Synthesis, Insecticidal Activity, and Molecular Docking Studies of Nitenpyram Analogues with a Flexible Ester Arm Anchored on Tetrahydropyrimidine Ring

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ABSTRACT: To make further researches on the structure−activity relationships (SARs) of our previous synthesized neonicotinoid compounds, a new series of nitenpyam analogues with flexible ester arm were synthesized. Preliminary bioassays indicated that all of our newly designed nitenpyam analogues exhibited good insecticidal activity at 100 mg/L, while analogues **4c** and **4d** afforded the best in vitro activity, and both of them had 100% mortality at 20 mg/L. The SAR studies suggested that their insecticidal potency was dual-controlled by the length of the ester arm and the size of the ester group. In addition, the molecular docking simulations revealed that the structural uniqueness of these analogues may lead to a unique molecular recognition and binding mode, which explained the SARs observed in vitro, and shed light on the novel insecticidal mechanism of these novel nitenpyam analogues.

KEYWORDS: Nitenpyram analogue, tetrahydropyrimidine, amino acid alkyl ester, flexible ester arm, size, insecticidal activities, molecular docking, dual control

■ INTRODUCTION

Neonicotinoid insecticides (NNSs), acting selectively on the insect nicotinic acetylcholine receptors (nAChRs),[1−3] are a relatively new class of synthetic organic insecticide as they combine unique properties, allowing them to be the fastest growing synthetic insecticides on the market. Since imidacloprid was introduced in the 1980s as an insecticide for crop protection,[4] NNSs have gained dramatic developments[5−10] and are remarkable for their significance in diverse disciplines including high insecticidal potency, low mammalian toxicity, broad insecticidal spectra, and no cross-resistance to conventional insecticide classes.[11−13] While they are fruitful in modern agricultural pest management[14] and environmental protection,[13] a potential problem facing all insecticides is the significant increase in resistance and its concomitant detrimental effect on agricultural productivity caused by the frequent applications of the NNSs.[15−17] Therefore, related research on neonicotinoids with new chemical structures and low resistance is an urgent requirement.

It is well-known that the structure optimization of commercial neonicotinoids is one of the effective resistance management tactics.[18−20] We have focused our attention on designing novel neonicotinoids, in which the nitenpyam structure was reserved and the amino acid alkyl ester was introduced into the leading compound through forming a tetrahydropyrimidine ring to fix the nitro group in the cis position (Figure 1). In our previous work,[21] a series of nitenpyam analogues **2** (Figure 1) were synthesized by introducing active ingredients L-α-amino acid methyl esters. These compounds exhibited good insecticide activities against *Nilaparvata lugens*. Structure−activity relationships (SARs) indicated that the length and flexibility of the substituent group at α- position had major impacts on the biological activities. It is worth pointing out that the anchorage length between the N atom on the tetrahydropyrimidine ring and

the methyl ester in nitenpyram analogues **2** is only one CH$_2$ group. Besides, only methyl ester was applied in the position of the ester group of nitenpyram analogues **2**. Why do we not change the way of controlling the flexibility and size of the molecular to manage the insecticidal activities of our designed compounds? To further research the SARs, more numerous nitenpyram analogues with diverse structural features and good activities are necessary.

Keeping the above idea in mind, starting from nitenpyram, three different length straight chain amino acids were introduced, and various ester groups were applied (Scheme 1), and the other novel nitenpyram analogues **3−5** described herein were designed and synthesized as shown in Figure 1. Parallel to our previous work,[21] microwave-assisted synthesis was also extended to the present work. As compared to conventional synthetic methods, controlled microwave heating had been shown to dramatically reduce reaction times, increase product yields, and enhance product purities by reducing unwanted side reactions.[22]

As expected, the nitenpyram analogues **3−5** exhibited various insecticide activities in a more controllable and rational manner by altering the length of the flexible ester arm and the size of ester group. Moreover, a preliminary bioassay against *N. lugens* showed that all nitenpyram analogues exhibited good insecticide activities at 500 and 100 mg/L, and the analogue **4d** showed the best activity at 20 mg/L. Their SARs were also discussed.

