Synthesis and *in vitro* dual calcium channel antagonist-agonist activity of some 1, 4-Dihydo-2,6-dimethyl-3-nitro and cyano-4-(*1-methyl-5-nitro-1H-imidazol-2-yl*)-5-pyridinecarboxylates

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ABSTRACT

Background and purpose of the study: The vasorelaxant action of the dihydropyridines (DHPs) provides many useful clinical indications. However, their negative effects on cardiac contractility is still of a great concern especially in patients with heart failure. Design and synthesis of dual action compounds, i. e. smooth muscle calcium channel antagonist/cardiac muscle calcium channel agonist provides better and safer compounds particularly in patients with compromised cardiac contractility. In the present study, dual cardioselective Ca^{2+} channel agonists / vascular selective smooth muscle Ca^{2+} channel antagonists as third generation of DHP drugs were synthesized by a reported method.

Methods: Synthetic procedure involved condensation of isopropyl-3-aminocrotonate with nitroacetone and 1-methyl-5-nitroimidazole2-carboxaldehyde and condensation of alkylacetoacetates with 3-aminocrotonitryl and 1-methyl-5-nitro-1H-imidazole-2-carbaldehyde for the preparation of 1,4-Dihydo-2,6-dimethyl-3-nitro and cyano-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-pyridinecarboxylates, respectively. The in vitro effects of the synthesized compounds were evaluated on longitudal Smooth Muscle (GPILSM) and Guinea Pig Left Atrium (GPLA) preparations and finally, their conformations and structure-activity relationships were assessed.

Results and major conclusion: All compounds showed calcium channel antagonist activity on isolated guinea pig ileum and some of them showed calcium channel agonist effects (or positive inotropic effect instead of calcium channel agonist effect) on isolated guinea-pig left atrium. QSAR and conformational analyses showed that conformation and charge of aryl substituents at C4 position have a main role in antagonistic activity while carbonyl group at C_5 position plays an important role in agonistic effects.

Keywords: Calcium channel antagonist-agonist activity, Dihydropyridines, QSAR

INTRODUCTION

Increase in cytosolic calcium concentration regulates many cellular functions. In many organs the major part of this increment is mediated by Ca^{2+} influx through voltage-gated channels so these channels are important targets for drug therapy of various disorders especially many cardiovascular diseases (1).

On the way of discovering effective compounds on calcium channels, the derivatives with 1,4-dihydropyridine (DHP) structure have been considered and many active compounds with calcium channel blocking effect have been obtained by making modifications on the nifedipine molecule (2).

In the previous study, it was shown that non-classical bioisosteric replacement of nitrophenyl by nitroimidazole at C-4 position of DHP could be a successful modification to increase the antagonist activity of some nifedipine analogues (3). Although the vasorelaxant action of the current available DHPs provides many useful clinical indications, their negative effects on cardiac contractility is still of a great concern especially in patients with heart failure (4). Therefore design and synthesis of dual action compounds, i. e. smooth muscle calcium channel antagonist/cardiac muscle calcium channel agonist will provide better and safer compounds particularly in patients with compromised cardiac contractility.

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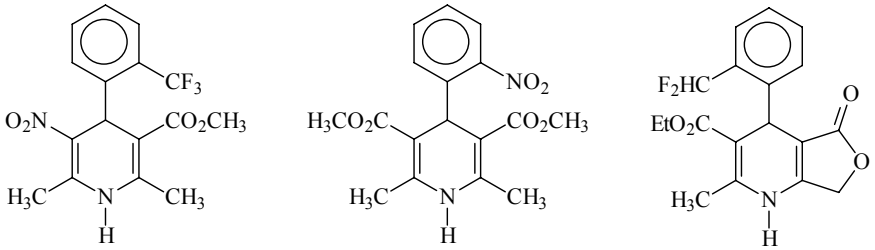


Bay k8644 Nifedipine CGP 28392

Figure 1. Chemical structures of Bay k8644, Nifedipine and CGP 28392

Up to now, few 1,4-dihydropyridine calcium channel agonists such as nitro compounds BAY K8644 (1), PN 202-791, LY 24993 and CGP 28392 (figure 1) have been introduced and it has been demonstrated that the presence of an electron withdrawing group such as nitro at C_5 position of DHP leads to compounds with Ca^{++} channel agonist activity (5-6).

