Synthesis and Smooth Muscle Calcium Channel Antagonist Effects of Dialkyl 1,4-Dihydro-2,6-dimethyl-4-aryl-3,5-pyridinedicarboxylates Containing a Nitrooxy or Nitrophenyl Moiety in the 3-Alkyl Ester Substituent

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Summary

A group of racemic 3-[2-nitrooxyethyl (1,3-dinitrooxy-2-propyl or 4-nitrophenylethyl)] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-[2-trifluoromethylphenyl (2-nitrophenyl or 3-nitrophenyl)]-3,5-pyridinedicarboxylates 13–15 were prepared using the Hantzsch reaction that involved the condensation of 2-nitrooxyethyl 9a, 1,3-dinitrooxy-2-propyl 9b or 4-nitrophenylethyl 9c acetoacetate with isopropyl 3-aminocrotonate 11 and 2-trifluoromethyl 12a, 2-nitro 12b or 3-nitro 12c benzaldehyde. *In vitro* calcium channel antagonist activities were determined using a guinea pig ileum longitudinal smooth muscle assay. Compounds 13–15 exhibited superior, or equipotent, calcium channel antagonist activity (10^{-8} to 10^{-10} M range) relative to the reference drug nifedipine (IC_{50} = 1.43×10^{-8} M). The R^1 C-3 ester substituent was a determinant of calcium channel antagonist activity where the potency order was $CH_2CH_2ONO_2 > CH_2CH_2\text{-}C_6H_4\text{-}4\text{-}NO_2 \geq CH(CH_2ONO_2)_2$. In contrast, the C-4 R^2-aryl substituent (2-CF_3-C_6H_4-, 2-O_2N-C_6H_4- or 3-O_2N-C_6H_4-) was not a major determinant of activity. Compounds 13a–15a, which possess a 3-(2-nitrooxyethyl) ester substituent exhibit superior calcium channel antagonist smooth muscle relaxant activity (IC_{50} = 10^{-10} M range) relative to nifedipine, could serve as potential probes to investigate the *in vivo* release of nitric oxide (NO) which induces vascular muscle relaxation.

Introduction

Organic nitrate compounds such as nitroglycerine (**1**), isosorbide-dinitrate (**2**) and nicorandil (**3**) activate guanylate cyclase to increase the level of cyclic guanosine monophosphate (cGMP) in various vascular smooth muscle tissues which then induces relaxation[1]. This class of compounds called *nitrovasodilators* has provided beneficial cardiovascular therapy for the treatment of angina pectoris[2,3], angina[4,5] and acute myocardial infarction[6]. Sala *et al.*[7] recently described a class of 3-[(nitrooxy)alkyl]-2*H*-1,3-benzoxazin-4(3*H*)-ones (**4**), which were designed to be devoid of the potassium channel agonist effect associated with nicorandil, that exert a preferential relaxant action on large coronary vessels[7]. Ogawa *et al.*[8] prepared a class of 1,4-dihydropyridines (**5**) containing a nitrooxy moiety at the C-3 ester position that increased femoral and vertebral arterial blood flow relative to nifedipine. The discovery that nitric oxide (NO)[9] is an endogenous activator of guanylate cyclase, the enzyme responsible for vascular muscle relaxation, and that organic nitrovasodilators act *in vivo* by by-passing the NO-generating system in the endothelium to deliver NO directly to muscle cells in the walls of the artery prompted us to acquire further structure-activity correlations for Hantzsch 1,4-dihydropyridine calcium channel antagonists having nitrooxy or nitrophenyl group(s) in the C-3 alkyl ester substituent. The nitrooxy compounds, which could also act as releasers of NO, have a number of potential applications including vasodilation, inhibition of platelet aggregation and adhesion, antineoplastic and antiparasitic effects.[9] In our ongoing program to develop structure-activity correlations for 1,4-dihydropyridine calcium channel antagonists, we now report the smooth muscle calcium channel antagonist activities of dialkyl 1,4-dihydro-2,6-dimethyl-4-aryl-3,5-pyridinedicarboxylates **13–15** having a nitrooxy or nitrophenyl moiety in the C-3 ester substituent.

