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Communication

Naphthalimide and quinoline derivatives as inhibitors for insect *N*-acetyl-*β*-D-hexosaminidase

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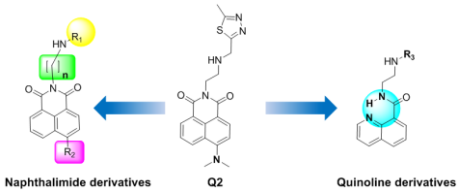
E-mail addresses: qingyang@dlut.edu.cn (Q. Yang), xhqian@ecust.edu.cn (X. Qian).
[1] These authors contributed equally to this work.

Graphical Abstract



A series of substituted naphthalimide and quinoline derivatives were designed, prepared and evaluated as potential inhibitors of *Of*Hex1. Compound **3m** was the most potent inhibitor with a K_i value of 0.34 μmol/L. Quinoline analogs with an intramolecular N-H hydrogen bond mimiced the naphthalimide configuration to maintain the inhibitory activity potency.

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ABSTRACT

Insect chitinolytic *β-N*-acetyl-D-hexosaminidase, such as *Of*Hex1 from *Ostrinia furnacalis*, is a potential target for insecticide design. Among the known *Of*Hex1 inhibitors, **Q2** is of great interest because it is the first non-carbohydrate inhibitor. In this study, we designed and synthesized a series of **Q2** derivatives by replacing the thiadiazole and naphthalimide groups and changing the linker length. Compound **3m** showed the best inhibitory activity with a K_i value of 0.34 μmol/L against *Of*Hex1, which is about one-quarter that of **Q2** (K_i = 1.4 μmol/L). Compound **6a** showed the best inhibitory activity among the quinoline-containing derivatives (K_i = 2.3 μmol/L). Molecular docking indicated that although **3m**, **6a**, and **Q2** binding the active pocket of *Of*Hex1 in similar mode, compound **3m** engaged better than the other compounds in intermolecular interaction with *Of*Hex1.

Chitin is the second most abundant biopolymer next to cellulose in nature, and is the major component of the extracellular matrix of some agricultural pests such as fungi [1], nematodes and insects [2]. Notably, chitin is completely absent from vertebrates and higher plants, and the key enzymes for chitin biosynthesis and biodegradation represent potential targets for pesticide development. For example, insect chitinolytic *β-N*-acetyl-D-hexosaminidase (Hex), which is responsible for hydrolyzing chitooligosaccharides to *N*-acetyl-D-glucosamine during chitin degradation, is considered to be an attractive target for pesticide development. Hex has been proved to be vital for insect survival in different insect species, such as *Tribolium castaneum* [3], *Ostrinia furnacalis* [4], *Locusta migratoria* [5], *Nilaparvata lugens* [6] and *Mamestra brassicae* [7]. Moreover, the crystal structure of *Of*Hex1 from *Ostrinia furnacalis* has been resolved and has provided a solid basis for the design of specific inhibitors [8].

Several kinds of *Of*Hex1 inhibitors have been reported, including TMG-chitotriomycin [9-14], PUGNAc [15,16], NAG-thiazoline and its derivative NMAGT [17,18], **Q2** [19], phlegmacin B1 [20], berberine [21] and thiazolylhydrazone derivatives [22]. Among these compounds, **Q2**, an unsymmetrical dyad of thiadiazole and 1,8-naphthalimide, is of great interest because it is the first non-carbohydrate inhibitor of *Of*Hex1 [19]. As part of our efforts toward the discovery and biological evaluation of new *Of*Hex1 inhibitor, we report here the synthesis and structure-activity relationships of a series of **Q2** derivatives formed by replacing the thiadiazole and naphthalimide

groups [23-25], and altering the linker length. Moreover, to improve the pharmacological profiles and solubilities of naphthalimide derivatives while maintaining their inhibitory activity, we replaced the naphthalimide moiety with quinoline carboxamide group *via* an intramolecular N-H hydrogen bond to mimic the naphthalimide configuration. We studied the inhibition mechanisms of these compounds using structure-based molecular docking.

