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ORIGINAL PAPER

Synthesis, evaluation of insecticidal activity, and crystal analysis of *cis*-nitenpyram analogs bearing 1,4-dihydropyridine

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Abstract A new series of nitenpyram analogs were designed by introducing 1,4-dihydropyridine to fix the pharmacophore (–C=C–NO_2) into the *cis*-configuration, as confirmed by X-ray diffraction. Crystal structure analysis showed that there was a homoconjugation effect on these *cis*-nitenpyram analogs, and a huge conjugated system comprising the 1,4-dihydropyridine scaffold and the ester group at the 3 position. Preliminary bioassays showed that most of the target compounds exhibit good insecticidal activities (>80 %) at 100 mg/dm^3 against *Aphis medicagini*, while a 4-fluorophenyl *cis*-nitenpyram analog afforded the best activity, with >90 % mortality at 20 mg/dm^3. These excellent insecticidal activities imply that this huge conjugated system results in an enhanced π–π interaction between the molecule and amino acid residues in receptors. Further studies on the mode of action of one of these *cis*-nitenpyram analogs and structural modifications are in progress.

Keywords Nitenpyram analog · 1,4-Dihydropyridine scaffold · Microwave-assisted synthesis · Structure–activity relationships · X-ray structure determination

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Introduction

The discovery of neonicotinoid insecticides (NNs) can be considered a milestone in agrochemical research over the past three decades [1]. NNs have recently received global interest in the fields of agricultural chemistry and medicine due to their broad spectrum of biological activities and high selectivity, which make them applicable to public health applications and crop protection [2]. The first neonicotinoid insecticide to find commercial use was imidacloprid (IMI). Since then, new generations of NNs have entered the market, and they account for one-fifth of the global insecticide market [3–6].

A well-recognized potential problem facing all insecticides is that insect populations gradually develop resistance to each insecticide [7, 8]. During the past decades, significant increases in resistance and cross-resistance have been observed in a range of species after the widespread and frequent use of NNs [9, 10]. In particular, some resistant species have increased in potency, with recently collected strains exhibiting more than 100-fold resistance to imidacloprid and comparable levels of resistance to thiamethoxam and nitenpyram [6, 11]. Therefore, concerted efforts must be made to synthesize new NNs and search for unique molecular recognition and binding modes in order to overcome this increased level of insecticide resistance [12, 13]. However, few studies have been focused on structural modifications of acyclic NNs such as nitenpyram.

As we all know, the electron-withdrawing nature of the nitro group plays an important role in its activities. On the other hand, due to the existence of the C=N double bond, neonicotinoids can exist as two isomers [14]. However, the nitro groups in all commercialized neonicotinoids have a *trans* configuration. Encouraged by the work of Li [15–17], who reported that many *cis*-imidacloprid analogs exhibited

low resistance from insects, we have found a new way to design a novel series of *cis*-nitenpyram analogs [18, 19] with a fixed tetrahydropyrimidine ring that show good insecticidal activities and a different mechanism from their *trans* configuration analogs. These results encouraged us to consider further structural derivatives of them. On the other hand, the reported structures **2** and **3** (Fig. 1), with highly conjugated systems, show relatively strong activities [2, 20]. It is worth pointing out that the *cis*-nitenpyram analogs that we synthesized in advance did not have any huge conjugated system. In 2007, Qian et al. suggested a new binding model that demonstrated the importance of hydrogen bonding and a cooperative π–π interaction between the molecule and amino acid residues [21]. This prompted the question: why not replace the tetrahydropyrimidine ring with a 1,4-dihydropyridine scaffold to fix the nitro group into the *cis*-configuration, thus producing a huge conjugated system, enhancing the π–π interaction, and improving the bioactivity of these new chemical entities?

In the study described in the present paper, *cis*-nitenpyram analogs bearing 1,4-dihydropyridine were synthesized though multicomponent reactions (MCRs), employing nitenpyram, aryl carbonyl compounds, and malononitrile as starting materials, and their insecticidal activity was assessed.

Result and discussion

Preparation of compounds

The nitenpyram analogs **6a–6t** bearing a 1,4-dihydropyridine scaffold were synthesized as shown in Scheme 1. The assembly of **6** occurs via the initial Michael addition of **7** to the ylidenic bond in **1b**, leading to the formation of an acyclic intermediate **8**, which cyclizes into the intermediate **9** via the nucleophilic attack of an NH group on a cyano carbon, followed by tautomerization to the final product **6**. Compound **7** is formed of through a Knoevenagel condensation reaction of malononitrile **4** and the appropriate aromatic aldehyde **5**. The MCRs proceed readily under microwave irradiation (whereas the starting materials must be refluxed in ethanol for 6 h under conventional conditions; see Table 1), thus representing a highly efficient method with good yields (63–82 %) and easy work-up.

Structure–activity relationship

As indicated in Table 2, compared with the *cis*-nitenpyram analogs that have a fixed tetrahydropyrimidine ring, the target compounds with a fixed 1,4-dihydropyridine ring have a broader spectrum of insecticidal activity, ranging from *Nilaparvata legen* to *Aphis medicagini*. Most of our target compounds exhibited significant insecticidal activities against *Aphis medicagini*, with >80 % mortality at 100 mg/dm^3. Among all of the analogs, **6b** showed the strongest (>90 %) activity at 20 mg/dm^3. The activities of the other compounds varied drastically, chiefly depending upon the type of substituent at the 4 position on 1,4-dihydropyridine. In general, as shown in Table 2, the insecticidal activities varied significantly when the doses were reduced to 100 and to 20 mg/dm^3. The introduction of a phenyl ring with electron-withdrawing groups, such as halogen atoms (**6a–6d**, **6g–6l**, **6m–6p**), yielded analogs with excellent inhibitory activities, even at 20 mg/dm^3, whereas appending electron-withdrawing groups such as a