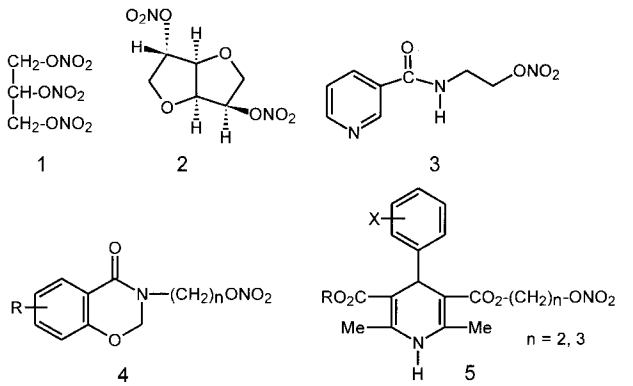

Figure 1. Structures of nitroglycerine (**1**), isosorbide-dinitrate (**2**), nicorandil (**3**), 3-[(nitrooxy)alkyl]-2*H*-1,3-benzoxazin-4(3*H*)-ones (**4**) and 3-nitrooxy analogs of Hantzsch 1,4-dihydropyridines (**5**).

Chemistry

1,3-Dinitrooxy-2-propyl acetoacetate (**9b**) was synthesized by the reaction of diketene (**6**) with 1,3-dibromo-2-propanol (**7**) to afford **8** which was then converted to the title compound upon reaction with silver nitrate in 40% overall yield. A related reaction of **6** with 4-nitrophenylethanol (**10**) yielded 4-nitrophenylethyl acetoacetate (**9c**, 81%). The 3-nitrooxyalkyl (or 4-nitrophenylethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(aryl)-3,5-pyridinedicarboxylates (**13–15**) were

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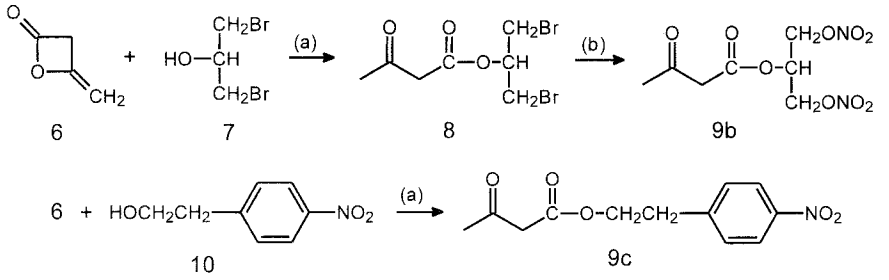
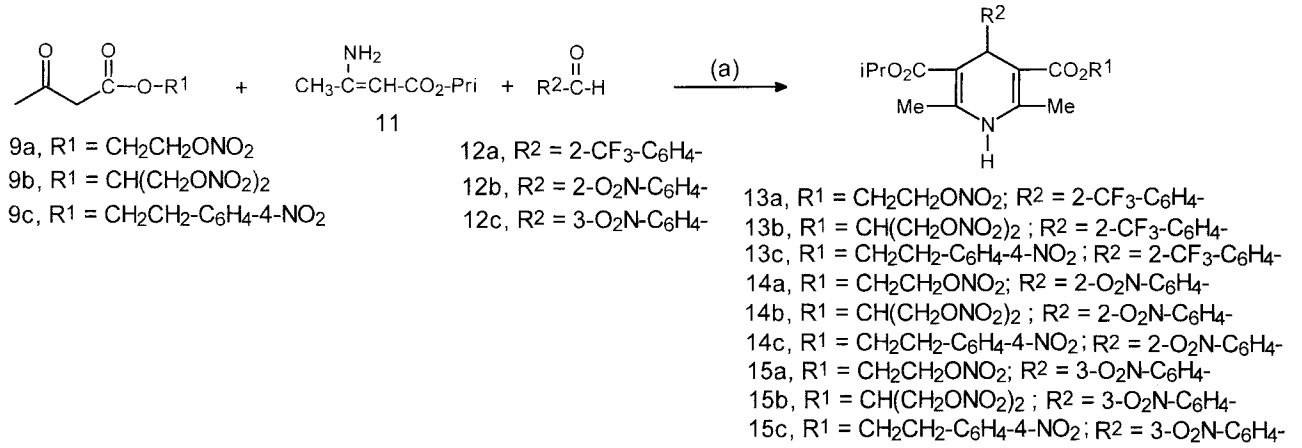


Scheme 1. Reagents and conditions: (a) Et_3N catalyst, 80 °C, 1 h; (b) $AgNO_3$, MeCN, 25 °C, 48 h.



