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Design, synthesis, insecticidal evaluation and molecular docking studies of *cis*-nitenpyram analogues bearing diglycine esters

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Based on the strategies of receptor structure-guided neonicotinoid design, a series of novel *cis*-nitenpyram analogues bearing diglycine esters were designed and synthesized. Preliminary bioassays indicated that the insecticidal spectra of the target compounds were expanded compared with our previous work, while all the target compounds presented excellent insecticidal activities against *Nilaparvata lugens* and *Aphis medicagini* at 100 mg/L. Among these analogues, **6b** showed 100% mortality against *Nilaparvata lugens* (LC_{50} = 0.163 mg/L) and 90% against *Aphis medicagini* at 4 mg/L. SARs suggested that the insecticidal potency of our designed *cis*-nitenpyram analogues was dual-controlled by the size and species of the ester groups. The molecular docking simulations revealed that the structural uniqueness of these analogues may lead to a unique molecular recognition and binding mode compared with the previously designed compounds. Introduction of the peptide bond gave rise to more significant hydrogen bonds between the nitenpyram analogues bonding with the amino acid residues of insect nAChRs. The docking results explained the SARs observed *in vitro*, and shed light on the novel insecticidal mechanism of these *cis*-nitenpyram analogues.

***cis*-nitenpyram analogues, hydrogen bonds, diglycine ester, synthesis, insecticidal activity, molecular docking, nAChRs, peptide bond**

1 Introduction

Recently, neonicotinoids began to replace pyrethroids, chlorinated hydrocarbons, organophosphates (OPs), carbamates and several other types of insecticides to control insect pests on major crops. Due to their efficient mode of action (MoA), neonicotinoids showed little cross-resistance to the conventional long-established insecticides, and they make up approximately one-fourth of the world insecticide market [1, 2]. As potent agonists, they selectively act on the insect nicotinic acetylcholine receptors (nAChRs), i.e., their molecular target site [3]. Neonicotinoids represented by imidacloprid [4] are the most important class of insecticides over the past three decades because of their potency, low mammalian toxicity, broad insecticidal spectra, and good systemic properties [5, 6]. However, the new mode of action, frequent applications in the field of pest control and structural similarity among neonicotinoids have led to the acquisition of resistance and cross-resistance in a range of species such as *Plutella xylostella*, *Tetranychus cinnabarinus* and *Aphis medicagini*s, *Nilaparvata lugens* [2, 7–13]. Especially, the brown planthopper, *Nilaparvata lugens*, a major rice pest in many parts of Asia, has developed strong resistance to imidacloprid [2, 9, 14]. As a result, development of new neonicotinoids with high insecticidal activities against resistance strains is highly desirable.

It is well-known that the structure optimization of commercial neonicotinoids is one of the effective strategies to manage tactics [15–17]. In our previous work [18], we have focused our attention on designing novel neonicotinoids with *cis*-configuration. Starting from nitenpyram, three lin-

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ear amino acids with different chain length were introduced, and various ester groups were applied (Figure 1). To this end, a new series of *cis*-configuration nitenpyram analogues **3**, **4**, **5** were synthesized by introducing 1, 2, 3, 6-tetrahydropyrimidine with flexible ester arms. These compounds have exhibited good insecticide activities against *Nilaparvata lugens*. The structure-activity relationships implied that the different 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 insecticidal potency depended greatly on the number of significant hydrogen bonds which are formed by the nitenpyram analogues and the amino acid residues of insect nAChRs. This observation provides us a rational guide to design novel *cis*-configuration nitenpyram analogues. In this context, it is very important to increase the number of significant hydrogen bonds between the nitenpyram analogues and the amino acid residues of insect nAChRs for the purpose of structure optimization or design.

In order to increase the number of significant hydrogen bonds between the nitenpyram analogues and the amino acid residues of insect nAChRs, based on our previous work (Figure 1), we inseted the peptide bond to the flexible ester arm of the nitenpyram analogues **5**, and designed and synthesized another novel nitenpyram analogues **6** as shown in Figure 2. The first reason to introduce the organic segment peptide bond is that it contains more electronegative atoms such as oxygen atoms and nitrogen atoms which can form more significant hydrogen bonds with the amino acid residues of insect nAChRs, which may result in improving the insecticidal activities. The second reason is that peptides are involved in important physiological and biochemical functions such as neuro transmission, neuro modulation, and act as hormones in receptor mediated signal transduction [19]. The increasing interests regarding the manifold actions of the bioactive peptides have made their structural studies an important aspect of research in pharmacology and medical sciences [20]. During drug development, identification of an 'active' part of a large peptide is critical for further improving its pharmacology.

As expected, preliminary bioassays indicated that not only the insecticidal spectra of the target compounds were expanded when compared with our previous work, but also all the target compounds presented excellent insecticidal activities against *Nilaparvata lugens* and *Aphis medicagini* at 100 mg/L. Among these analogues, **6b** showed 100% mortality against *Nilaparvata lugens* (LC_{50} = 0.163 mg/L) and 90% against *Aphis medicagini* at 4 mg/L. Their structure-activity relationships indicated that insecticide activities were dual-controlled by the size and species of the ester group. To further investigate their binding interactions, molecular docking simulations were carried out. The results showed that the active analogues exhibited significant hydrogen bonding interactions with the amino acid residues of insect nAChRs, where the peptide bonds displayed an important role. These results confirmed our ideas. The docking results explained the structure-activity relationships observed *in vitro*, and shed a light on the novel insecticidal

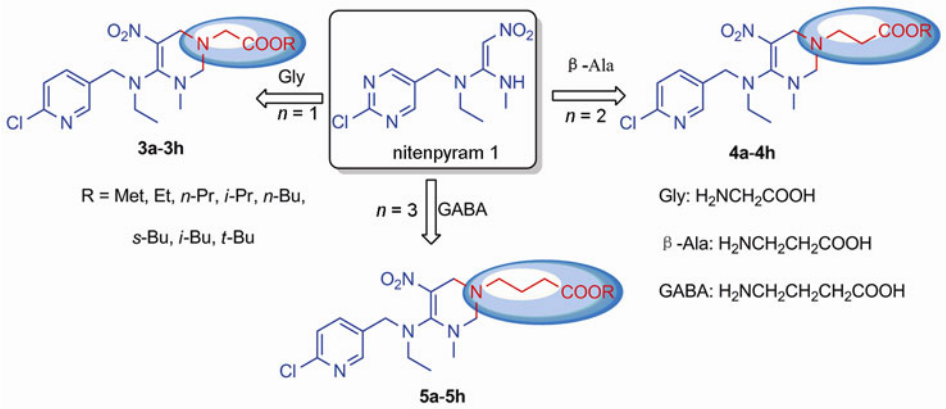


Figure 1 Reaction of nitenpyram with different lengths of amino acid alkyl esters in our previous work.

