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Communication

Thiazolylhydrazone dervatives as inhibitors for insect *N*-acetyl-*β*-D-hexosaminidase and chitinase

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ABSTRACT

Insect chitinase and *N*-acetyl-*β*-D-hexosaminidases (Hex) are potential targets for developing new pesticides. Here, a series of thiazolylhydrazones **I** (with substituted group R_1 at N3) and **II** (with substituted group R_1 at N2) were designed, synthesised and evaluated as competitive inhibitors of *Of*Hex1 and *Of*Chi-h, from the agricultural pest *Ostrinia furnacalis*. Derivatives **I-3d** and **II-3d**, with phenoxyethyl group at R_1, demonstrated the best inhibitory activities against *Of*Hex1 and *Of*Chi-h. Molecular docking analysis indicated that the branched conformation compound **II-3d** (K_i = 1.5 μmol/L) formed more hydrogen bonds with *Of*Hex1 than the stretched conformation compound **I-3d** (K_i = 5.9 μmol/L). The differences in compounds' binding conformations with *Of*Chi-h explained differences in inhibitory activity of compounds **I-3d** (K_i = 1.9 μmol/L) and **II-3d** (K_i = 4.1 μmol/L). This work suggests a novel scaffold for developing specific Hex and Chi-h inhibitors.

Heterocyclic compounds are an important role building block in medicinal chemistry. Thiazolidinones have been extensively investigated over recent decades for a varied range of biological activities, including antifungal [1], antiparasitic [2], antimicrobial [3], antioxidant [4], anticonvulsant [5], anti-HIV [6], anti-inflammatory [7], anti-tuberculosis [8], and anti-tumour activities [9]. Thiazolylhydrazone derivatives, with a R_1R_2C = N-N = substituent in the 2-position of thiazolidin-4-one, have been studied for their biological properties, such as antiparasitic [2,10], antifungal [11], antiurease [12] and antiviral activities [13].

Chitin, a linear homopolymer of *N*-acetyl-*β*-D-glucosamines and a major structural component of insect cuticles, plays an important role in the molting [14–16]. In the degradation system of insect chitin, two glycoside hydrolase family members, the glycosyl hydrolase family 18 (GH18) chitinase (EC 3.2.1.14) [17] and the glycosyl hydrolase family 20 (GH20) *β*-*N*-acetylhexosaminidase (Hex; EC 3.2.1.52) [18], cooperate with each other to facilitate the degradation of chitin. The chitinase can act on random positions of the chitin polymer chain to produce chitooligosaccharides [19,20], while Hex is responsible for hydrolyzing chitooligosaccharides to *N*-acetyl-D-glucosamine during chitin degradation [18,21]. Because of the absence of chitin from vertebrates and higher plants, small molecules inhibitors against chitinase and Hex are promising potential targets for pesticide development.

Reported GH20 inhibitors have varied chemical structures. Some are carbohydrate-based inhibitors, such as DNJNAc and its derivatives [22–25], GDL and its derivative PUGNAC [26–29], NTA-glucal [30], 6-Ac-CAS [31,28], NAG-thiazoline and its derivative NMAGT [32,33], LNB-thiazoline [34] and TMG-chitotriomycin [35]. Non-carbohydrate-based GH20 inhibitors include naphthalimides [36], pyrimethamine and its derivatives [37]. Small molecules against GH18 chitinases have been reported. The pseudotrisaccharide allosamidin isolated from the *Streptomyces sp.* [38], cyclopentapeptides argifin isolated from *Gliocladium sp*. FTD-0668 [39], argadin isolated from *Clonostachys sp*. FO-7314 [40], cyclo(L-Arg-D-Pro) from *Pseudomonas sp*. IZ208 [41], chitobiose and chitotriose thiazolines [42], xanthine derivatives [43], and CI-4 [44,45], all exhibit effective inhibitory activities. However, it is