Scheme 2. Reagents and conditions: (a) *i*PrOH, reflux, 3 h.

prepared using the Hantzsch reaction. Thus, condensation of the respective acetoacetate analog (**9a–c**) with isopropyl 3-aminocrotonate (**11**) and the respective aldehyde (**12a–c**) afforded the title compounds in 11–40% yields as illustrated in Scheme 2 and summarized in Table 1.

Results and Discussion

The concomitant use of a calcium channel antagonist and a nitrate vasodilator increases antihypertensive activity with few side effects[10]. In view of the encouraging results of Ogawa *et al.*[8], a group of 1,4-dihydropyridine compounds **13–15** was investigated to determine whether incorporating both a *nitro-like* and a calcium channel antagonist moiety into a hybrid molecule would provide superior calcium channel smooth muscle relaxant activity. It was anticipated that compounds possessing a nitrooxy moiety could also serve as releasers of nitric oxide which has a number of potential therapeutic applications as described previously[9].

The *in vitro* calcium channel activities of compounds **13–15** were determined using guinea pig ileum longitudinal smooth muscle (GPILSM). The calcium channel antagonist activities of **13–15**, determined as the concentration required to produce 50% inhibition of GPILSM contractility,[11] are presented in Table 1. Compounds **13–15** exhibited superior/equipotent calcium channel antagonist activity (10^{-8} to 10^{-10} M range) relative to the reference drug nifedipine ($IC_{50} = 1.43 \times 10^{-8}$ M). A comparison of the relative potency order for **13a–c** (R^2 = 2-CF_3-C_6H_4-), **14a–c** (R^2 = 2-O_2N-C_6H_4-) and **15a–c** (R^2 = 3-O_2N-C_6H_4-) showed that the R^1-substituent was a determinant of calcium channel antagonist activity where the activity profile was $CH_2CH_2ONO_2$ > CH_2CH_2-C_6H_4-4-NO_2 ≥ $CH(CH_2ONO_2)_2$, [**13a** > **13c** > **13b**; **14a** > **14c** ≥ **14b**; **15a** > **15c** ≥ **15b**]. In contrast, the R^2-substituent (2-CF_3-C_6H_4-, 2-O_2N-C_6H_4- or 3-O_2N-C_6H_4-) was not a major determinant of activity since the differences in activity between **13a–15a** (R^1 = $CH_2CH_2ONO_2$) and **13c–15c** (R^1 = CH_2CH_2-C_6H_4-4-NO_2) were generally small, although the activities of **13b–15b** [R^1 = $CH(CH_2ONO_2)_2$] varied over a one-log unit range. These results indicate that compounds **13a–15a** possessing a R^1 = $CH_2CH_2ONO_2$ substituent exhibit superior calcium channel antagonist smooth muscle relaxant activity ($IC_{50} = 10^{-10}$ M range) relative to nifedipine. Compounds **13a–15a** (R^1 = $CH_2CH_2ONO_2$) could serve as potential probes to investigate the *in vivo* release of nitric oxide which induces vascular muscle relaxation[9].

Acknowledgments

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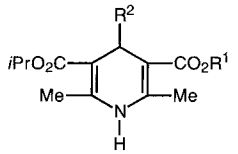
Experimental

Melting points were determined using a Thomas-Hoover capilliary apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of $[D_2]H_2O$. Infrared spectra were acquired using

Table 1. Physical and calcium channel antagonist activities of 3-nitrooxyalkyl (or 4-nitrophenylethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(aryl)-3,5-pyridinedicarboxylates (**13–15**).



