Nitenpyram analogues with 1,4 – dihydropyridine fixed *cis*-configuration: synthesis, insecticidal activities and molecular docking studies

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Abstract: A novel series of Nitenpyram analogues(I_a – I_j) with 1,4 – dihydropyridine fixed *cis*-configuration were designed and synthesized. Preliminary bioassays showed that most of them exhibited good insecticidal activities against *Aphis medicagini* and *Brown rice planthopper* at 500 mg/L and 100 mg/L. The analogue I_j afforded the best activity *in vitro*, that had 100% mortality at 4 mg/L against *Brown rice planthopper* and *Aphis medicagin*. In addition, the molecular docking simulations revealed that the structural uniqueness of these analogues may lead to a unique molecular recognition and binding mode, and the results explained the SARs observed *in vitro*, which shed light on the novel insecticidal mechanism of these novel nitenpyam analogues.

Key words: *cis*-Nitenpyram analogue; 1,4 – dihydropyridine; synthesis; insecticidal acvitity; molecular docking

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1 Introduction

Neonicotinoid insecticides (NNSs) act selectively on the insect nicotinic acetylcholine receptor (nAChR) [1-2], which represent a new generation of synthetic insecticides as they have some specific properties that allow them to be the fastest growing synthetic insecticides on the market [3-5]. Since imidacloprid (IMI) was first introduced to the market in 1991, many new neonicotinoid insecticides (NNSs) are now on the market with their own prominence. As the second of the chloronicotinyl subclass, Nitenpyram, which was brought to market in 1995, was characterized with much lower toxicity against the mammals than imidacloprid. Besides, Nitenpyram also had higher selectivity and better systemic properties against mammals, birds, aquatic life than insects, due to the differential binding affinities with the nAChR of their neurosystem. However, frequent applications of structural analogues of neonicotinoids have led to the acquisition of resistance and cross-resistance in a range of species. Hence, the development of new neonicotinoids with novel structures and high insecticidal activities against resistance is an urgent requirement [6]. It is well known that hanging the configura-

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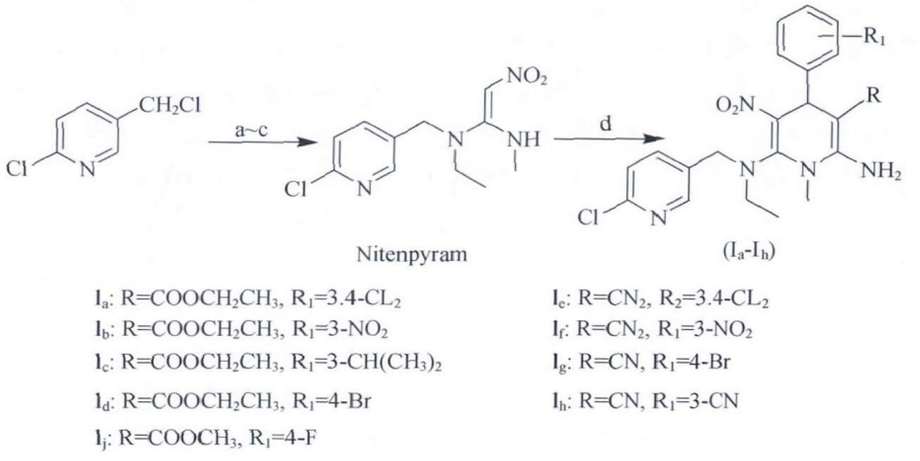
tion of commercial neonicotinoids´ pharmacophore is one of the effective resistance-management tactics [7-8].

The nitro groups in all commercialized neonicotinoids have a *trans*-configuration, on which three proposals formodes of action are based [9]. However, in the late 1980s, Bayer and Nihon Tokushu Noyaku Seizo Co. reported that several cis-configuration neonicotinoids showed high insecticidal activity, which implied that neonicotinoids in the cis-configuration might bind to the receptor in a different way, and their insecticidal activities may benefit from it. In addition, considering the pharmacophoric moieties, these commercialized neonicotinoids can be divided into two groups: the cyclic NNSs and the acyclic NNSs, which are different in their molecular characteristics. Until recently, most of the structural optimization of NNSs are based on cyclic neonicotinoid insecticides, such as imidacloprid. However, few studies have been focused on the structural modification of acyclic NNSs, such as Nitenpyram.