In the previous paper,[21] the result of molecular docking simulations had shown that the analogue **2** might recognize and bind at the nAChR in a unique way. To further explore the influential factors for the bioactivities of our designed nitenpyram analogues, in the rest of this paper, the molecular docking
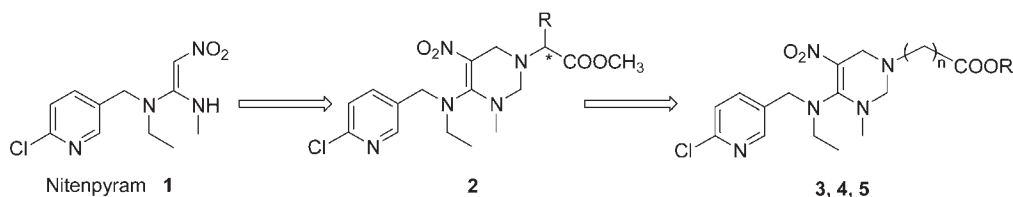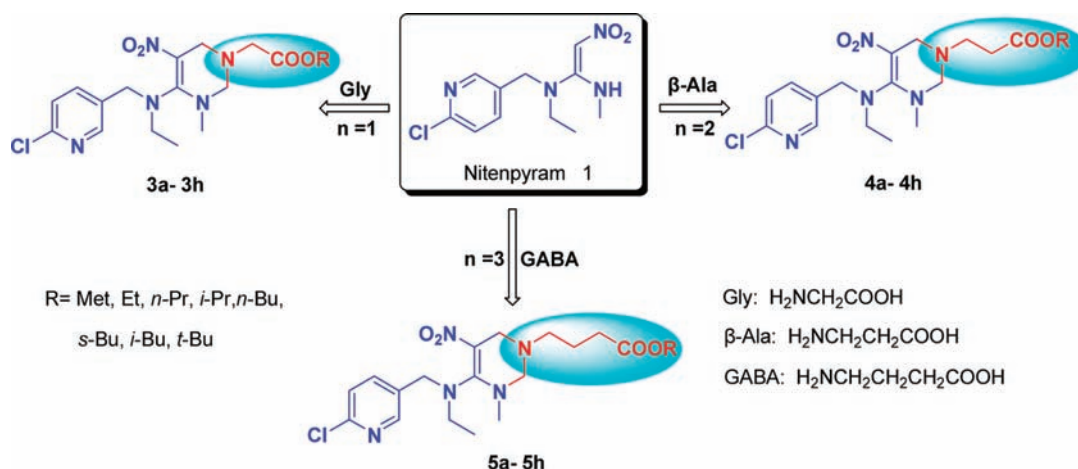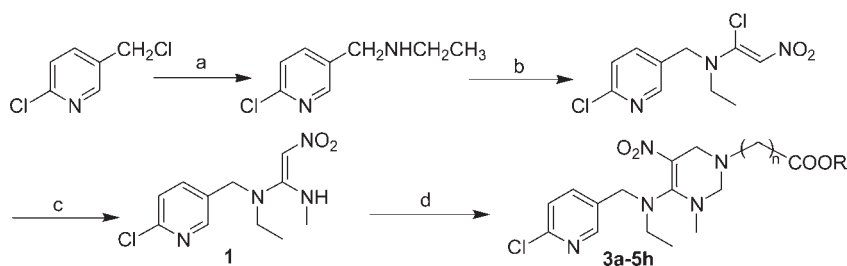
Figure 1. Development of novel nitenpyram analogues in our group.

Scheme 1. Reaction of Nitenpyram with Different Lengths of Amino Acid Alkyl Esters

Scheme 2. General Synthetic Route for the Target Compounds[a]

[a] Reagents and conditions: (a) Ethanamine (42%). (b) 1,1,1-Trichloro-2-nitroethane/CHCl$_3$ 2−7 °C (65%). (c) Methanamine 3−7 °C (58%). (d) Amino acid alkyl ester hydrochloride, HCHO, Et$_3$N/EtOH (71−79%).

investigation was carried out by docking the nitenpyram analogues into the active site of nAChR. The results of molecular docking suggest that the analogues 3−5 with various flexibility and size show their different binding affinities to the insect nAChR. This study examined the hypothesis that NNSs with ester groups might bind in a unique way at the nAChR. It is therefore fascinating to consider that the structural uniqueness of our newly designed nitenpyam analogues led to a unique molecular recognition and binding mode.

■ MATERIALS AND METHODS

Instruments. Melting points were measured using an uncorrected RK-1 microscopic melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker AVANCE (400 MHz) spectrometer with DMSO-d_6 as the solvent and tetramethylsilane as the internal standard.

The IR spectra were obtained from KBr discs in the range 4000−400 cm^{-1} on a Nicolet 5DXFT-IR spectrophotometer. Combustion analyses for elemental composition were made with a Perkin-Elmer 2400 instrument. All microwave experiments were performed using YL8023B1 microwave reactor possessing a single-mode microwave cavity producing controlled irradiation at 2.45 GHz.

Synthetic Procedures. Synthesis procedures for the title compounds are summarized in Scheme 2. Unless otherwise noted, reagents and solvents were of analytical reagent grade or were chemically pure and used as received without further purification.

General Synthetic Procedures for Target Compounds 3a−5h. A mixture of compound 1 (2.71 g, 10.0 mmol), amino acid alkyl ester hydrochloride (12.0 mmol), Et$_3$N (1.7 mL), and formaldehyde (1.95 mL, 37%) in ethanol (20 mL) was heated to 65 °C for 5 min in a microwave reactor and stirred for 20 min at the temperature. The reaction mixture was concentrated under reduced pressure and treated with 20 mL of water. Then, the solution was extracted three times with

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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 to afford pure products.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid Methyl Ester (**3a**).* Yield, 72.5%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.0 Hz, 1H, Py-H), 7.71 (dd, J = 8.1, 2.0 Hz, 1H, Py-H), 7.31 (d, J = 8.0 Hz, 1H, Py-H), 4.50 (d, J = 15.2, 1H, Py-CH$_2$), 4.19 (d, J = 15.2 Hz, 1H, Py-CH$_2$), 3.78–3.81 (m, 4H), 3.73 (s, 3H, COOCH$_3$), 3.60 (d, J = 2.8 Hz, 1H, NCH$_2$CO), 3.25–3.26 (m, 1H), 2.99 (s, 3H, NCH$_3$), 2.88–2.92 (dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz 2H), 1.14 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2951, 2872, 1744, 1546, 1303, 1250. Anal. calcd for C$_{16}$H$_{22}$ClN$_5$O$_4$: C, 50.07; H, 5.78; N, 18.25. Found: C, 50.03; H, 5.81; N, 18.27 (see ref 23).

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid Ethyl Ester (**3b**).* Yield, 72.0%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.0 Hz, 1H, Py-H), 7.71 (dd, J = 8.0, 2.4 Hz, 1H, Py-H), 7.30 (d, J = 8.4 Hz, 1H, Py-H), 4.51 (d, J = 15.2, 1H, Py-CH$_2$), 4.17 (d, J = 15.2 Hz, 1H, Py-CH$_2$), 4.14 (q, J = 7.2 Hz, 2H, COOCH$_2$), 3.65–3.75 (m, 4H), 3.37 (s, 2H, NCH$_2$COO), 3.14–3.23 (m, 1H), 2.98 (s, 3H, NCH$_3$), 2.81–2.89 (m, 1H), 1.22 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_3$), 1.10 (t, J = 7.2 Hz, 3H, COOCH$_2$CH$_3$) ppm. IR (KBr, cm^{-1}): 2964, 2870, 1741, 1551, 1450, 1388. Anal. calcd for C$_{17}$H$_{24}$ClN$_5$O$_4$: C, 51.32; H, 6.08; N, 17.60. Found: C, 51.41; H, 6.10; N, 17.57 (see ref 24).

