminocrotonate and 1-methyl-5-nitroimidazole-2-carboxaldehyde (**17**). In vitro calcium channel antagonist activities were determined using a guinea pig ileum longitudinal smooth muscle assay. Compounds **18**–**29** exhibited superior, or equipotent, calcium antagonist activity ($IC_{50} = 10^{-11}$–10^{-13} M range) relative to the reference drug nifedipine ($IC_{50} = 1.07 \pm 0.12 \times 10^{-11}$ M), which could serve as potential probes to investigate the in vivo release of nitric oxide which induces vascular muscle relaxation. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Dihydropyridine; Organic nitrate; Calcium channels; Nitrooxyalkyl; Nitroimidazole

1. 1.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 [1–4]. The discovery that the 1,4-dihydropyridine (Nifedipine, Isradipine) 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 [5–7] (Fig. 1). Changes in substitution pattern at the C-3, C-4 and C-5 positions of nifedipine alter activity and tissue selectivity [8,9].

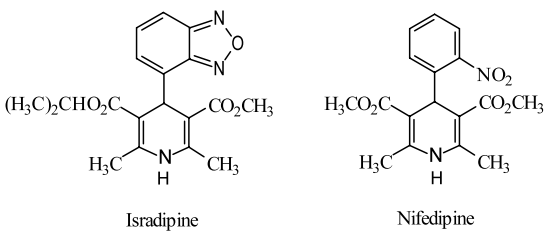

Fig. 1. Some dihydropyridine calcium channel antagonist.

On the other hand, organic nitrate compounds such as Nitroglycerin, Isosorbide dinitrate and Nicorandil activate guanylate cyclase to increase the level of cyclic guanosine 5-monophosphate (cGMP) in various vascular smooth muscle tissues and promote relaxation [10,11] (Fig. 2).

Simultaneous uses of calcium antagonist and nitrate compounds enhance the antihypertensive action with little side effects [12,13]. So the combination of