Cmpd	R^1	R^2	Cryst. solvent	mp, °C	% Yield	Formula	Anal.[a]	Calcium channel antagonist act:IC_{50}(M)[b]
13a	$CH_2CH_2ONO_2$	2-CF_3-C_6H_4–	NA[c]	oil	21	$C_{21}H_{23}F_3N_2O_7$	C,H,N	$9.51 \pm 0.12 \times 10^{-10}$(3)
13b	$CH(CH_2ONO_2)_2$	2-CF_3-C_6H_4-	NA[c]	oil	11	$C_{22}H_{24}F_3N_3O_{10}$	C,H,N	$2.29 \pm 0.27 \times 10^{-8}$ (3)
13c	$(CH_2)_2$-C_6H_4-4-NO_2	2-CF_3-C_6H_4-	NA[c]	40	30	$C_{27}H_{27}F_3N_2O_6$	C,H,N[d]	$5.81 \pm 0.35 \times 10^{-9}$ (3)
14a	$CH_2CH_2ONO_2$	2-O_2N-C_6H_4-	CH_2Cl_2-*i*Pr_2O	145	40	$C_{20}H_{23}N_3O_9$	C,H,N	$6.59 \pm 0.87 \times 10^{-10}$(3)
14b	$CH(CH_2ONO_2)_2$	2-O_2N-C_6H_4-	NA[c]	72	24	$C_{21}H_{24}N_4O_{12}$	C,H,N[e]	$6.01 \pm 0.66 \times 10^{-9}$ (3)
14c	$(CH_2)_2$-C_6H_4-4-NO_2	2-O_2N-C_6H_4-	NA[c]	60	22	$C_{26}H_{27}N_3O_8$	C,H,N	$3.91 \pm 0.12 \times 10^{-9}$ (3)
15a	$CH_2CH_2ONO_2$	3-O_2N-C_6H_4-	EtOAc-hexane	128–129[f]	35	$C_{20}H_{23}N_3O_9$	C,H,N	$5.77 \pm 0.37 \times 10^{-10}$(3)
15b	$CH(CH_2ONO_2)_2$	3-O_2N-C_6H_4-	EtOAc-hexane	130–131	26	$C_{21}H_{24}N_4O_{12}$	C,H,N[g]	$2.65 \pm 0.03 \times 10^{-9}$ (3)
15c	$(CH_2)_2$-C_6H_4-4-NO_2	3-O_2N-C_6H_4-	*i*Pr_2O	95–97	33	$C_{26}H_{27}N_3O_6$	C,H,N	$1.01 \pm 0.03 \times 10^{-9}$ (3)
Nifedipine								$1.43 \pm 0.38 \times 10^{-8}$ (8)

[a] Microanalytical analyses were within ± 0.4% of theoretical values, unless otherwise indicated.

[b] The molar concentration of antagonist test compound causing a 50% decrease in the slow component, or tonic contractile response, (IC_{50} ± SEM) in guinea pig ileal longitudinal smooth muscle by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose-response curves. The number of experiments is shown in brackets.

[c] NA = not applicable, since the compound undergoes partial decomposition in solution during attempted recrystallization.

[d] 1/2 molecule of water of hydration.

[e] N: calcd, 10.68; found, 10.21.

[f] Lit. mp 126–127 °C[8].

[g] C: calcd, 48.10; found, 48.59.

a Nicolet 5DX-FT spectrometer. Silica gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica gel. Microanalyses were within ± 0.4% of theoretical values for all elements listed, unless otherwise stated. 2-Nitrooxyethyl acetoacetate (**9a**) was prepared according to the reported procedure.[8] Diketene (**6**) and isopropyl 3-aminocrotonate (**11**) were purchased from the Aldrich Chemical Co.

*1,3-Dinitrooxy-2-propyl Acetoacetate (**9b**)*

Diketene (0.84 g, 10 mmol) was added dropwise with stirring to 1,3-dibromo-2-propanol (2.17 g, 10 mmol) preheated to 50–60 °C in the presence of a catalytic amount of Et_3N (5 drops). 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 1,3-dibromo-2-propyl acetoacetate **8** which was used immediately in the subsequent reaction (bp 150 °C/1 mm, 2.15 g, 71%); IR (neat): ν = 1754, 1720 cm^{-1} (C=O).– ^1H NMR ($CHCl_3$-d_1): δ 2.26 (s, 3 H, *Me*), 3.50–3.66 (m, 6 H, CH_2), 5.17 (quint, *J* = 6 Hz, 1 H, C*H*). Silver nitrate (4.07 g, 24 mmol) was added to a solution of **8** (3.01 g, 10 mmol) in acetonitrile (20 ml) and the reaction was allowed to proceed at 25 °C for 48 h with stirring. Removal of the precipitate by filtration, washing the precipitate with acetonitrile (10 ml) and then removal of the solvent *in vacuo* from the combined filtrates gave a residue which was purified by silica gel column chromatography using EtOAc-hexane (30:70, *v/v*) as eluent to afford **9b** as a pale yellow oil (1.5 g, 56%); IR (neat): ν = 1761, 1728 cm^{-1} (C=O).– ^1H NMR ($CHCl_3$-d_1): δ 2.26 (s, 3 H, *Me*), 3.54 (s, 2 H, $COCH_2$), 4.60 (dd, J_{gem} = 12, J_{vic} = 6 Hz, 2 H, $CH_aH_bONO_2$), 4.74 (dd, J_{gem} =12, J_{vic} = 4 Hz, 2 H, $CH_{a'}H_{b'}ONO_2$), 5.40–5.48 (m, 1 H, CO_2CH).