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid Propyl Ester (**3c**).* Yield, 78.7%; mp 90–92 °C. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 2.1 Hz, 1H, Py-H), 7.72 (dd, J = 8.2, 2.4 Hz, 1H, Py-H), 7.32 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.09 (t, J = 6.7 Hz, 2H, COOCH$_2$), 3.83–3.68 (m, 4H), 3.43 (d, J = 3.8 Hz, 2H, NCH$_2$COO), 3.29–3.19 (m, 1H, NCH$_2$CH$_3$), 3.05 (s, 3H, NCH$_3$), 2.96–2.87 (m, 1H, NCH$_2$CH$_3$), 1.71–1.62 (m, 2H, CH$_2$CH$_2$CH$_3$), 1.16 (d, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.94 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2953, 2865, 1739, 1553, 1305, 1249. Anal. calcd for C$_{18}$H$_{26}$ClN$_5$O$_4$: C, 52.49; H, 6.36; N, 17.00. Found: C, 52.41; H, 6.44; N, 17.11.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid 1-Methylethyl Ester (**3d**).* Yield, 77.9%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33 (d, J = 2.1 Hz, 1H, Py-H), 7.74 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.34 (d, J = 8.3 Hz, 1H, Py-H), 5.14–5.04 (m, 1H, COOCH), 4.54 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 14.9 Hz, 1H, Py-CH$_2$), 3.83–3.72 (m, 4H), 3.41 (d, J = 1.6 Hz, 2H, NCH$_2$COO), 3.32–3.22 (m, 1H, NCH$_2$CH$_3$), 3.07 (s, 3H, NCH$_3$), 2.98–2.87 (m, 1H, NCH$_2$CH$_3$), 1.29 (d, J = 6.3 Hz, 6H, CHCH$_3$), 1.19 (t, J = 7.1 Hz, 1H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2966, 2868, 1733, 1552, 1368, 1300. Anal. calcd for C$_{18}$H$_{26}$ClN$_5$O$_4$: C, 52.49; H, 6.36; N, 17.00. Found: C, 52.35; H, 6.48; N, 16.91.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid Butyl Ester (**3e**).* Yield, 74.2%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33 (d, J = 2.3 Hz, 1H, Py-H), 7.73 (dd, J = 8.3, 2.4 Hz, 1H, Py-H), 7.35 (d, J = 8.2 Hz, 1H, Py-H), 4.54 (d, J = 14.9 Hz, 1H, Py-CH$_2$), 4.21–4.15 (m, 3H), 3.84–3.72 (m, 4H), 3.44 (d, J = 1.0 Hz, 2H, NCH$_2$COO), 3.33–3.23 (m, 1H, NCH$_2$CH$_3$), 3.07 (s, 3H, NCH$_3$), 2.98–2.89 (m, 1H, NCH$_2$CH$_3$), 1.69–1.62 (m, 2H, COOCH$_2$CH$_2$), 1.44–1.36 (m, 2H, CH$_2$CH$_2$CH$_3$), 1.19 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.96 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2931, 2875, 1742, 1553, 1304, 1249. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.49; H, 6.71; N, 16.52.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid 1-Methylpropyl Ester (**3f**).* Yield, 75.1%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33

(d, J = 1.9 Hz, 1H, Py-H), 7.74 (dd, J = 8.2, 2.0 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.97–4.89 (m, 1H, COOCH), 4.54 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 15.2 Hz, 1H, Py-CH$_2$), 3.84–3.73 (m, 4H), 3.43 (s, 2H, NCH$_2$COO), 3.32–3.23 (m, 1H, NCH$_2$CH$_3$), 3.08 (s, 3H, NCH$_3$), 2.97–2.89 (m, 1H, NCH$_2$CH$_3$), 1.67–1.55 (m, 2H, COOCHCH$_2$), 1.26 (d, J = 6.3 Hz, 3H, COOCHCH$_3$), 1.19 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.92 (t, J = 7.5 Hz, 3H, CHCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2949, 2870, 1737, 1548, 1305, 1254. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.49; H, 6.71; N, 16.52.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid 2-Methylpropyl Ester (**3g**).* Yield, 73.6%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33 (d, J = 2.4 Hz, 1H, Py-H), 7.74 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.54 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.1 Hz, 1H, Py-CH$_2$), 3.95 (d, J = 6.7 Hz, 2H, COOCH$_2$CH), 3.85–3.73 (m, 4H), 3.47 (s, 2H, NCH$_2$COO), 3.33–3.23 (m, 1H, NCH$_2$CH$_3$), 3.07 (s, 3H, NCH$_3$), 2.98–2.89 (m, 1H, NCH$_2$CH$_3$), 2.01–1.93 (m, 1H, CH$_2$CHCH$_3$), 1.19 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.96 (d, J = 6.7 Hz, 6H, CHCH$_3$). IR (KBr, cm^{-1}): 2959, 2874, 1734, 1550, 1302, 1253. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.66; H, 6.59; N, 16.38.