On the basis of the above mentioned reports, in order to search for lead compounds of neonicotinoid insecticides with novel structural features, high activity, less resistance and broad insecticidal spectra, we developed a new design strategy by introducing a 1,4 – dihydropyridine ring into nitenpyram and fixing the nitro group in *cis*-configuration. A new series of Nitenpyram analogues ($\mathbf{I}_a - \mathbf{I}_j$) described herein were synthesized via reactions of Nitenpyram, substituted aromatic aldehydes and ethyl cyanoacetate or malononitrile in piperidine/anhydrous alcohol under microwave irradiation (Fig. 1). Preliminary bioassay against *Aphis medicagini* and *Brown planthopper* showed that all these analogues exhibited excellent insecticide activities, and their structure-activity relationships were discussed. To further investigate their binding interactions, molecular docking simulations were carried out. As expected, active analogues exhibited significant hydrogen bonding interactions with the nAChR target. The docking results explained the SARs observed *in vitro*, and shed a light on the novel insecticidal mechanism of these new analogues, which may provide some useful information for future design of new insecticides.



Reagents and conditions (a) ethanamine (42%) ,(b) 1,1,1 – trichloro – 2 – nitroethane/$CHCl_3$ 2 – 7 ℃(65%) , (c) methanamine 3 – 7 ℃(58%) ,(d) substituted aromatic aldehydes, ethyl cyanoacetate or malononitrile, piperidine/anhydrous alcohol, M. W 65 ℃(65.5% – 86.0%)

Figure 1　Synthesis of Nitenpyram analogues($\mathbf{I}_a - \mathbf{I}_j$) cotaining 1,4 – dihydropyridine ring.

2　Experimental

2.1　Materials and physical measurements

Unless otherwise noted, reagents and solvents were of analytical reagent grade or were chemically pure and

used as received without further purification. Melting points were measured using an uncorrected RK^{-1} microscopic melting point apparatus. ^1H 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 4000 to 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 a YL8023B1 microwave reactor possessing a single-mode microwave cavity producing controlled irradiation at 2.45 GHz.

2.2 General Synthetic Procedures for Target Compounds (I_a – I_j) (exemplified by I_a)

Starting from 2 – chloro – 5 – chloromethylpridine, Nitenpyram were prepared based on the procedures in the literature [9].

A mixture of ethyl cyanoacetate (18 mmol), 3,4 – dichlorobenzaldehyde (18 mmol), piperidine (0.15 mmol), and Nitenpyram (15 mmol) in anhydrous alcohol (30 mL) was heated to 60 – 75 °C for 5 min in a microwave reactor, and stirred for 30 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, and the combined extracts were dried over $MgSO_4$. The organic phase was evaporated under reduced pressure, and crude product was subjected to flash chromatography on silica gel, eluting with ethyl acetate/petroleum ether (3∶1) to afford pure products I_a.

The syntheses of I_b – I_j were carried out by the similar method. The analytical data for the compounds I_a – I_j were summarized as follows:

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N*-ethyl] amino – 3 – ethoxycarbonyl – 1 – methyl – 4 – (3,4 – dichlorophenyl) – 5 – nitro – 1,4 – dihydropyridine (I_a)**

Yellow crystals, yield 67.2%. m. p. 228 – 229℃; ^1H NMR ($CDCl_3$, 400 MHz) δ8.13 (s, 1H, Pyridine), 7.81 (s, 1H, Pyridine), 7.38 – 7.26 (m, 1H, Ph-H), 7.18 – 7.14 (m, 1H, Ph-H), 7.07 – 7.5 (d, J = 8.1 Hz, 1H, Ph-H), 6.89-6.87 (d, J = 8.0 Hz, 1H, Pyridine), 6.24 – 6.06 (br, 2H, $-NH_2$), 5.39 (s, 1H, –CH), 4.35 – 4.31 (d, J = 14.6 Hz, 1H, $-NCH_2CH_3$), 4.13 – 4.12 (m, 2H, $-COOCH_2CH_3$), 4.09 – 4.05 (d, J = 14.9 Hz, 1H, $-NCH_2CH_3$), 3.29 – 3.27 (m, 1H), 3.26 (s, 3H, $-NCH_3$), 3.17 – 3.10 (m, 1H), 1.33 – 1.29 (t, J = 7.0 Hz, 3H, $-COOCH_2CH_3$), 1.23 – 1.20 (t, J = 7.0 Hz, 3H, $-NCH_2CH_3$); IR (KBr, cm^{-1}) v_{max} 3301, 3237 (NH_2), 2995, 2943, 2901 (C = O), 1342 (NO_2), 1293 (v_{as} C – O – C), 1235 (v_a C – O – C); MS (ESI) m/z: 540 ([M – H]$^-$). Anal. calcd for $C_{23}H_{24}Cl_3N_5O_4$: C 51.08, H 4.47, N 12.95; found C 51.21, H 4.50, N 12.81.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 3 – ethoxycarbonyl – 1 – methyl – 4 – (3 – nitrophenyl) – 5 – nitro – 1,4 – dihydropyridine (I_b)**