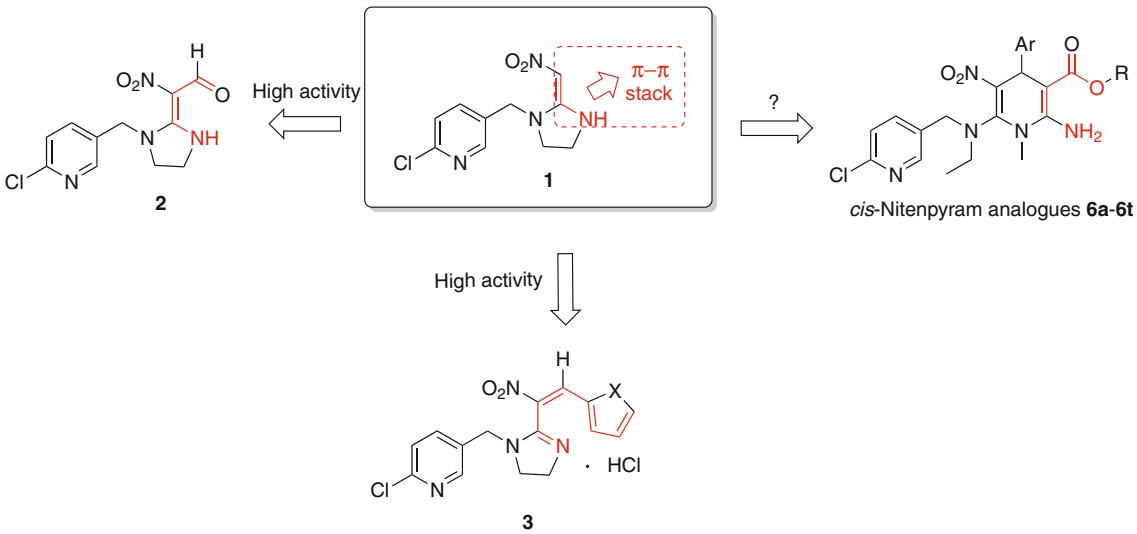
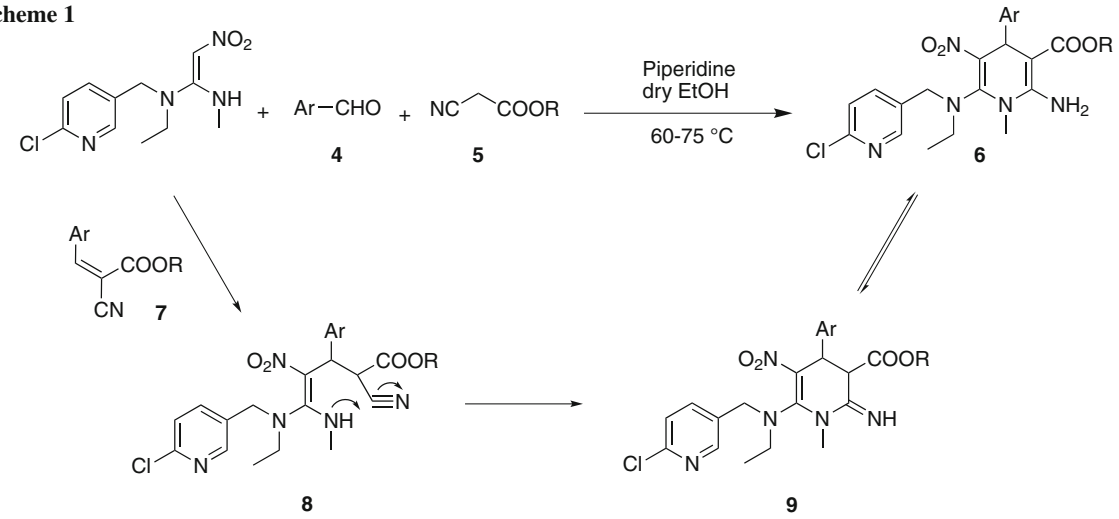


Fig. 1 Our tactic for modifying *cis*-configuration nitenpyram analogs

Scheme 1



methoxy group or methyl (**6e**, **6f**, **6r**–**6t**) had no obvious influence on the insecticide activities of the resulting analogs.

Moreover, the position of the substituent on the phenyl group also influences the insecticidal activity, with inhibitory potency decreasing in the order 4 position (**6k**) > 2 position (**6i**) > 3 position (**6j**), and increasing in the order 3 position (**6m**) > 2 position (**6l**) > 4 position (**6n**). On the other hand, the size of the R substituent does not appear to be as strongly related to the activity. These variations in activity may be related to differences in affinity to the nAChR target, such as π–π stacking interactions with the active-site residues. These results may provide useful information for the design of new insecticides.

Table 1 Yields of the title compounds **6a**–**6t** under conventional conditions and microwave irradiation (Scheme 1)

Compound	R	Ar	Yield/%	
			Conventional	Microwave
6a	Me	3-F–C_6H_4	55.2	71.2
6b	Me	4-F–C_6H_4	57.6	74.7
6c	Me	2,4-di-Cl–C_6H_3	55.4	67.4
6d	Me	3,4-di-Cl–C_6H_3	64.7	73.2.
6e	Me	4-CH_3–C_6H_4	62.4	72.2
6f	Me	2-CH_3O–C_6H_4	67.2	72.2
6g	Et	4-Cl–C_6H_4	61.1	65.5
6h	Pr	C_6H_5	65.8	81.2
6i	Pr	2-F–C_6H_4	64.8	75.7
6j	Pr	3-F–C_6H_4	63.8	72.8
6k	Pr	4-F–C_6H_4	64.7	76.4
6l	Pr	2-Cl–C_6H_4	67.8	75.3
6m	Pr	3-Cl–C_6H_4	65.2	74.9
6n	Pr	4-Cl–C_6H_4	64.5	71.7
6o	Pr	2,4-di-Cl–C_6H_3	64.3	77.2
6p	Pr	3,4-di-Cl–C_6H_3	65.2	79.6
6q	Pr	4-Br–C_6H_4	77.9	82.2
6r	Pr	2-CH_3O–C_6H_4	51.8	83.5
6s	Pr	4-CH_3O–C_6H_4	53.6	78.1
6t	Pr	4-CH_3–C_6H_4	70.3	71.9

Table 2 Insecticidal activities of compounds **6a–6t** and nitenpyram against *Aphis medicagini*

Compound	R	Ar	Concentration/mg dm^{-3}		
			500	100	20
6a	Me	3-F–C_6H_4	++++++	++++	+++
6b	Me	4-F–C_6H_4	++++++	+++++	+++++
6c	Me	2,4-di-Cl–C_6H_3	++++++	++++	++
6d	Me	3,4-di-Cl–C_6H_3	++++++	++++	++
6e	Me	4-CH_3–C_6H_4	++++++	++	nt
6f	Me	2-CH_3O–C_6H_4	++++++	+	nt
6g	Et	4-Cl–C_6H_4	++++++	++++	++
6h	Pr	C_6H_5	++++++	+++	+
6i	Pr	2-F–C_6H_4	++++++	++++	+
6j	Pr	3-F–C_6H_4	++++++	++++	–
6k	Pr	4-F–C_6H_4	++++++	++++	++
6l	Pr	2-Cl–C_6H_4	++++++	++++	+
6m	Pr	3-Cl–C_6H_4	++++++	++++	–
6n	Pr	4-Cl–C_6H_4	++++++	++++	+++
6o	Pr	2,4-di-Cl–C_6H_3	++++++	++++	–
6p	Pr	3,4-di-Cl–C_6H_3	++++++	+++++	+
6q	Pr	4-Br–C_6H_4	++++++	+++++	nt
6r	Pr	2-CH_3O–C_6H_4	++++++	++	–
6s	Pr	4-CH_3O–C_6H_4	++++++	+++	–
6t	Pr	4-CH_3–C_6H_4	++++++	+++	–
1			++++++	++++++	++++++