*4-Nitrophenylethyl Acetoacetate (**9c**)*

Reaction of diketene (0.84 g, 10 mmol) with 4-nitrophenylethanol (10 mmol) in the presence of Et_3N (5 drops) and distillation of the product, using the procedure described for the preparation of **9b**, afforded **9c** as a pale yellow oil (2.04 g, 81%), bp 180 °C/1.5 mm; IR (neat): ν = 1750, 1720 cm^{-1} (C=O), 1417, 1320 (NO_2).– ^1H NMR ($CHCl_3$-d_1): δ 2.18 (s, 3 H, *Me*), 3.03 (t, *J* = 7 Hz, 2 H, $CO_2CH_2CH_2$), 3.42 (s, 2 H, $COCH_2$), 4.36 (t, *J* = 7 Hz, 2 H, $CO_2CH_2CH_2$), 7.36 (d, *J* = 8 Hz, 2 H, phenyl H-2, H-6), 8.08 (d, *J* = 8 Hz, 2 H, phenyl H-3, H-5).

General Method for the Preparation of 3-[2-Nitrooxyethyl (1,3-Dinitrooxy-2-propyl or 4-Nitrophenylethyl)] 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(aryl)-3,5-pyridinedicarboxylates **13–15**

A mixture of the respective acetoacetate **9a, 9b,** or **9c** (5.0 mmol), isopropyl 3-aminocrotonate (0.71 g, 5.0 mmol) and the respective aldehyde **12a, 12b** or **12c** (5.0 mmol) in isopropanol (25 ml) was refluxed for 3 h with stirring. Removal of the solvent *in vacuo* afforded a residue which was purified by silica gel column chromatography using hexane-EtOAc (60:40, *v/v*) as eluent to yield the respective product. The recrystallization solvent when applicable, mp when applicable, and % yield of products **13-15** are summarized in Table 1.

3-(2-Nitrooxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate **13a**.

IR (neat): ν = 3328 cm^{-1} (NH), 1679 (C=O), 1640, 1285 (ONO_2).– UV (EtOH): λ_{max} (log ε) 206 nm (4.54), 236 (4.37), 355 (3.85).– ^1H NMR ($CHCl_3$-d_1): δ 1.06 and 1.18 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, $CHMe_2$), 2.21 (s, 6 H, C-2 and C-6 *Me*), 4.17 (dt, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, $CH_aH_bONO_2$), 4.38 (dt, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, $CH_aH_bONO_2$), 4.56 (t, *J* = 6 Hz, 2 H,

CO_2CH_2), 4.93 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.52 (s, 1 H, H-4), 6.41 (br s, 1 H, NH), 7.19 (dd, $J_{3,4}$ = 8, $J_{4,5}$ = 8 Hz, 1 H, phenyl H-4), 7.37 (dd, $J_{4,5}$ = 8, $J_{5,6}$ = 8 Hz, 1 H, phenyl H-5), 7.43–7.51 (m, 2 H, phenyl H-3 and H-6).