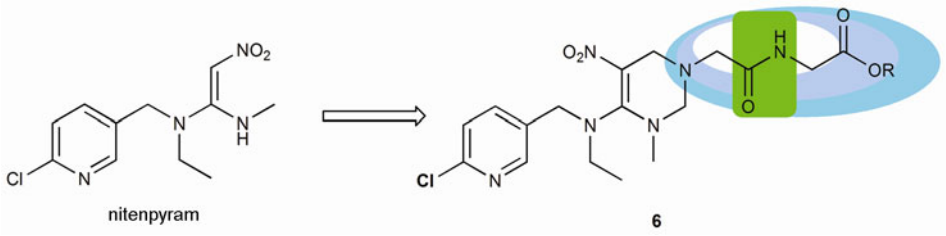


Figure 2 *cis*-nitenpyram analogues containing different diglycine esters.

mechanism of these new analogues, which may provide some useful information for future design of new insecticides.

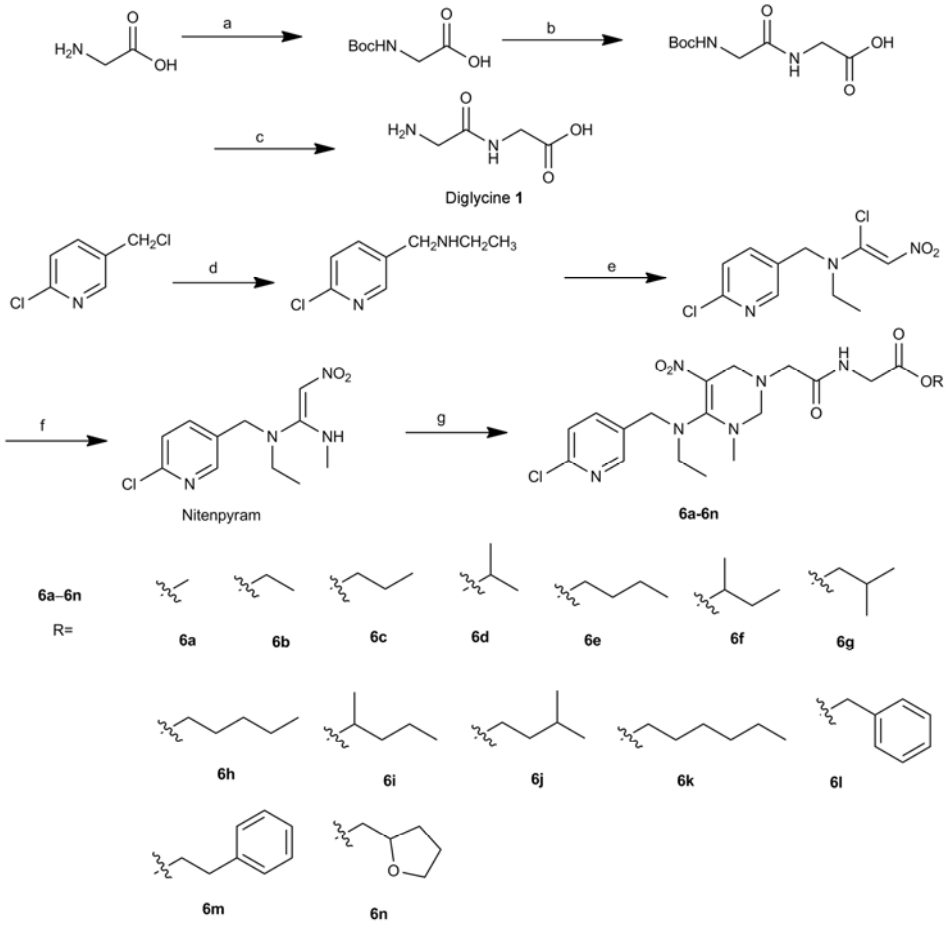
2 Chemistry

To prepare a series of *cis*-nitenpyram analogues bearing the diglycine ester, an efficient synthesis approach was developed as depicted in Scheme 1. Diglycine **1** was synthesized according to Ref. [21], and was then converted to the intermediate of diglycine hydroxyalkyl ester hydrochloride according to the procedures given in Ref. [22]. Nitenpyram, which was prepared based on the procedures previously reported [23], was reacted with formaldehyde and a variety of substituted diglycine ester hydrochloride in ethanol to afford the desired compounds **6a–6n** with *cis*-configuration fixed by 1, 2, 3, 6-tetrahydropyrimidine bearing the diglycine ester. Parallel to our previous work [18], the microwave-assisted synthesis was also extended to the present work. As compared to the conventional synthetic methods, the controlled microwave heating had been shown to dramatically reduce reaction time, increase product yields, and enhance product purities by reducing unwanted side reactions [24].

3 Experimental

3.1 Materials and apparatus

Unless otherwise noted, reagents and solvents were of analytical reagent grade or were chemically pure and used as received without further purification. ^{1}H NMR spectrum ($CDCl_3$) was recorded on a Bruker AVANCE-400 MHz with TMS as an internal standard. Coupling constants (*J* values) are in Hertz. The IR spectra were obtained from KBr discs in the range of 4000–400 cm^{-1} on a Nicolet 5DXFT-IR spectrophotometer. Combustion analyses for elemental composition were conducted on a Perkin-Elmer 2400 instrument. All microwave experiments were performed using a YL8023B1 microwave reactor possessing a single-mode microwave cavity producing controlled irradiation at 2.45 GHz.