Based on these findings and in pursuit of ongoing program to design tissue selective calcium channel modulators, herein the synthesis and in vitro calcium channel modulating activities of a series of 1,4-Dihydo-2,6-dimethyl-3-nitro and cyano-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-pyridinecarboxylates derivatives and their in vitro effects on longitudal Smooth Muscle (GPILSM) and Guinea Pig Left Atrium (GPLA) preparations are described.

METHODS & MATERIALS

Chemistry

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AM-300 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. Infrared spectra were acquired using a Nicplet-IR 500 Series II spectrometer. Silica gel column chromatography was carried out using Merck 7734 (60-20 mesh) silica gel. The progress of reactions was monitored using Macherey-Nagel Polygram® Sil G/UV$_{254}$ plates (0.25 mm diameter). 2,2,6-Trimethyl-4H-1,3-dioxine-4-one **V**, isopropyl 3-aminocrotonate **III**, methyl (ethyl) acetoacetates and 3-aminocrotonitryl **VII** were purchased from the Aldrich Chemical Co. (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Nitroacetone **II** was prepared according to the reported procedure (7).

Pharmacology

a) Investigation of agonist activity on isolated left atria of guinea pigs

Healthy male guinea-pigs, weighing 300-400 g, were killed by a blow on the head and decapitation. The heart was excised and left atrium was dissected free and suspended in an organ bath filled with physiologic solution. The isolated tissue was connected to an isometric transducer (K30, Hugo Sachs Electronic, Germany). In order to induce contractions in the left atrium, the tissue was stimulated with a stimulator (stimulator type 215, Hugo Sachs Electronic, Germany) and a bipolar platinum electrode. To study the effect of synthesized compounds on the contractility of rat atrium, increasing concentrations of each compound were added to the organ bath and changes in contraction force were recorded. The pEC130 (the negative logarithm of the concentration which increased the contractions 30 % above the base line) value for each compound was calculated from concentration-response curves and compared with that of Bay K 8644 (as a reference compound) (8-9).

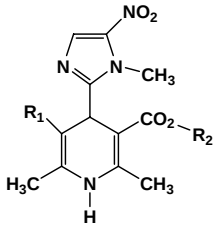
b) Investigation of antagonist activity on isolated ileum of guinea pigs:

Male guinea pigs, weighing 300-400 g, were killed by a blow on the head. The non-terminal part of the ileum was removed and cut into segments of 10-15 mm length. Each ileal segment was suspended in organ bath and connected to an isometric transducer (K30, Hugo Sachs Electronic, Germany). Contractions of the ileal segments were recorded, using an amplifier (Plugsys, Hugo Sachs Electronic, Germany) and a recorder (Graphtec, model WR3320). From concentration-response curves, the pIC_{50} value of each compound was calculated and compared with that of nifedipine as a reference compound (10).

Statistical analyses

Results are expressed as Means ± S.E.M by comparison of pEC_{130} values of compounds on isolated guinea-pig atrium and their pIC_{50} values on isolated giunea-pig ileum were performed using one-way ANOVA followed by Dunnet test. A P value < 0.05 was considered to be significant.

Table 1. Physical properties and *In vitro* calcium channel antagonist (IC_{50}) and agonist (EC_{130}) activity of compounds **VIII$_{a-g}$** (n=3-6)



Comp.	R_1	R_2	MP(°C)	Yield (%)	IC_{50}±SEM	EC_{130}±SEM
VIII$_a$	NO_2	$CH(CH_3)_2$	260-262	44	$(2.5\pm 0.29)\times10^{-8}$	-
VIII$_b$	CN	CH_2CH_3	266-268	49	$(6.9\pm0.03)\times10^{-6}$	-
VIII$_c$	CN	CH_2CH_2CN	225-228	42	$(5.4\pm0.06)\times10^{-6}$	$(1\pm0.08)\times10^{-7}$
VIII$_d$	CN	$CH(CH_3)_2$	272-274	57	$(2.1\pm 0.21)\times10^{-6}$	-
VIII$_e$	CN	$CH_2CH_2CH_3$	197-199	35	$(7\pm 0.050)\times10^{-7}$	$(3.2\pm0.03)\times10^{-6}$
VIII$_f$	CN	$CH_2CH_2CH_2CH_3$	200-203	43	$(5.3\pm0.07)\times10^{-6}$	$(3.6\pm0.33)\times10^{-6}$
VIII$_g$	CN	CH_3	282-284	53	$(1.8\pm0.05)\times10^{-6}$	$(1.5\pm0.19)\times10^{-6}$
Nifedipine	-	-	-	-	$(6.31\pm 0.08)\times10^{-9}$	-
Bay k8644	-	-	-	-	-	$(3\pm0.28)\times10^{-7}$

