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Design, Synthesis, and Insecticidal Activity of Novel Doramectin Derivatives Containing Acylurea and Acylthiourea Based on Hydrogen Bonding

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ABSTRACT

Our recent investigation on the insecticidal activities of several doramectin derivatives preliminarily revealed that the presence of hydrogen bonds at the C4″ position of the molecule with target protein GABA receptor was crucial for retaining high insecticidal activity. As a continuation of our research work on the development of new insecticides, two series of novel acylurea and acylthiourea doramectin derivatives were designed and synthesized. The bioassay results indicated that the newly synthesized compounds (**5o**, **5t**, and **6t**) exhibited higher insecticidal activity against diamondback moth, oriental armyworm, and corn borer than the controls compounds doramectin, commercial avermectins, chlorbenzuron, and the lead compound **3g** in our laboratory. Specifically, compound **5t** was identified as the most promising insecticide against diamondback moth with a final mortality rate of 80.00% at the low concentration of 12.50 mg/L, showing approximately 7.75-fold higher potency than the parent doramectin (LC_{50} value of 48.1547 mg/L), 6.52-fold higher potency than commercial avermectins (LC_{50} value of 40.5507 mg/L), and 3.98-fold higher potency than **3g** (LC_{50} value of 24.7742 mg/L). Additionally, molecular docking simulations revealed that **5t** (2.17, 2.20, 2.56, and 2.83 Å) displayed stronger hydrogen bond action in binding with GABA receptor, better than that of **5o** (1.64 and 2.15 Å) and **6t** (2.20 and 2.31 Å) at the C4″ position. This work demonstrated that these compounds containing hydrogen bond groups might contribute to the improvement of insecticidal activity and supplies certain hints toward structure optimization design for the development of new insecticides.

KEYWORDS: *Acylurea, acylthiourea*, *insecticidal activities*, *molecular docking*, *oriental armyworm*, *diamondback moth*, and *corn borer.*

INTRODUCTION

Currently, Lepidoptera (diamondback moth, oriental armyworm, corn borer, etc.) is becoming a major threat to the agricultural system.[1] To obtain higher food production, many chemical and biological pesticides have been widely applied for control of insect pests.[2-8] In the quest for new insect control agents, natural products have been and remain an excellent source of novel chemistry and inspiration for insecticides. Among natural product-based insecticides, avermectin, a macrocyclic lactone with high efficiency and without cross-resistance, is an important insecticide that has been commercialized in crop protection and plays an important role in modern agriculture by increasing both crop quality and yield while improving living standards due to a unique mechanism of action.[9] To further identify more stable, efficient, low-toxicity and broad-spectrum chemical compounds, scientists have made a large number of structural modifications using natural avermectin components as parent compounds, and obtained many highly active derivatives.[10-15] Among the avermectin-based insecticides, doramectin, a third generation of the avermectins family with a cyclohexyl group at C25 position in lieu of the sec-butyl or isopropyl of avermectins, can aid in the identification of new mode of action and might be used as a lead structure to derive new potent pesticides. Therefore, doramectin might be a promising template for the discovery of new insecticide candidates.

The gamma-aminobutyric acid (GABA) receptor (GABAR) is a crucial target for

insecticide action. Studies suggested that the target of avermectin and doramectin was the GABA-gated chloride channel, and this targeting caused hyperpolarization of nerve membrane potential and inhibited nerve membrane, thus blocking normal nerve conduction and paralyzing insects.[16] In previous papers from our laboratory, we reported that with the introduction of carbamate, ester, sulfonate active groups to replace the hydroxyl group at the C4″ position of doramectin, compound **3g** (Figure 1) containing cyclopropyl carbamate exhibited excellent insecticidal activities.[17] A molecular docking study indicated that **3g** could form the N−H···O H bond, which was crucial to the binding of the macrolide and GABAR, and the NH group on the C4″ position of doramectin was necessary to retaining higher insecticidal activity. In fact, Yamamoto,[18] Kagabu,[19] Casida,[20] and Qian[21] demonstrated the importance of the hydrogen bond between pesticide molecules and target proteins in various modes. To the best of our knowledge, use of acylureas and acylthioureas has reported for many years in insecticidal, herbicidal, fungicidal applications for crop protection.[22-24] Acylureas and acylthioureas contain two NH groups, which are also highly susceptible to additional hydrogen bonds with the target protein.[25, 26] With this characteristic in mind, we speculated whether the insecticidal activity could be enhanced by forming additional hydrogen bonds through the introduction of acylurea and acylthiourea moieties.

Herein, as a continuation of our work and to verify our hypothesis, a series of novel acylurea and acylthiourea derivatives were rationally designed and synthesized by replacing the hydroxyl group at the C4″ position with acylurea and acylthiourea

active groups (Figure 1). The insecticidal activities of these target compounds were tested against diamondback moth, oriental armyworm, and corn borer. Furthermore, structure-activity relationship (SAR) studies and docking analysis were also discussed.

MATERIALS AND METHODS

Chemicals. Doramectin was purchased from Chongqing Qiantai Biopharmaceutical Co., Ltd. (Chongqing, China). Imidazole, triphosgene, tert-butyldimethylsiyl chloride (TBDMS-Cl), and *N*, *N*-dimethypyridin-4-amine (DMAP) were purchased from Energy Chemical Ltd. (Beijing, China). All chemical materials amides and acid chlorides, phenyl dichlorophosphate, sodium cyanoborohydride, ammonium thiocyanate, oxalyl chloride, and dry dichloromethane (DCM) were purchased from J&K Chemical Ltd. (Beijing, China). Triethylamine, *p*-toluenesulfonic acid (TsOH), sodium carbonate, magnesium sulfate, methanol, dichloromethane (DCM), and dimethyl sulfoxide (DMSO) were purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China). All reagents and solvents were reagent grade without further purification.

Instruments. The melting points were determined on an X-6 precision micro-melting point apparatus (Beijing Fukui Technology Development Co., Ltd). ^{1}H NMR (400 MHZ) and ^{13}C NMR (100 MHZ) spectra were obtained using a Bruker 400 spectrometer in $CDCl_3$ solution with tetramethylsilane as the internal standard. Reaction progress was monitored according to thin-layer chromatography (TLC) on silica gel GF254 with ultraviolet (UV) detection. High resolution mass spectra

(HRMS) data were recorded on a Micro Q-TOF II mass spectrometer (HR-ESI-MS, Bruker, Germany) in the negative ion detection mode.

General Synthesis. The silica gel chromatography was performed with a column of 254 mm × 26 mm i.d. (Synthware glass Co. Ltd., Beijing, China) using 100-140 mesh silica gel (Sinopharm Chemical reagent Co. Ltd., Shanghai, China). The general synthetic methods for doramectin derivatives containing acylurea (**5a-5v)** and acylthiourea **(6a-6v)** groups are shown in **Schemes 1** and **Schemes 2**, and their structures are listed in **S-1** (Supporting Information). Acyl isocyanate and acyl isothiocyanate were prepared according to the methods in the literature.[27, 28]

General Procedure for Synthesis of Acylurea Derivatives of Doramectin (5a-5v) (Scheme 1).