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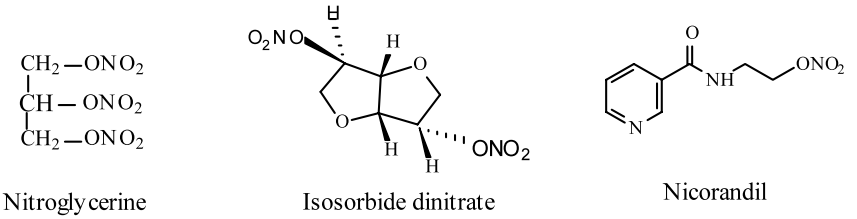
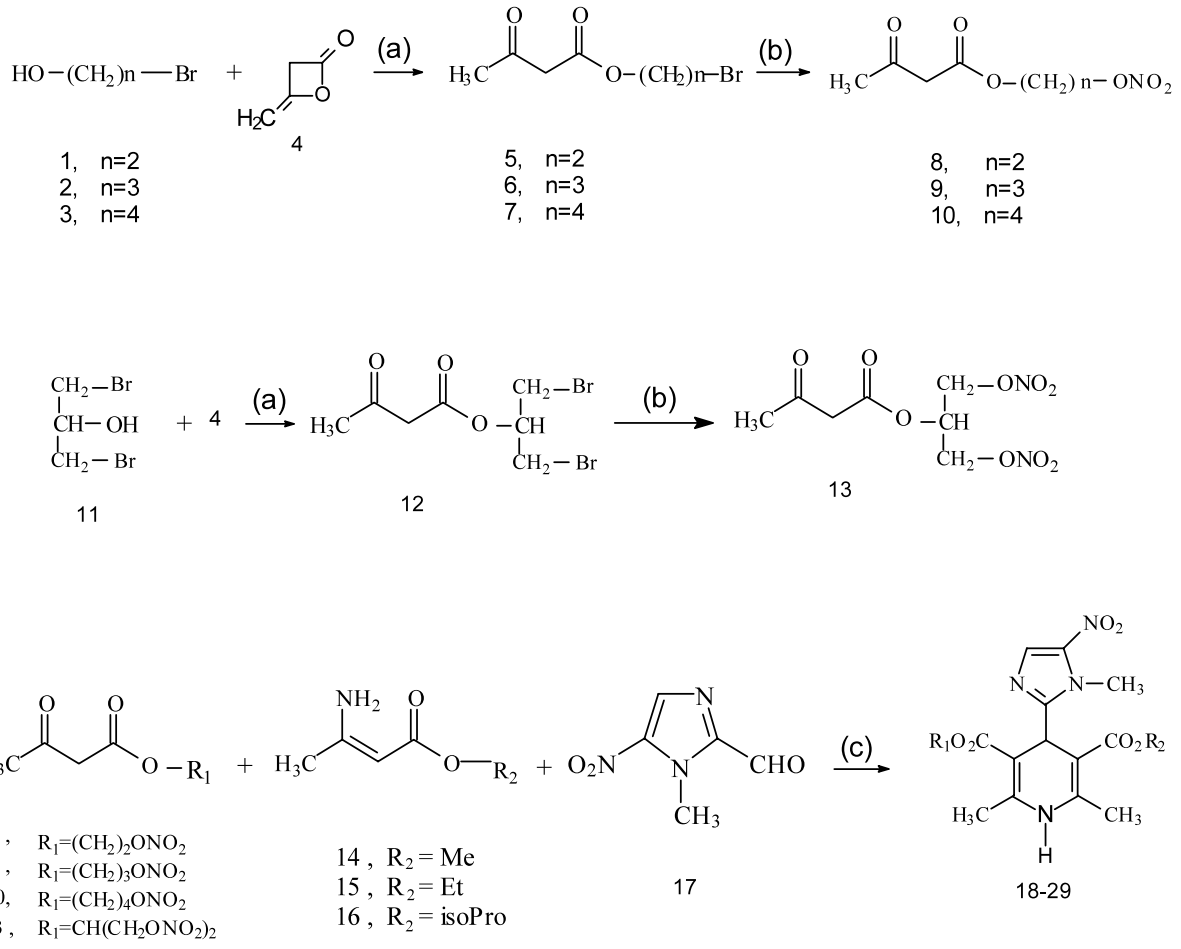


Fig. 2.



Scheme 1. Reagents and conditions: (a) Et_3N catalyst, 80 °C, 1 h; (b) $AgNO_3$, MeCN, 25 °C, 48 h; (c) EtOH, reflux, 12 h.

nitrate-like and calcium blocking action in a single molecule was expected to have a potential vasodilating activity superior to that of known 1,4-dihydropyridines [14]. Previously, we reported that 1-methyl-5-nitroimidazole was bioisoster of nitrophenyl in Nifedipine analogues [15].

It was of interest to determine the effects of C-3 different nitrooxyalkyl substituents, in conjugation with C-4 1-methyl-5-nitro-2-imidazolyl substituents, on calcium channel antagonist activity. We now report the synthesis and calcium channel antagonist activities of dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate containing nitrooxy moiety in the 3-alkyl ester substituent.

2. Chemistry

Nitrooxy alkyl acetoacetate **8**–**10** were synthesized by the reaction of diketene **4** with bromoalcohols **1**–**3** to afford **5**–**7** which were then converted to the title compounds upon reaction with silver nitrate in 40–51% overall yield (Scheme 1). Also reaction of **4** with 1,3-dibromo-2-propanol **11** yielded 1,3-dibromo-2-propyl acetoacetate **12** which was then converted to **13** upon reaction with $AgNO_3$ in 42% yield [16,17].

The unsymmetrical analogues **18**–**29** were synthesized by a modified Hantsch reaction using a procedure reported by Iwanami. Thus condensation of alkyl-3-aminocrotonate **14**–**16**, acetoacetic ester **9**–**10**, **13** and

1-methyl-5-nitroimidazole-2-carboxaldehyde **17** afforded the required products in 27–53% yield [18–21].