*2-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Acetic Acid 1,1-Dimethylethyl Ester (**3h**).* Yield, 71.5%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 2.1 Hz, 1H, Py-H), 7.74 (dd, J = 8.2, 2.4 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.54 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 3.82–3.74 (m, 4H), 3.42 (s, 2H, NCH$_2$COO), 3.28–3.19 (m, 1H, NCH$_2$CH$_3$), 3.06 (s, 3H, NCH$_3$), 2.95–2.86 (m, 1H, NCH$_2$CH$_3$), 1.45 (s, 9H, CCH$_3$), 1.18 (d, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2951, 2887, 1731, 1553, 1375, 1257. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.52; H, 6.68; N, 16.49.

*3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid Methyl Ester (**4a**).* Yield, 78.2%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.1 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.3 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.51 (d, J = 14.9 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.2 Hz, 1H, Py-CH$_2$), 3.71 (s, J = 7.5 Hz, 3H, COOCH$_3$), 3.69–3.59 (m, 4H), 3.33–3.22 (m, 1H, NCH$_2$CH$_3$), 2.98 (s, 3H, NCH$_3$), 2.96–2.87 (m, 1H, NCH$_2$CH$_3$), 2.87–2.78 (m, 2H, NCH$_2$CH$_2$), 2.57 (t, J = 6.7 Hz, 2H, CH$_2$CH$_2$COO), 1.19 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2951, 2872, 1745, 1548, 1303, 1251. Calcd for C$_{17}$H$_{24}$ClN$_5$O$_4$: C, 51.32; H, 6.08; N, 17.60. Found: C, 51.27; H, 6.15; N, 17.56.

*3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid Ethyl Ester (**4b**).* Yield, 76.9%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.29 (d, J = 2.2 Hz, 1H, Py-H), 7.69 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.31 (d, J = 8.2 Hz, 1H, Py-H), 4.50 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.21–4.10 (m, 3H), 3.69–3.56 (m, 4H), 3.31–3.18 (m, 1H, NCH$_2$CH$_3$), 2.97 (s, 3H, NCH$_3$), 2.96–2.87 (m, 1H, NCH$_2$CH$_3$), 2.87–2.75 (m, 2H, NCH$_2$CH$_2$), 2.54 (t, J = 6.9 Hz, 2H, CH$_2$CH$_2$COO), 1.26 (t, J = 7.1 Hz, 3H, COOCH$_2$CH$_3$), 1.17 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2949, 2873, 1741, 1552, 1305, 1252. Anal. calcd for C$_{18}$H$_{26}$ClN$_5$O$_4$: C, 52.49; H, 6.36; N, 17.00. Found: C, 52.45; H, 6.43; N, 17.11.

*3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid Propyl Ester (**4c**).* Yield, 75.5%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.3 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.3 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.07 (t, J = 6.6 Hz, 2H, COOCH$_2$), 3.69–3.60 (m, 4H), 3.31–3.22 (m, 1H, NCH$_2$CH$_3$), 2.98 (s, 3H, NCH$_3$), 2.96–2.89

(m, 1H, NCH$_2$CH$_3$), 2.87–2.78 (m, 2H, NCH$_2$CH$_2$), 2.57 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$COO), 1.71–1.62 (m, 2H, COOCH$_2$CH$_2$), 1.18 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.95 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2958, 2865, 1738, 1549, 1305, 1254. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.52; H, 6.70; N, 16.49.

3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid 1-Methylethyl Ester (4d). Yield, 74.4%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.30 (d, J = 2.3 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.3 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 5.09–4.99 (m, 1H, COOCH), 4.51 (d, J = 15.1 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 3.69–3.59 (m, 4H), 3.31–3.22 (m, 1H, NCH$_2$CH$_3$), 2.98 (s, 3H, NCH$_3$), 2.97–2.90 (m, 1H, NCH$_2$CH$_3$), 2.88–2.78 (m, 2H, NCH$_2$CH$_2$), 2.53 (t, J = 6.4 Hz, 2H, CH$_2$CH$_2$COO), 1.25 (d, J = 6.3 Hz, 6H, CHCH$_3$), 1.19 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2949, 2870, 1734, 1548, 1370, 1305. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.67; H, 6.55; N, 16.52.

3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid Butyl Ester (4e). Yield, 73.1%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.5 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.12 (t, J = 6.6 Hz, 2H, COOCH$_2$), 3.69–3.60 (m, 4H), 3.31–3.22 (m, 1H, NCH$_2$CH$_3$), 2.98 (s, 3H, NCH$_3$), 2.97–2.89 (m, 1H, NCH$_2$CH$_3$), 2.88–2.78 (m, 2H, NCH$_2$CH$_2$), 2.56 (t, J = 6.9 Hz, 2H, CH$_2$CH$_2$COO), 1.66–1.59 (m, 2H, COOCH$_2$CH$_2$), 1.44–1.35 (m, 2H, CH$_2$CH$_2$CH$_3$), 1.19 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_3$), 0.94 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2877, 1742, 1553, 1304, 1249. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.49; H, 6.95; N, 15.85.

3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid 1-Methylpropyl Ester (4f). Yield, 72.8%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.30 (d, J = 2.4 Hz, 1H, Py-H), 7.71 (dd, J = 7.7, 2.3 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 4.93–4.84 (m, 1H, COOCH), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 14.9 Hz, 1H, Py-CH$_2$), 3.71–3.59 (m, 4H), 3.31–3.21 (m, 1H, NCH$_2$CH$_3$), 2.97 (s, 3H, NCH$_3$), 2.96–2.89 (m, 1H, NCH$_2$CH$_3$), 2.88–2.77 (m, 2H, NCH$_2$CH$_2$), 2.55 (t, J = 6.5 Hz, 2H, CH$_2$CH$_2$COO), 1.65–1.51 (m, 2H, COOCHCH$_2$), 1.21 (d, J = 6.3 Hz, 3H, COOCHCH$_3$), 1.18 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_3$), 0.90 (t, J = 7.4 Hz, 3H, CHCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2963, 2864, 1738, 1552, 1307, 1257. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.67; H, 6.98; N, 16.81.