Yellow crystals, yield 59.2%. m. p. 213 – 214℃; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.13 – 8.11 (d, J = 8.1 Hz, 1H, Pyridine), 8.06 – 8.04 (d, J = 8.3 Hz, 2H, Ph – H), 7.42 – 7.40 (d, J = 8.0 Hz, 1H, Pyridine), 7.17 – 7.12 (dd, J = 12.5, 5.3 Hz, 2H, Ph – H), 6.95 – 6.93 (d, J = 8.1 Hz, 1H, Pyridine), 6.34 – 6.18 (br, 2H, $-NH_2$), 5.48 (s, 1H, –CH), 4.35 – 4.31 (d, J = 14.6 Hz, 1H, $-NCH_2CH_3$), 4.17 – 4.14 (m, 2H, $-COOCH_2CH_3$), 4.09 – 4.05 (dd, J = 12.3, 5.3 Hz, 1H, $-NCH_2CH_3$), 3.38 – 3.33 (dd, J = 13.7, 6.7 Hz, 1H), 3.30 (s, 3H, $-NCH_3$), 3.23 – 3.16 (m, 1H) 1.38 – 1.32 (t, J = 7.1 Hz, 3H, $-COOCH_2CH_3$), 1.19 – 1.16 (t, J = 7.1 Hz, 3H, $-NCH_2CH_3$); IR (KBr, cm^{-1}) v_{max} 3385, 3297 (NH_2), 3083, 2980, 2935 (C = O), 1349 (NO_2), 1300 (v_{as} C – O – C), 1237 (v_a C – O – C); MS (ESI) m/z: 515 ([M – H]$^-$). Anal. calcd for $C_{23}H_{25}ClN_6O_6$: C 53.45, H 4.91, N 16.31; found C 53.44, H 4.87, N 16.26.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 3 – ethoxycarbonyl – 1 – methyl – 4 – (4 – isopropylphenyl) – 5 – nitro – 1,4 – dihydropyridine (I_c)**

Yellow crystals,yield 61.3%. m. p. 153 – 154℃; NMR ($CDCl_3$,400 MHz) δ 8.15 (s,1H,Pyridine) , 7.35 (s,1H,Pyridine) ,7.11 (d,J = 6.6 Hz,3H,PhH) ,7.04 (d,J = 7.4 Hz,1H,Pyridine) ,6.94 (d, J = 7.5 Hz,1H,PhH) ,5.03 (s,1H,CH) ,4.50 (d,J = 18.2 Hz,2H,NH_2) ,4.37 (d,J = 13.8 Hz,1H) , 4.34 – 4.30 (d,J = 14.1 Hz,1H, – NCH_2CH_3) ,4.20 – 4.16 (m,2H, – $COOCH_2CH_3$) ,4.10 – 4.07 (dd,J = 12.2,5.6 Hz,1H, – NCH_2CH_3) ,4.03 (d,J = 14.6 Hz,1H) ,3.20 (s,1H) ,3.14 (s,3H, NCH_3) ,3.09 – 2.97 (m,1H) ,2.93 – 2.82 (m,1H,$CH(CH_3)_2$) ,1.32 – 1.25 (m,3H,NCH_2CH_3) , 1.23 (d,J = 6.6 Hz,6H,$CH(CH_3)_2$) . IR (KBr, cm^{-1}) ν_{max} 3329, 3194 (NH_2) , 3080, 2981, 2933 (C = O) ,1460,1411 (NO_2) ,1302 (vasC – O – C) ,1230 (vaC – O – C) ; MS (ESI) m/z: 512 ([M – H] –) . Anal. calcd for $C_{26}H_{32}ClN_5O_4$: C 60.75,H 6.90,N 16.62; found C 60.71,H 6.78,N 16.64.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 3 – ethoxycarbonyl – 1 – methyl – 4 – (4 – bromophenyl) – 5 – nitro – 1,4 – dihydropyridine (I_d)**