*Synthesis of [5-O-(tert-butyldimethylsilyl)doramectin]**(2)***.[29] To a solution of doramectin (20.00 g, 22.24 mmol) in dry dichloromethane (200.00 mL), imidazole (15.10 g, 222.00 mmol), *N*, *N*-dimethypyridin-4-amine (DMAP, 271.00 mg, 2.22 mmol), and tert-butyldimethylsiyl chloride (TBDMS-Cl, 11.72 g, 77.77 mmol) were added. The mixture was stirred for 15 h at room temperature. When the reaction was completed according to TLC analysis, water (200.00 mL) and dichloromethane (200.00 mL) were added to the mixture. The aqueous phase was extracted with dichloromethane (3 × 100.00 mL), and the combined organic phases were washed with saturated brine (3 × 100.00 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated by evaporation under vacuum to yield a white solid. The residue was purified by column chromatography on silica gel eluted with petroleum

ether/ethyl acetate (2: 1, v/v) to produce 19.48 g (86 %) of compound **2** as a white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88-5.80 (m, 1H, H9), 5.79-5.66 (m, 3H, H10, H11, H23), 5.53 (dd, *J* = 9.9, 2.6 Hz, 1H, H22), 5.41-5.32 (m, 3H, H3, H19, H1″), 5.04-4.97 (m, 1H, H15), 4.78 (dd, *J* = 4.0, 1.3 Hz, 1H, H1′), 4.68 (dd, *J* = 14.5, 2.4 Hz, 1H, H8a-a), 4.58 (dd, *J* = 14.5, 2.3 Hz, 1H, H8a-b), 4.46-4.39 (m, 1H, H4″), 4.12 (d, *J* = 13.1 Hz, 1H, H13), 3.93 (s, 1H, H7-OH), 3.90-3.72 (m, 4H, H17, H5′, H5″, H5), 3.61 (ddd, *J* = 11.0, 8.4, 4.6 Hz, 1H, H3′), 3.51-3.44 (m, 1H, H3″), 3.41 (d, *J* = 2.9 Hz, 6H, H3′-OMe, H3″-OMe), 3.39 (d, *J* = 2.4 Hz, 1H, H6), 3.33-3.11 (m, 3H, H2, H25, H4′), 2,58 (s, 1H, H4″-OH), 2.55-2.47 (m, 1H, H12), 2.37-2.19 (m, 5H, H16, H18a, H2′a, H2″a), 2.04-1.96 (m, 1H, H20a), 1.83-1.75 (m, 6H, H4a-CH_3, H27a, H30a, H31a), 1.73-1.55 (m, 5H, H24, H28a, H26, H29), 1.52-1.44 (m, 4H, H14a-CH_3, H20b), 1.32-1.10 (m, 15H, H2″b, H27b, H28b, H30b, H31b, H2′b, H5′-Me, H5″-Me, H12a-CH_3), 0.98-0.79 (m, 13H, H24a-CH_3, H18b, H-C$(CH_3)_3$), 0.13 (s, 6H, H-Si$(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 140.1, 137.6, 137.5, 136.1, 135.1, 127.7, 124.7, 119.4, 118.2, 117.2, 98.4, 95.7, 94.8, 81.8, 80.4, 80.2, 80.1, 79.3, 78.2, 77.2, 76.1, 69.5, 68.3, 68.2, 68.1, 67.9, 67.2, 56.5, 56.4, 45.7, 40.3, 39.6, 38.7, 36.6, 34.6, 34.4, 34.1, 31.4, 30.0, 27.0, 26.9, 26.6, 26.5, 25.8(3-C), 25.5, 20.3, 20.0, 18.4, 17.6, 16.6, 15.2, -4.5, -4.8.

Synthesis of [4″-O-5-O-(tert-butyldimethylsilyl)doramectin].[30] Dimethyl sulfoxide (DMSO, 25.00 mL) and triethylamine (TEA, 60.00 mL) were added to a solution of compound **2** (15.00 g, 14.80 mmol) in 100.00 mL of dry dichloromethane. A solution of $PhOPOCl_2$ (22.00 mL) in CH_2Cl_2 (100.00 mL) was

added dropwise over 60 minutes to the mixture at -15 °C, which was stirred at -15 °C overnight until TLC indicated that the reaction was completed. The reaction solution was poured into 1% H_3PO_4, the aqueous phase was extracted with dichloromethane (3 × 100.00 mL), and the combined organic layers were washed with saturated brine (3 × 100.00 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to yield a yellow solid (14.21 g), and the product was not purified for use in the next step.

Synthesis of [4″-epi-NH_2-5-O-(tert-butyldimethylsilyl)doramectin].[30] A mixture of compound **3** (14.00 g, 13.8 mmol), methanol (200.00 mL), and ammonium acetate (5.32 g, 69.00 mmol) was stirred 1 h at room temperature, and sodium cyanoborohydride (4.33 g, 69.00 mmol) was added. The mixture was stirred for 4 h at room temperature until the reaction was completed according to TLC analysis. Water (100.00 mL), saturated sodium carbonate (100.00 mL), and ethyl acetate (200.00 mL) were added, the aqueous phase was extracted with ethyl acetate (3 × 100.00 mL), and the combined organic phases were washed with saturated sodium chloride solution (3 × 100.00 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to yield a yellow solid. The residue was purified by column chromatography on silica gel eluted with dichloromethane/methanol (20: 1, v/v) to produce 5.38 g (38 %) of intermediate compound **4** as a yellow solid.

Synthesis of (4″-N- benzoylurea doramectin) ***(5a).***[27, 29] A mixture of benzoylamide (1.21 g, 10.00 mmol), 1,2-dichloroethane (1.75 mL, 20.00 mmol), and oxalyl chloride (1.75 mL, 20.00 mmol) was stirred overnight at 84 °C under an N_2 atmosphere. The

177 residue was concentrated in vacuo to benzoyl isocyanate (1.12 g) as a colorless liquid
178 and was not purified for use in the next step.

179 Benzoyl isocyanate (218.00 mg, 1.48 mmol) was added to a solution of compound
180 **4** (300.00 mg, 0.29 mmol) and *N*, *N*-dimethypyridin-4-amine (DMAP, 3.60 mg) in dry
181 dichloromethane (20.00 mL). The mixture was stirred for 2 h at room temperature
182 until the reaction was completed according to TLC analysis. Saturated sodium
183 bicarbonate (20.00 mL) was added, the aqueous phase was extracted with
184 dichloromethane (3 × 20.00 mL), and the combined organic phases were washed with
185 saturated sodium chloride solution (3 × 20.00 mL), dried over anhydrous magnesium
186 sulfate, filtered, and concentrated in vacuo. The residue was purified by column
187 chromatography on silica gel eluted with petroleum ether/ethyl acetate (4: 1, v/v) to
188 produce 280.00 mg as a yellow solid. A deprotection reagent solution of 7.50 mL of
189 *p*-toluenesulfonic acid-methanol complex (0.02 g/mL) was added dropwise to a
190 solution of yellow solid (280.00 mg) in methanol (15.00 mL). The mixture was stirred
191 1 h at room temperature until the reaction was completed according to TLC analysis.
192 Saturated sodium bicarbonate (30.00 mL) and dichloromethane (30.00 mL) were
193 added, the aqueous phase was extracted with dichloromethane (3 × 10.00 mL), and
194 the combined organic phases were washed with saturated brine (3 × 10.00 mL), dried
195 over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The crude
196 product was purified by column chromatography on silica gel eluted with petroleum
197 ether/ethyl acetate (4: 1, v/v) to produce 195.21 mg (78 %) of compound **5a**. ^{1}H NMR
198 (400 MHz, $CDCl_3$) δ 8.95 (d, J = 9.8 Hz, 1H, CONHCO), 8.45 (s, 1H, CONH), 7.86