3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid 2-Methylpropyl Ester (4g). Yield, 73.7%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.30 (d, J = 2.4 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.4 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 4.51 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.17 (d, J = 15.2 Hz, 1H, Py-CH$_2$), 3.90 (d, J = 6.6 Hz, 2H, COOCH$_2$CH), 3.70–3.59 (m, 4H), 3.31–3.21 (m, 1H, NCH$_2$CH$_3$), 2.97 (s, 3H, NCH$_3$), 2.95–2.88 (m, 1H, NCH$_2$CH$_3$), 2.87–2.77 (m, 2H, NCH$_2$CH$_2$), 2.58 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$COO), 1.99–1.90 (m, 1H, CH$_2$CHCH$_3$), 1.18 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.94 (d, J = 6.7 Hz, 6H, CHCH$_3$). IR (KBr, cm^{-1}): 2974, 2872, 1735, 1551, 1311, 1259. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.55; H, 6.93; N, 15.87.

3-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Propionic Acid 1,1-Dimethylethyl Ester (4h). Yield, 72.6%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33 (d, J = 2.1 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.1 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.07 (t, J = 6.6 Hz, 2H, COOCH$_2$), 3.69–3.60 (m, 4H), 3.32 – 3.23 (m, 1H, NCH$_2$CH$_3$), 2.99 (s, 3H, NCH$_3$), 2.97–2.90 (m, 1H, NCH$_2$CH$_3$), 2.84–2.76 (m, 2H, NCH$_2$CH$_2$), 2.49 (t, J = 7.1 Hz, 2H, CH$_2$CH$_2$COO), 1.47 (s, 9H, CCH$_3$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2944, 2889, 1732, 1553, 1369, 1254. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.51; H, 6.96; N, 15.82.

4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid Methyl Ester (5a). Yield, 78.1%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.5 Hz, 1H, Py-H), 7.70 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.51 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 3.68 (s, 3H, COOCH$_3$), 3.67–3.57 (m, 4H), 3.32–3.21 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H, NCH$_3$), 2.98–2.90 (m, 1H, NCH$_2$CH$_3$), 2.57–2.46 (m, 2H, NCH$_2$CH$_2$), 2.43 (t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.92–1.84 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2875, 1744, 1552, 1302, 1252. Anal. calcd for C$_{18}$H$_{26}$ClN$_5$O$_4$: C, 52.49; H, 6.36; N, 17.00. Found: C, 52.38; H, 6.46; N, 16.92.

4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid Ethyl Ester (5b). Yield, 78.8%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 2.1 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.4 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.1 Hz, 1H, Py-CH$_2$), 4.21–4.12 (m, 3H), 3.67–3.56 (m, 4H), 3.32–3.22 (m, 1H, NCH$_2$CH$_3$), 2.99 (s, 3H, NCH$_3$), 2.97–2.92 (m, 1H, NCH$_2$CH$_3$), 2.56–2.46 (m, 2H, NCH$_2$CH$_2$), 2.41 (t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.92–1.82 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.27 (t, J = 7.1 Hz, 3H, COOCH$_2$CH$_3$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2872, 1742, 1550, 1303, 1254. Anal. calcd for C$_{19}$H$_{28}$ClN$_5$O$_4$: C, 53.58; H, 6.63; N, 16.44. Found: C, 53.66; H, 6.54; N, 16.57.

4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid Propyl Ester (5c). Yield, 75.7%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 1.8 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.1 Hz, 1H, Py-H), 7.33 (d, J = 8.2 Hz, 1H, Py-H), 4.50 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.03 (t, J = 6.7 Hz, 2H, COOCH$_2$), 3.69–3.54 (m, 4H), 3.29–3.21 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H, NCH$_3$), 2.98–2.89 (m, 1H, NCH$_2$CH$_3$), 2.55–2.45 (m, 2H, NCH$_2$CH$_2$), 2.40 (t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.92–1.82 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.68–1.62 (m, J = 14.2, 6.9 Hz, 2H, CH$_2$CH$_2$CH$_3$), 1.18 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.94 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2873, 1738, 1551, 1300, 1251. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.52; H, 6.99; N, 16.01.

4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid 1-Methylethyl Ester (5d). Yield, 76.4%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (s, 1H, J = 2.1 Hz,Py-H), 7.72 (d, J = 7.9, 2.1 Hz, 1H, Py-H), 7.34 (d, J = 7.7 Hz, 1H, Py-H), 5.06–4.97 (m, 1H, COOCH), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.18 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 3.68–3.56 (m, 4H), 3.33–3.22 (m, 1H, NCH$_2$CH$_3$), 3.01 (s, 3H, NCH$_3$), 2.98–2.91 (m, 1H, NCH$_2$CH$_3$), 2.57–2.46 (m, 2H, NCH$_2$CH$_2$), 2.38 (t, J = 6.7 Hz, 2H, CH$_2$CH$_2$COO), 1.91–1.82 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.25 (d, J = 6.2 Hz, 6H, CHCH$_3$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2949, 2870, 1735, 1552, 1369, 1305. Anal. calcd for C$_{20}$H$_{30}$ClN$_5$O$_4$: C, 54.60; H, 6.87; N, 15.92. Found: C, 54.53; H, 6.96; N, 16.03.