3. Experimental

3.1. Chemistry

Melting points were determined on a kofler hot stage apparatus and are uncorrected.^{1}H NMR spectra were run at a Varian Unity Plus 400 MHz spectrometer. 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 at 70 eV. The IR spectra were obtained by using a Nicolet 50X-FT spectrometer (KBr disks). All spectra were consistent with the assigned structures. Elemental analyses (C, H, N) were within acceptable limits of $\pm$0.4% of theory. Diketene **4** and methyl (ethyl or isopropyl) 3-aminocrotonate **14**–**16** were purchased from the Aldrich Chemical Co.

3.1.1. General procedure for the synthesis of bromoalkylacetoacetate derivatives *5*–*7*, *12*

Diketene (4.2 g, 50 mmol) was added dropwise with stirring to the respective bromoalcohol **1**–**3**, **11** (50 mmol) preheated to 50–60 °C in the presence of a catalytic amount of Et_3N (0.3 ml, 5.5 mmol). Diketene was added at a rate such that the temperature of the reaction mixture did not exceed 80 °C, and then the reaction was allowed to proceed for 1 h at 80 °C. Distillation of the mixture afforded **5**–**7**, **12** that were used immediately in subsequent reaction.

3.1.1.1. 2-Bromoethyl acetoacetate (5). ^{1}H NMR ($CDCl_3$): δ 4.32 (t, $J = 6.1$ Hz, 2H, CO_2CH_2), 3.43 (t, $J = 6.1$ Hz, 2H, CH_2Br), 3.39 (s, 2H, $COCH_2CO_2$), 2.16 (s, 2H,CH_3CO).

IR (KBr): ν 1742 (C=O, ester), 1712 cm^{-1} (C=O, ketone).

3.1.1.2. 3-Bromopropyl acetoacetate (6). ^{1}H NMR ($CDCl_3$): δ 4.49 (t, $J = 6.3$ Hz, 2H, CO_2CH_2), 3.45 (t, $J = 6.5$ Hz, 2H, CH_2Br), 3.41 (s, 2H, $COCH_2CO_2$), 2.19 (s, 2H,CH_3CO), 2.05 (m, 2H, CH_2).

IR (KBr): ν 1755 (C=O, ester), 1719 cm^{-1} (C=O, ketone).

3.1.1.3. 4-Bromobutyl acetoacetate (7). ^{1}H NMR ($CDCl_3$): δ 4.19 (t, $J = 5.9$ Hz, 2H, CO_2CH_2), 3.51 (t, $J = 6.1$ Hz, 2H, CH_2Br), 3.41 (s, 2H, $COCH_2CO_2$), 2.20 (s, 2H, CH_3CO), 1.77 (m, 4H, CH_2–CH_2).

IR (KBr): ν 1744 (C=O, ester), 1712 cm^{-1} (C=O, ketone).

3.1.1.4. 1.3-Dibromo-2-propyl acetoacetate (12). ^{1}H NMR ($CDCl_3$): δ 5.15 (q, $J = 5.2$ Hz, 1H, CO_2CH), 3.58 (d, $J = 5.2$ Hz, 4H, CH_2Br), 3.49 (s, 2H, $COCH_2CO_2$), 2.25 (s, 2H,CH_3CO).

IR (KBr): ν 1754 (C=O, ester), 1718 cm^{-1} (C=O, ketone).

3.1.2. General procedure for the synthesis of nitrooxyalkylacetoacetate derivatives *8*–*10*, *13*

Silver nitrate [10.2 g, 60 mmol for **5**–**7** and 20.4 g, 120 mmol for **12**] was added to solution of **5**–**7**, **12** (50 mmol) in acetonitrile (50 ml) and the reaction was allowed to proceed at 25 °C for 48 h with stirring. Removal of precipitate by filtration, washing the precipitate with acetonitrile and removal of solvent in vacuo from the combined filtrate gave a residue which was purified by silica gel column chromatography using EtOAc–hexane (30:70, v/v) as eluent to afford **8**–**10**, **13** as oil.