(d, J = 7.1 Hz, 2H, Ph), 7.63-7.56 (m, 1H, Ph), 7.49 (dd, J = 8.4, 7.0 Hz, 2H, Ph), 5.90 (d, J = 10.1 Hz, 1H, H9), 5.83-5.68 (m, 3H, H10, H11, H23), 5.54 (dd, J = 9.9, 2.5 Hz, 1H, H22), 5.50-5.32 (m, 3H, H3, H19, H1″), 5.01 (d, J = 10.6 Hz, 1H, H15), 4.79 (d, J = 3.8 Hz, 1H, H1′), 4.69 (t, J = 2.9 Hz, 2H, H8a), 4.42-4.35 (m, 1H, H4″), 4.30 (t, J = 7.3 Hz, 1H, H5), 4.16-4.09 (m, 1H, H5″), 4.07 (s, 1H, H7-OH), 3.97 (d, J = 6.2 Hz, 1H, H6), 3.94 (s, 1H, H13), 3.92-3.81 (m, 2H, H17, H5′), 3.74 (dt, J = 12.2, 4.4 Hz, 1H, H3″), 3.66-3.57 (m, 1H, H3′), 3.44 (s, 3H, H3′-OMe), 3.43 (s, 3H, H3″-OMe), 3.35-3.20 (m, 3H, H2, H25, H4′), 2.59-2.46 (m, 1H, H12), 2.37 (d, J = 8.2 Hz, 1H, H5-OH), 2.33-2.19 (m, 4H, H16, H24, H2″a), 2.09-1.97 (m, 2H, H2′a, H20a), 1.88 (d, J = 2.1 Hz, 3H, H4a-CH_3), 1.83-1.72 (m, 3H, H27a, H30a, H31a), 1.74-1.64 (m, 2H, H18a, H28a), 1.56 (d, J = 11.1 Hz, 3H, H26, H29), 1.49 (d, J = 8.1 Hz, 4H, H14a-CH_3, H20b), 1.31-1.14 (m, 15H, H2″b, H27b, H28b, H30b, H31b, H2′b, H5′-Me, H5″-Me, H12a-CH_3), 0.93 (d, J = 7.1 Hz, 3H, H24a-CH_3), 0.88 (d, J = 12.4 Hz, 1H, H18b). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 168.1, 154.8, 139.5, 138.0, 137.8, 136.2, 135.0, 132.9, 132.5, 128.7(2-C), 127.8(2-C), 124.7, 120.5, 118.2, 118.1, 98.7, 95.7, 94.9, 81.8, 81.0, 80.4, 79.2, 73.9, 68.4, 68.3, 68.2, 67.7, 67.1, 65.4, 56.7, 56.0, 52.5, 49.8, 45.7, 41.1, 40.4, 39.7, 38.6, 36.6, 34.6, 34.3, 31.8, 31.4, 30.0, 29.7, 26.9, 26.6, 26.5, 25.5, 20.2, 19.9, 18.3, 17.3, 16.6, 15.1. HRMS (ESI) m/z calcd. for $C_{58}H_{80}O_{15}N_2Na$: $(M+Na)^+$, 1067.5451; found 1067.5416.

The target compounds (**5b-5v**) were synthesized according to a procedure similar to that used for compound **5a.** Their HRMS, ^{1}H NMR, and ^{13}C NMR data can be found in Supporting Information.

General Procedure for Synthesis of Acylthiourea Derivatives of Doramectin (6a-6v) (Scheme 2).

Synthesis of benzoyl isothiocyanate.[28] A mixture of benzoyl chloride (703.00 mg, 5.00 mmol), acetone (15.00 mL), and ammonium thiocyanate (381.00 mg, 5.00 mmol) was stirred 1 h at room temperature under an N_2 atmosphere. The mixture was filtered, and concentrated in vacuo to yield 600.00 mg of light yellow liquid, which was not purified for use in the next step.

Synthesis of (4″-N- benzoylthiourea doramectin) ***(6a).***[28, 29] Benzoyl isothiocyanate (322.32 mg, 1.98 mmol) was added to a solution of compound **4** (400.00 mg, 0.39 mmol) and *N*, *N*-dimethypyridin-4-amine (DMAP, 4.80 mg, 0.04 mmol) in dry dichloromethane (20.00 mL). The mixture was stirred for 1 h at room temperature until the reaction was completed according to TLC analysis. Saturated brine (20.00 mL) was added, the aqueous phase was extracted with dichloromethane (3 × 20.00 mL), and the combined organic phases were washed with saturated brine (3 × 20.00 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (7:1, v/v) to produce 401.00 mg as a yellow solid. A deprotection reagent solution of 15.00 mL of *p*-toluenesulfonic acid-methanol complex (0.02 g/mL) was added dropwise to a solution of yellow solid (401.00 mg) in methanol (15.00 mL). The mixture was stirred for 1 h at room temperature until the reaction was completed according to TLC analysis. Saturated sodium bicarbonate (50.00 mL) and dichloromethane (50.00 mL) were added, the aqueous phase was

243 extracted with dichloromethane (3 × 10.00 mL), and the combined organic phases
244 were washed with saturated brine (3 × 10.00 mL), dried over anhydrous magnesium
245 sulfate, filtered, and concentrated in vacuo. The crude product was purified by column
246 chromatography on silica gel eluted with petroleum ether/ethyl acetate (2.5: 1, v/v) to
247 produce 254.00 mg (70 %) of compound **6a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.11 (d,
248 *J* = 9.8 Hz, 1H, CONHCS), 9.07 (s, 1H, CSNH), 7.88-7.82 (m, 2H, Ph), 7.64-7.59 (m,
249 1H, Ph), 7.51 (t, *J* = 7.6 Hz, 2H, Ph), 5.92-5.85 (m, 1H, H9), 5.83-5.67 (m, 3H, H10,
250 H11, H23), 5.57-5.50 (m, 2H, H22, H1″), 5.47-5.36 (m, 2H, H3, H19), 5.09 (dd, *J* =
251 10.0, 3.6 Hz, 1H, H4″), 5.03-4.98 (m, 1H, H15), 4.79 (d, *J* = 3.8 Hz, 1H, H1′),
252 4.73-4.62 (m, 2H, H8a), 4.33-4.24 (m, 1H, H5), 4.22-4.13 (m, 1H, H5″), 4.06 (s, 1H,
253 H7-OH), 3.97 (d, *J* = 6.2 Hz, 1H, H6), 3.94 (s, 1H, H13), 3.92-3.78 (m, 3H, H17, H5′,
254 H3″), 3.62 (ddd, *J* = 11.2, 8.5, 4.8 Hz, 1H, H3′), 3.52 (s, 3H, H3″-OMe), 3.43 (s, 3H,
255 H3′-OMe), 3.34-3.21 (m, 3H, H2, H25, H4′), 2.57-2.49 (m, 1H, H12), 2.41 (s, 1H,
256 H5-OH), 2.27 (dddd, *J* = 18.9, 11.4, 5.8, 2.2 Hz, 4H, H16, H24, H2″a), 2.14 (dd, *J* =
257 13.7, 5.0 Hz, 1H, H2′a), 2.03-1.97 (m, 1H, H20a), 1.87 (t, *J* = 2.0 Hz, 3H, H4a-CH_3),
258 1.84-1.74 (m, 3H, H27a, H30a, H31a), 1.71-1.63 (m, 2H, H18a, H28a), 1.56 (d, *J* =
259 8.2 Hz, 3H, H26, H29), 1.49 (d, *J* = 7.9 Hz, 4H, H14a-CH_3, H20b), 1.30-1.14 (m,
260 15H, H2″b, H27b, H28b, H30b, H31b, H2′b, H5′-Me, H5″-Me, H12a-CH_3), 0.92 (d,
261 *J* = 7.2 Hz, 3H, H24a-CH_3), 0.87-0.82 (m, 1H, H18b). ^{13}C NMR (100 MHz, $CDCl_3$) δ
262 181.2, 173.5, 166.9, 139.5, 138.0, 137.8, 136.2, 135.0, 133.4, 131.8, 129.0(2-C),
263 127.7, 127.5(2-C), 124.7, 120.5, 118.2, 118.1, 98.6, 95.7, 94.8, 81.8, 81.0, 80.4, 79.2,
264 79.2, 73.8, 68.4, 68.2, 68.2, 67.7, 67.0, 65.7, 57.1, 56.6, 55.7, 45.7, 40.3, 39.7, 38.6,