QSAR & conformation studies

Chemical structure of each molecule was built by Hyperchem software (ver 7.1, 2002, Hypercube Inc) (11) and Gaussian98 was used to optimize the molecular structure (12). The structures were optimized by ab initio calculations at the level of RHF/STO-3G. Local charges (LC) at each atom were calculated according to Mulliken population method. Some theoretical QSAR descriptors including physicochemical properties, constitutional descriptors, geometrical descriptors and topological indices were calculated by DRAGON software (13). The MLR was performed by the SPSS software using the stepwise selection and elimination method for variable selection (14). For conformation analysis, optimized molecules in Gaussian 98 were reloaded to Hyperchem (11).

RESULTS AND DISCUSION

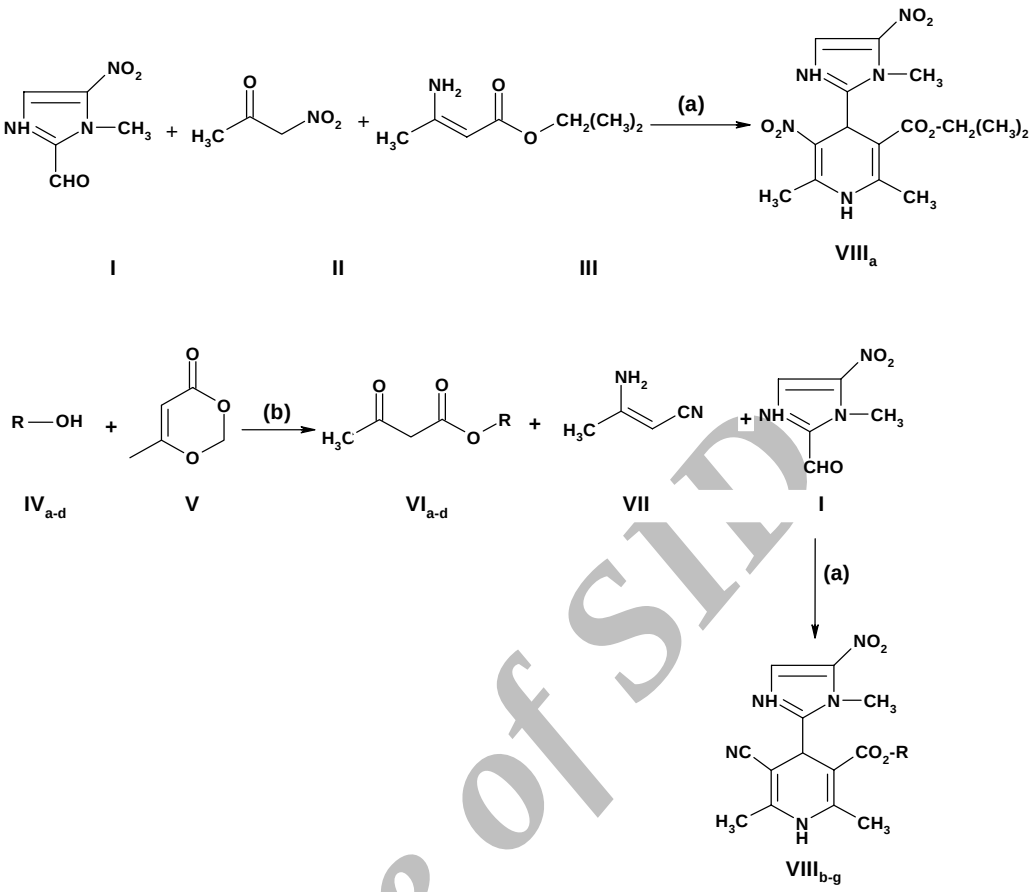
The unsymmetrical analogues **VIII$_{a-g}$** (table 1) were synthesized by a modified Hansch reaction using a procedure reported by Iwanami (15). 1,4-Dihydo-2,6-dimethyl-3-nitro analogue **VIII$_a$** (yield=43%) was synthesized by condensation of alkyl-3- aminocrotonate **III**, nitroacetone **II** and 1-methyl-5-nitro-1H-imidazole-2-carbaldehyde **I**. Reaction of alcohols **IV$_{a-d}$** with 2, 2, 6-trimethyl-4H-1, 3-dioxin-4-one **V** afforded the corresponding acetoacetic esters **VI$_{a-d}$** in 85-95% yields (16). Condensation of acetoacetic esters **VI$_{a-d}$,** 3-aminocrotonitryl **VII** and 1-methyl-5-nitroimidazole-2-carboxaldehyde **I** afforded the required products (**VIII$_{b-g}$**) in 35-57% yields. (Scheme 1)