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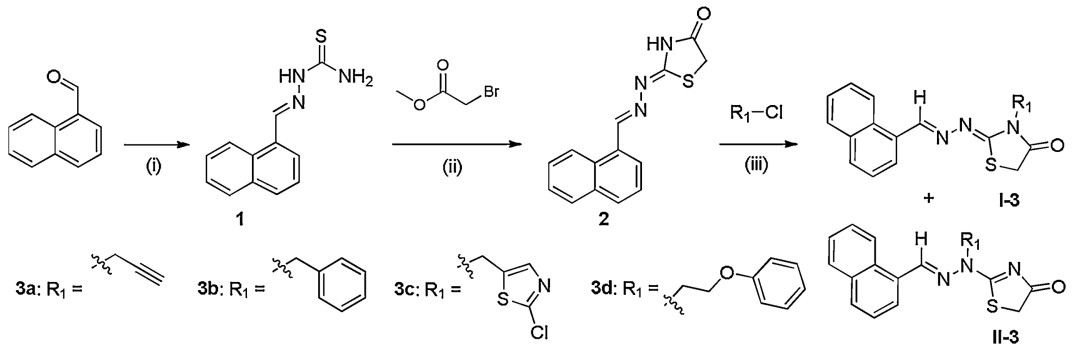
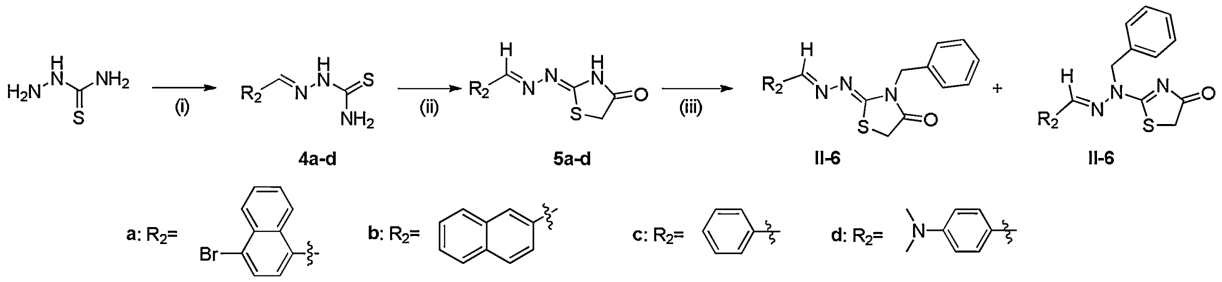
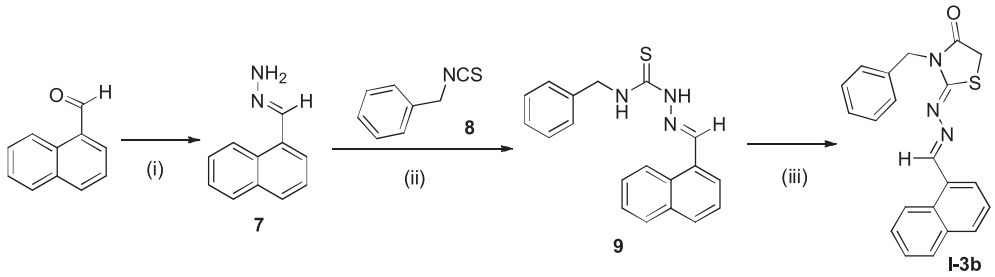


Scheme 1. Overview of synthetic methods for **3a**–**d**. Reagents and conditions: (i) thiosemicarbazide, EtOH, acetic acid (cat.), r.t., 15 h; (ii) MeOH, CH_3COONa, reflux, 5 h; (iii) CH_3CN, K_2CO_3, reflux, 5 h.



Scheme 2. Overview of synthetic methods for **6a**–**d**. Reagents and conditions: (i) R_2CHO, EtOH, acetic acid (cat.), r.t., 15 h; (ii) $BrCH_2COOEt$, MeOH, CH_3COONa, reflux, 5 h; (iii) chloromethylbenzene, CH_3CN, K_2CO_3, reflux, 5 h.