36.7, 34.6, 34.3, 32.3, 31.4, 30.0, 29.6, 26.9, 26.6, 26.5, 25.5, 20.2, 19.9, 18.3, 17.2, 16.6, 15.1. HRMS (ESI) m/z calcd. for $C_{58}H_{80}O_{14}SN_2Na$: $(M+Na)^+$, 1083.5222; found 1083.5187.

The target compounds (**6b-6v**) were prepared by following the same procedure as for **6a.** Their HRMS, ^1H NMR, and ^{13}C NMR data are list in Supporting Information.

Biological Assay and Insecticidal Test. Diamondback moth, oriental armyworm, and corn borer were used to examine the insecticidal activities of the target compounds (**5a-5v** and **6a-6v**) according to the previously reported methods.[31-33] For each compound, 30 larvae (10 larvae per group) were used. The percentage of mortalities was evaluated 2 days after treatment. Each bioassay was repeated in triplicate at 25 ± 1 °C. For comparative purposes, doramectin, commercial avermectins, chlorbenzuron, diafenthiuron, and our previous work **3g** were tested under the same condition. Assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula.[34] Percentage mortalities were based on a percentage scale of 0-100, where 0 was no activity, 100 was total kill.

Homology Modeling and Molecular Docking.[17] In our previously reported study, the crystal structure of human glycine receptor alpha-3 (PDB ID 5TIN) was chosen as the template to build the 3D structure of *Plutella xylostella* GABA receptor. Schrodinger-Glide was used to investigate the binding mode between compounds and the target protein. The best-scoring pose judged by the Glide docking score was chosen and analyzed using Schrodinger software.

RESULTS AND DISCUSSION

Chemical Synthesis. As shown in **Scheme 1** and **Scheme 2**, two series of novel acylurea and acylthiourea derivatives of doramectin (**5a–5v** and **6a–6v**) modified at position C4″-OH were prepared. As an initial material, doramectin **1** was reacted with tert-butylchlorodimethylsilane (TBDMS-Cl) for selective protection of the hydroxyl group at the 5-position of doramectin to prepare 5-*O*-TBDMS doramectin **2**, as described previously.[29] In an attempt to introduce the C4″ acyl isocyanate and acyl isothiocyanate side chain, the protected intermediate **2** was first oxidized using diphenyl phosphate dichloride ($PhOPOCl_2$) in DMSO at low temperature to produce 4″-oxo-5-*O*- TBDMS doramectin (**3**). Subsequently, C4″=O was reductively aminated to C4″-NH_2 with ammonium acetate and sodium cyanoborohydride ($NaCNBH_3$) to produce the key intermediate 4″-*epi*-NH_2-5-*O*-TBDMS doramectin (**4**) at 38 % yield. The amine intermediate **4** was first reacted with different newly prepared acyl isocyanate (RCONCO) or acyl isothiocyanate (RCONCS) using *N*, *N*-dimethypyridin-4-amine (DMAP) as a catalyst in a solution of dichloromethane, which was further deprotected using *p*-toluenesulfonic acid-methanol complex, according to published procedures.[30] All target compounds were purified by column chromatography, and their structures were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS.

Insecticidal Activities. Forty-four newly synthesized acylurea and acylthiourea doramectin derivatives (**5a-5v** and **6a-6v**) were evaluated for insecticidal activities against diamondback moth and oriental armyworm, and a few representative compounds **5l**, **5o**, **5t**, **6l**, **6o**, **6n**, **6p**, and **6t** were further evaluated for insecticidal

efficacy against corn borer, and the results are listed in **Tables 1**, **2** and **3**. The LC_{50} values were calculated using five different concentrations (200.00 mg/L, 100.00 mg/L, 50.00 mg/L, 25.00 mg/L, and 12.50 mg/L) and are listed in **Table 4**. Doramectin, commercial avermectins, chlorbenzuron, diafenthiuron, and **3g** were used as positive controls. Leaves treated with acetone were used as a blank control group.

Insecticidal Activity against Diamondback Moth. As shown in **Table 1**, acylurea and acylthiourea derivatives of doramectin except for **5r**, **5u**, **6b**, **6e**, **6h**, **6i**, **6p**, **6q**, and **6u,** exhibited insecticidal activities against diamondback moth at the concentration of 100.00 mg/L. Moreover, the acylurea derivatives (**5a-5e**, **5g-5j**, **5m-5q**, and **5t-5v**) had higher insecticidal activities than the corresponding acylthiourea series (**6a-6e**, **6g-6j**, **6m-6q**, and **6t-6v**). Furthermore, among all derivatives, compounds **5j**, **5o**, **5t**, **6l**, and **6o** exhibited insecticidal activities equal to or higher than that of the positive control, doramectin, commercial avermectins, and chlorbenzuron. Particularly, **5j**, **5o**, **5t**, and **6l** displayed high insecticidal activities (≥ 80%) against diamondback moth at a concentration of 100.00 mg/L. When the concentration was reduced to 12.50 mg/L, **5o** and **5t** exhibited desirable insecticidal activities, and compound **5t** still had 80.00% mortality. The insecticidal activity of **5t** against diamondback moth was much higher than that of the control compounds, including doramectin, commercial avermectins, chlorbenzuron, and **3g** (the most active compound in our group), which was parallel to that of diafenthiuron at the same concentration of 12.50 mg/L. The LC_{50} value of **5t** (LC_{50}= 6.2123 mg/L) was

6.52-fold that of avermectins (LC_{50}= 40.5507 mg/L), 7.75-fold that of doramectin (LC_{50}= 48.1547 mg/L), and 3.98-fold that of **3g** (LC_{50}= 24.7742 mg/L).