Yellow crystals,yield 51.7%. m. p. 208 – 209℃; ^{1}H NMR ($CDCl_3$,400 MHz) δ8.12 (s,1H,Pyridine) , 7.80 (s,1H,Pyridine) ,7.36 (s,1H,Pyridine) ,7.33 – 7.31 (d,J = 8.1 Hz,1H,Ph – H) ,7.10 – 7.08 (d,J = 6.4 Hz,1H,Ph – H) ,7.03 – 7.10 (d,J = 8.1 Hz,1H,Ph – H) ,6.93 – 6.91 (d,J = 6.4 Hz, 1H,Ph – H) ,6.06 – 6.23 (br,2H, – NH_2) ,5.41 (s,1H, – CH) ,4.34 – 4.31 (d,J = 14.6 Hz,1H, – NCH_2CH_3) ,4.16 – 4.12 (m,2H, – $COOCH_2CH_3$) ,4.09 – 4.06 (d,J = 14.9 Hz,1H, – NCH_2CH_3) , 3.30 – 3.24 (dd,J = 13.8,7.0 Hz,1H) ,3.20 (s,3H, – NCH_3) ,3.16 – 3.09 (dd,J = 14.0,7.1 Hz, 1H) ,1.33 – 1.29 (t,J = 7.0 Hz,3H, – $COOCH_2CH_3$) ,1.21 (t,J = 7.1 Hz,3H, – NCH_2CH_3) ; IR (KBr, cm^{-1}) ν_{max} 3399,3291 (NH_2) ,2976,2929,(C = O) ,1358 (NO_2) ,1305 (ν_{as}C – O – C) ,1275 (ν_aC – O – C) ; MS (ESI) m/z: 550 ([M – H] –) . Anal. calcd for $C_{23}H_{25}BrClN_5O_4$: C 50.15,H 4.57,N 12.71; found C 50.18,H 4.54,N 12.74.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 3 – cyano – 4 – (3,4 – dichlorophenyl) – 1 – methyl – 5 – nitro – 1,4 – dihydropyridine (I_e)**

Yellow crystals,yield 63.3%. m. p. 107 – 108℃; ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.12 (s,1H,Pyridine) ,7.77 (d,J = 12.0 Hz,1H,Pyridine) ,7.34 (d,J = 8.1 Hz,1H,Pyridine) ,7.23 – 6.84 (m,3H, PhH) ,5.01 (s,1H) ,4.62 (s,1H,CH) ,4.37 (d,J = 14.4 Hz,2H,NH_2) ,4.09 (d,J = 15.5 Hz,1H) , 3.34 – 3.25 (m,1H) ,3.23 (s,3H,NCH_3) ,3.17 (dd,J = 10.5,4.4 Hz,1H) ,1.37 – 1.20 (m,3H,NCH_2CH_3) . IR (KBr,cm^{-1}) ν_{max} 2970,2931(CH_3) ,3323,3195, (NH_2) ,2186 (CN) ,1464,1412 (NO_2) ,1648, 1610,1557 (benzene) ; MS (ESI) m/z: 491 ([M – H] –) . Anal. calcd for $C_{21}H_{19}Cl_3N_6O_2$: C 51.08,H 3.88,N 17.02; found C 50.12,H 3.78,N 17.14.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 3 – cyano – 1 – methyl – 4 – (3 – nitrophenyl) – 5 – nitro – 1,4 – dihydropyridine (I_f)**