Scheme 1 Synthesis of the target compounds (**6a–6n**). Reagents and conditions: a) Boc_2O, NaOH/THF/H_2O, rt; b) N_2 atmosphere, CDI/THF, 60 °C; c) CF_3COOH/CH_2Cl_2, –5 °C; d) ethanamine; e) 1, 1, 1-trichloro-2-nitroethane/$CHCl_3$, 2–7 °C; f) methanamine, 3–7 °C; g) diglycine ester hydrochloride, HCHO, Et_3N/EtOH, 60–75 °C.

3.2 Insecticidal activity assay

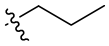
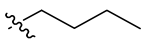
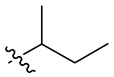
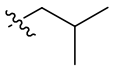
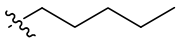
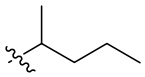
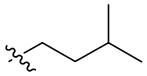
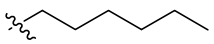
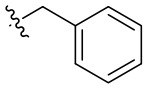
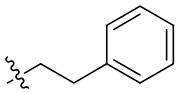
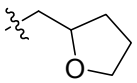
The insecticidal activities of compounds **6a–6n** were measured against *Nilaparvata lugens* and *Aphis medicagini* according to the standard test [25] with a slight modification. The test analogues were dissolved in DMF and serially diluted with water containing Triton X-80 (0.1 mg/L) to obtain the required concentrations. The insects were reared at 25 (±1) °C, and groups of 10 were transferred to glass Petri dishes and sprayed with the aforementioned solutions using a Potter sprayer. Assessments were made after 72 h by the number and size of the live insects relative to those in the negative control, and evaluations are based on a percentage scale of 0–100. The mortality rates were subjected to probit analysis [26]. The reference compounds were nitenpyram, while water containing Triton X-80 (0.1 mg/L) was used as a negative control. All experiments were carried out in three replicates for the purpose of statistic requirements, and the results are shown in Table 1.

3.3 General procedure

3.3.1 General synthetic procedures for diglycine

NaOH (33 mmol, 0.87 g), followed by Boc_2O (11 mmol, 2.4 g) was added to a stirred solution of glycine (12 mmol, 0.90 g) in THF/H_2O (30 mL of each solvent) at room temperature and the resulting mixture was stirred for 18 h. THF was removed under vacuum and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The aqueous layer was acidified with hydrochloric acid (1 mol/L) to pH 4, and then extracted with CH_2Cl_2 (4 × 15 mL). The organic phase was combined and dried over Na_2SO_4 and the solvent was evap-

Table 1 Insecticidal activities of *cis*-nitenpyram analogues (**6a–6n**) against *Nilaparvata lugens* and *Aphis medicagini*

Compd.	R	Mortality (%) at different concentrations (mg/L)					
		Nilaparvata lugens			*Aphis medicagini*		
		100	20	4	100	20	4
6a		100	100	98	100	100	88
6b		100	100	100 [a)]	100	100	90
6c		100	100	95	100	90	85
6d		100	100	88	100	88	70
6e		100	95	80	100	85	63
6f		100	85	72	95	75	50
6g		100	90	75	95	80	55
6h		100	90	60	90	70	48
6i		100	80	50	90	65	42
6j		100	80	45	85	60	35
6k		90	60	40	80	53	24
6l		100	100	75	100	84	70
6m		100	100	70	100	80	65
6n		100	100	80	100	90	75
5b		100	85	70	90	70	34
	nitenpyram	100	100	100 [b)]	100	100	100

a) LC_{50} = 0.163 mg/L; b) LC_{50} = 0.129 mg/L.

orated under vacuum. The resulting crude Boc-glycine was used in the next step without further purification.

A 100 mL round bottom flask was charged with Boc-glycine (10 mmol, 1.75g), carbonyldimidazole (6 mmol, 0.97 g) and freshly distilled THF (20 mL) under the nitrogen atmosphere and was heated to 60 °C for 1 h. The mixture was cooled to room temperature and glycine (10 mmol, 0.75 g) was added by syringe. The mixture was stirred for 3–4 h to get the intermediates solution, then the intermediates solution was concentrated and subjected to flash column chromatography on silica gel to afford Boc-diglycine (ethyl acetate/petroleum ether (*v*/*v*)= 3/2 as eluent).

CF_3CO_2H (15 mmol) was added dropwise to the solution of Boc-diglycine (10 mmol, 2.48g) in CH_2Cl_2 (10 mL) in ice bath. The mixture was stirred at –5 °C and the progress of the reaction was tracked by TLC. The digycline that broke off from the Boc was obtained. Methanol (10 mL) was added to the reaction mixture and the reaction mixture was concentrated under reduced pressure. This process was repeated four times in order to remove the residual CF_3CO_2H, and the resulting oil liquid was used in the next step without further purification.

3.3.2 General synthetic procedures for target compounds ***6a–6n***

A mixture of nitenpyram (2.71 g, 10.0 mmol), diglycine ester hydrochloride (10.0 mmol), Et_3N (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 ethyl acetate (3 × 30 mL), and the combined extracts were dried over $MgSO_4$. The organic phase was evaporated under reduced pressure, and the residue was subjected to flash column chromatography on silica gel, eluting with ethyl acetate/petroleum ether (*v*/*v* = 3:1) to afford pure products.