Rating system for the mortality percentage: ++++++, 100 %; +++++, ≥90 %; ++++, ≥80 %; +++, ≥70 %; ++, ≥60 %; +, ≥50 %; –, <50 %

nt not tested

*Single-crystal structure of compound **6g***

To confirm the *cis* configuration using precise three-dimensional information, the single-crystal structure of compound **6g** (Figs. 2, 3) was investigated and determined by X-ray diffraction analysis (CCDC number: 824407). The dihedral angle of N2–C9–C13–N4 in the two independent molecules is 32.0(7)°, so the nitro group in **6g** is obviously in the *cis* configuration, as anticipated. In addition, the 1,4-dihydropyridine ring of compound **6g** adopts a skew boat conformation. Interestingly, the C–C bond lengths in the 1,4-dihydropyridine ring, such as 1.507(5) Å (C(12)–C(11)) and 1.507(6) Å (C(12)–C(13)), are obviously shorter than the pure C–C single bond (1.54 Å) [22]. Similarly, the bond lengths of C–N on the 1,4-dihydropyridine ring, such as 1.403(5) (C(10)–N(3)) and 1.382(5) Å (C(9)–N(3)), are obviously shorter than that of the pure C–N single bond (1.47 Å) [23]. On the

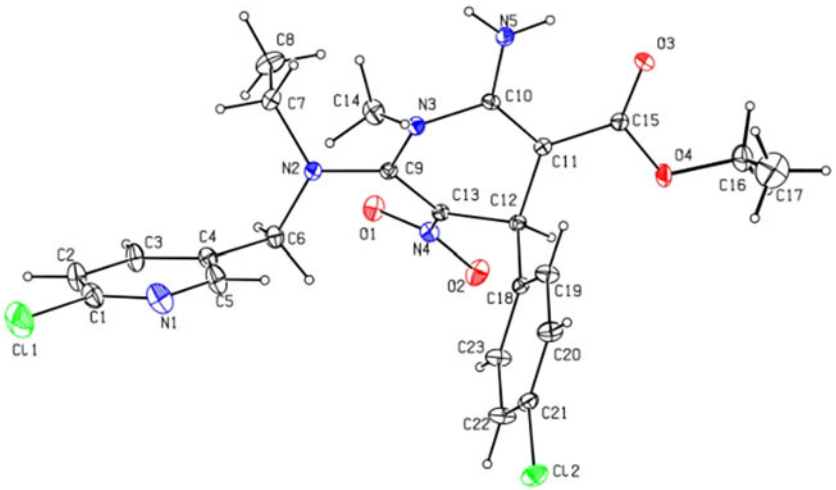


Fig. 2 Molecular structure of compound **6g**, with atom labeling

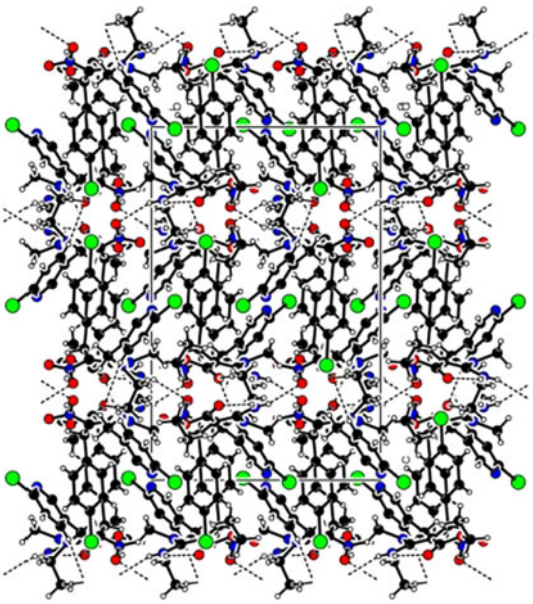


Fig. 3 Packing diagram of compound **6g**

contrary, the bond lengths of C(13)=C(9) and C(11)=C(10) (1.386(6) and 1.364(6) Å, respectively) are longer than that of the pure C=C bond (1.34 Å) [15]. Based on these data, there is a homoconjugation effect on the 1,4-dihydropyridine scaffold in **6g**, and a huge conjugated system is formed by the 1,4-dihydropyridine scaffold and the ester group at the 3 position.

Conclusions

In summary, a new series of *cis*-configuration nitenpyram analogs with a fixed 1,4-dihydropyridine scaffold were synthesized and tested for insecticidal activity. Crystal structure analysis showed that there is a homoconjugation effect on the 1,4-dihydropyridine scaffold in compound **6g**, which formed a huge conjugated system with the ester group at the 3 position. Preliminary bioassays showed that most of the target compounds exhibited good insecticidal activity at 100 mg/dm^3 against *Aphis medicagini*, while **6a** exhibited the best inhibitory activity, displaying 90 % mortality at 20 mg/dm^3. These bioassay data confirm that the huge conjugated system results in an enhanced π–π interaction between the molecule and amino acid residues in receptors, which plays a positive role in the promotion of insecticidal activity. Moreover, compared with other *cis*-nitenpyram analogs with a fixed tetrahydropyrimidine ring that we designed previously, their spectrum of insecticidal activity is broader, ranging from *Nilaparvata legen* to *Aphis medicagini*. Further studies on the mode of action (MoA) of **6g** and structural modifications are in progress.

Experimental

All reactions were performed in oven-dried glassware with magnetic stirring. All of the chemical reagents purchased were of analytical grade and used without further purification, except for toluene, which was dried by refluxing in the presence of sodium and distilled prior to use. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany). ^{1}H NMR spectra were recorded on a Bruker (Rheinstetten, Germany) Avance 400 (400 MHz) spectrometer, using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. The chemical shift values (δ) are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard; s = singlet, d = doublet, m = multiplet. Melting points were determined by an RK1 microscopic melting apparatus. IR spectra were obtained on a Nicolet (Madison, WI, USA) 5DX FT-IR spectrophotometer in the region 4,000–400 cm^{-1} using KBr discs. MS spectra were recorded on a Finnigan (San Jose, CA, USA) Trace DSQ mass spectrograph.

*General procedure for synthesis of **6a–6t***

A mixture of methyl cyanoacetate (12 mmol), 3-fluorobenzaldehyde (12 mmol), piperidine (0.1 mmol), and nitenpyram (10 mmol) in 20 cm^3 anhydrous alcohol was heated to 60–75 °C for 5 min in a microwave reactor and stirred for 30 min at 65 °C. The reaction mixture was concentrated under reduced pressure and treated with 20 cm^3 of water. The solution was extracted three times with ethyl acetate, and the combined extracts were dried over $MgSO_4$. The organic phase was evaporated under reduced pressure, and the crude product was subjected to flash chromatography on silica gel, eluting with ethyl acetate/petroleum ether (3:1 v/v) to afford the pure product **6a**. Compounds **6b–6t** were synthesized analogously.