Insecticidal Activity against Oriental Armyworm. The insecticidal activities of all compounds against oriental armyworm were evaluated, and the results are shown in **Table 2**. Acylurea and acylthiourea doramectin derivatives, except for **5a**, **5d**, **5e**, **5f**, **5h**, **5k**, **5r**, **5s** and **5v**, exhibited insecticidal activities against oriental armyworm at concentrations of 200.00-50.00 mg/L. Unlike the insecticidal efficacy against diamondback moth, the acylthiourea series were more active than the corresponding acylurea series at the same concentration. Most acylthiourea derivatives displayed good to excellent insecticidal activities at high concentration (≥ 50.00 mg/L) and certain insecticidal activities at low concentration (12.50 mg/L), whereas a few acylurea derivatives (**5e**, **5f**, **5k**, and **5v)** displayed no insecticidal activities against oriental armyworm at 200.00 mg/L, and only three compounds (**5l**, **5o**, and **5t**) had insecticidal activities at the concentration of 12.50 mg/L. In addition, compounds **5l**, **5o**, **5t**, **6l**, **6n**, and **6p** exhibited good insecticidal activities against oriental armyworm with mortality rates of 56.67%, 50.00%, 60.00%, 56.67%, 53.67%, and 50.00%, respectively, at 12.50 mg/L, which were superior to those of doramectin (20.00%), avermectins (23.33%), and chlorbenzuron (40.00%) under the same concentration. Interestingly, compounds **6o** and **6t** with mortality rates of 66.67% and 73.33% showed a much more potent inhibitory effect against oriental armyworm than the most effective **3g** (60.00%), as reported in our previous paper. The insecticidal activity of compound **6t** against oriental armyworm was the highest, and the LC_{50}

353 value of **6t** (LC_{50}= 6.2816 mg/L) was 5.08-fold that of avermectins (LC_{50}= 31.9488
354 mg/L), 6.21-fold that of doramectin (LC_{50}= 39.0069 mg/L), and 1.29-fold that of **3g**
355 (LC_{50}= 8.1391 mg/L).

356 *Insecticidal Activity against Corn Borer*. From **Table 1** and **Table 2**, several
357 compounds (**5l**, **5o**, **5t**, **6l**, **6o**, **6n**, **6p**, and **6t)** exhibited excellent insecticidal activities
358 against diamondback moth or oriental armyworm among all synthesized compounds.
359 For further evaluation of the insecticidal efficacy of these compounds, the results of
360 insecticidal activities against corn borer are shown in **Table 3**. From **Table 3,** we
361 found that all compounds had insecticidal efficacy at concentrations from 200.00 to
362 25.00 mg/L, and only compound **6p** lost insecticidal activity at a concentration of
363 12.50 mg/L. Additionally, compounds **5o**, **5t** and **6t** exhibited insecticidal activities
364 higher than those of doramectin, avermectins, chlorbenzuron and diafenthiuron.
365 Specifically, compound **5o** showed the highest insecticidal activity with a mortality
366 rate of 60.00% at a concentration of 12.50 mg/L, which was superior to those of **5t**
367 (43.33%), **6t** (40.00%), and the contrast **3g** (36.67%) at the same concentration. The
368 LC_{50} values of compounds **5o**, **5t**, **6t** and **3g** against corn borer were 10.0931,
369 22.7966, 26.7018 and 23.0991 mg/L, respectively. Compound **5o** exhibited better
370 insecticidal activity than **3g**.

371 **Structure-Activity Relationship (SAR)**. **A** comparison of the insecticidal
372 activities of different types of doramectin acylurea and acylthiourea derivatives is
373 shown in **Table 1**, **Table 2**, and **Table 3**. The overall contrasting trends are given as
374 follows: (1) insecticidal activities against diamondback moth: acylurea > acylthiourea

groups, such as, **5t >> 6t**, **5o > 6o**; **4**-aromatic-substituted compounds (**5g**, **5j**) are superior to **2**-aromatic-substituted compounds (**5b**-**5d**) and **3**-aromatic-substituted compounds (**5e**, **5f**); cyclopropyl-substituted compound (**5o**) is better than other alkyl-substituted compounds (**5l**-**5n**, **5p**, **5q**); (2) insecticidal activities against oriental armyworm: acylthiourea > acylurea groups, such as, **6t > 5t**, **6o > 5o**, **6p >> 5p**; alkyl-substituted acylthiourea compounds (**6l**, **6n-6p**) are superior to monosubstituted aromatic compounds (**6a-6k**); compound **6t** has the highest activity; (3) insecticidal activities against corn borer: **5o > 5t > 6t**.

Docking Analysis. To better elucidate the effect of the acylurea and acylthiourea moieties on insecticidal activity against diamondback moth, we selected the sequence of *Plutella xylostella* Rdl-1 for homology modeling to investigate the binding mode between three compounds (**5o**, **5t**, and **6t**) and the target protein using Schrodinger-Glide. Compounds **5o**, **5t**, and **6t** were docked into human glycine receptor alpha-3, and the detailed interactions of the compounds and receptor are shown in **Figure 2**. Due to the different substituents at C4″ position, different types of hydrogen bonds were formed. The acylureas and acylthioureas contained two NH groups and were highly susceptible to more hydrogen bonds with the target protein. As shown in **Figure 2**, two N−H···O H-bonds (1.64 and 2.15 Å for **5o**; 2.20 and 2.30 Å for **6t**) were formed between two NH groups on acylurea and the oxygen atom of Asp165. Impressively, four hydrogen bonds were formed between the acylurea of compound **5t** and the target protein. Two NH groups on acylurea of compound **5t** formed N−H···O H-bonds (2.17 and 2.56 Å) with the oxygen atom of Asp165, and

two CO groups on acylurea also formed O···H H-bonds (2.20 and 2.83 Å) with the hydrogen atom of Asn164. Among the three compounds, the hydrogen bonds between compound **5t** and the receptor protein in the docking simulation were the most numerous and strongest. Accordingly, compound **5t**, with the strongest H-bonding strengths, exhibited the highest insecticidal activity against diamondback moth compared with **5o** and **6t**.

To better explain the structure-activity relationship, we also calculated the binding free energy, as shown in **Table 5**, and compared the binding conformations of **5o** and **5t** with those of the lead compound **3g** (described in our previous study) and **6t**, respectively. The results (**Table 5**) showed that the binding free energy of **5o** (-3.637 kcal mol^{-1}) was comparable to that of **3g** (-3.964 kcal mol^{-1}), and the binding free energy of **5t** (-1.758 kcal mol^{-1}) was much lower than that of **6t** (-0.821 kcal mol^{-1}). Additionally, the H-bonding strengths of **5o** (1.64 and 2.15 Å) were comparable to those of **3g** (2.12 and 2.31 Å), whereas the H-bonding strengths of **5t** (2.17, 2.20, 2.56 and 2.83 Å) were much better than those of **6t** (2.20 and 2.30 Å) at the C4″ position. The docking results of compounds **5o**, **5t**, **6t** and **3g** were in good agreement with our insecticidal assays; the activity of **5o** with acylurea was comparable to that of **3g** with carbamate and the activity of **5t** with acylurea was better than that of **6t**. From the results above, it can be preliminarily concluded that the insecticidal activity of our synthesized compounds had a positive correlation with the H-bonds at the C4″ position.

In conclusion, forty-four novel doramectin derivatives containing acylurea and

419 acylthiourea groups were designed and synthesized based on hydrogen bonds. The
420 results from bioassays of the insecticidal activities were evaluated. Compounds **5t, 6t**,
421 and **5o** exhibited excellent insecticidal activities against diamondback moth, oriental
422 armyworm and corn borer, respectively. Molecular docking also showed that
423 compound **5t** formed stronger hydrogen bonds with the target protein, and the docking
424 result was consistent with the corresponding insecticidal activity test result. Therefore,
425 the rationality of the designed novel doramectin derivatives based on hydrogen
426 bonding was fully supported in our study. A future detailed study on the structural
427 optimization and mechanism of action are in progress at our laboratory.

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431 Sciences (No. KFJ-BRP-008).