4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid Butyl Ester (5e). Yield, 74.9%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.31 (d, J = 2.1 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.34 (d, J = 8.3 Hz, 1H, Py-H), 4.51 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.09 (t, J = 6.7 Hz, 2H, COOCH$_2$), 3.66–3.57 (m, 4H), 3.32–3.22 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H, NCH$_3$), 2.98–2.89 (m, 1H, NCH$_2$CH$_3$), 2.58–2.46 (m, 2H, NCH$_2$CH$_2$), 2.41 (t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.91–1.84 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.66–1.59 (m, 2H, COOCH$_2$CH$_2$), 1.44–1.34 (m, 2H, CH$_2$CH$_2$CH$_3$), 1.20

Table 1. Insecticidal Activities of Nitenpyram Analogues against *N. lugen*

compd	n, R	mortality (%) at different concentrations (mg/L)		
		500	100	20
3a	1, Me	100	94	86
3b	1, Et	100	100	94
3c	1, ^{n}Pr	100	96	73
3d	1, ^{i}Pr	100	85	76
3e	1, ^{n}Bu	100	75	67
3f	1, ^{s}Bu	100	79	53
3g	1, ^{i}Bu	100	100	68
3h	1, ^{t}Bu	100	73	47
4a	2, Me	100	100	95
4b	2, Et	100	100	93
4c	2, ^{n}Pr	100	100	100[a]
4d	2, ^{i}Pr	100	100	100[b]
4e	2, ^{n}Bu	100	92	78
4f	2, ^{s}Bu	100	100	84
4g	2, ^{i}Bu	100	100	52
4h	2, ^{t}Bu	100	81	66
5a	3, Me	100	100	79
5b	3, Et	100	100	85
5c	3, ^{n}Pr	100	100	43
5d	3, ^{i}Pr	100	100	58
5e	3, ^{n}Bu	100	100	82
5f	3, ^{s}Bu	100	93	59
5g	3, ^{i}Bu	100	100	54
5h	3, ^{t}Bu	100	68	36
nitenpyram[c]		100	100	100
imidacloprid[d]		100	100	100

[a] LC_{50} = 0.194 mg/L. [b] LC_{50} = 0.162 mg/L. [c] LC_{50} = 0.129 mg/L. [d] LC_{50} = 0.108 mg/L.

(t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.95 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2875, 1739, 1553, 1309, 1255. Anal. calcd for C$_{21}$H$_{32}$ClN$_5$O$_4$: C, 55.56; H, 7.11; N, 15.43. Found: C, 55.47; H, 7.22; N, 15.36.

*4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid 1-Methylpropyl Ester (**5f**).* Yield, 75.1%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 2.0 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.90–4.82 (m, 1H, COOCH), 4.52 (d, J = 15.1 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 15.1 Hz, 1H, Py-CH$_2$), 3.67–3.57 (m, 4H), 3.31–3.20 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H, NCH$_3$), 2.98–2.90 (m, 1H, NCH$_2$CH$_3$), 2.59–2.45 (m, 2H, NCH$_2$CH$_2$), 2.39 (t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.92–1.83 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.62–1.52 (m, 2H, COOCHCH$_2$), 1.22–1.18 (m, 6H), 0.91 (t, J = 7.5 Hz, 3H, CHCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2964, 2864, 1741, 1553, 1310, 1259. Anal. calcd for C$_{21}$H$_{32}$ClN$_5$O$_4$: C, 55.56; H, 7.11; N, 15.43. Found: C, 55.65; H, 7.21; N, 15.33.

*4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid 2-Methylpropyl Ester (**5g**).* Yield, 74.2%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.32 (d, J = 1.8 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.0 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 14.9 Hz, 1H, Py-CH$_2$), 3.87 (d, J = 6.1 Hz, 2H, COOCH$_2$CH), 3.67–3.57 (m, 4H), 3.32–3.22 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H), 2.98–2.90 (m, 1H, NCH$_2$CH$_3$), 2.57–2.48 (m, 2H, NCH$_2$CH$_2$), 2.43

(t, J = 7.2 Hz, 2H, CH$_2$CH$_2$COO), 1.99–1.92 (m, 1H, CH$_2$CHCH$_3$), 1.91–1.84 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$), 0.95 (d, J = 6.7 Hz, 6H, CHCH$_3$). IR (KBr, cm^{-1}): 2975, 2874, 1737, 1551, 1303, 1256. Anal. calcd for C$_{21}$H$_{32}$ClN$_5$O$_4$: C, 55.56; H, 7.11; N, 15.43. Found: C, 55.64; H, 7.23; N, 15.34.

*4-[(4Z)-4-[[(6-Chloro-3-pyridinyl)methyl]ethylamino]-3-methyl-5-nitro-1,2,3,6-tetrahydropyrimidin-1-yl] Butyric Acid 1,1-Dimethylethyl Ester (**5h**).* Yield, 71.5%; yellow oil. ^{1}H NMR (ppm, DMSO-d_6): δ 8.33 (s, 1H, J = 2.1 Hz, Py-H), 7.71 (d, J = 8.2, 2.1 Hz, 1H, Py-H), 7.34 (d, J = 8.2 Hz, 1H, Py-H), 4.52 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 4.19 (d, J = 15.0 Hz, 1H, Py-CH$_2$), 3.66–3.56 (m, 4H), 3.32–3.21 (m, 1H, NCH$_2$CH$_3$), 3.00 (s, 3H, NCH$_3$), 2.96–2.89 (m, 1H, NCH$_2$CH$_3$), 2.54–2.45 (m, 2H, NCH$_2$CH$_2$), 2.39 (t, J = 6.8 Hz, 2H, CH$_2$CH$_2$COO), 1.90–1.81 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.46 (s, 9H, CCH$_3$), 1.20 (t, J = 7.1 Hz, 3H, NCH$_2$CH$_3$). IR (KBr, cm^{-1}): 2949, 2890, 1730, 1550, 1371, 1261. Anal. calcd for C$_{21}$H$_{32}$ClN$_5$O$_4$: C, 55.56; H, 7.11; N, 15.43. Found: C, 55.45; H, 7.20; N, 15.44.