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(3-fluorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6a**, $C_{22}H_{23}ClFN_5O_4$)
Yield 71.2 %; yellow solid; m.p.: 154–155 °C; R_f = 0.60 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20 (d, 1H, J = 16.8 Hz, Py-H), 7.82 (s, 1H, Py-H), 7.19 (d, 1H, J = 16.8 Hz, Py-H), 7.15–7.10 (m, 3H, Ph-H), 6.95 (d, 1H, J = 16.8 Hz, Ph-H), 6.21 (s, 2H, NH_2), 5.68 (s, 1H, CH), 4.38 (d, J = 14.8 Hz, 1H, Py-CH_2), 4.12 (d, 2H, J = 14.9 Hz, Py-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.27–3.23 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.10 (m, 1H, NCH_2CH_3), 1.32 (t, J = 6.8 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 25.5, 29.9, 42.4, 50.8, 51.1, 82.2, 91.1, 114.5, 115.5, 122.2, 123.5, 131.3, 132.1, 138.7, 144.2, 145.3, 148.8, 151.3, 163.1,

166.8, 169.4 ppm; IR (KBr): $\bar{\nu}$ = 3,311, 3,222, 2,984, 2,935, 2,870, 1,343, 1,299, 1,235 cm^{-1}; HRMS: *m/z* = 476.1423 [$(M + H)^+$].

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(4-fluorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6b**, $C_{22}H_{23}ClFN_5O_4$)
Yield 74.7 %; yellow solid; m.p.: 161–163 °C; R_f = 0.58 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (d, 1H, *J* = 16.8 Hz, Py-H), 7.82 (s, 1H, Py-H), 7.19 (d, 1H, *J* = 16.8 Hz, Py-H), 7.15–7.10 (m, 2H, Ph-H), 6.95–6.93 (m, 2H, Ph-H), 6.23 (s, 2H, NH_2), 5.67 (s, 1H, CH), 4.38 (d, *J* = 14.8 Hz, 1H, Py-CH_2), 4.12 (d, 2H, *J* = 14.9 Hz, Py-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.27–3.23 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.10 (m, 1H, NCH_2CH_3), 1.32 (t, *J* = 6.8 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.9, 24.5, 28.5, 42.1, 50.8, 51.1, 82.2, 91.1, 114.9, 116.1, 122.2, 123.5, 131.3, 133.6, 136.7, 144.2, 146.3, 148.8, 151.3, 163.1, 165.2, 168.4 ppm; IR (KBr): $\bar{\nu}$ = 3,311, 3,222, 2,984, 2,935, 2,870, 1,343, 1,299, 1,235 cm^{-1}; HRMS: *m/z* = 476.1423 [$(M + H)^+$].

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(2,4-dichlorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6c**, $C_{22}H_{22}Cl_3N_5O_4$)
Yield 67.4 %; yellow solid; m.p.: 203–204 °C; R_f = 0.60 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.18 (s, 1H, Py-H), 7.82 (s, 1H, Py-H), 7.19 (d, 1H, *J* = 16.8 Hz, Py-H), 7.16–7.14 (m, 1H, Ph-H), 7.07 (d, *J* = 8.1 Hz, 1H, Ph-H), 6.89 (m, 1H, Ph-H), 6.21 (s, 2H, NH_2), 5.68 (s, 1H, CH), 4.38 (d, *J* = 14.8 Hz, 1H, Py-CH_2), 4.12 (d, 2H, *J* = 14.9 Hz, Py-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.27–3.23 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.10 (m, 1H, NCH_2CH_3), 1.32 (t, *J* = 6.8 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.8, 24.5, 28.6, 43.2, 50.8, 52.5, 83.2, 91.1, 114.9, 116.1, 122.2, 126.3, 131.3, 134.3, 135.5, 144.2, 146.3, 148.8, 149.1, 155.2, 167.2, 169.4 ppm; IR (KBr): $\bar{\nu}$ = 3,311, 3,256, 2,975, 2,921, 2,870, 1,343, 1,301, 1,247 cm^{-1}; HRMS: *m/z* = 527.0737 [$(M + H)^+$].

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(3,4-dichlorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6d**, $C_{22}H_{23}Cl_3N_5O_4$)
Yield 73.2 %; yellow solid; m.p.: 180–182 °C; R_f = 0.61 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1H, Py-H), 7.82 (s, 1H, Py-H), 7.19 (s, 1H, Py-H), 7.16–7.12 (m, 1H, Ph-H), 7.06 (d, 1H, *J* = 8.1 Hz, 1H, Ph-H), 6.24 (s, 2H, NH_2), 5.67 (s, 1H, CH), 4.34 (d, *J* = 14.3 Hz, 1H, Py-CH_2), 4.12 (d, 2H, *J* = 14.9 Hz, Py-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.27–3.23 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.10 (m, 1H, NCH_2CH_3), 1.32 (t, *J* = 6.8 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 24.5, 28.5, 42.1, 50.8, 51.0, 84.3, 91.1, 114.9, 116.1, 123.9, 124.5, 131.3, 133.6, 136.7, 144.2, 146.3, 149.9, 151.3, 163.1, 164.2, 167.4 ppm; IR (KBr): $\bar{\nu}$ = 3,311, 3,222, 2,984, 2,935, 2,870, 1,343, 1,299, 1,235 cm^{-1}; HRMS: *m/z* = 527.0737 [$(M + H)^+$].

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-1-methyl-4-(4-methylphenyl)-5-nitro-3-pyridinecarboxylate (**6e**, $C_{23}H_{26}ClN_5O_4$)
Yield 72.2 %; yellow solid; m.p.: 173–175 °C; R_f = 0.62 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (s, 1H, Py-H), 7.76 (s, 1H, Py-H), 7.32 (d, *J* = 7.6 Hz, 1H, Py-H), 7.08 (d, *J* = 8.2 Hz, 2H, Ph-H), 6.93 (d, *J* = 8.2 Hz, 2H, Ph-H), 6.13 (s, 2H, NH_2), 5.42 (s, 1H, CH), 4.21 (d, *J* = 14.4 Hz, 1H, Py-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.31–3.22 (m, 1H, NCH_2CH_3), 3.17 (s, 3H, NCH_3), 3.11 (m, 1H, NCH_2CH_3), 2.62 (s, 3H, Ph-CH_3), 1.34 (t, *J* = 7.1 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 26.5, 26.9, 42.1, 45.8, 53.8, 56.7, 82.2, 91.1, 114.9, 116.1, 119.7, 120.8, 122.5, 133.6, 136.7, 143.8, 146.3, 148.8, 151.3, 163.1, 164.8, 167.4 ppm; IR (KBr): $\bar{\nu}$ = 3,327, 3,200, 2,984, 2,935, 2,870, 1,343, 1,308, 1,235 cm^{-1}; HRMS: *m/z* = 472.9417 [$(M + H)^+$].

Methyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-4-(2-methoxyphenyl)-1-methyl-5-nitro-3-pyridinecarboxylate (**6f**, $C_{23}H_{26}ClN_5O_5$)
Yield 69.1 %; yellow solid; m.p.: 134–135 °C; R_f = 0.60 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1H, Py-H), 7.81 (s, 1H, Py-H), 7.27 (s, 1H, Py-H), 7.17–7.10 (m, 3H, Ph-H), 6.95–6.94 (m, 1H, Ph-H), 6.24 (s, 2H, NH_2), 5.49 (s, 1H, CH), 4.36 (d, *J* = 14.5 Hz, 1H, Py-CH_2), 4.07–4.03 (m, 1H, Py-CH_2), 3.80 (s, 3H, OCH_3), 3.65 (s, 3H, $COOCH_3$), 3.23–3.11 (m, 5H, NCH_3, NCH_2CH_3), 3.19–3.09 (m, 2H), 3.11 (dd, *J* = 13.7, 6.9 Hz, 1H), 1.21 (t, *J* = 7.1 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.9, 24.5, 28.5, 42.1, 50.8, 51.1, 84.3, 94.5, 109.3, 116.1, 121.9, 122.7, 131.3, 133.6, 139.2, 142.5, 144.2, 146.3, 147.2, 151.3, 163.1, 165.2, 166.4 ppm; IR (KBr): $\bar{\nu}$ = 3,327, 3,200, 2,984, 2,935, 2,870, 1,343, 1,308, 1,235 cm^{-1}; HRMS: *m/z* = 488.9325 [$(M + H)^+$].

Ethyl 2-amino-4-(4-chlorophenyl)-6-[(6-chloro-3-pyridinylmethyl)ethylamino]-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6g**, $C_{23}H_{26}ClN_5O_5$)
Yield 65.5 %; yellow solid; m.p.: 134–135 °C; R_f = 0.63 (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1H, Py-H), 7.81 (s, 1H, Py-H), 7.27 (s, 1H, Py-H), 7.10 (d, *J* = 8.2 Hz, 2H, Ph-H), 6.93 (d, *J* = 8.2 Hz, 2H, Ph-H), 6.24 (s, 2H, NH_2), 5.49 (s, 1H, CH), 4.36 (d, *J* = 14.5 Hz, 1H, Py-CH_2), 4.14–4.08 (m, 2H, $COOCH_2CH_3$), 4.07–4.03 (m, 1H, Py-CH_2), 3.23 (s, 3H, NCH_3), 3.19 (d, *J* = 7.3 Hz, 1H, NCH_2CH_3), 3.07 (dd,

J = 13.7, 6.9 Hz, 1H), 1.30 (dd, J = 13.3, 6.2 Hz, 3H, $COOCH_2CH_3$), 1.21 (t, J = 7.1 Hz, 3H, NCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.7, 25.1, 29.4, 42.4, 50.9, 51.5, 61.9, 83.1, 91.6, 115.1, 114.9, 122.3, 122.9, 131.3, 132.1, 138.7, 144.3, 145.7, 148.2, 151.7, 163.4, 167.2, 168.5 ppm; IR (KBr): $\bar{\nu}$ = 3,384, 3,298, 3,065, 2,981, 2,933, 1,337, 1,294, 1,267 cm^{-1}; HRMS: m/z = 505.1321 [$(M + H)^+$].

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-1-methyl-5-nitro-4-phenyl-3-pyridinecarboxylate (**6h**, $C_{24}H_{28}ClN_5O_4$)
Yield 81.2 %; yellow solid; m.p.: 167–168 °C; R_f = 0.62 (ethyl acetate/petroleum ether 3:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.08 (s, 1H, Py-H), 7.82 (s, 1H, Py-H), 7.19 (m, 3H, Ph-H), 7.05 (s, 2H, Ph-H), 6.98 (s, 1H, Py-H), 6.19 (s, 2H, NH_2), 5.49 (s, 1H, CH), 4.32 (d, J = 14.6 Hz, 1H, Py-CH_2), 4.16–4.12 (m, 2H, $COOCH_2CH_2CH_3$), 4.06 (d, J = 14.8 Hz, 1H, Py-CH_2), 3.27–3.23 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.10 (dt, J = 13.9, 7.0 Hz, 1H, NCH_2CH_3), 1.59 (d, J = 7.0 Hz, 2H, $COOCH_2CH_2CH_3$), 1.23 (t, J = 7.0 Hz, 3H, NCH_2CH_3), 0.82 (t, J = 7.4 Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.3, 13.5, 22.1, 26.5, 29.2, 41.9, 50.6, 67.9, 80.1, 90.2, 123.4, 123.5, 125.8, 128.7, 128.7, 131.4, 137.8, 138.5, 145.9, 149.4, 150.3, 159.9, 167.2, 170.4 ppm; IR (KBr): $\bar{\nu}$ = 3,327, 3,200, 2,984, 2,935, 2,870, 1,343, 1,308, 1,235 cm^{-1}; HRMS: m/z = 486.9641 [$(M + H)^+$].

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(2-fluorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6i**, $C_{24}H_{27}ClFN_5O_4$)
Yield 69.5 %; yellow solid; m.p.: 121–123 °C; R_f = 0.61 (ethyl acetate/petroleum ether 3:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.26 (s, 1H, Py-H), 7.87 (s, 1H, Py-H), 7.35 (d, J = 7.8 Hz, 1H, Py-H), 7.18 (d, J = 7.2 Hz, 1H, Ph-H), 7.05 (t, J = 7.8 Hz, 2H, Ph-H), 6.92–6.85 (m, 1H, Ph-H), 6.20 (s, 2H, NH_2), 5.38 (s, 1H, CH), 4.35 (d, J = 14.4 Hz, 1H, Py-CH_2), 4.14–3.99 (m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.37–3.35 (m, 1H, NCH_2CH_3), 3.30 (s, 3H, NCH_3), 3.19–3.07 (m, 1H, NCH_2CH_3), 1.64 (dd, J = 7.1, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 0.88 (t, J = 7.3 Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 12.5, 23.5, 27.4, 29.2, 41.9, 50.6, 67.9, 81.2, 92.3, 115.4, 115.4, 125.8, 130.7, 130.7, 132.7, 138.9, 140.2, 145.9, 149.4, 150.3, 159.9, 167.2, 169.5 ppm; IR (KBr): $\bar{\nu}$ = 3,371, 3,277, 2,972, 2,912, 2,855, 1,356, 1,299, 1,244 cm^{-1}; HRMS: m/z = 504.1679 [$(M + H)^+$].