432 **Supporting Information**

433 Supplementary data associated with this article can be found in Supporting
434 Information. ^{1}H NMR and ^{13}C NMR data and spectrums of compounds **5a-5v**, **6a-6v**
435 were available free of charge via the Supporting Information.

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Figure captions

Figure 1. Design of target compounds.

Figure 2. Molecular docking of compounds **5o**, **5t**, and **6t**.

Scheme 1. General Procedure for Synthesis of Acylurea Derivatives of Doramectin (**5a-5v**).

Scheme 2. General Procedure for Synthesis of Acylthiourea Derivatives of Doramectin (**6a-6v**).

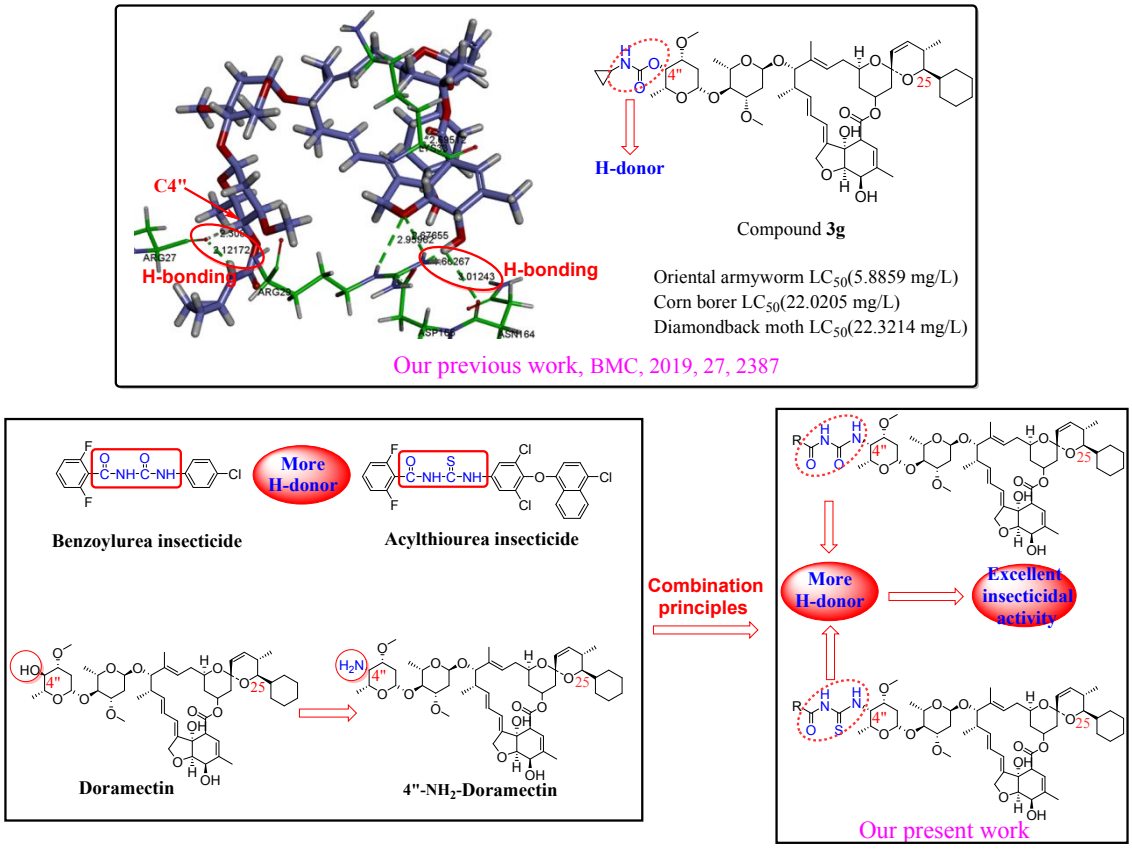

Figure 1. Design of target compounds.

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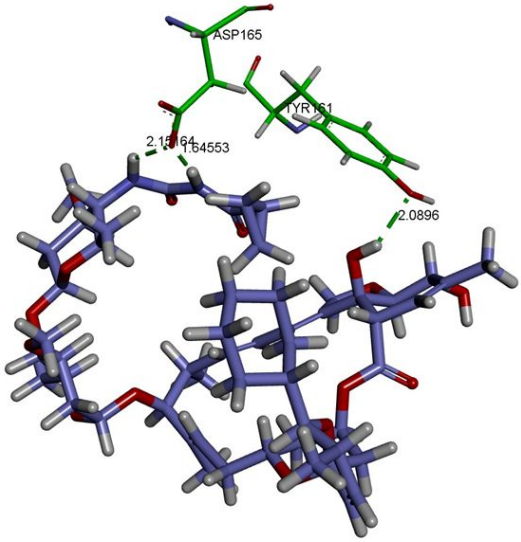
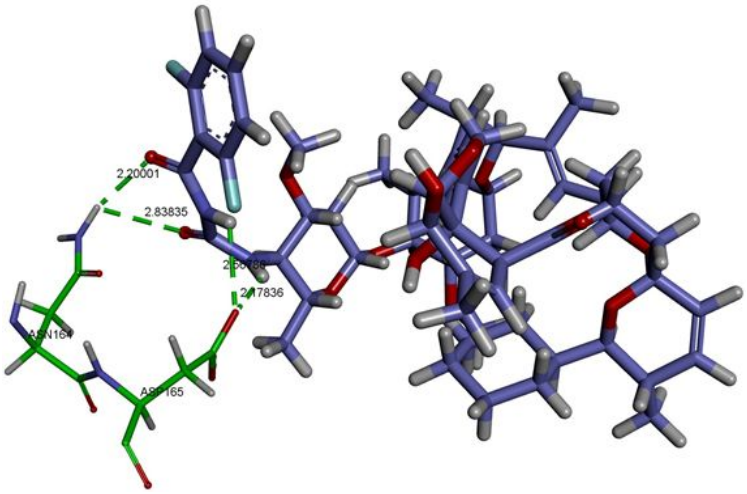


571

572 **5o**



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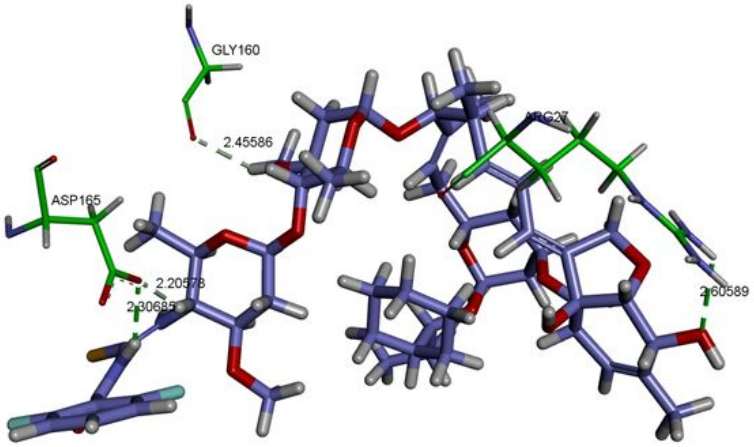
574 **5t**