3-(1,3-Dinitrooxy-2-propyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate **13b**

IR (neat): ν = 3344 cm^{-1} (NH), 1696 (C=O), 1638, 1282 (ONO_2).– UV (EtOH): λ_{max} (log ε) 206 nm (4.48), 352 (3.49).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.14 and 1.21 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.22 and 2.31 (two s, 3 H each, C-2 and C-6 *Me*), 4.35 (dd, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, CH-CH_aH$_b$), 4.46–4.60 (m, 2 H, CH-CH$_a$CH_b, CH-CH_a'H$_b$'), 4.68 (dd, J_{gem} = 12, J_{vic} = 4 Hz, 1 H, CH-CH$_a$'H_b'), 4.98 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.30–5.38 (m, 1 H, CO_2CH), 5.49 (s, 1 H, H-4), 6.07 (br s, 1 H, NH), 7.25 (dd, $J_{3,4}$ = 8, $J_{4,5}$ = 8 Hz, 1 H, phenyl H-4), 7.42 (dd, $J_{4,5}$ = 8, $J_{5,6}$ = 8 Hz, 1 H, phenyl H-5), 7.50 (dd, $J_{3,4}$ = $J_{5,6}$ = 8 Hz, 2 H, phenyl H-3, H-6).

3-(4-Nitrophenylethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate **13c**

IR (KBr): ν = 3336 cm^{-1} (NH), 1687 (C=O), 1491, 1351 (NO_2).– UV (EtOH): λ_{max} (log ε) 204 nm (4.37), 237 (4.23), 269 (4.06), 354 (3.73).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.02 and 1.14 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.12 and 2.18 (two s, 3 H each, C-2 and C-6 *Me*), 2.84–3.02 (m, 2 H, $CO_2CH_2CH_2$), 4.16–4.36 (m, 2 H, $CO_2CH_2CH_2$), 4.92 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.48 (s, 1 H, H-4), 6.86 (br s, 1 H, NH), 7.15–7.98 (m, 8 H, aryl hydrogens).

3-(2-Nitrooxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate **14a**

IR (KBr): ν = 3336 cm^{-1} (NH), 1696 (C=O), 1638, 1491, 1365, 1277 (ONO_2, NO_2).– UV (EtOH): λ_{max} (log ε) 205 nm (4.34), 226 (4.22), 277 (3.75), 314 (3.65).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.00 and 1.24 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.31 and 2.36 (two s, 3 H each, C-2 and C-6 *Me*), 4.18–4.26 (m, 1 H, CH_aH$_b$ONO$_2$), 4.33–4.41 (m, 1 H, CH$_a$$H_bONO_2$), 4.54–4.67 (m, 2 H, CO_2CH_2), 4.97 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.73 (br s, 1 H, NH), 5.86 (s, 1 H, H-4), 7.26 (ddd, $J_{3,4}$ = $J_{4,5}$ = 8.5, $J_{4,6}$ = 1.5 Hz, 1 H, phenyl H-4), 7.44–7.55 (m, 2 H, phenyl H-5, H-6), 7.76 (dd, $J_{3,4}$ = 8.5, $J_{3,5}$ = 1.5 Hz, 1 H, phenyl H-3).

3-(1,3-Dinitrooxy-2-propyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate **14b**

IR (KBr): ν = 3385 cm^{-1} (NH), 1687 (C=O), 1646, 1482, 1359, 1277 (ONO_2, NO_2).– UV (EtOH): λ_{max} (log ε) 206 nm (4.31), 236 (4.13), 323 (3.49), 371 (3.42).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.07 and 1.21 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.32 and 2.38 (two s, 3 H each, C-2 and C-6 *Me*), 4.35 (dd, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, CH-CH_aH$_b$), 4.50 (dd, J_{gem} = 12, J_{vic} = 4 Hz, 1 H, CH-CH$_a$CH_b), 4.61–4.74 (m, 2 H, CH_2), 4.98 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.32–5.40 (m, 1 H, CO_2CH), 5.76 (br s, 1 H, NH), 5.88 (s, 1 H, H-4), 7.24–7.76 (m, 4 H, aryl hydrogens).

3-(4-Nitrophenylethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate **14c**

IR (KBr): ν = 3377 cm^{-1} (NH), 1687 (C=O), 1482, 1343 (NO_2).– UV (EtOH): λ_{max} (log ε) 204 nm (4.29), 236 (4.10), 267 (3.95), 324 (3.56).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.01 and 1.24 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.25 and 2.37 (two s, 3 H, each, C-2 and C-6 *Me*), 2.92–3.10 (m, 2 H, $CO_2CH_2CH_2$), 4.18–4.26 (m, J_{gem} = 7.5, J_{vic} = 4 Hz, 1 H, CO_2CH_aH$_b$), 4.30–4.38 (m, J_{gem} = 7.5, J_{vic} = 4 Hz, 1 H, CO_2CH_aH$_b$), 4.98 (sept, J = 6 Hz, 1 H, CHMe$_2$), 5.68 (br s, 1 H, NH), 5.82 (s, 1 H, H-4), 7.22–8.06 (m, 8 H, aryl hydrogens).