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(3-fluorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6j**, $C_{24}H_{27}ClFN_5O_4$)
Yield 82.5 %; yellow solid; m.p.: 145–147 °C; R_f = 0.60 (ethyl acetate/petroleum ether 3:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.21 (s, 1H, Py-H), 7.76 (s, 1H, Py-H), 7.35 (d, J = 7.8 Hz, 1H, Py-H), 7.18 (d, J = 7.2 Hz, 1H, Ph-H), 7.05–6.95 (m, 3H, Ph-H), 6.20 (s, 2H, NH_2), 5.37 (s, 1H, CH), 4.35 (d, J = 14.4 Hz, 1H, Py-CH_2), 4.14–3.99 (m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.37–3.35 (m, 1H, NCH_2CH_3), 3.30 (s, 3H, NCH_3), 3.19–3.08 (m, 1H, NCH_2CH_3), 1.64 (dd, J = 7.1, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 0.87 (t, J = 7.2 Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9, 12.1, 22.8, 28.2, 29.9, 43.5, 51.2, 68.5, 83.1, 92.9, 115.9, 115.9, 124.8, 131.5, 131.5, 132.3, 140.4, 142.7, 147.7, 148.4, 153.2, 159.1, 167.9, 170.2 ppm; IR (KBr): $\bar{\nu}$ = 3,371, 3,277, 2,972, 2,912, 2,855, 1,356, 1,299, 1,244 cm^{-1}; HRMS: m/z = 504.167 [$(M + H)^+$].

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(4-fluorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6k**, $C_{24}H_{27}ClFN_5O_4$)
Yield 65.0 %; yellow solid; m.p.: 126–127 °C; R_f = 0.57 (ethyl acetate/petroleum ether 3:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.12 (s, 1H, Py-H), 7.82 (s, 1H, Py-H), 7.35 (d, J = 7.6 Hz, 1H, Py-H), 7.17–7.15 (m, 2H, Ph-H), 6.98–6.96 (m, 2H, Ph-H), 6.21 (s, 2H, NH_2), 5.43 (s, 1H, CH), 4.32 (d, J = 14.4 Hz, 1H, Py-CH_2), 4.06 (dt, J = 13.1, 5.2 Hz, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.32–3.23 (m, 1H, NCH_2CH_3), 3.21 (s, 3H, NCH_3), 3.14 (dt, J = 14.2, 7.2 Hz, 1H, NCH_2CH_3), 1.59 (dd, J = 13.9, 7.3 Hz, 2H, $COOCH_2CH_2CH_3$), 1.31 (dd, J = 12.4, 5.3 Hz, 3H, NCH_2CH_3), 0.83 (t, J = 7.4 Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.7, 13.1, 22.9, 27.9, 28.2, 41.2, 51.6, 68.8, 81.1, 92.1, 116.3, 116.3, 124.9, 131.4, 131.4, 133.6, 137.9, 141.1, 146.2, 149.7, 151.1, 159.1, 168.1, 168.7 ppm; IR (KBr): $\bar{\nu}$ = 3,369, 3,273, 2,977, 2,933, 2,855, 1,356, 1,311, 1,244 cm^{-1}; HRMS: m/z = 504.1679 [$(M + H)^+$].

Propyl 2-amino-4-(2-chlorophenyl)-6-[(6-chloro-3-pyridinylmethyl)ethylamino]-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6l**, $C_{24}H_{27}Cl_2N_5O_4$)
Yield 64.7 %; yellow solid; m.p.: 157–158 °C; R_f = 0.59 (ethyl acetate/petroleum ether 3:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.20 (s, 1H, Py-H), 7.83 (s, 1H, Py-H), 7.34 (s, 1H, Py-H), 7.23 (d, J = 6.9 Hz, 1H, Ph-H), 7.13 (dd, J = 16.4, 8.1 Hz, 2H, Ph-H), 7.04 (d, J = 8.0 Hz, 1H, Ph-H), 6.26 (s, 2H, NH_2), 5.71 (s, 1H, CH), 4.37 (d, J = 14.3 Hz, 1H, Py-CH_2), 4.17–4.11 (m, 3H, Py-CH_2,

$COOCH_2$ CH_2 CH_3), 3.33–3.31 (m, 1H, NCH_2CH_3), 3.25 (s, 3H, NCH_3), 3.10–3.05 (m, 1H, NCH_2CH_3), 1.63 (dd, $J = 7.0$, 3.6 Hz, 2H, $COOCH_2CH_2CH_3$), 1.33 (t, $J = 6.9$ Hz, 3H, NCH_2CH_3), 0.84 (t, $J = 7.4$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.7$, 12.5, 23.5, 27.9, 29.4, 41.1, 51.3, 67.3, 81.5, 92.1, 114.9, 114.7, 125.2, 130.5, 130.6, 132.1, 138.1, 141.2, 145.3, 148.8, 151.2, 159.4, 166.5, 169.4 ppm; IR (KBr): $\bar{\nu} = 3{,}365$, 3,241, 2,966, 2,912, 2,855, 1,356, 1,303, 1,255 cm^{-1}; HRMS: $m/z = 520.4098$ $[(M + H)^+]$.

Propyl 2-amino-4-(3-chlorophenyl)-6-[(6-chloro-3-pyridinylmethyl)ethylamino]-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6m**, $C_{24}H_{27}Cl_2N_5O_4$)
Yield 66.7 %; yellow solid; m.p.: 164–165 °C; $R_f = 0.60$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.10$ (s, 1H, Py-H), 7.82 (d, 1H, $J = 8.0$ Hz, Py-H), 7.31 (s, 1H, Py-H), 7.19–7.18 (m, 3H, Ph-H), 6.98 (d, $J = 8.0$ Hz, 1H, Ph-H), 6.11 (s, 2H, NH_2), 5.49 (s, 1H, CH), 4.32 (d, $J = 14.6$ Hz, 1H, Py-CH_2), 4.16–4.09 (m, 2H, $COOCH_2CH_2CH_3$), 4.06 (d, $J = 14.8$ Hz, 1H, Py-CH_2), 3.27–3.20 (m, 1H, NCH_2CH_3), 3.16 (s, 3H, NCH_3), 3.09 (m, 1H, NCH_2CH_3), 1.63 (m, 2H, $COOCH_2CH_2CH_3$), 1.31 (t, $J = 7.0$ Hz, 3H, NCH_2CH_3), 0.84 (t, $J = 7.4$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.4$, 11.9, 23.5, 27.4, 29.2, 41.5, 51.1, 67.9, 81.9, 92.8, 115.2, 115.4, 125.8, 131.2, 131.1, 133.3, 138.9, 140.2, 146.6, 149.5, 150.5, 159.9, 166.2, 169.8 ppm; IR (KBr): $\bar{\nu} = 3{,}370$, 3,241, 2,944, 2,905, 2,855, 1,356, 1,311, 1,268 cm^{-1}; HRMS: $m/z = 520.4098$ $[(M + H)^+]$.