Biology Assay. The bioassay was measured according to the standard test[25] with a slight modification, and all analogues were tested against *N. lugens* to evaluate their insecticidal activities. The compounds were dissolved in dimethyl formamide (DMF) and serially diluted with water containing Triton X-80 (0.1 mg/L) to get the required test concentrations. All experiments were carried out in three replicates according to statistical requirements. The insects were reared at 25 (±1) °C, 25 (±2) % relative humidity, and 12 h light photoperiod. Groups of 12 were transferred to glass Petri dishes and sprayed with the aforementioned solutions using a Potter sprayer. After they were air-dried, they were kept in a special room for normal cultivation. Assessments weremade after 72 h by the number of killed and size of live insects relative to that in the negative control, and evaluations were based on a percentage scale of 0–100, in which 100 was total kill and 0 was no activity. The mortality rates were subjected to probit analysis.[26] All results are shown in Table 1. The reference compound was nitenpyram, and water containing DMF (0.5 mg/L) and Triton X-80 (0.1 mg/L) was used as a negative control.

Experimental Protocol of Docking Study. The high nAChR inhibitory activity of compound **2d** was chosen to understand the ligand–protein interactions in detail, and AutoDock 4.0[27] was used to carry out the molecular modeling study. Because the amino acids forming the active pockets are both structurally and functionally consistent in the diverse nAChRs and AchBPs, the crystal structure of the *Lymnaea stagnalis* AchBP (*Ls*-AChBP) complexed with imidacloprid (PDB code: 2zju)[28] was used as the template to construct the models. The receptor was prepared for docking by the addition of hydrogen atoms and the removal of cocrystallizedmolecules. The putative active binding site was characterized by selecting all residues within a 12 Å radius of the original binding substrate in the X-ray structure. Each ligand was iteratively minimized and assigned the Gasteiger–Hückel charges.

The tested compounds were flexibly docked automatically in the active site of nAChR. The AMBER force field was used to calculate a three-dimensional grid of interaction energies for the target nAChR by AutoGrid (Component of the AutoDock 4.0 program), and these grids were precomputed to store the electrostatic and van der Waals values. Default values were used for all docking parameters with 20 independent docking runs for each ligand. Intermolecular energy, torsional free energy, and intermolecular hydrogen bonds were included to evaluate their binding free energy. Cluster analysis was performed on the docked results using a root-mean-square deviation (rmsd) tolerance of 0.75 Å . For each cluster, the conformation with the lowest binding energy in the binding site was chosen for further analysis and comparison. AccelrysDS visualizer 2.5 [Accelrys Inc., San Diego, CA (2009)] was used for molecular modeling to determine their binding orientations and interactions.
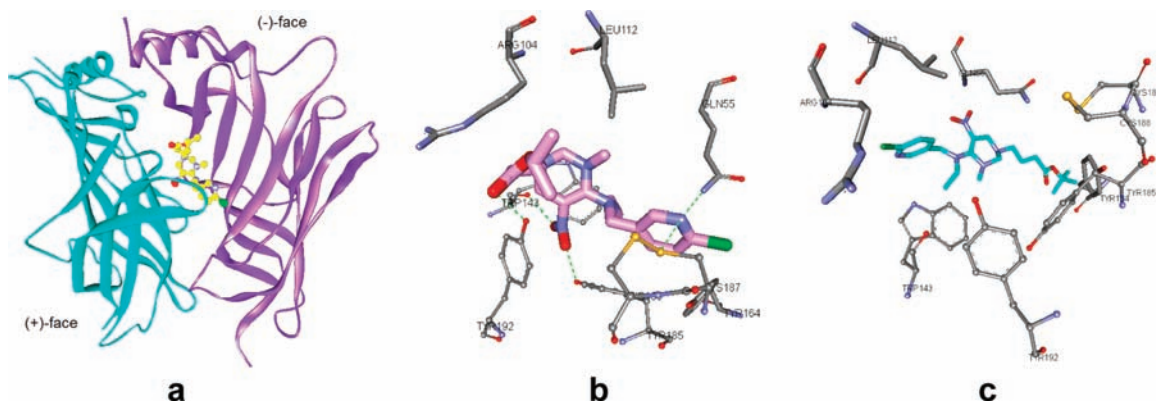
4832

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Figure 2. View of the binding modes and interactions of analogues **4d** and **5h** in the binding site of Ls-AChBP structural surrogate of the insect nAChR, suggested by molecular docking studies. (a) Analogue **4d** is bound into the subunit interfacial binding pocket between two faces of adjacent subunits. For clarity, only two of the five subunits are extracted and shown from the pentameric nAChR structure, and the corresponding interfacial binding pocket of interest is displayed. (b) Zoomed-in view of the interactions between analogue **4d** and amino acids from the active site of the receptor. (c) The predicted binding mode of analogue **5h** with relatively low activity. Key H-bonds are indicated by green dotted lines.

■ RESULTS AND DISCUSSION

Synthesis. Amino acids were converted to the intermediates of amino acid alkyl ester hydrochlorides according to the procedures given in the literature.[29] Starting from 2-chloro-5-chloromethylpridine, a set of (E)-N-(6-chloro-3-pyridylmethyl)-N-ethyl-1-chloro-2-nitroethylene-1-amine and **1** were prepared based on the procedures in the literatures.[30,31] The further reaction of **1** with amino acid alkyl ester hydrochlorides could proceed readily under microwave irradiation (Scheme 2), which was a highly efficient way that gave good yields (71−79%) and had easy post-treatments.