*N-methoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6a**)*

Yellow oil, yield 76.4%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.34 (d, *J* = 2.2 Hz, 1H, Py-H), 7.71 (dd, *J* = 8.2, 2.5 Hz, 1H, Py-H), 7.39 (d, *J* = 5.1 Hz, 1H,NH), 7.35 (s, 1H, Py-H), 4.52 (d, *J* = 14.9 Hz, 1H, Py-CH_2), 4.21 (d, *J* = 15.0 Hz, 1H, Py-CH_2), 4.14 (q, *J* = 5.1 Hz, 2H, NCH_2CO), 3.85–3.72 (m, 7H), 3.35–3.28 (m, 1H, NCH_2), 3.26 (d, *J* = 3.2 Hz, 2H, NCH_2CONH), 3.10 (s, 3H, NCH_3), 3.03–2.93 (m, 1H, NCH_2), 1.23 (q, *J* = 7.2 Hz, 3H, NCH_2CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3200 (N–H), 2951, 2872, 1744, 1540 (C=O), 1546 (NO_2), 1303 ($\nu_{as\ (C–O–C)}$),1251 ($\nu_{a\ (C–O–C)}$). Anal. calcd. for $C_{18}H_{25}ClN_6O_5$: C, 49.04; H, 5.72; N, 19.06. Found: C, 49.11; H, 5.68; N, 19.10. ESI-MS (M + H) *m*/*z*: 441.16.

*N-ethoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6b**)*

Yellow oil, yield 78.9%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, *J* = 2.3 Hz, 1H, Py-H), 7.71 (dd, *J* = 8.2, 2.5 Hz, 1H, Py-H), 7.40 (t, *J* = 5.2 Hz, 1H, NH), 7.35 (d, *J* = 8.2 Hz, 1H, Py-H), 4.51 (d, *J* = 15.0 Hz, 1H, Py-CH_2), 4.22 (dd, *J* = 11.6, 4.6 Hz, 2H, CH_2COO), 4.12 (dd, *J* = 9.3, 4.3 Hz, 2H,OCH_2), 4.06 (d, *J* = 5.6 Hz, 1H, Py-CH_2), 3.82–3.71 (m, 4H), 3.30 (d, *J* = 9.0 Hz, 1H), 3.26 (d, *J* = 3.6 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 3.02–2.94 (m, 1H), 1.31 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.22 (t, *J* = 7.1 Hz, 3H, NCH_2CH_3). FTIR(KBr, cm^{-1}) ν_{max}: 3210 (N–H), 2951, 2872, 1744, 1541 (C=O), 1544 (NO_2), 1303 ($\nu_{as\ (C–O–C)}$), 1251 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{19}H_{27}ClN_6O_5$: C, 50.16; H, 5.98; N, 18.47. Found: C, 50.18; H, 5.96; N, 18.50. ESI-MS (M + H) *m*/*z*: 454.17.

*N-propoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6c**)*

Yellow oil, yield 75.8%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (d, *J* = 1.5 Hz, 1H, Py-H), 7.70 (dd, *J* = 8.1, 2.1 Hz, 1H,P y-H), 7.40 (s, 1H, NH), 7.34 (d, *J* = 8.2 Hz, 1H, Py-H), 4.51 (d, *J* = 14.9 Hz, 1H, Py-CH_2), 4.20 (d, *J* = 15.0 Hz, 1H, Py-CH_2), 4.12 (s, 2H, $NHCH_2$), 3.81 (t, *J* = 8.0 Hz, 2H, COO CH_2), 3.77–3.66 (m, 4H), 3.35–3.27 (m, 1H), 3.25 (d, *J* = 2.8 Hz, 2H, NCH_2CO), 3.07 (s, 3H, NCH_3), 2.96 (td, *J* = 14.1, 7.1 Hz, 1H), 1.70 (dt, *J* = 14.2, 7.1 Hz, 2H, CH_2CH_3), 1.21 (t, *J* = 5.7 Hz, 3H, NCH_2CH_3), 0.96 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2CH_3$). FTIR (KBr, cm^{-1}) ν_{max}: 3211 (N–H), 2951, 2872, 1744, 1540 (C=O), 1546 (NO_2), 1302 ($\nu_{as\ (C–O–C)}$), 1250 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{20}H_{29}ClN_6O_5$: C, 51.23; H, 6.23; N, 17.92. Found: C, 51.18; H, 6.32; N, 17.88. ESI-MS (M + H) *m*/*z*: 469.19.

*N-(1-methyl)ethoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6d**)*

Yellow oil, yield 76.7%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, *J* = 2.3 Hz, 1H, Py-H), 7.71 (dd, *J* = 8.2, 2.5 Hz, 1H, Py-H), 7.40 (s, 1H, NH), 7.35 (d, *J* = 8.2 Hz, 1H, Py-H), 4.51 (d, *J* = 15.0 Hz, 1H, Py-CH_2), 4.27–4.22 (m, 2H, CH_2COO), 4.18 (dd, *J* = 14.0, 7.0 Hz, 2H, NCH_2COO), 4.14–4.09 (m, 1H, $OCH(CH_3)_2$), 4.05 (d, *J* = 9.9 Hz, 1H, Py-CH_2), 3.85–3.66 (m, 4H), 3.30 (d, *J* = 7.6 Hz, 1H), 3.26 (d, *J* = 3.6 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 2.96 (dt, *J* = 14.1, 7.1 Hz, 1H), 1.31 (dd, *J* = 9.0, 5.3 Hz, 3H, NCH_2CH_3), 1.29 (d, *J* = 6.9 Hz, 2H, CH_2CH_3), 1.19–1.26 (m, 6H, $CH(CH_3)_2$). FTIR (KBr, cm^{-1}) ν_{max}: 3217 (N–H), 2952, 2873, 1749, 1541 (C=O), 1540(NO_2), 1301 ($\nu_{as\ (C–O–C)}$), 1255 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{20}H_{29}ClN_6O_5$: C, 51.23; H, 6.23; N, 17.92. Found: C, 51.20; H, 6.25; N, 17.86. ESI-MS (M + H) *m*/*z*: 469.19.