The *in vitro* antagonist and agonist activities of the target compounds are represented in Table 1. According to the obtained results, all the synthesized compounds showed lower channel antagonistic activity than nifedipine as the reference drug. The compound **VIII$_a$** containing nitro at C-3 and isopropyl at C-5 ester moiety was significantly more active than other synthesized compounds. The order of activity for this series of compounds was:

VIII$_a$>VIII$_e$>VIII$_g$>VIII$_d$>VIII$_c$=VIII$_f$> VIII$_b$, implying that, in addition to the function of an electron withdrawing group at C-3, and the presence of a lipophilic or nonlipophilic substituent at C-5 ester moiety, the changes in antagonist activity could be due to hydrophobic interactions at ligand receptor site (17).

For the agonist activity, it was found, that compounds **VIII$_c$** and **VIII$_g$**, had positive inotropic effects. A comparison of EC130 values of the synthesized compounds revealed that while compound **VIII$_c$** showed the highest agonist activity, compounds **VIII$_a$**, **VIII$_b$** and **VIII$_d$** did not show any agonist activity in guinea pig left atrium.

The best multi-linear regression equation was obtained by the stepwise selection methods of MLR subroutine of SPSS software. The correlation coefficient (R^2), standard error of regression (*SE*), and correlation coefficient for leave-one-out cross-validation significance (Q^2_{LOO}) were employed to judge the validity of regression equation.



Scheme 1. Regents and Conditions a) dry EtOH, reflux, 12h, b) Xylene, reflux, 30min

An appropriate equation for Ca^{2+} channel blocking activity by calculated parameters was obtained as follows:

pIC_{50}= 0.770(±0.162) (LC_{C1})* − 5.841(±0.150)

N=7, R=0.904, R^2=0.818, Q^2_{LOO}=0.858, F=22.46, SE=0.39

* Local charge on C_1 of nitroimidazole group

In this equation the values in the parenthesis represent the standard deviation of the coefficients. N, R, Se and F are number of components, correlation coefficient, standard error of regression and Fisher's *F*-ratio, respectively. In this one parametric equation Local charge on C_1 of nitroimidazole group implies the importance of charge parameters of aryl group at the C_4 position activity. This findings is in agreement with the results of previous findings in which it has been shown that aryl group of C_4 position have great impact on Ca^{2+} channel blocking activity of Dihydropyridines (18,19). Also the positive relation of parameter and activity revealed that this parameter has a positive effect on calcium channel activity.

The optimized 3D structure of molecules obtained by *ab initio* calculations at the level of STO-3G is presented in Figure 2 and the data related to two dihedral angles represent positions of aryl substituent at C_4 and carbonyl group at C_5 compared to DHP ring.

Table 2. Two geometrical features of optimized derivatives

Copmound	Φ1[a]	Φ2[b]
$VIII_a$	60.35	171.68
$VIII_b$	-108.12	172.00
$VIII_c$	-107.73	-179.82
$VIII_d$	-108.05	173.00
$VIII_e$	-108.02	173.00
$VIII_f$	-108.10	172.00
$VIII_g$	-107.08	175.00
Nifedipine	70.63	-169.43
Bay k8644	-118.59	-179.43

[a] Dihedral angle between nitroimidazole and DHP ring
[b] Dihedral angle between C-5-CO and DHP ring

It is obvious that the conformation of nitroimidazolyl moiety has an important role in their Ca^{2+} channel blocking activity, since only compound **$VIII_a$** which showed highest