3-(2-Nitrooxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate **15a**

IR (KBr): ν = 3435 cm^{-1} (NH), 1696 (C=O), 1645, 1480, 1335, 1277 (ONO_2, NO_2).– UV (EtOH): λ_{max} (log ε) 206 nm (4.37), 237 (4.32), 356 (3.71).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.12 and 1.27 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.34 and 2.35 (two s, 3 H each, C-2 and C-6 *Me*), 4.25–4.39 (m, 2 H, CH_2), 4.62 (t, J = 6 Hz, 2 H, CH_2), 4.96 (sept, $J_{CH,Me}$ = 6 Hz, 1 H, CHMe$_2$), 5.05 (s, 1 H, H-4), 5.86 (br s, 1 H, NH), 7.38 (dd, $J_{4,5}$ = $J_{5,6}$ = 8 Hz, 1 H, phenyl H-5), 7.62 (d, $J_{5,6}$ = 8 Hz, 1 H, phenyl H-6), 8.04 (dd, $J_{4,5}$ = 8, $J_{2,4}$ = 2 Hz, 1 H, phenyl H-4), 8.12 (d, $J_{2,4}$ = 2 Hz, 1 H, phenyl H-2).

3-(1,3-Dinitrooxy-2-propyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate **15b**

IR (KBr): ν = 3361 cm^{-1} (NH), 1687 (C=O), 1646, 1482, 1351, 1280 (ONO_2, NO_2).– UV (EtOH): λ_{max} (log ε) 206 nm (4.45), 238 (4.48), 361 (3.88).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.12 and 1.26 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.36 and 2.38 (two s, 3 H each, C-2 and C-6 *Me*), 4.20 (dd, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, CH-CH_aH$_b$ONO$_2$), 4.52 (dd, J_{gem} = 12, J_{vic} = 4 Hz, 1 H, CH-CH$_a$CH_bONO$_2$), 4.62 (dd, J_{gem} = 12, J_{vic} = 6 Hz, 1 H, CHCH_a'H$_b$'ONO$_2$), 4.74 (dd, J_{gem} = 12, J_{vic} = 4 Hz, 1 H, CHCH$_a$'H_b'ONO$_2$), 4.90–5.06 (m, 2 H, H-4, CHMe$_2$), 5.30–5.38 (m, 1 H, CO_2CH), 5.85 (br s, 1 H, NH), 7.39 (dd, $J_{4,5}$ = $J_{5,6}$ = 8 Hz, 1 H, phenyl H-5), 7.62 (d, $J_{5,6}$ = 8 Hz, 1 H, phenyl H-6), 8.04 (dd, $J_{4,5}$ = 8, $J_{2,4}$ = 2 Hz, 1 H, phenyl H-4), 8.10 (d, $J_{2,4}$ = 2 Hz, 1 H, phenyl H-2).

3-(4-Nitrophenylethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate **15c**

IR (KBr): ν = 3361 cm^{-1} (NH), 1687 (C=O), 1482, 1351 (NO_2).– UV (EtOH): λ_{max} (log ε) 205 nm (4.52), 237 (4.43), 353 (3.79).– ^{1}H NMR ($CHCl_3$-d$_1$): δ 1.08 and 1.12 (two d, $J_{CH,Me}$ = 6 Hz, 3 H each, CHMe_2), 2.30 and 2.32 (two s, 3 H, each, C-2 and C-6 *Me*), 3.03 (t, J = 7 Hz, 2 H, $CO_2CH_2CH_2$), 4.34 (t, J = 7 Hz, 2 H, $CO_2CH_2CH_2$), 4.88–5.00 (m, 2 H, CHMe$_2$, H-4), 6.27 (br s, 1 H, NH), 7.26–8.08 (m, 8 H, aryl hydrogens).

In Vitro Calcium Channel Antagonist Assay

The calcium channel antagonist activities of compounds **13–15** were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{+2} dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure reported previously.[11] The IC_{50} value (± SEM) was determined graphically from the dose-response curve.

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