Yellow crystals,yield 63.3%. m. p. 111 – 112℃; ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.31 (s,1H,Pyridine) ,8.07 (s,1H,Pyridine) ,7.36 (s,1H,Pyridine) ,7.23 – 6.56 (m,5H,PhH) ,5.06 (s,1H,CH) ,4.69 (s,2H,NH_2) ,4.35 (d,J = 14.3 Hz,1H) ,4.09 (d,J = 14.8 Hz,1H) ,3.36 – 3.24 (m,1H) ,3.21 (s,3H, NCH_3) ,3.16 (d,J = 6.5 Hz,1H) ,1.36 – 1.27 (m,3H,NCH_2CH_3) . IR (KBr,cm^{-1}) ν_{max} 2973 (CH_3) , 3203 (NH_2) ,2187 (CN) ,1460,1416 (NO_2) ,1660,1607,1525 (benzene) ; MS (ESI) m/z: 468 ([M – H] –) . Anal. calcd for $C_{21}H_{20}ClN_7O_4$: C 53.68,H 4.29,N 20.87; found C 53.62,H 4.38,N 20.84.

cis **– 2 – amino – 6 – [*N* – (6 – chloro – 3 – pyridinylmethyl) – *N* – ethyl] amino – 4 – (4 – bromo–**

phenyl) -3-cyano-1-methyl-5-nitro-1,4-dihydropyridine (I_g)

Yellow crystals, yield 76.1%. m. p. 123-124℃; ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.11 (s, 1H, Pyridine), 7.78 (s, 1H, Pyridine), 7.38 (d, J = 7.5 Hz, 2H, Pyridine, PhH), 7.16, 6.86 (m, 3H, PhH), 5.01 (s, 1H, CH), 4.60 (s, 2H, NH2), 4.35 (d, J = 13.6 Hz, 1H), 4.09 (d, J = 14.1 Hz, 1H), 3.27 (d, J = 7.2 Hz, 1H), 3.20 (s, 3H, NCH_3), 3.08 (d, J = 5.0 Hz, 1H), 1.29 (t, J = 10.7 Hz, 3H, NCH_2CH_3); IR (KBr, cm^{-1}) v_{max} 2973 (CH_3), 3330, 3199 (NH_2), 2185 (CN), 1465-1412 (NO_2), 1648, 1611, 1556 (benzene); MS (ESI) m/z: 503 ([M-H]$^-$). Anal. calcd for $C_{21}H_{20}BrClN_6O_2$: C 50.07, H 4.00, N 16.68; found C 50.12, H 4.02, N 16.59.

***cis*-2-amino-6-[*N*-(6-chloro-3-pyridinylmethyl)-*N*-ethyl]amino-3-cyano-4-(4-cyanophenyl)-1-methyl-5-nitro-1,4-dihydropyridine (I_h)**

Yellow crystals, yield 85.5%. m. p. 102-103℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (s, 1H, Pyridine), 8.09 (s, 1H, Pyridine), 7.78 (s, 1H, Pyridine), 7.21 (d, J = 7.3 Hz, 1H, PhH), 7.10 (d, J = 6.8 Hz, 1H, PhH), 7.01 (d, J = 7.8 Hz, 1H, PhH), 6.94 (d, J = 7.5 Hz, 1H, PhH), 5.01 (s, 1H, CH), 4.51 (s, 2H, NH_2), 4.33 (d, J = 14.2 Hz, 1H), 4.10 (dd, J = 16.9, 10.8 Hz, 1H), 3.25 (m, 1H), 3.16 (s, 3H, NCH_3), 3.14 (m, 1H), 1.35-1.20 (m, 3H, NCH_2CH_3); IR (KBr, cm^{-1}) v_{max} 2927 (CH_3), 3447, 3319, 3196 (NH_2), 2184 (CN), 1485-1413 (NO_2), 1648, 1608, 1557 (benzene); MS (ESI) m/z: 448 ([M-H]$^-$). Anal. calcd for $C_{22}H_{20}ClN_7O_2$: C 58.73, H 4.48, N 21.79; found C 58.82, H 4.35, N 21.62.