Scheme 3. Overview of synthetic methods for **I-3b**. Reagents and conditions: (i) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 6 h; (ii) isothiocyanatomethylbenzene, EtOH, reflux, 3 h; (iii) $BrCH_2COOEt$, MeOH, CH_3COONa, reflux, 5 h.

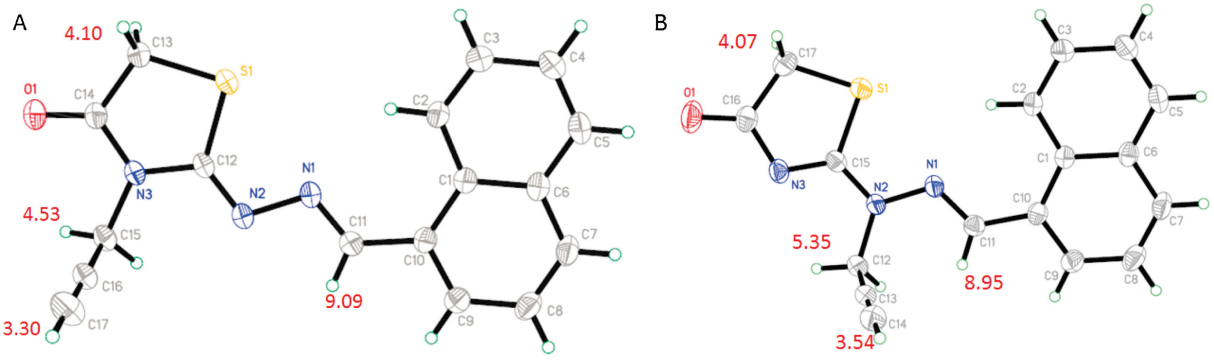
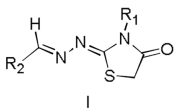


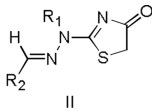
Fig. 1. The crystal structures and chemical shifts of ^1H NMR of compounds **I-3a** (A) and **II-3a** (B).

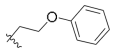
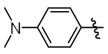
more challenging to drive these leads into large-scale application because of poor physicochemical properties or complex synthetic chemistry.

*Of*Hex1 [46] and *Of*Chi-h [47], from the agricultural pest *Ostrinia furnacalis*, have been demonstrated to function exclusively in chitin degradation. Previously, our group reported N3 substituted thiazolylhydrazone compounds as *Of*Hex1 inhibitors [48], and we were surprised to observe the by-product N2 substituted thiazolylhydrazone compounds during the synthesis of N3 substituted thiazolylhydrazone. Only relatively few researches

Table 1
Inhibitory activities of compounds **I** and **II** against *Of*Hex1 and *Of*Chi-h.


I


II

R_1	R_2	Compd.	Inhibitory rate (%, 10 μmol/L)		Inhibitory rate (%, 1 μmol/L)	
			*Of*Hex1	*Of*Chi-h	*Of*Hex1	*Of*Chi-h
		**I-3b**	62.6 ± 0.3	62.0 ± 3.2	25.7 ± 3.1	26.0 ± 1.8
		II-3b	72.8 ± 1.7	60.1 ± 3.0	31.5 ± 7.1	27.9 ± 2.3
		**I-3c**	20.6 ± 6.3	18.0 ± 1.0	6.8 ± 2.9	1.9 ± 1.5
		II-3c	67.7 ± 0.4	23.1 ± 0.7	30.3 ± 0.2	5.3 ± 0.2
		**I-3d**	65.8 ± 3.4	79.6 ± 0.1	29.0 ± 4.5	38.7 ± 1.2
		II-3d	81.3 ± 0.5	59.2 ± 0.5	42.9 ± 1.6	28.6 ± 2.4
		**I-6a**	38.3 ± 0.2	62.3 ± 1.8	10.3 ± 3.3	30.1 ± 1.9
		II-6a	67.9 ± 0.2	33.8 ± 3.8	29.9 ± 2.5	12.6 ± 2.0
		**I-6b**	50.2 ± 3.4	53.7 ± 0.7	18.1 ± 0.9	23.1 ± 1.3
		II-6b	55.8 ± 0.1	11.1 ± 1.9	25.9 ± 0.6	2.3 ± 2.1
		**I-6c**	37.4 ± 0.9	23.7 ± 1.4	11.2 ± 1.8	7.0 ± 0.6
		II-6c	47.7 ± 6.4	37.8 ± 5.0	20.8 ± 0.3	9.9 ± 1.3
		**I-6d**	45.5 ± 0.6	32.9 ± 0.0	16.3 ± 0.8	11.0 ± 2.1
		II-6d	44.4 ± 0. 1	11.4 ± 0.3	15.5 ± 3.0	1.3 ± 0.8