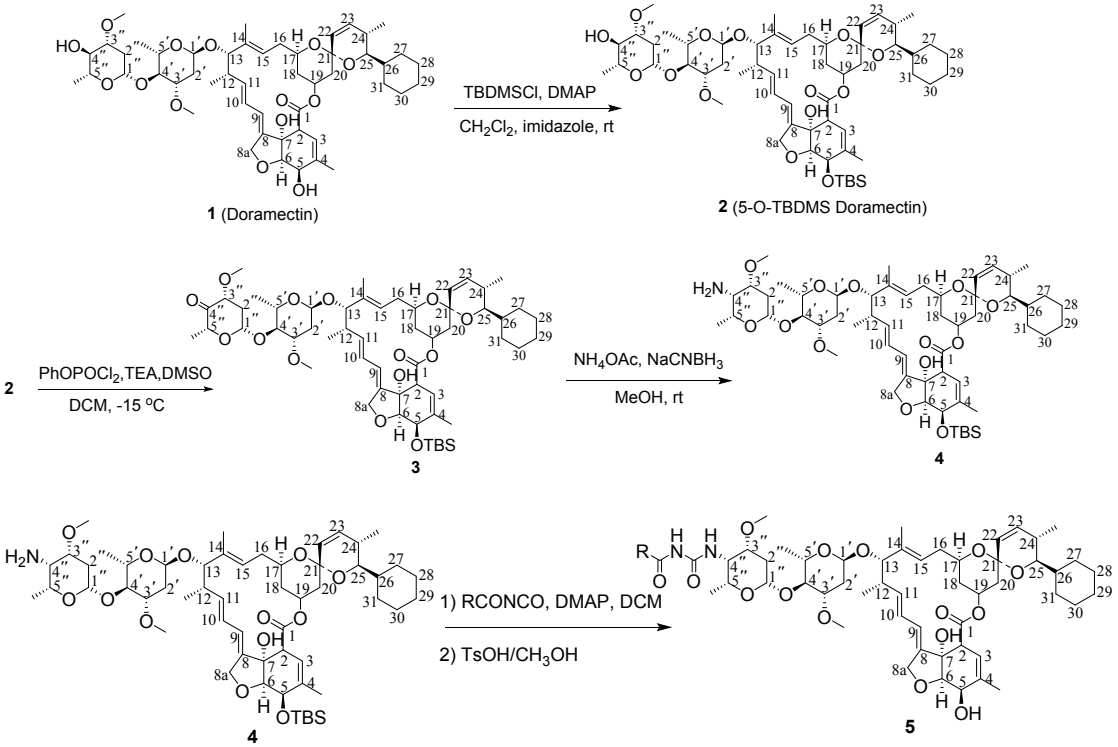


575

576 **6t**

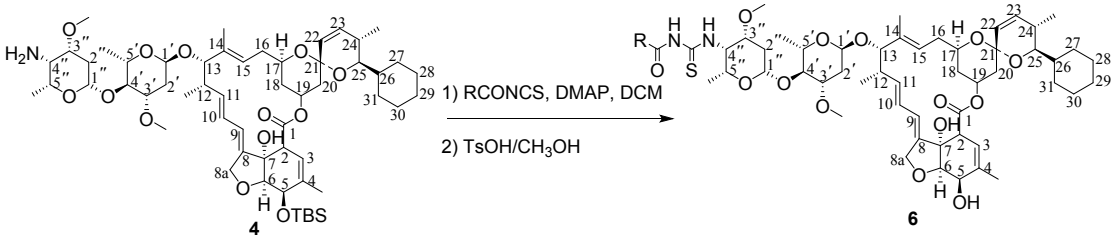
Figure 2. Molecular docking of compounds **5o**, **5t**, and **6t**. The H-bonding distance of **5o** (1.64, 2.15 Å), **5t** (2.17, 2.20, 2.56, 2.83 Å), and **6t** (2.20, 2.30 Å) at the C4″ position.

Scheme 1. General Procedure for Synthesis of Acylurea Derivatives of Doramectin (5a-5v).



5a: Ph;	**5g**: 4-F-Ph;	**5m**: CH_3CH_2;	**5s**: 2,5-difluorophenyl;
5b: 2-F-Ph;	**5h**: 4-Cl-Ph;	**5n**: $CH_3CH_2CH_2$;	**5t**: 2,6-difluorophenyl;
5c: 2-Cl-Ph;	**5i**: 4-Br-Ph;	**5o**: C_3H_5;	**5u**: 3,5-dimethoxyphenyl;
5d: 2-CF_3-Ph;	**5j**: 4-OCH_3-Ph;	**5p**: C_6H_{11};	**5v**: 3,4,5-trimethoxyphenyl
5e: 3-CH_3-Ph;	**5k**: 4-CF_3-Ph;	**5q**: $CH_3CH_2CH_2CH_2CH_2$;	
5f: 3-CF_3-Ph;	**5l**: CH_3;	**5r**: 2,4-difluorophenyl;	

Scheme 2. General Procedure for Synthesis of Acylthiourea Derivatives of Doramectin (6a-6v).



6a: Ph;	**6g**: 4-F-Ph;	**6m**: CH_3CH_2;	**6s**: 2,5-difluorophenyl;
6b: 2-F-Ph;	**6h**: 4-Cl-Ph;	**6n**: $CH_3CH_2CH_2$;	**6t**: 2,6-difluorophenyl;
6c: 2-Cl-Ph;	**6i**: 4-Br-Ph;	**6o**: C_3H_5;	**6u**: 3,5-dimethoxyphenyl;
6d: 2-CF_3-Ph;	**6j**: 4-OCH_3-Ph;	**6p**: C_6H_{11};	**6v**: 3,4,5-trimethoxyphenyl;
6e: 3-CH_3-Ph;	**6k**: 4-CF_3-Ph;	**6q**: $CH_3CH_2CH_2CH_2CH_2$;	
6f: 3-CF_3-Ph;	**6l**: CH_3;	**6r**: 2,4-difluorophenyl;	

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593 **Table 1.** Insecticidal Activities of Doramectin Derivatives Against Diamondback Moth.

Compd.	200.00 (mg/L)	100.00 (mg/L)	50.00 (mg/L)	25.00 (mg/L)	12.50 (mg/L)
5a	50.00%	36.67%	30.00%	10.00%	0
5b	56.67%	33.33%	0	0	0
5c	70.00%	60.00%	30.00%	23.33%	0
5d	100.00%	60.00%	30.00%	0	0
5e	40.00%	10.00%	0	0	0
5f	43.33%	20.00%	13.33%	0	0
5g	80.00%	60.00%	50.00%	36.66%	30.00%
5h	46.67%	20.00%	10.00%	0	0
5i	76.67%	60.00%	33.33%	20.00%	10.00%
5j	100.00%	83.33%	70.00%	40.00%	30.00%
5k	30.00%	10.00%	0	0	0
5l	33.33%	20.00%	0	0	0
5m	90.00%	80.00%	63.33%	30.00%	0
5n	70.00%	50.00%	36.67%	23.33%	10.00%
5o	90.00%	80.00%	63.33%	50.00%	40.00%
5p	63.33%	43.33%	40.00%	23.33%	10.00%
5q	40.00%	20.00%	10.00%	0	0
5r	0	0	0	0	0
5s	36.67%	20.00%	0	0	0
5t	**100.00%**	**100.00%**	**100.00%**	**90.00%**	**80.00%**
5u	0	0	0	0	0
5v	50.00%	20.00%	0	0	0
6a	30.00%	26.67%	20.00%	0	0
6b	0	0	0	0	0
6c	30.00%	13.67%	0	0	0
6d	50.00%	30.00%	0	0	0
6e	0	0	0	0	0
6f	70.00%	53.33%	40.00%	33.33%	0
6g	73.33%	40.00%	30.00%	10.00%	0
6h	0	0	0	0	0
6i	0	0	0	0	0
6j	40.00%	33.33%	0	0	0
6k	63.33%	46.67%	20.00%	10.00%	0
6l	90.00%	80.00%	53.33%	30.00%	20.00%
6m	50.00%	36.33%	30.00%	0	0
6n	56.67%	50.00%	10.00%	0	0
6o	76.67%	50.00%	40.00%	36.33%	20.00%
6p	0	0	0	0	0