3.1.2.1. 2-Nitrooxyethyl acetoacetate (8). ^{1}H NMR ($CDCl_3$): δ 4.69 (m, 2H, CO_2CH_2), 4.44 (m, 2H, $CH_aCH_bONO_2$), 3.53 (s, 2H, $COCH_2CO_2$), 2.30 (s, 2H, CH_3CO).

IR (KBr): ν 1746 (C=O, ester), 1724 (C=O, ketone), 1636 cm^{-1} (NO, nitroxy).

3.1.2.2. 3-Nitrooxypropyl acetoacetate (9). ^{1}H NMR ($CDCl_3$): δ 4.59 (t, $J = 5.0$ Hz, 2H, CO_2CH_2), 4.28 (m, 2H, $CH_aCH_bONO_2$), 3.50 (s, 2H, $COCH_2CO_2$), 2.28 (s, 2H, CH_3CO), 2.04 (m, 2H, CH_2).

IR (KBr): ν 1755 (C=O, ester), 1719 (C=O, ketone), 1631 cm^{-1} (NO, nitroxy).

3.1.2.3. 4-Nitrooxybutyl acetoacetate (10). ^{1}H NMR ($CDCl_3$): δ 4.43 (t, $J = 5 = 6.0$ Hz, 2H, CO_2CH_2), 4.14 (m, 2H, $CH_aCH_bONO_2$), 3.43 (s, 2H, $COCH_2CO_2$), 2.21 (s, 2H,CH_3CO), 1.74 (m, 4H, CH_2–CH_2).

IR (KBr): ν 1752 (C=O, ester), 1721 (C=O, ketone), 1628 cm^{-1} (NO, nitroxy).

3.1.2.4. 1.3-Dinitrooxy-2-propyl acetoacetate (13). ^{1}H NMR ($CDCl_3$): δ 5.44 (m, 1H, CO_2CH), 4.74 (dd, $J_{gem} = 12$ Hz, $J_{vic} = 6$Hz, 4H, $CH_aCH_bONO_2$), 4.60 (dd, $J_{gem} = 12$ Hz, $J_{vic} = 6$ Hz, 4H, $CH_aCH_bONO_2$), 3.54 (s, 2H, $COCH_2CO_2$), 2.26 (s, 2H,CH_3CO).

IR (KBr): ν 1755 (C=O, ester), 1720 (C=O, ketone), 1634 cm^{-1} (NO, nitroxy).

3.1.3. General procedure for the synthesis 3-nitrooxyalkyl, 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate derivatives *18*–*29*

A mixture of the respective acetoacetic ester **8**–**10**, **13** (5.0 mmol), 1-methyl-5-nitro-imidazole-2-carboxaldehyde **17** (0.78 g, 5 mmol) and the respective alkyl

3-aminocrotonate (5.0 mmol) **14**–**16** in absolute ethanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold ethanol, and then dried in vacuo. Recrystallization form methanol gave **18**–**29** (27–53%) as yellow or white crystals.

*3.1.3.1. 3-(2-Nitrooxyethyl), 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**18**).* ^{1}H NMR ($CDCl_3$): δ 8.74 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4–H), 4.61 (t, $J = 4.8$ Hz, 2H, CO_2CH_2), 4.31 (m, 2H, $CH_aCH_bONO_2$), 4.21 (s, 3H, N–CH_3), 3.67 (s, 3H, CO_2CH_3), 2.22 (s, 6H, C_2–CH_3 and C_6–CH_3).

IR (KBr): ν 3282 (NH), 1698 (C=O), 1633, 1285 cm^{-1} (ONO_2).

*3.1.3.2. 3-(2-Nitrooxyethyl), 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**19**).* ^{1}H NMR ($CDCl_3$): δ 8.24 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.14 (s, 1H, C_4–H), 4.61 (t, $J = 4.8$ Hz, 2H, CO_2CH_2), 4.43 (m, 2H, $CH_aCH_bONO_2$), 4.21 (s, 3H, N–CH_3), 4.10 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 2.26 (s, 6H, C_2–CH_3 and C_6–CH_3), 1.24 (t, $J = 7.2$ Hz, 3H, CH_3).