on the exocyclic N substituted thiazolylhydrazone compounds have been reported. In a continued effort in search for biologically active molecules, here we report the synthesis of thiazolylhydrazone derivatives and their inhibitory activities against *Of*Hex1 and *Of*Chi-h. We also investigated the inhibitory mechanisms of these compounds using structure-based molecular docking.

General experimental, synthesis, characterizations of new compounds can be found in Supporting information. The N2 substituted thiazolylhydrazone **II** were isolated as by-product during the synthesis of thiazolyhydrazone **I** according to previously reported method [48] (Schemes 1 and 2). Compounds **I-3a**, **I-3c** and **I-3d** had been reported in our previous work [48]. The R_f values of thiazolyllhydrazone **I** and **II** is about 0.8 and 0.2 in solvent of ethyl acetate-petroleum ether (1:1), respectively, and the ratio of thiazolylhydrazone **I** to **II** afforded was approximately 9:1. To confirm the regiochemistry, the N3 substituted thiazolylhydrazones were synthesised by the route illustrated in Scheme 3. The geometric configuration of double bond and the regiochemistry were further established by X-ray analysis of compounds **I-3a** (CCDC No. 1851329) and **II-3a** (CCDC No. 1912786). As shown in Fig. 1, for thiazolylhydrazone **I**, the substituted group R_1 was at N3 position of thiazoline ring, and the configurations of the double bonds N(2)=C(12) and N(1)=C(11) were *Z* and *E*, respectively. For thiazolylhydrazone **II**, the substituted group R_1 was at the exocyclic N2 position, and the configuration of the N(1)=C(11) double bonds was *E*. When comparing the ^{1}H NMR data of compounds **II** with **I**, the chemical shift of $\underline{CH_2}$ protons of substituted R_1 of **II** (such as **II-3a**, 5.35 ppm) appeared at a higher frequency due to the conjugative effect of the N(1)=C(11) double bonds with naphthalene ring than one of compound **I** (such as **I-3a**, 4.53 ppm).

The inhibitory activities of thiazolylhydrazone derivatives **I** and **II** against *Of*Hex1 and *Of*Chi-h are outlined in Table 1. Some of the compounds exhibited good inhibitory activities at 1 μmol/L and 10 μmol/L. Our previous work investigating the structure-activity relationships of the thiazolylhydrazone derivatives **I** against *Of*Hex1 reported the stretched conformation. Interestingly, the branched conformation of thiazolylhydrazone derivatives **II**, with aromatic groups at the N2 atom, such as benzyl (**3b**) and phenoxyethyl (**3d**), exhibited potent inhibitory activity. Further studies then focused on the naphthalene ring R_2. The inhibitory activities of the resultant derivatives were weakly influenced by either the substitutes or their position on the naphthalene ring (compare **3b** with **6a** and **6b**). However, replacement of the naphthalene ring with benzene, as in compounds **6c** and **6d**, resulted in a significant inhibitory activity decrease. Gratifyingly, it was found that the thiazolylhydrazone derivatives **I** and **II** exhibited promising inhibitory activity against *Of*Chi-h. In

particular, compounds **I-3d** and **II-3d** were found to display considerable activity against *Of*Hex1 and *Of*Chi-h (Fig. 2).