*N-butoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6e**)*

Yellow oil, yield 78.8%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (d, J = 1.4 Hz, 1H, Py-H), 7.72 (dd, J = 8.0, 2.0 Hz, 1H,P y-H), 7.41 (s, 1H, NH), 7.34 (d, J = 8.0 Hz, 1H, Py-H), 4.50 (d, J = 14.9 Hz, 1H, Py-CH_2), 4.21 (d, J = 14.6 Hz, 1H, Py-CH_2), 4.12 (s, 2H, $NHCH_2$), 3.81 (t, J = 8.0 Hz, 2H, $COOCH_2$), 3.78–3.65 (m, 4H), 3.32–3.25(m, 1H), 3.24 (d, J = 2.8 Hz, 2H, NCH_2CO), 3.07 (s, 3H, NCH_3), 2.96 (td, J = 14.1, 7.1 Hz, 1H), 1.70–1.33 (m, 4H, CH_2CH_2), 0.96 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$). FTIR (KBr, cm^{-1}) ν_{max}: 3210 (N–H), 2952, 2870, 1751, 1542 (C=O), 1546 (NO_2), 1312 ($\nu_{as\ (C–O–C)}$), 1252 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{21}H_{31}ClN_6O_5$: C, 52.22; H, 6.47; N, 17.40. Found: C, 52.25; H, 6.49; N, 17.48. ESI-MS (M + H) *m/z*: 483.20.

*N-(1-methyl)propoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6f**)*

Yellow oil, yield 75.3%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (s, 1H, Py-H), 7.71 (d, J = 8.1 Hz, 1H, Py-H), 7.40 (t, sJ = 14.9 Hz, 1H, Py-CH_2), 4.20 (d, J = 15.1 Hz, 1H, Py-CH_2), 4.13 (t, J = 5.0 Hz, 2H, $NHCH_2COO$), 3.95 (d, J = 6.7 Hz, 2H, OCH_2), 3.80 (d, J = 4.5 Hz, 2H), 3.75 (d, J = 7.5 Hz, 2H), 3.34–3.28 (m, 1H), 3.25 (d, J = 2.8 Hz, 2H, NCH_2), 3.09 (s, 3H, NCH_3), 2.97 (dq, J = 14.6, 7.4 Hz, 1H), 2.02–1.92 (m, 1H, $CH(CH_3)_2$), 1.21 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 0.95 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$). FTIR (KBr, cm^{-1}) ν_{max}: 3213 (N–H), 2950, 2874, 1750, 1540 (C=O), 1542 (NO_2), 1310 ($\nu_{as\ (C–O–C)}$), 1256 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{21}H_{31}ClN_6O_5$: C, 52.22; H, 6.47; N, 17.40. Found: C, 52.20; H, 6.45; N, 17.45. ESI-MS (M + H) *m/z*: 483.20.

*N-(2-methyl)propoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6g**)*

Yellow oil, yield 76.3%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (d, J = 2.1 Hz, 1H, Py-H), 7.74 (dd, J = 8.0, 2.5 Hz, 1H, Py-H), 7.42 (s, 1H, NH), 7.34 (d, J = 8.0 Hz, 1H, Py-H), 4.52 (d, J = 15.3 Hz, 1H, Py-CH_2), 4.28–4.23 (m, 2H, CH_2COO), 4.19 (dd, J = 14.2, 7.1 Hz, 2H, NCH_2COO), 4.13–4.09(m, 1H, OCH), 4.05 (d, J = 9.9 Hz, 1H, Py-CH_2), 3.85–3.66 (m, 4H), 3.30 (d, J = 7.6 Hz, 1H), 3.26 (d, J = 3.6 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 2.96 (dt, J = 14.1, 7.1 Hz, 1H), 1.31 (dd, J = 9.0, 5.3 Hz, 3H, NCH_2CH_3), 1.29 (d, J = 6.9 Hz, 2H, CH_2CH_3), 1.19 (d, J = 7.0 Hz, 3H, $CHCH_3$). 0.96 (t, J = 6.9 Hz, CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3218 (N–H), 2952, 2873, 1749, 1541 (C=O), 1542 (NO_2), 1305 ($\nu_{as(C–O–C)}$), 1250 ($\nu_{a(C–O–C)}$). Anal. calcd for $C_{21}H_{31}ClN_6O_5$: C, 52.22; H, 6.47; N, 17.40. Found: C, 52.20; H, 6.50; N, 17.43. ESI-MS (M + H) *m/z*: 483.20.

*N-pentoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6h**)*

Yellow oil, yield 75.7%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, J = 2.4 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.5 Hz, 1H, Py-H), 7.39 (t, J = 5.2 Hz, 1H, NH), 7.35 (d, J = 8.2 Hz, 1H, Py-H), 4.51 (d, J = 15.0 Hz, 1H, Py-CH_2), 4.22 (s, 1H, Py-CH_2), 4.17 (t, J = 6.8 Hz, 2H, $NHCH_2$), 3.81 (t, J = 8.3 Hz, 2H, $COOCH_2$), 3.74 (dt, J = 14.0, 6.3 Hz, 4H), 3.34–3.27 (m, 1H), 3.26 (d, J = 3.5 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 2.97 (dd, J = 14.1, 7.1 Hz, 1H), 1.67 (dt, J = 13.8, 6.9 Hz, 2H, $COOCH_2CH_2$), 1.38–1.32 (m, 4H, $CH_2CH_2CH_3$), 1.23–1.20 (m, 3H, NCH_2CH_3), 0.95–0.89 (m, 3H, CH_2CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3210 (N–H), 2952, 2870, 1750, 1541 (C=O), 1548 (NO_2), 1312 ($\nu_{as\ (C–O–C)}$), 1260 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{22}H_{33}ClN_6O_5$: C, 53.17; H, 6.69; N, 16.91. Found: C, 53.20; H, 6.65; N, 16.97. ESI-MS (M + H) *m/z*: 497.22.