***cis*-2-amino-6-[*N*-(6-chloro-3-pyridinylmethyl)-*N*-ethyl]amino-4-(4-fluorophenyl)-1-methyl-3-methoxycarbonyl-5-nitro-1,4-dihydropyridine (I_j)**

Yellow crystals, yield 83.2%, m. p. 231-232°C ^{1}H NMR (δ, ppm, $CDCl_3$): 8.21 (d, J = 16.8 Hz, Py-H, 1H), 7.19-7.09 (m, Ph-H, 4H), 7.03 (d, J = 7.9 Hz, Py-H, 1H), 6.96 (d, J = 7.0 Hz, Py-H, 1H), 6.22 (s, $-NH_2$, 2H), 5.74 (d, J = 17.1 Hz, $-CH$, 1H), 4.37 (d, J = 14.8 Hz, $-NCH_2CH_3$, 1H), 4.11 (d, J = 14.9 Hz, $-NCH_2CH_3$, 1H), 3.63 (s, $-COOCH_3$, 3H), 3.27 (s, 1H), 3.21 (s, $-NCH_3$, 3H), 3.11-3.05 (m, 1H), 1.32 (t, J = 6.8 Hz, $-NCH_2CH_3$, 3H). IR (potassium bromide, cm^{-1}) v_{max} 3364, 3278 (NH_2), 2981, 2945, 2876 (C=O), 1342 (NO_2), 1309 (vasC-O-C), 1235 (vaC-O-C). Anal. calcd for $C_{22}H_{23}FClN_5O_4$: C 55.52, H 4.87, N 14.72; found C 55.66, H 4.82, N 14.68. ESI-MS (M+H) m/z: 475.14.

2.3 Biology Assay

The bioassay was measured according to the standard test [10] with a slight modification, and all analogues were tested to evaluate their insecticidal activities. The compounds were dissolved in dimethylformamide (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 air dried, they were kept in a special room for normal cultivation. Assessments were made after 72 h by the killed number of and size of live insects relative to those 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. 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.

2.4 Experimental Protocol of Docking Study

To understand the ligand protein interactions in detail, we chose the high nAChR inhibitory activity of

compound $\mathbf{I}_j$ with AutoDock 4.0 [11] 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) [12-13] was used as the template to construct the models. The docking was carried out through the graphical user interface AUTODOCKTOOLS (ADT 1.4.6).

3 Results and Discussion

3.1 Evaluation of insecticidal activities

The insecticidal activities of the target *cis*-Nitenpyram analogues were evaluated against *Aphis medicagini* and *Brown rice planthopper*. As shown in Table 1, most of the target *cis*-Nitenpyram analogues exhibited good insecticidal activities against *Aphis medicagini* and *Brown rice planthopper* at 500 and 100 mg/L. Among them, analogue $\mathbf{I}_j$ afforded the best activity *in vitro*, which had 100% mortality at 4 mg/L against *Brown rice planthopper* and *Aphis medicagin*.

When different substituents R and R_1 were introduced to the 1,4 – dihydropyridine ring, the insecticidal activities varied greatly. When the R_1 group was the same, the insecticidal activities increased in the order $\mathbf{I}_e$, $\mathbf{I}_f$, $\mathbf{I}_g$ (R = CN) < $\mathbf{I}_a$, $\mathbf{I}_b$, $\mathbf{I}_d$ (R = $COOCH_2CH_3$) respectively. As for the substituent R($COOCH_2CH_3$), the insecticidal activities increased in the order $\mathbf{I}_c$ (4 – $CH(CH_3)_2$) < $\mathbf{I}_d$ (4 – Br) < $\mathbf{I}_b$ (3 – NO_2) < $\mathbf{I}_a$ (3,4 – Cl_2), which may be related to their different affinities to the nAChR target, such as the number of hydrogen bonds.

Table 1　Insecticidal activities of *cis*-Nitenpyram analogues ($\mathbf{I}_a$ – $\mathbf{I}_j$) against *Aphis medicagini and Brown rice planthopper*

analogues	R	R_1	mortality(%) at different concentrations (mg/L) against *Aphis medicagini*				mortality(%) at different concentrations (mg/L) against *Brown rice planthopper*			
			500	100	20	4	500	100	20	4
$\mathbf{I}_a$	$COOCH_2CH_3$	3,4 – Cl_2	100	100	100	100	100	100	95	80
$\mathbf{I}_b$	$COOCH_2CH_3$	3 – NO_2	100	90	70	10	95	85	75	50
$\mathbf{I}_c$	$COOCH_2CH_3$	4 – $CH(CH_3)_2$	100	60	10	n.t	85	70	40	20
$\mathbf{I}_d$	$COOCH_2CH_3$	4 – Br	100	70	50	5	90	80	75	45
$\mathbf{I}_e$	CN	3,4 – Cl_2	100	100	90	70	95	95	80	65
$\mathbf{I}_f$	CN	3 – NO_2	100	80	65	5	95	80	65	45
$\mathbf{I}_g$	CN	4 – Br	100	60	50	n.t	90	75	50	20
$\mathbf{I}_h$	CN	3 – CN	100	70	50	n.t	95	80	50	30
$\mathbf{I}_j$	$COOCH_3$	4 – F	100	100	100	100	100	100	100	100
	Nitenpyram		100	100	100	100	100	100	100	100