Propyl 2-amino-4-(4-chlorophenyl)-6-[(6-chloro-3-pyridinylmethyl)ethylamino]-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6n**, $C_{24}H_{27}Cl_2N_5O_4$)
Yield 75.8 %; yellow solid; m.p.: 132–133 °C; $R_f = 0.63$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.12$ (s, 1H, Py-H), 7.82 (s, 1H, Py-H), 7.35 (d, $J = 7.6$ Hz, 1H, Py-H), 7.12 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.96 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.21 (s, 2H, NH_2), 5.43 (s, 1H, CH), 4.32 (d, $J = 14.4$ Hz, 1H, Py-CH_2), 4.06–4.01 (m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.32–3.23 (m, 1H, NCH_2CH_3), 3.21 (s, 3H, NCH_3), 3.14 (m, 1H, NCH_2CH_3), 1.59 (dd, $J = 13.9$, 7.3 Hz, 2H, $COOCH_2CH_2CH_3$), 1.31 (dd, $J = 12.4$, 5.3 Hz, 3H, NCH_2CH_3), 0.83 (t, $J = 7.4$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.1$, 12.5, 23.7, 27.5, 28.2, 41.5, 50.1, 68.2, 81.7, 92.7, 115.9, 115.7, 125.5, 130.2, 130.1, 132.7, 138.9, 141.1, 145.7, 149.9, 151.2, 159.5, 167.7, 169.8 ppm; IR (KBr): $\bar{\nu} = 3{,}361$, 3,241, 2,944, 2,905, 2,877, 1,356, 1,311, 1,263 cm^{-1}; HRMS: $m/z = 520.4098$ $[(M + H)^+]$.

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(2,4-dichlorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6o**, $C_{24}H_{26}Cl_3N_5O_4$)
Yield: 77.2 %; yellow solid; m.p.: 136–138 °C; $R_f = 0.61$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.27$ (s, 1H, Py-H), 7.78 (s, 1H, Py-H), 7.35 (s, 1H, Py-H), 7.14 (d, $J = 14.2$ Hz, 2H, Ph-H), 6.83 (s, 1H, Ph-H), 6.24 (s, 2H, NH_2), 5.32 (d, $J = 13.2$ Hz, 1H, CH), 4.42 (d, $J = 13.2$ Hz, 1H), 4.13–4.02 (m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.30–3.25 (m, 4H, NCH_3, NCH_2CH_3), 3.14 (d, $J = 2.0$ Hz, 1H, NCH_2CH_3), 1.64 (dd, $J = 7.1$, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, $J = 7.1$ Hz, 3H, NCH_2CH_3), 0.88 (t, $J = 7.3$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.4$, 11.6, 23.2, 27.9, 30.1, 42.2, 49.9, 67.9, 81.2, 92.2, 115.9, 115.9, 125.8, 130.2, 130.3, 131.5, 138.5, 141.2, 145.4, 149.3, 150.3, 159.8, 167.2, 170.2 ppm; IR (KBr): $\bar{\nu} = 3{,}320$, 3,211, 2,951, 1,477, 1,413, 1,552, 152, 1,577 cm^{-1}; HRMS: $m/z = 555.8533$ $[(M + H)^+]$.

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-4-(3,4-dichlorophenyl)-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6p**, $C_{24}H_{26}Cl_3N_5O_4$)
Yield 79.6 %; yellow solid; m.p.: 175–177 °C; $R_f = 0.59$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.33$ (d, $J = 8.0$ Hz, 1H, Py-H), 7.84 (s, 1H), 7.37 (s, 1H), 7.20–7.17 (m, 2H, Ph-H), 6.93–6.77 (m, 1H, Ph-H), 6.24 (s, 2H, NH_2), 5.30 (s, 1H, CH), 4.44 (s, 1H, Py-CH_2), 4.09 (dt, $J = 13.1$, 5.2 Hz, 1H, Py-CH_2), 4.05–4.03 (m, 2H, $COOCH_2CH_2CH_3$), 3.31–3.18 (m, 4H, NCH_3, NCH_2CH_3), 3.10 (m, 1H, NCH_2CH_3), 1.59 (dd, $J = 13.9$, 7.3 Hz, 2H, $COOCH_2CH_2CH_3$), 1.28 (dd, $J = 13.5$, 6.4 Hz, 3H, NCH_2CH_3), 0.83 (t, $J = 7.4$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.3$, 12.3, 23.9, 26.8, 29.2, 41.9, 51.2, 67.9, 81.1, 92.3, 116.1, 116.3, 125.7, 130.7, 130.8, 132.1, 137.9, 140.5, 145.6, 149.5, 151.1, 159.3, 167.3, 169.5 ppm; IR (KBr): $\bar{\nu} = 3{,}322$, 3,199, 2,963, 1,652, 1,612, 1,577, 1,457, 1,417 cm^{-1}; HRMS: $m/z = 555.8533$ $[(M + H)^+]$.

Propyl 2-amino-4-(4-bromophenyl)-6-[(6-chloro-3-pyridinylmethyl)ethylamino]-1,4-dihydro-1-methyl-5-nitro-3-pyridinecarboxylate (**6q**, $C_{24}H_{27}BrClN_5O_4$)
Yield 82.2 %; yellow solid; m.p.: 179–181 °C; $R_f = 0.58$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.11$ (s, 1H, Py-H), 7.77 (s, 1H, Py-H), 7.34 (d, $J = 7.6$ Hz, 1H, Py-H), 7.10 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.95 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.11 (s, 2H, NH_2), 5.43 (s, 1H, CH), 4.25 (d, $J = 14.4$ Hz, 1H, Py-CH_2), 4.01–3.95

(m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.31–3.22 (m, 1H, NCH_2CH_3), 3.17 (s, 3H, NCH_3), 3.11 (m, 1H, NCH_2CH_3), 1.60 (dd, $J = 13.9$, 7.3 Hz, 2H, $COOCH_2CH_2CH_3$), 1.25 (dd, $J = 12.4$, 5.3 Hz, 3H, NCH_2CH_3), 0.82 (t, $J = 7.4$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.3$, 12.9, 22.8, 27.1, 29.9, 40.9, 51.2, 68.7, 81.5, 92.5, 115.9, 115.8, 126.3, 130.5, 130.4, 132.2, 138.3, 141.1, 144.9, 150.3, 150.9, 159.5, 167.5, 168.7 ppm; IR (KBr): $\bar{\nu} = 3{,}355$, 3,233, 2,935, 2,905, 2,877, 1,366, 1,311, 1,261 cm^{-1}; HRMS: $m/z = 564.8576$ $[(M + H)^+]$.