6q	0	0	0	0	0
6r	40.00%	30.00%	26.67%	10.00%	0
6s	50.00%	26.67%	0	0	0
6t	66.67%	53.33%	40.00%	30.00%	13.33%
6u	0	0	0	0	0
6v	50.00%	10.00%	0	0	0
3g	100.00%	86.67%	63.33%	50.00%	33.33%
doramectin	93.33%	66.67%	46.67%	30.00%	16.67%
avermectins	90.00%	70.00%	53.33%	40.00%	20.00%
chlorbenzuron	60.00%	30.00%	0	0	0
diafenthiuron	100.00%	100.00%	100.00%	80.00%	80.00%

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619 **Table 2.** Insecticidal Activities of Doramectin Derivatives Against Oriental Armyworm.

Compd.	200.00 (mg/L)	100.00 (mg/L)	50.00 (mg/L)	25.00 (mg/L)	12.50(mg/L)
5a	60.00%	40.00%	0	0	0
5b	90.00%	66.67%	20.00%	0	0
5c	73.33%	60.00%	33.33%	10.00%	0
5d	30.00%	20.00%	0	0	0
5e	0	0	0	0	0
5f	0	0	0	0	0
5g	40.00%	20.00%	10.00%	0	0
5h	30.00%	0	0	0	0
5i	56.67%	40.00%	13.33%	0	0
5j	80.00%	60.00%	46.33%	0	0
5k	0	0	0	0	0
5l	100.00%	86.67%	80.00%	63.33%	56.67%
5m	80.00%	56.33%	40.00%	20.00%	0
5n	100.00%	80.00%	50.00%	0	0
5o	100.00%	83.33%	80.00%	60.00%	50.00%
5p	36.67%	30.00%	20.00%	0	0
5q	76.33%	60.00%	40.00%	0	0
5r	50.00%	26.67%	0	0	0
5s	50.00%	30.00%	0	0	0
5t	100.00%	100.00%	86.67%	70.00%	60.00%
5u	70.00%	60.00%	20.00%	0	0
5v	0	0	0	0	0
6a	100.00%	80.00%	56.67%	46.67%	16.67%
6b	90.00%	70.00%	53.33%	26.67%	10.00%
6c	100.00%	80.00%	60.00%	43.33%	20.00%
6d	80.00%	60.00%	40.00%	30.00%	10.00%
6e	73.33%	60.00%	50.00%	30.00%	16.67%
6f	100.00%	73.33%	60.00%	30.00%	0
6g	90.00%	73.33%	40.00%	30.00%	20.00%
6h	100.00%	80.00%	56.67%	40.00%	10.00%
6i	76.67%	70.00%	60.00%	30.00%	0
6j	80.00%	63.33%	46.67%	20.00%	0
6k	90.00%	73.33%	60.00%	40.00%	0
6l	100.00%	90.00%	80.00%	60.00%	56.67%
6m	70.00%	60.00%	30.00%	0	0
6n	100.00%	90.00%	76.67%	60.00%	53.67%
6o	100.00%	100.00%	83.33%	76.67%	66.67%

6p	100.00%	90.00%	76.67%	56.67%	50.00%
6q	100.00%	70.00%	50.00%	40.00%	10.00%
6r	100.00%	60.00%	50.00%	36.67%	0
6s	50.00%	23.33%	20.00%	0	0
6t	**100.00%**	**100.00%**	86.67%	**80.00%**	**73.33%**
6u	63.33%	43.33%	30.00%	0	0
6v	90.00%	80.00%	46.67%	30.00%	10.00%
3g	100.00%	86.67%	80.00%	76.67%	60.00%
doramectin	100.00%	83.33%	46.67%	30.00%	20.00%
avermectins	100.00%	90.00%	53.33%	40.00%	23.33%
chlorbenzuron	100.00%	90.00%	73.33%	50.00%	40.00%
diafenthiuron	100.00%	100.00%	100.00%	100.00%	100.00%

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621 **Table 3.** Insecticidal Activities of Doramectin Derivatives Against Corn Borer.

Compd.	200.00 (mg/L)	100.00 (mg/L)	50.00 (mg/L)	25.00 (mg/L)	12.50 (mg/L)
5l	83.33%	70.00%	50.00%	36.67%	20.00%
5o	**100.00%**	**86.67%**	**80.00%**	**66.67%**	**60.00%**
5t	93.33%	73.33%	60.00%	50.00%	43.33%
6l	63.33%	43.33%	30.00%	20.00%	16.67%
6o	60.00%	46.67%	36.67%	20.00%	10.00%
6n	60.00%	40.00%	33.33%	20.00%	10.00%
6p	70.00%	56.67%	36.67%	20.00%	0
6t	90.00%	66.67%	60.00%	46.67%	40.00%
3g	100.00%	83.33%	73.33%	46.67%	36.67%
doramectin	90.00%	73.33%	60.00%	43.33%	20.00%
avermectins	86.67%	53.33%	43.33%	36.67%	13.33%
chlorbenzuron	70.00%	56.67%	30.00%	10.00%	0
diafenthiuron	70.00%	60.00%	40.00%	26.67%	10.00%

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623 **Table 4.** LC_{50} Values of **5t**, **6t**, **5o**, **3g**, Doramectin, and Avermectins against Oriental armyworm，
624 Diamondback moth and Corn borer.

	Diamondback moth		Oriental armyworm		Corn borer	
	LC_{50}(mg/L)	toxic ratio	LC_{50}(mg/L)	toxic ratio	LC_{50}(mg/L)	toxic ratio
5t	6.2123	**6.52**	11.2172	2.84	22.7966	2.54
6t	83.5867	0.48	6.2816	**5.08**	26.7018	2.17
5o	22.8235	1.77	14.6415	2.18	10.0931	**5.74**
3g	24.7742	1.63	8.1391	3.92	23.0991	2.50
doramectin	48.1547	0.84	39.0069	0.81	36.2447	1.59
avermectins	40.5507	1.00	31.9488	1.00	57.9459	1.00

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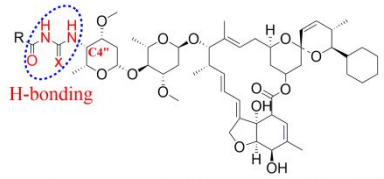
626 **Table 5.** Glide Docking Score of **3g**, **5o**, **5t**, and **6t**.

Compound	Glide Docking Score (kcal mol^{-1})
3g	-3.964
5o	-3.637
5t	-1.758
6t	-0.821

627 Table of Contents Graphic:



Compound	H-bonding	Insecticidal activity
5o (R=  X=O)	(1.64, 2.15Å)	Corn borer $\mathbf{LC_{50}}$(10.0931 mg/L)
5t (R= X=O)	(2.17, 2.20, 2.56, 2.83Å)	Diamondback moth $\mathbf{LC_{50}}$(6.2123 mg/L)
6t (R=  X=S)	(2.20, 2.30Å)	Oriental armyworm $\mathbf{LC_{50}}$(6.2816 mg/L)

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