3.2 Molecular docking study

As a result, all active analogues exhibited significant hydrogen bonding interactions with the nAChR target. As expected, the compound $\mathbf{I}_j$ is nicely accommodated within the subunit interfacial binding pocket between the two faces of adjacent subunits. Its binding conformation exhibited one important hydrogen bond between the O30 of its ester group and the H-O of Tyr185 and its chloropyridine interacts primarily with the side chain of Glu190. The hydrogen-bonds also exits between its chlorophenyl growp and the side chain of Gln55. Moreover, $\mathbf{I}_j$ showed the important additional H bonding interaction with Trp143 at the interface of two adjacent nAChR subunits (Fig. 2). In addition, due to the novel structure of compound $\mathbf{I}_j$. All these interactions above may greatly enhance the binding affinity of inhibitor $\mathbf{I}_j$, and account for its high inhibitory potency. Hence, the structure-activity relationships observed in vitro have been explained by the observations herein.

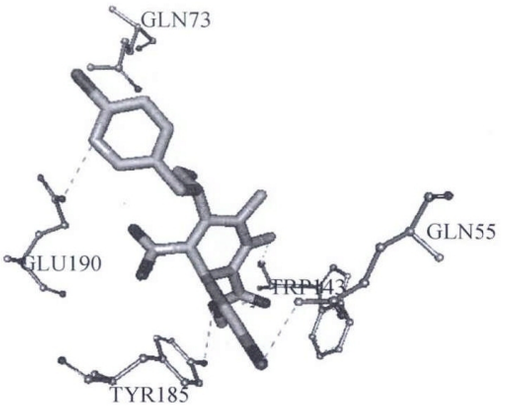


Figure 2 Modeling of the docking results of compounds $\mathbf{I}_j$ in the extracellular domain of insect nAChR
(The hydrogen-bonding between $\mathbf{I}_j$ and the active site residues of nAChR)

4 Conclusion

In conclusion, a new series of *cis*-Nitenpyram analogues ($\mathbf{I}_a - \mathbf{I}_j$) were designed and synthesized. All the target analogues presented good insecticidal activities against *Aphis medicagini* and *Brown planthopper* at 500 mg/L and 100 mg/L *in vitro*. Among them $\mathbf{I}_j$ afforded the best activity, and had 100% mortality against *Brown rice planthopper* and *Aphis medicagin* at 4mg/L. Structure-activity relationships indicated that insecticidal activities varied greatly when different substituents R and R_1 were introduced to the 1,4 - dihydropyridine ring. In addition, molecular docking investigation was also 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 Nitenpyram, and the docking scores were in good agreement with their high insecticidal potential, which also explained the structure-activity relationships observed *in vitro*. The study herein may prompt structure-guided future attempts to design and develop novel insecticides with less resistance and better selectivity. Studies on much more test objects and further structural modification of Nitenpyram are underway.

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用 1，4 - 二氢吡啶环固定顺式构型的烯啶虫胺类似物: 合成、杀虫活性和分子对接研究

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摘　要: 合成了一系列未见文献报道的用 1，4 - 二氢吡啶环固定顺式构型的烯啶虫胺类似物(I_a - I_j). 初步的杀虫活性测试结果表明: 大多数目标化合物在 500 mg/L 和 100 mg/L 的浓度下，对苜蓿蚜和褐飞虱有高效杀虫活性，其中类似物 I_j 在 4 mg/L 时对苜蓿蚜和褐飞虱的致死率都达到 100%. 分子对接模拟结果显示，标题类似物具有独特的与靶标的结合模式，并初步解释了构效关系.

关键词: 顺式烯啶虫胺类似物; 1，4 - 二氢吡啶; 合成; 杀虫活性; 分子对接模拟

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