To further explore possible binding modes of these compounds, molecular docking studies were carried out using the *Of*Hex1 (PDB code: 3NSN) and *Of*Chi-h (PDB code: 5GQB) as templates. The predicted docking results reveal that the thiazolylhydrazone derivatives bind in the active sites of *Of*Hex1 and *Of*Chi-h with different modes (Fig. 3). As shown in Fig. 3A, compound **I-3d** binds the entire active pocket of *Of*Hex1 *via* hydrogen bonds, π-π stacking and van der Waals interactions. The naphthalene and thiazolinone rings binds the −1 and +1 subsites and stack with Trp524 and Trp490, respectively. The oxygen atom of the thiazolinone ring forms a hydrogen bond with the Glu328 and Glu526 residue. The phenoxyethyl group binds a subpocket formed by Trp483 and Asn489 on Loop478-496, and Gln527 *via* van der Waals interactions (Fig. 3A). In the docked structure of *Of*Hex1 in complex with **II-3d**, the N1 and N2 atoms of the linker form hydrogen bonds with the Glu328, Glu526 and Trp490 residues, respectively. The N3 atom forms a hydrogen bond with Glu526, and the oxygen atom of thiazolinone ring forms a hydrogen bond with the Gln527 and Asn489 residues (Fig. 3B). Compound **II-3d** forms more hydrogen bonds with *Of*Hex1 than compound **I-3d**. The superimposition of compound **I-3d** and **II-3d** reveals that two compounds bind in the −1 and +1 subsites of *Of*Hex1 (Fig. 3C). The predicted different binding modes of **I-3d** (K_i = 5.9 μmol/L, Fig. 2A) and **II-3d** (K_i = 1.5 μmol/L, Fig. 2B) could explain the difference in inhibitory activity.

Interestingly, experiments showed that compound **I-3d** (K_i = 1.9 μmol/L, Fig. 2C) has better inhibitory activity against *Of*Chi-h than compound **II-3d** (K_i = 4.1 μmol/L, Fig. 2D). As shown in Fig. 3D, the compound **I-3d** binds the entire active pocket of *Of*Chi-h in a stretched conformation. The naphthalene ring is sandwiched by Trp268 and Trp389, and the phenoxyethyl group occupies the hydrophobic patch comprised by Phe184, Thr269 and Leu270. The oxygen atom of the phenoxyethyl group forms a hydrogen bond with the Trp268 residue. The N1 and N2 atoms form hydrogen bonds with Glu308, stabilizing the binding conformations. While the mechanism of interaction of **II-3d** with *Of*Chi-h is different from that of **I-3d**, the naphthalene ring occupied the hydrophobic patch comprised by Phe184, Thr269 and Leu270, the oxygen atom of the thiazolinone ring only forms a hydrogen bond with the Leu352 residue (Fig. 3E). The superimposition of compound **I-3d** and **II-3d** reveals that the differences in the compounds' binding conformations with *Of*Chi-h explained the differences in their inhibitory activity (Fig. 3F).

In summary, we have designed, prepared and evaluated a series of thiazolylhydrazone derivatives **I** and **II** as potential inhibitors of *Of*Hex1 or *Of*Chi-h. To further explore possible binding modes of