IR (KBr): ν 3248 (NH), 1704 (C=O), 1636, 1279 cm^{-1} (ONO_2).

*3.1.3.3. 3-(2-Nitrooxyethyl), 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**20**).* ^{1}H NMR ($CDCl_3$): δ 8.97 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.11 (s, 1H, C_4–H),4.93 (m, 1H, CO_2CH), 4.58 (t, $J = 5.4$ Hz, 2H, CO_2CH_2), 4.25 (m, 2H, $CH_aCH_bONO_2$), 4.22 (s, 3H, N–CH_3), 2.24 (s, 6H, C_2–CH_3 and C_6–CH_3), 1.25 and 1.17 (two d, $J = 4.2$ Hz, 3H each, $CH(CH_3)_2$).

IR (KBr): ν 3282 (NH), 1709 (C=O), 1643, 1272 cm^{-1} (ONO_2).

*3.1.3.4. 3-(3-Nitrooxypropyl), 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**21**).* ^{1}H NMR ($CDCl_3$): δ 8.82 (br s, 1H, NH), 7.91 (s, 1H, imidazole H-4), 5.16 (s, 1H, C_4–H), 4.46 (t, $J = 6.1$ Hz, 2H, CO_2CH_2), 4.26 (m, 2H, $CH_aCH_bONO_2$), 4.22 (s, 3H, N–CH_3), 3.67 (s, 3H, CO_2CH_3), 2.22 (s, 6H, C_2–CH_3 and C_6–CH_3), 2.06 (m, 2H, CH_2).

IR (KBr): ν 3328 (NH), 1706 (C=O), 1641, 1279 cm^{-1} (ONO_2).

*3.1.3.5. 3-(3-Nitrooxypropyl), 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**22**).* ^{1}H NMR ($CDCl_3$): δ 8.48 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.14 (s, 1H, C_4–H), 4.46 (t, $J = 5.8$ Hz, 2H, CO_2CH_2), 4.22 (s, 3H, N–CH_3), 4.10 (m, 4H, $CH_aCH_bONO_2$ and CO_2CH_2), 2.26 (s, 6H, C_2–CH_3 and C_6–CH_3), 2.06 (m, 2H, CH_2), 1.24 (t, $J = 7.1$ Hz, 3H, CH_3).

IR (KBr): ν 3313 (NH), 1714 (C=O), 1629, 1281 cm^{-1} (ONO_2).

*3.1.3.6. 3-(3-Nitrooxypropyl), 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**23**).* ^{1}H NMR ($CDCl_3$): δ 8.37 (br s, 1H, NH), 7.92 (s, 1H, imidazole H-4), 5.12 (s, 1H, C_4–H), 4.95 (m, 1H, CO_2CH), 4.49 (t, $J = 6.1$ Hz, 2H, CO_2CH_2), 4.25 (m, 2H, $CH_aCH_bONO_2$), 4.23 (s, 3H, N–CH_3), 2.24 (s, 6H, C_2–CH_3 and C_6–CH_3), 2.06 (m, 2H, CH_2), 1.27 and 1.16 (two d, $J = 4.6$ Hz, 3H each, $CH(CH_3)_2$).

IR (KBr): ν 3279 (NH), 1711 (C=O), 1651, 1291 cm^{-1} (ONO_2).

*3.1.3.7. 3-(4-Nitrooxybutyl), 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**24**).* ^{1}H NMR ($CDCl_3$): δ 8.74 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4–H), 4.44 (t, $J = 5.3$ Hz, 2H, CO_2CH_2), 4.22 (s, 3H, N–CH_3), 4.19 (m, 2H, $CH_aCH_bONO_2$), 3.68 (s, 3H, CO_2CH_3), 2.22 (s, 6H, C_2–CH_3 and C_6–CH_3), 2.03 (m, 4H, CH_2–CH_2).

IR (KBr): ν 3331 (NH), 1711 (C=O), 1645, 1281 cm^{-1} (ONO_2).