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-4-(2-methoxyphenyl)-1-methyl-5-nitro-3-pyridinecarboxylate (**6r**, $C_{25}H_{30}ClN_5O_5$)
Yield 83.5 %; yellow solid; m.p.: 192–194 °C; $R_f = 0.63$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (s, 1H, Py-H), 7.81 (s, 1H, Py-H), 7.27 (s, 1H, Py-H), 7.22 (d, $J = 8.0$ Hz, 2H, Ph-H), 7.03 (d, $J = 8.0$ Hz, 2H, Ph-H), 6.24 (s, 2H, NH_2), 5.68 (s, 1H, CH), 4.36 (d, $J = 14.5$ Hz, 1H, Py-CH_2), 4.09 (s, 1H, Py-CH_2), 4.05–4.03 (m, 2H, $COOCH_2CH_2CH_3$), 3.80 (s, 3H, OCH_3), 3.27–3.25 (m, 1H, NCH_2CH_3), 3.23 (s, 3H, NCH_3), 3.11 (dd, $J = 13.7$, 6.9 Hz, 1H, NCH_2 CH_3), 1.64 (dd, $J = 7.1$, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, $J = 7.1$ Hz, 3H, NCH_2 CH_3), 0.88 (t, $J = 7.3$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.1$, 12.5, 23.5, 27.4, 29.2, 44.5, 46.2, 50.6, 67.9, 83.2, 92.3, 114.3, 117.4, 127.6, 130.7, 133.7, 135.8, 139.9, 145.4, 146.9, 147.9, 154.1, 157.9, 168.2, 169.9 ppm; IR (KBr): $\bar{\nu} = 3{,}323$, 3,211, 2,963, 1,652, 1,612, 1,577, 1,477, 1,413 cm^{-1}; HRMS: $m/z = 516.9875$ $[(M + H)^+]$.

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-4-(4-methoxyphenyl)-1-methyl-5-nitro-3-pyridinecarboxylate (**6s**, $C_{25}H_{30}ClN_5O_5$)
Yield 78.1 %; yellow solid; m.p.: 201–202 °C; $R_f = 0.62$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (s, 1H, Py-H), 7.81 (s, 1H, Py-H), 7.27 (s, 1H, Py-H), 7.22 (d, $J = 8.0$ Hz, 2H, Ph-H), 7.03 (d, $J = 8.0$ Hz, 2H, Ph-H), 6.24 (s, 2H, NH_2), 5.68 (s, 1H, CH), 4.36 (d, $J = 14.5$ Hz, 1H, Py-CH_2), 4.09 (s, 1H, Py-CH_2), 4.05–4.03 (m, 2H, $COOCH_2CH_2CH_3$), 3.80 (s, 3H, OCH_3), 3.27–3.25 (m, 1H, NCH_2CH_3), 3.23 (s, 3H, NCH_3), 3.11 (dd, $J = 13.7$, 6.9 Hz, 1H, NCH_2CH_3), 1.64 (dd, $J = 7.1$, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, $J = 7.1$ Hz, 3H, NCH_2CH_3), 0.88 (t, $J = 7.3$ Hz, 3H, $COOCH_2CH_2$ CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.3$, 12.5, 23.5, 27.4, 29.2, 44.5, 48.9, 50.6, 67.9, 83.2, 92.3, 114.3, 117.4, 125.8, 130.7, 131.4, 132.7, 139.9, 143.2, 146.9, 147.9, 154.1, 157.9, 168.2, 169.5 ppm; IR (KBr): $\bar{\nu} = 3{,}323$, 3,211, 2,963, 1,477, 1,413, 1,652, 1,612, 1,577 cm^{-1}; HRMS: $m/z = 516.9875$ $[(M + H)^+]$.

Propyl 2-amino-6-[(6-chloro-3-pyridinylmethyl)-ethylamino]-1,4-dihydro-1-methyl-4-(4-methylphenyl)-5-nitro-3-pyridinecarboxylate (**6t**, $C_{25}H_{30}ClN_5O_4$)
Yield 71.9 %; yellow solid; m.p.: 189–190 °C; $R_f = 0.59$ (ethyl acetate/petroleum ether 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.10$ (s, 1H, Py-H), 7.76 (s, 1H, Py-H), 7.32 (d, $J = 7.6$ Hz, 1H, Py-H), 7.08 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.93 (d, $J = 8.2$ Hz, 2H, Ph-H), 6.13 (s, 2H, NH_2), 5.42 (s, 1H, CH), 4.21 (d, $J = 14.4$ Hz, 1H, Py-CH_2), 4.01–3.95 (m, 3H, Py-CH_2, $COOCH_2CH_2CH_3$), 3.31–3.22 (m, 1H, NCH_2CH_3), 3.17 (s, 3H, NCH_3), 3.11 (m, 1H, NCH_2CH_3), 2.61 (s, 3H, Ph-CH_3), 1.64 (dd, $J = 7.1$, 3.1 Hz, 2H, $COOCH_2CH_2CH_3$), 1.34 (t, $J = 7.1$ Hz, 3H, NCH_2CH_3), 0.87 (t, $J = 7.2$ Hz, 3H, $COOCH_2CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.3$, 12.5, 23.5, 27.4, 29.2, 44.5, 48.9, 50.6, 67.9, 82.2, 92.3, 115.3, 117.4, 125.8, 130.7, 131.4, 132.7, 139.9, 143.2, 146.9, 149.4, 153.1, 159.9, 167.2, 169.5 ppm; IR (KBr): $\bar{\nu} = 3{,}327$, 3,200, 2,984, 2,935, 2,870, 1,343, 1,308, 1,235 cm^{-1}; HRMS: $m/z = 500.9871$ $[(M + H)^+]$.

Biological assay

The insecticidal activities of compounds **6a–6t** were measured against *Aphis medicagini* according to the standard test [24, 25], with a slight modification. The test analogs were dissolved in DMF and serially diluted with water containing Triton X-80 (0.1 mg/dm^3) to get the required concentrations. The insects were reared at 25(±1) °C, and groups of ten were transferred to glass Petri dishes and sprayed with the aforementioned solutions using a Potter sprayer. Assessments were made after 72 h based on the number and size of live insects relative to those sprayed with the negative control, and evaluations were based on a percentage scale of 0–100, in which 100 is total kill and 0 is no activity. The mortality rates were subjected to probit analysis. The reference compound was nitenpyram, while water containing Triton X-80 (0.1 mg/dm^3) was used as a negative control. All experiments were carried out in three replicates according to statistical requirements, and the results are shown in Table 1.

X-ray data for compound ***6g***

$C_{23}H_{25}Cl_2N_5O_4$, unit cell parameters: $a = 19.8308(16)$ Å, $b = 12.8128(10)$ Å, $c = 19.8844(15)$ Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$; space group Pbca. The crystallographic data for **6g** reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 824407. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uk).

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