*N-(2-methyl)butoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6i**)*

Yellow oil, yield 72.8%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.33 (d, J = 2.0 Hz, 1H, Py-H), 7.73 (dd, J = 8.1, 2.4 Hz, 1H, Py-H), 7.40 (s, 1H, NH), 7.33 (d, J = 8.0 Hz, 1H, Py-H), 4.53 (d, J = 15.3 Hz, 1H, Py-CH_2), 4.27–4.22 (m, 2H, CH_2COO), 4.20 (dd, J = 14.0, 7.0 Hz, 2H, NCH_2COO), 4.13–4.09 (m, 1H,OCH), 4.05 (d, J = 9.9 Hz, 1H, Py-CH_2), 3.85–3.66 (m, 4H), 3.30 (d, J = 7.6 Hz, 1H), 3.26 (d, J = 3.6 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 2.96 (dt, J = 14.1, 7.1 Hz, 1H), 1.31 (dd, J = 9.0, 5.3 Hz, 3H, NCH_2CH_3), 1.29 (m, 2H, $CHCH_2$), 1.10 (m, 2H, CH_2CH_3), 1.19 (d, J = 7.0 Hz, 3H, $CHCH_3$). 0.96 (t, J = 6.9 Hz, CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3215 (N–H), 2950, 2871, 1749, 1541 (C=O), 1540 (NO_2), 1305 ($\nu_{as\ (C–O–C)}$), 1251 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{22}H_{33}ClN_6O_5$: C, 53.17; H, 6.69; N, 16.91. Found: C, 53.20; H, 6.89; N, 16.93. ESI-MS (M + H) *m/z*: 497.22.

*N-(3-methyl)butoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6j**)*

Yellow oil, yield 76.4%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (s, 1H, Py-H), 7.70 (d, J = 8.2 Hz, 1H, Py-H), 7.42 (t, J = 5.2 Hz, 1H, NH), 7.34 (d, J = 8.0 Hz, 1H, Py-H), 4.52 (d, J = 14.8 Hz, 1H, Py-CH_2), 4.22 (d, J = 15.2 Hz, 1H, Py-CH_2), 4.12 (t, J = 5.1 Hz, 2H, $NHCH_2COO$), 3.92 (d, J = 6.6 Hz, 2H, OCH_2), 3.80 (d, J = 4.5 Hz, 2H), 3.75 (d, J = 7.5 Hz, 2H), 3.34–3.28 (m, 1H), 3.25 (d, J = 2.8 Hz, 2H, NCH_2), 3.09 (s, 3H, NCH_3), 2.97 (dq, J = 14.6, 7.4 Hz, 1H), 2.01–1.83 (m, 1H, $CH(CH_3)_2$), 1.53–1.22 (m, 2H, CH_2CH), 1.21 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 1.01 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$). FTIR(KBr, cm^{-1}) ν_{max}: 3213 (N–H), 2950, 2874, 1750, 1540 (C=O), 1542 (NO_2), 1310 ($\nu_{as\ (C–O–C)}$), 1256 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{22}H_{33}ClN_6O_5$: C, 53.17; H, 6.69;

N, 16.91. Found: C, 53.15; H, 6.71; N, 16.94. ESI-MS (M + H) *m/z*: 497.22.

*N-hexoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl) ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6k**)*

Yellow oil, yield 75.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J = 2.1 Hz, 1H, Py-H), 7.71 (dd, J = 8.2, 2.4 Hz, 1H, NH), 7.38 (t, J = 5.4 Hz, 1H, Py-H), 7.35 (d, J = 8.2 Hz, 1H, Py-H), 4.51 (d, J = 14.9 Hz, 1H, Py-CH_2), 4.22 (s, 1H, Py-CH_2), 4.18 (d, J = 6.8 Hz, 2H, $NHCH_2$), 4.14–4.09 (m, 2H, $COOCH_2$), 3.78 (dd, J = 16.0, 6.1 Hz, 4H), 3.33–3.27 (m, 1H), 3.25 (d, J = 3.1 Hz, 2H, NCH_2CO), 3.10 (s, 3H, NCH_3), 1.40–1.28 (m, 8H, $(CH_2)_4CH_3$), 1.22 (t, J = 7.1 Hz, 3H, NCH_2CH_3), 0.90 (t, J = 6.7 Hz,3H, $(CH_2)_4CH_3$). FTIR (KBr, cm^{-1}) ν_{max}: 3212 (N–H), 2953, 2872, 1753, 1544 (C=O), 1542 (NO_2), 1310 ($\nu_{as\ (C–O–C)}$), 1262 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{21}H_{30}ClN_6O_5$: C, 52.33; H, 6.27; N, 17.44. Found: C, 52.30; H, 6.25; N, 17.46. ESI-MS (M + H) m/z: 511.24.

*N-phenmethoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6l**)*

Yellow oil, yield 71.7%; ^{1}H NMR (400 MHz, DMSO) δ 8.32 (s, 1H, Py-H), 7.70 (d, J = 8.2 Hz, 1H, Py-H), 7.41 (d, J = 6.0 Hz, 1H, NH), 7.40–7.34 (m, 5H, Ph-H), 7.33 (s, 1H, Py-H), 5.20 (s, 2H, $COOCH_2$), 4.50 (d, J = 15.0 Hz, 1H, Py-CH_2), 4.22 (s, 1H, Py-H), 4.20–4.14 (m, 2H, $NHCH_2$), 3.83–3.69 (m, 4H), 3.29 (d, J = 6.7 Hz, 1H), 3.25 (d, J = 2.7 Hz, 2H, NCH_2), 3.06 (s, 3H, NCH_3), 2.97 (dq, J = 13.9, 7.0 Hz, 1H), 1.21(t, J = 7.1 Hz, 3H, NCH_2CH_3). FTIR (KBr, cm^{-1}) ν_{max} 3213 (N–H), 2954, 2871, 1750, 1541 (C=O), 1549 (NO_2), 1680, 1580, 1500 (C=C), 1312 ($\nu_{as\ (C–O–C)}$), 1260 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{22}H_{24}ClN_6O_5$: C, 54.16; H, 4.96; N, 17.22. Found: C, 54.20; H, 4.93; N, 17.25. ESI-MS (M + H) *m/z*: 517.19.