*3.1.3.8. 3-(4-Nitrooxybutyl), 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**25**).* ^{1}H NMR ($CDCl_3$): δ 8.49 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.14 (s, 1H, C_4–H), 4.44 (t, $J = 5.8$ Hz, 2H, CO_2CH_2), 4.23 (s, 3H, N–CH_3), 4.10 (m, 4H, $CH_aCH_bONO_2$ and CO_2CH_2), 2.25 (s, 6H, C_2–CH_3 and C_6–CH_3), 2.06 (m, 4H, CH_2–CH_2), 1.24 (t, $J = 7.2$ Hz, 3H, CH_3).

IR (KBr): ν 3332 (NH), 1704 (C=O), 1614, 1279 cm^{-1} (ONO_2).

*3.1.3.9. 3-(4-Nitrooxybutyl), 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**26**).* ^{1}H NMR ($CDCl_3$): δ 8.31 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.12 (s, 1H, C_4–H), 4.92 (m, 1H, CO_2CH), 4.44 (t, $J = 5.3$ Hz, 2H, CO_2CH_2), 4.24 (s, 3H, N–CH_3), 4.16 (m, 2H, $CH_aCH_bONO_2$), 2.21 (s, 6H, C_2–CH_3 and C_6–CH_3), 1.74 (m, 4H, CH_2–CH_2), 1.25 and 1.17 (two d, $J = 4.6$ Hz, 3H each, $CH(CH_3)_2$).

IR (KBr): ν 3316 (NH), 1709 (C=O), 1655, 1224 cm^{-1} (ONO_2).

*3.1.3.10. 3-(1,3-Dinitrooxy-2-propyl), 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 9.03 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 4.41 (m, 1H, CO_2CH), 5.15 (s, 1H, C_4–H), 4.61 (m, 4H,

$CH_aCH_bONO_2$), 4.23 (s, 3H, N–CH_3), 3.69 (s, 3H, CO_2CH_3), 2.24 and 2.21 (two s, 3H each, C_2–CH_3 and C_6–CH_3).

IR (KBr): *v* 3328 (NH), 1721 (C=O), 1637, 1278 cm^{-1} (ONO_2).

*3.1.3.11. 3-(1,3-Dinitrooxy-2-propyl), 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**28**).* ^1H NMR ($CDCl_3$): δ 9.07 (br s, 1H, NH), 7.97 (s, 1H, imidazole H-4), 4.41 (m, 1H, CO_2CH), 5.12 (s, 1H, C_4–H), 4.65 (m, 4H, $CH_aCH_bONO_2$), 4.23 (s, 3H, N–CH_3), 4.11 (q, $J = 7.2$ Hz, 2H, CO_2CH_2), 2.23 and 2.20 (two s, 3H each, C_2–CH_3 and C_6–CH_3), 1.24 (t, $J = 7.2$ Hz, 3H, CH_3).

IR (KBr): *v* 3332 (NH), 1714 (C=O), 1614, 1281 cm^{-1} (ONO_2).

*3.1.3.12. 3-(1,3-Dinitrooxy-2-propyl), 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (**29**).* ^1H NMR ($CDCl_3$): δ 8.72 (br s, 1H, NH), 7.93 (s, 1H, imidazole H-4), 5.44 (m, 1H, CO_2CH), 5.10 (s, 1H, C_4–H), 4.89 (m, 1H, $CH(CH_3)_2$), 4.58 (m, 4H, $CH_aCH_bONO_2$), 4.24 (s, 3H, N–CH_3), 2.23 and 2.20 (two s, 3H each, C_2–CH_3 and C_6–CH_3), 1.25 and 1.15 (two d, $J = 4.1$ Hz, 3H each, $CH(CH_3)_2$).

IR (KBr): *v* 3323 (NH), 1716 (C=O), 1661, 1231 cm^{-1} (ONO_2).

3.2. Pharmacology

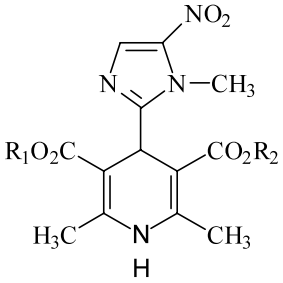
Compounds **18**–**29** were investigated pharmacologically.