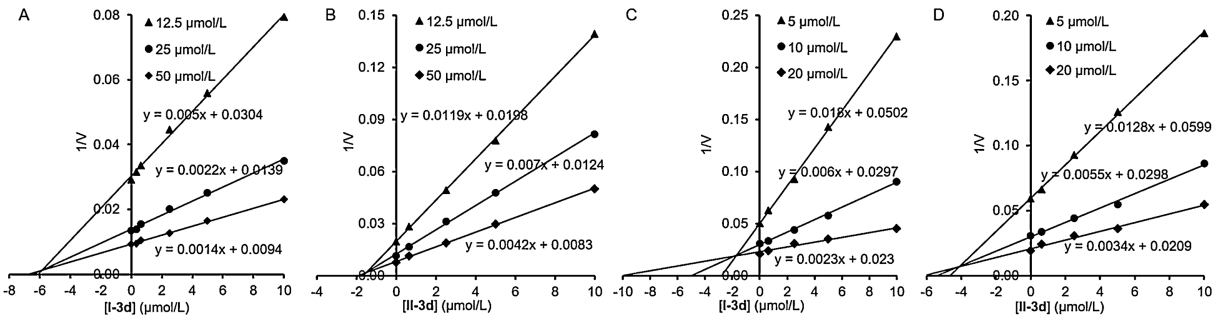


Fig. 2. Inhibitory kinetics of compounds **I-3d** (A), **II-3d** (B) against *Of*Hex1 and **I-3d** (C), **II-3d** (D) against *Of*Chi-h.

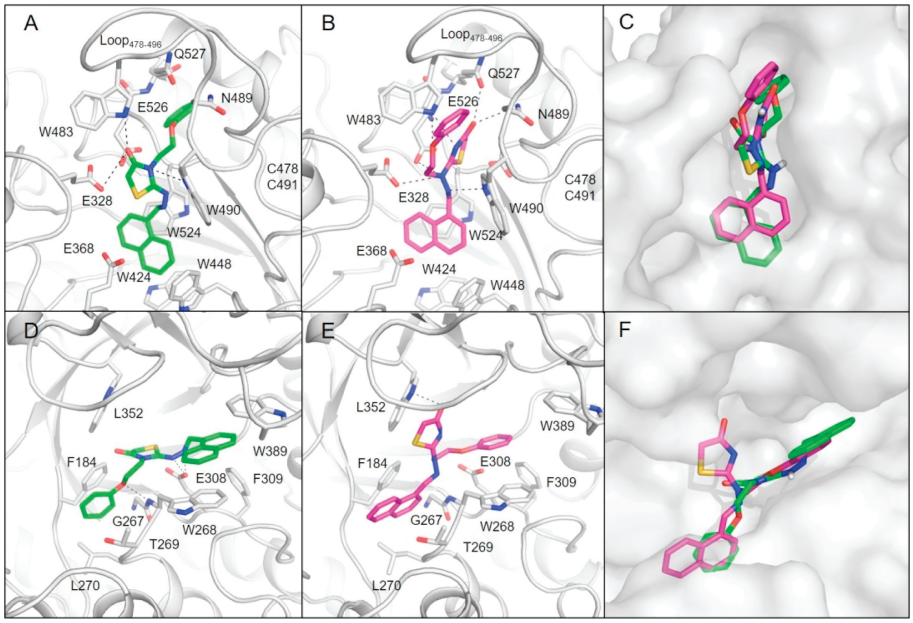


Fig. 3. Proposed binding modes of compounds **I-3d** and **II-3d** to the enzymes *Of*Hex1 and *Of*Chi-h respectively: (A) Binding mode **I-3d** in the active pocket of *Of*Hex1. The compound **I-3d** was shown in green. The hydrogen bonds were shown in black dashes. (B) Binding mode of **II-3d** in the active pocket of *Of*Hex1. The compound **II-3d** was shown in magenta. (C) Superimposition of compound **I-3d** and **II-3d** in the active pocket of *Of*Hex1. (D) Binding mode **I-3d** in the active pocket of *Of*Chi-h. (E) Binding mode of **II-3d** in the active pocket of *Of*Chi-h. (F) Superimposition of compounds **I-3d** and **II-3d** in the active pocket of *Of*Chi-h.

these compounds, molecular docking studies were carried out using the *Of*Hex1 and *Of*Chi-h as templates. Docking results reveal the mechanism of improved inhibitory activity against *Of*Hex1 and *Of*Chi-h of compounds **I-3d** and **II-3d**. This work provides a novel scaffold for developing specific Hex and Chi-h inhibitors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.11.035.

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