SAR. The insecticidal activities of the new nitenpyam analogues against *N. lugens* were listed in Table 1. All of the analogues had 100% mortality at 500 mg/L, and most of them exhibited good insecticidal activities at 100 mg/L. Among these analogues, **4c** and **4d** afforded the best in vitro inhibitory activities and had 100% mortality at 20 mg/L. In general, as shown in Table 1, the analogues with different length of the ester arms exhibited equivalent activities at high dose (500 mg/L). However, the insecticidal activities showed significant differences when the doses were reduced to 100 and 20 mg/L. Clearly, the analogues of **4** (n = 2, **4a−h**) displayed better insecticidal potencies than those of the analogues of **3** (n = 1, **3a−h**) and analogues of **5** (n = 3, **5a−h**). These differences were likely to depend upon the flexibility of the molecular. It appeared that as the aster arm was lengthened, and the flexibility increased at the same time, whereas the size of the molecular also increased. So, we found that the analogues of **4** (n = 2), in which the lengths of ester arms were moderate, had the best average insecticidal potency as compared to the analogues of **3** and **5**. These observations suggest that the length of the ester arm is one of the important factors influencing the potency of the nitenpyam analogues.

As for the size of the ester group, there was no remarkable difference in the insecticidal activities when various alkyl esters were introduced into the nitenpyam analogues at a higher dose (500 and 100 mg/L). Expectedly, the analogues bearing the smaller ester groups (**3a,b**, **4a,b**, and **5a,b**) had shown relatively better insecticidal potency in the respective series, when the dose was reduced to 20 mg/L. In contrast, the analogues **3f,h**, **4g,h**, and **5g,h**, in which the bigger ester groups were applied, showed much less activity.

Considering the discussion above, we found the insecticidal potencies of our designed nitenpyam analogues were dual-controlled by altering the length of the ester arm and the size of the ester group. Only when a molecule can keep a good balance between the flexibility and the size will it attain the best insecticidal activity, such as the analogues **4c** and **4d**. The results of insecticidal activities further suggested that small differences between the structures could lead to large differences in the overall activities. As compared with the best one of analogues **2** (LC_{50} = 0.216 mg/L) in the previous paper, the LC_{50} values of analogues **4c** and **4d** were 0.194 and 0.162 mg/L, respectively, which implied that the nitenpyam analogues were more comparable to those of nitenpyram, and the approach presented in this paper is both practical and feasible.

Molecular Docking Study. To further explore the structural requirement for better activities, binding site interactions of these analogue nitenpyam were simulated with *Ls*-AChBP based on its structure cocrystallized with bound imidacloprid. As expected, the most potent analogue **4d** was nicely accommodated within the subunit interfacial binding pocket between the principal or (+)-face subunit and the complementary or (−)-face subunit (Figure 2a), with its backbone and chains nicely nestled. As compared with our previous work, the binding conformation of analogue **4d** in this docking simulation showed the similar binding mode. However, at the same time, unexpectedly, the molecular recognition mode of analogue **4d** was different from that of the analogues **2** with a more rational way.

As illustrated in Figure 2b, chloropyridine N(24) substantially interacts with S of Cys187/Cys188 on loop C and exhibits a hydrogen bond with NH of Gln55 on loop D, while the nitro O(17) and O(18) interact with OH of Trp143 on loop B and OH of Tyr185 on loop C, respectively, which suggest that chloropyridine and nitro moieties are presumably important for recognition of the analogues. In addition, its binding conformation exhibits another important hydrogen bond between O24 of its ester and NH of Gln73. Other interactions in this area may be mediated via water(s) as these residues are near the protein surface. These observations have also explained why the analogue **4d** attained the highest score.

Furthermore, most of the other active analogues shared a quite similar binding mode with analogue **4d**. Many of these analogues

exhibited more than two hydrogen bonds between its O17, O18, O24, and N17 and different amino acid residues from the *Ls*-AChBP including Gln55, Gln73, Arg104, Trp 143, Thr144, Cys187/Cys188, Glu190, and Tyr 192, which strengthen the *n*AChR—insecticide interaction actually and accounted for a novel mechanism of the insecticidal effect by these new analogues again. The results of docking also indicated that the analogues bearing shorter or longer chain and smaller or bigger ester will not show the best insecticidal potency, for little hydrogen bonds and other interactions could be found in their best binding conformations, such as the predicated binding mode of analogue **5h** (Figure 2c). Thereby, the newly introduced ester chains with moderate length and size presumably play important roles in ligand recognition and binding interactions, which may further enhance their activities and contribute to the selectivity as well. Consequently, our docking results were consistent with the experimental activities and explained the SARs observed in vitro, which may provide some useful information for future design of new insecticides.

In conclusion, a series of novel nitenpyram analogues were designed and synthesized by introducing different straight chain amino acid alkyl esters into nitenpyram. The analogues exhibited various insecticide activities in a more controllable and rational manner by altering the length of the flexible ester arm and the size of ester group. All of the test analogues presented good insecticidal activity at 100 mg/L, and the analogues **4c** and **4d** afforded the best in vitro activity and had 100% mortality at 20 mg/L. SARs suggested that the insecticidal potency of our designed nitenpyam analogues was dual-controlled by the flexibility and size of the molecular. In addition, molecular docking studies were also carried out to model the ligand—nAChR complexes and analyze their interactions between the analogues and the key residue in *Ls*-AChBP ligand binding domain. The docking results revealed that the structural uniqueness of our newly designed nitenpyram analogues led to a unique molecular recognition and binding mode and were in good agreement with their high insecticidal potential, which also explained the SARs observed in vitro. In some degree, these findings, combined with the SARs results, could explain the novel mechanism of the insecticidal effect by these new analogues. The study herein may prompt structure-guided future attempts to design and develop novel insecticides with less resistance and better selectively. Studies on much more test objects and further structural modification of nitenpyram are underway.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This manuscript was originally published on the web on April 15, 2011, with an error to the spelling of tetrahydropyrimidine in the title and Introduction. The corrected version was reposted on May 4, 2011.

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