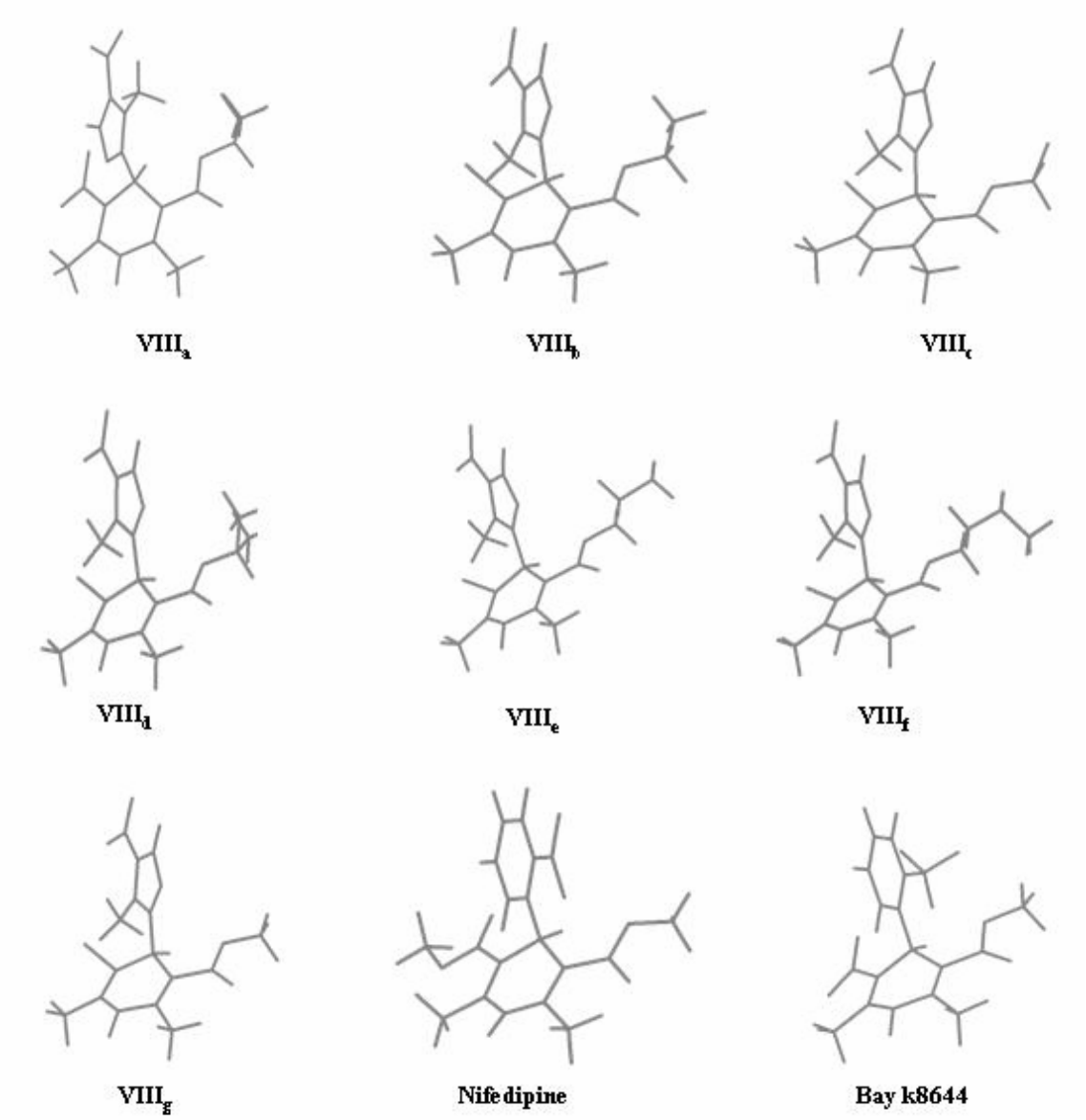


Figure 2. Optimized Structures of DHP derivatives, Nifedipine and Bay k8644

antagonistic activity among synthesized compounds had a conformation similar to nifedipine in this region. This is in agreement with previous findings which showed that conformation of substituent at C_4 position have a great impact on antagonistic activity (18-19).
Second dihedral angle between carbonyl group at C_5 and DHP ring revealed that C_5 substituent might have a role in agonistic activity, since b_3 had a similar angle toward BAY k8644 which had higher agonistic effects compared to other compounds (Table 2).