*N-phenethoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6m**)*

Yellow oil, yield 74.9%; ^{1}H NMR (400 MHz, DMSO) δ 8.30 (s, 1H, Py-H), 7.78 (d, J = 8.2 Hz, 1H, Py-H), 7.40 (d, J = 6.1 Hz, 1H, NH), 7.42–7.32 (m, 5H, Ph-H), 7.34 (s, 1H, Py-H), 5.21 (s, 2H, $COOCH_2$), 4.53 (d, J = 15.0 Hz, 1H, Py-CH_2), 4.23 (s, 1H, Py-H), 4.20–4.14 (m, 2H, $NHCH_2$), 3.83–3.69 (m, 4H), 3.30 (d, J = 6.6 Hz, 1H), 3.24 (d, J = 2.5 Hz, 2H, NCH_2), 3.03 (s, 3H, NCH_3), 2.97 (dq, J = 13.9, 7.0 Hz, 1H), 2.83 (t, J = 2.3 Hz, 2H, Ph-CH_2), 1.21 (t, J = 7.1 Hz, 3H, NCH_2CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3214 (N–H), 2953, 2870, 1754, 1540 (C=O), 1545 (NO_2), 1684, 1582, 1508 (C=C), 1313 ($\nu_{as\ (C–O–C)}$), 1261 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{23}H_{26}ClN_6O_5$: C, 55.04; H, 5.22; N, 16.74. Found: C, 55.06; H, 5.00; N, 16.75. ESI-MS (M + H) *m/z*: 531.20.

*N-(2-tetrahydrofuryl)methoxycarbonylmethyl-2-[[4-(6-chloro-3-pyridinylmethyl)ethylamino]-3-methyl-5-nitro-1-(1,2,3,6-tetrahydropyrimidinyl)]acetamide (**6n**)*

Yellow oil, yield 72.3%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (s, 1H, Py-H), 7.71 (d, J = 8.2 Hz, 1H, Py-H), 7.39 (d, J = 6.1 Hz, 1H, NH), 7.33 (s, 1H, Py-H), 7.30–6.19 (m, 3H, tetrahydrofury-H), 5.20 (s, 2H, $COOCH_2$), 4.50 (d, J = 15.0 Hz, 1H, Py-CH_2), 4.22 (s, 1H, Py-H), 4.20–4.14 (m, 2H, $NHCH_2$), 3.83–3.69 (m, 4H), 3.29 (d, J = 6.7 Hz, 1H), 3.25 (d, J = 2.7 Hz, 2H, NCH_2), 3.06 (s, 3H, NCH_3), 2.97 (dq, J = 13.9, 7.0 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H, NCH_2CH_3). FTIR (KBr, cm^{-1}) ν_{max}: 3211 (N–H), 2950, 2870, 1755, 1542 (C=O), 1546 (NO_2), 1312 ($\nu_{as\ (C–O–C)}$), 1260 ($\nu_{a\ (C–O–C)}$). Anal. calcd for $C_{20}H_{26}ClN_6O_6$: C, 49.85; H, 5.44; N, 17.44. Found: C, 49.87; H, 5.42; N, 17.46. ESI-MS (M + H) *m/z*: 511.20.

4 Results and discussion

4.1 Evaluation of insecticidal activities

By analyzing the data of Table 1, we found that all the designed analogues have better insecticidal performance than **5b** which had the best insecticidal activities in the previous work. Most of our designed analogues exhibited excellent insecticide activities against *Nilaparvata lugen* and *Aphis medicagini* at 100 mg/L (Table 1), while the target compounds had higher insecticidal activities against *Nilaparvata lugen* than *Aphis medicagini* at a lower dose. Among these analogues, **6a** and **6b** afforded the best *in vitro* inhibitory activities and had 98% and 100% mortality at 4 mg/L, respectively. The LC_{50} value of **6b** was 0.163 mg/L, which was more comparable to nitenpyram (LC_{50} = 0.129 mg/L) than **5b**.

The insecticidal activities showed significant differences with the change in the size of the ester groups R. When R was smaller, the insecticidal potency was improved. As for the different R groups, their insecticidal activities increased in the order: ethyl (**6b**) > methyl (**6a**) > propyl (**6c**) > butyl (**6e**) > pentyl (**6h**) > hexyl (**6k**). These observations suggest that the size of the ester group is one of the important factors that influence the potency of the nitenpyram analogues. As for the species of the R groups, the insecticidal activities of the target compounds were higher when R was a linear aliphatic alkyl group than those when R was a branched aliphatic alkyl group. As an example, the insecticidal activities of the target compounds increased in the order: *n*-propyl (**6c**) > isopropyl (**6d**), *n*-butyl (**6e**) > *sec*-butyl (**6g**) > *iso*-butyl (**6f**), *n*-pentyl (**6h**) > *sec*-pentyl (**6i**) > *iso*-pentyl (**6j**).

Furthermore, the nitenpyram analogues containing aromatic esters (**6l**, **6m**) had lower activities than the saturated aliphatic esters (**6b**, **6c**), and the insecticidal activities of tetrahydrofuryl ester (**6n**) which contains an oxygen atom was relatively high. These results insecticidal activities fur-

ther suggested that small structure differences could lead to large differences in the overall activities, which implies further possibilities of lead compound development.

4.2 Molecular docking study

To further explore the structural features of the target compounds for better activities, models of these new compounds–receptor complexes were investigated by docking studies with CDOCK [27]. Since the amino acids formed at the active sites are both structurally and functionally consistent in the diverse nAChRs and AchBPs, the published crystal structure of a Lymnaea stagnalis-AChBP (Ls-AChBP) co-crystallized with imidacloprid (PDB ID: 2zju) [28] was used as the template of receptor. The docking study was carried out through the graphical user interface CDOCKTOOLS. The only modification was the number of docking runs that was set to 200 (previously 100) for higher accuracy.