3.2.1. Material and methods

Male albino guinea pigs (300–450 g) were killed by a blow to the head. The intestine removed above the ileocecal junction. Smooth muscle segments of about 2 cm length were mounted under a resting tention of 500 mg and were maintained at 37 °C in a 20 ml jacketed organ bath containing oxygenated (95%O_2 and 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. 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 by accumulative amounts 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 [20–22].

The IC_{50} values were graphically determined from the contraction–response curve.

Table 1
Physical properties and calcium channel antagonist activity of compounds **18**–**29**



Comp.	R_1	R_2	m.p. (°C)	Yield (%)	Calcium channel antagonist activity $IC_{50} \pm SEM$, $n = 5$
18	$(CH_2)_2ONO_2$	Me	246–248	44	$1.16 \pm 0.44 \times 10^{-11}$
19	$(CH_2)_2ONO_2$	Et	226–227	51	$2.54 \pm 0.30 \times 10^{-12}$
20	$(CH_2)_2ONO_2$	isoPro	240–242	31	$9.49 \pm 1.19 \times 10^{-13}$
21	$(CH_2)_3ONO_2$	Me	212–215	53	$7.62 \pm 0.92 \times 10^{-11}$
22	$(CH_2)_3ONO_2$	Et	196–198	47	$4.78 \pm 1.02 \times 10^{-12}$
23	$(CH_2)_3ONO_2$	isoPro	206–209	37	$1.77 \pm 0.51 \times 10^{-12}$
24	$(CH_2)_4ONO_2$	Me	226–230	51	$1.34 \pm 0.46 \times 10^{-12}$
25	$(CH_2)_4ONO_2$	Et	217–220	41	$2.53 \pm 0.92 \times 10^{-12}$
26	$(CH_2)_4ONO_2$	isoPro	222–226	38	$2.11 \pm 0.55 \times 10^{-12}$
27	$CH(CH_2ONO_2)_2$	Me	199–204	27	$3.86 \pm 0.82 \times 10^{-12}$
28	$CH(CH_2ONO_2)_2$	Et	211–215	34	$2.17 \pm 0.42 \times 10^{-12}$
29	$CH(CH_2ONO_2)_2$	isoPro	186–188	30	$2.43 \pm 0.69 \times 10^{-12}$
	Nifedipine				$1.07 \pm 0.12 \times 10^{-11}$

3.2.2. Statistics

The results obtained were presented as means and evaluated statistically using Student's *t*-test.

4. Results and discussion

The in vitro calcium channel antagonist activities (IC_{50}) of compounds **18**–**29** were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbacol, 1.67×10^{-7} M) Ca^{+2} dependent contraction (tonic response) of guinea pig ileal longitudinal smooth muscle (GPILSM), and are presented in Table 1.

These results indicate that compounds **18**–**29** possessing a nitrooxy substituent exhibit superior or equipotent calcium channel antagonist activity (10^{-11}–10^{-13} M) relative to the reference drug Nifedipine ($IC_{50} = 1.07 \pm 0.12 \times 10^{-11}$ M).

The R_1C-3 ester substituent was a determinant of calcium channel antagonist activity where the potency order was: $(CH_2)_2ONO_2 > (CH_2)_3ONO_2 > (CH_2)_4$-$ONO_2 > CH(CH_2ONO_2)_2$. In addition, the R_2C-5 ester substituent was another determinant of activity where the potency order was: isopropyl > ethyl > methyl.

The comparison of the activities of the compounds **18**–**29** with the compounds reported by Shafiee et al. [15] having the same structure without nitrooxy group, reveals that the presence of a nitrooxy group substituted on C-3 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 Ogawa et al. [14], indicated that 1,4-dihydropyridine compounds with nitrooxy substituted on C-3 or C-5 ester position of the ring have more activity than similar compounds without nitrooxy substitute and could serve as potential probes to investigate the in vivo release of nitric oxide (NO) which induces vascular muscle relaxation.

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