CONCLUSION

In the present study, the dual calcium channel antagonist-agonist activity of some newly synthesized derivatives of DHP was evaluated. All compounds showed calcium antagonist activity on GPILSM but some of them showed agonist activity on guinea-pig atrium. QSAR and conformational analysis revealed that conformation and charge of aryl substituent at C_4 position have a main role in antagonistic activity while carbonyl group at C_5 position interferes with agonistic effect significantly.

EXPERIMENTAL

*Preparation of 3-isopropyl-5-nitro-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3- carboxylate (***VIII$_a$***)*

A solution of nitroacetone **II** (1.0 g, 10 mmol) in absolute EtOH (3 ml) was added to a solution of isopropyl 3-aminocrotonate **III** (1.43 g, 10 mmol)

and 1-methyl-5-nitroimidazole-3-carboxaldehyde **I** (1.55 g, 10 mmol) in absolute EtOH (5 ml) during five min period while steering and the resulting solution was heated at reflux for 12 h. The solvent was removed in vacuo and the residue obtained purified by silica gel column chromatography using ethyl acetate-hexane (3:1 v/v). (Scheme 1)

^{1}H NMR ($CDCl_3$): δ 9.84 (br s, 1H, NH), 7.92 (s, 1H, imidazole H-4), 5.53 (s, 1H, C_4-H), 5.05 (m, 1H, CO_2CH), 4.31 (s, 3H, N-CH_3), 2.43 and 2.23 (two s, 3H each, C_2-CH_3 and C_6-CH_3), 1.25 and 1.17 (two d, J=4.0 Hz, 3H each,$CH(CH3)_2$).

IR (KBr): ν 3323 (NH), 1716 (CO_2), 1516, 1327 (ONO_2, NO_2) cm^{-1}.

MS: m/z (%) 365(M^+,6), 284(16), 319(39), 259(100), 233(40), 196(66) and 150(69).

General procedure for the synthesis of acetoacetic esters ***VI$_{a-d}$.***

A solution of alcohols **IV$_{a-d}$** (50 mmol) and 2,2,6-trimethyl-4-H-1,3-dioxine-4-one **V** (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 preheated to 150 °C, and the solution was vigorously stirred. The evolution of acetone became apparent within several minutes and heating was continued for a total 30 min. The reaction was cooled and the xylene was removed by evaporator. Distillation of the mixture afforded acetoacetic esters **VI$_{a-d}$** which were used immediately in subsequent reactions (20).

*Synthesis of cyano-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-pyridinecarboxylates (***VIII$_{b-g}$***)*

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and respective acetoactic esters **VI$_{a-d}$** (5 mmol) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_{b-g}$** (35-57%). (Scheme 2)

3-ethyl-5-cyano-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_b$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and ethyl acetoacetate (5 mmol, 0.65 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_b$** (49%).

^{1}H NMR ($CDCl_3$): δ 8.74 (br s, 1H, NH), 7.97 (s, 1H, imidazole H-4), 4.94 (s, 1H, C_4-H), 4.17 (s, 3H, N-CH_3), 3.95 (q, J=7.3 Hz, 2H, CO_2CH_2), 2.50 (s, 3H, C_2-CH_3), 2.06 (s, 3H, C_6-CH_3), 1.14 (t, J=7.3 Hz, 3H, CH_3).

IR (KBr): ν 3358 (NH), 2246 (CN), 1734 (CO_2), 1531, 1307 (NO_2) cm^{-1}.

MS: m/z (%) 331 (M^+, 51), 269(15), 258(100), 205(66), and 177(74).

3-(2-cyanoethyl)-5-cyano-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_c$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and cyanoethyl acetoacetate (5 mmol, 0.775 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_c$** (42%).

^{1}H NMR ($CDCl_3$): δ 8.91 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 4.95 (s, 1H, C_4-H), 4.31 (t, J=6.3 Hz, 2H, CO_2CH_2), 4.13 (s, 3H, N-CH_3), 2.59 (t, J=6.3 Hz, 2H, CH_2-CN), 2.39 (s, 3H, C_2-CH_3) and 2.08 (s, 3H, C_6-CH_3), 1.14 (t, J=7.3 Hz, 3H, CH_3).