The scoring function of the docking program ranked the compounds in the same general order observed experimentally (data not shown), and all the active analogues exhibited significant hydrogen bonding interactions with the nAChRs target. As expected, the most potent compound **6b** is nicely accommodated within the subunit interfacial binding pocket between the two faces of adjacent subunits (Figure 3(a)), with its backbone and chains nicely nestled. The binding conformation of **6b** in this docking simulation revealed an intriguing molecular binding mode at the active site of nAChRs, with a very low docked energy. As compared with our previous work, the binding conformation of analogue **6b** in this docking simulation showed the similar binding mode. However, unexpectedly, the molecular binding site of analogue **6b** was different from that of the analogues **3**, **4, 5** with a more rational way.

As illustrated in Figure 3(b), analogue **6b** exhibits two hydrogen bonds via its nitro O (30) and O (31) with the backbone H and side chain H of Tyr192, respectively, and the N (17) of its tetrahydropyrimidine interacts with the side chain H of Gln55. Besides, its binding conformation exhibited three important hydrogen bonds between the O (22) of the diglycine ester and H–O of Tyr164, H–O of Gln155, respectively. In addition, another important hydrogen bond between HN (23) of its peptide bond and H–S of Met114 was displayed. 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 **6b** attained the highest score.

Furthermore, most of the other active analogues (**6a**, **6c**, and **6n**) shared a quite similar binding mode with **6b**, and many of them exhibited more than four hydrogen bonds with different amino acids of the active pocket between the nAChRs subunits, which is consistent with their high insecticidal activities. However, consistent with the SARs observed *in vitro*, the binding interactions of analogues with low inhibitory potency were not satisfactory, so did their docking scores. No significant formation of hydrogen bonds and hydrophilic or hydrophobic contact could be found in their best binding conformations, such as the predicted binding mode of compound **6k** (Figure 3(c)). Our docking results coincided well with the experimental activities, which indicated that different insecticidal potency depended on the number of significant hydrogen bonds formed by the nitenpyram analogue binding with the amino acid residues. Thereby, the binding model proposed here may provide an alternative way close to the actual binding features of these

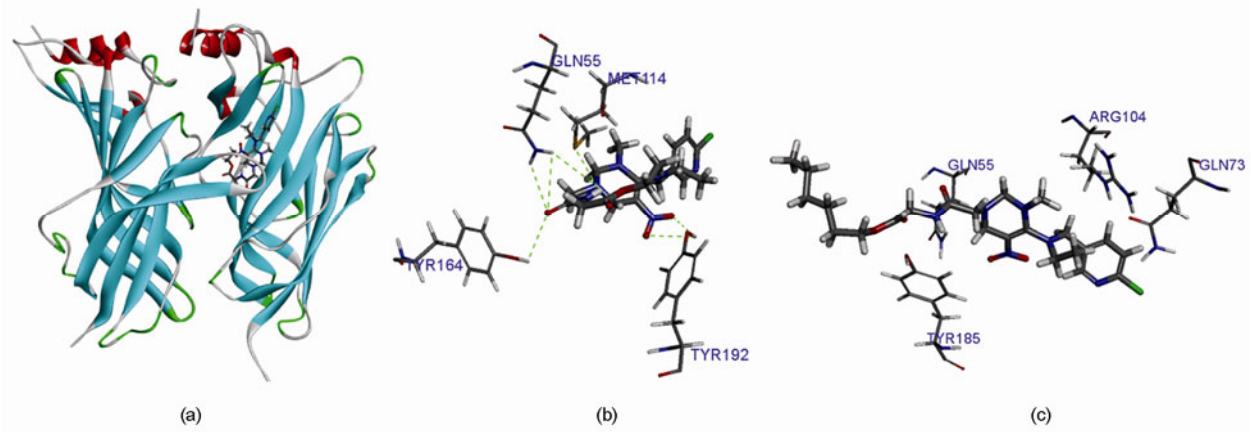


Figure 3 Binding site interactions of analogue **6b** with the extracellular domain of nAChRs (protein data bank code: 2zju). (a) Compound **6b** is bound into the subunit interfacial binding pocket between two faces of adjacent subunits. For clarity, only two of five subunits are extracted and shown from the pentameric nAChRs structure, and the corresponding interfacial binding pocket of interest is displayed; (b) zoomed-in view of the interactions between compound **6b** and amino acids from the active site of the receptor; (c) the predicted binding mode of compound **6k** with relatively low activity. Key H-bonds are indicated by green dotted lines.

new series of neonicotinoids analogues, which may provide some useful information for future receptor structure-guided design of novel insecticides.

5 Conclusions

Based on the strategy of receptor structure-guided neonicotinoid design, a new series of *cis*-configuration nitenpyram analogues (**6a–6n**) were designed and synthesized by introducing the tetrahydropyrimidine ring bearing diverse diglycine esters, which inset the peptide bond to the flexible ester arm. The insecticidal spectra of the target compounds were expanded when compared to our previous work, and all the target compounds presented excellent insecticidal activities against *Nilaparvata lugens* and *Aphis medicagini* at 100 mg/L. Among these analogues, **6b** afforded the best activity, and had 100% mortality against *Nilaparvata lugens* (LC_{50} = 0.163 mg/L) and 90% against *Aphis medicagini* at 4 mg/L. SARs suggested that the insecticidal potency of our designed *cis*-nitenpyram analogues was dual-controlled by the size and species of the ester group. The molecular docking investigation was carried out to model the ligand-receptor complexes and analyze their interactions for improved activity. The docking results revealed a unique binding mode other than the previously designed compounds, because introduction of the peptide bond gave rise to more significant hydrogen bonds between the nitenpyram analogues and the amino acid residues of insect nAChRs. Additionally, the docking scores were in good agreement with their high insecticidal potential, which suggested that the more significant the hydrogen bonds were, the better the active would become. The results of molecular docking also explained the structure-activity relationships observed *in vitro*. Further studies are ongoing to verify the molecular nAChRs target and evaluate their inhibitory activities against resistant insect species. The study herein has shed a light on the mechanism of the function of these *cis*-configuration neonicotinoid analogues when interacted with the amino acid residues of insect nAChRs, and may facilitate receptor structure-guided design of novel insecticidal compounds with less resistance and better selectivities.

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