IR (KBr): ν 3365 (NH), 2242 (CN), 1759 (CO_2), 1531, 1643 (C=C), 1519 and 1378 (NO_2) cm^{-1}.

MS: m/z (%) 357 (M^+,61), 302(16), 259(100), 177(63) and 131(54).

3-isopropyl-5-Cyano-2,-6-dimethyl-4-(1-methyl-5-nitro-1H-imidazole-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_d$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and isopropyl acetoacetate (5 mmol, 0.72 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_d$** (57%).

^{1}H NMR ($CDCl_3$): δ 8.86 (br s, 1H, NH), 7.98 (s, 1H, imidazole H-4), 5.00 (s, 1H, C_4-H), 4.93 (m, 1H, CO_2CH), 4.17 (s, 3H, N-CH_3), 2.25 (s, 3H, C_2-CH_3), 2.01 (s, 3H, C_6-CH_3), 1.18 and 1.04 (dd, J=5.6 Hz, 3H each, $CH(CH_3)_2$).

IR (KBr): ν 3347 (NH), 2243 (CN), 1716 (CO_2), 1519, 1327 (NO_2) cm^{-1}.

MS: m/z (%) 345 (M^+, 32), 284(16), 258(100), 240(27), 176(88) and 131(51).

3-propyl-5-cyano-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_e$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and N-propyl acetoacetate (5 mmol, 0.72 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_e$** (35%).

^{1}H NMR ($CDCl_3$): δ 9.59 (br s, 1H, NH), 7.91 (s, 1H, imidazole H-4), 5.01 (s, 1H, C_4-H), 4.21 (s, 3H, N-CH_3), 4.03 (s, 2H, CO_2CH_2), 2.51 (s, 3H, C_2-CH_3), 2.11 (s, 3H, C_6-CH_3) 2.51 (m, 2H, $\underline{CH_2}CH_3$) 0.98 (t, J=7.1 Hz, 3H, CH_3).

IR (KBr): ν 3360 (NH), 2251 (CN), 1738 (CO_2), 1536, 1321 (NO_2) cm^{-1}.

MS: m/z (%) 365 (M^+, 11), 291(51), 259(100), 231(30), and 150(31)

3-butyl-5-cyano-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_f$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and N-butyl acetoacetate (5 mmol, 0.79 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_f$** (43%).

^{1}H NMR ($CDCl_3$): δ 9.81 (br s, 1H, NH), 7.82 (s, 1H, imidazole H-4), 4.94 (s, 1H, C_4-H), 4.17 (s, 3H, N-CH_3), 4.11 (t, 2H, CO_2CH_2), 2.49 (s, 3H, C_2-CH_3), 2.15 (s, 3H, C_6-CH_3), 1.62 (m, 2H, $CO_2CH_2\underline{CH_2}$) 1.35 (m, 2H, $CH_2\underline{CH_2}CH_3$) 0.75 (t, J=7.2 Hz, 3H each, CH_3).

IR (KBr): ν 3401 (NH), 2243 (CN), 1745 (CO_2), 1541, 1343 (NO_2) cm^{-1}.

MS: m/z (%) 379 (M^+, 9), 323(28), 259(100), 232(28), 164(16) and 150 (41).

3-methyl-5-cyano-2,6-dimethyl-4-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,4-dihydropyridine-3-carboxylate ***VIII$_g$***

A mixture of 3-aminocrotonitryl **VII** (5 mmol, 0.41 g), 1-methyl-5-nitroimidazolyl-2-carboxaldehyde **I** (5 mmol, 0.77 g) and methyl acetoacetate (5 mmol, 0.68 g) in methanol (25 ml) was refluxed for 12 h with stirring. After cooling, the precipitated product was filtered off, washed with cold methanol, and then dried in vacuo. Recrystallization from methanol gave the appropriate product **VIII$_g$** (53%).

^{1}H NMR ($CDCl_3$): δ 9.61 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 4.95 (s, 1H, C_4-H), 4.16 (s, 3H, N-CH_3), 3.64 (s, 3H, CO_2CH_3), 2.29 (s, 3H, C_2-CH_3), and 2.06 (s, 3H, C_6-CH_3).

IR (KBr): ν 3335 (NH), 2273 (CN), 1732 (CO_2), 1563, 1319 (NO_2) cm^{-1}.

MS: m/z (%) 317(M^+,6), 258(56), 190(100), 159(27), and 131(38).

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