The preparation of substituted naphthalimide derivatives **3a**-**m** is summarized in Scheme 1. The dimethylamine-substituted intermediate **1b** can be obtained from the corresponding commercially available 4-bromo-1,8-naphthalic anhydride **1a**. Intermediates **2** were synthesized by reacting a suspension of intermediates **1** in ethanol with excess Boc-protected amines, followed by deprotection in acidic condition. Compounds **3** were synthesized from intermediates **2** with a halogenated compound or aryl acyl chloride under base conditions [26].

The quinoline derivatives were prepared according the method described in Scheme 2. The quinoline acyl chloride **4**, which was prepared from quinoline carboxylic acid, was reacted with the Boc-protected amine and then deprotected under acidic condition to obtain the intermediate **5**. Compounds **6** were synthesized from intermediate **5** with the halogenated compounds or aryl acyl chloride under base conditions. Details for the synthetic procedures, physical characteristics and the results of ^{1}H NMR, ^{13}C NMR and MS for all the synthesized compounds are listed in the Supporting information.

The inhibitory activities of naphthalimide derivatives **3a**-**m** toward *Of*Hex1 are outlined in Table 1. For substitution of NH (R_1), a comparison of **3a** with **3b** showed that small rings, such as thiazole, gave better inhibitory activity than the pyridine ring. The carbonyl group was introduced to increase the hydrogen bond between the compound and the enzyme, which could improve binding affinity. However, a comparison of **3a** with **3c**–**i** showed that replacement of methylene with a carbonyl or sulfone group did not remarkably improve the inhibitory activity.

Compound **3a** had an acceptable level of inhibitory activity, and the raw material of **3a**, 2-chloro-5-(chloromethyl)thiazole, is cheap and easy to get. Thus, we used (2-chlorothiazol-5-yl)methyl as R_1 and focused our attention on the effects of the substitution of the naphthalimide and elongation of the alkyl chain (n). Replacement of the dimethylamino group with bromine or hydrogen at the 5-position (R_2; compound **3j** and **3k**) decreased the activity against *Of*Hex1. A comparison of the inhibitory activities of compounds **3a**, **3l** and **3m** showed a large enhancement with elongation of the alkyl chain (n). Compound **3m** stood out with comparatively higher activity with a K_i value of 0.34 μmol/L (Fig. 1A), and exhibited about 4-fold increase in inhibitory activity when compared with compound **Q2** (K_i = 1.4 μmol/L).

To improve the pharmacological profiles of naphthalimide derivatives but maintain the inhibitory activity, we replaced the naphthalimide moiety with quinoline carboxamide group, which contains an intramolecular N-H hydrogen bond and could mimic the naphthalimide while improving the solubility. The quinoline analogues showed obvious better inhibitory activities against *Of*Hex1, regardless of their content of a six-membered ring or five-membered ring (Table 2). Compounds containing a thiazole ring, including **6a**, **6e** and **6j**, exhibited more potent inhibitory activity (>70%) than compounds containing other heterocycle ring. Compound **6a** showed the best inhibitory activity with a K_i value of 2.3 μmol/L against *Of*Hex1 (Fig. 1B).

To elucidate the inhibition mechanism of **3m**, molecular docking was performed using *Of*Hex1 as template. We found that **3m** occupied the entire substrate binding pocket of *Of*Hex1 and interacted *via* hydrogen bonds (Fig. 2A). The molecular docking study revealed good binding of the linker and the thiazole group of **3m** at the subsite −1. The linker of **3m** was bent into a curved conformation and the secondary nitrogen atom formed a hydrogen bond with the catalytic residue Glu368 and the residue Glu328. The N3 atom formed a hydrogen bond with the phenolic hydroxyl group of Tyr475. Interestingly, the length of the linker region had a strong effect on the inhibition mechanism. Elongation of the alkyl chain resulted in tight binding of the 4-dimethylaminonaphthalimide group of **3m**. A hydrophobic patch comprising Trp483, Trp490 and carbonyl groups from the 4-dimethylaminonaphthalimide formed hydrogen bonds with Val327, Glu328 and Glu526.

The binding mode of compound **6a** in *Of*Hex1 was studied by molecular docking. As shown in Fig. 2B, binding of compound **6a** occurred in the entire active pocket of *Of*Hex1 in a similar manner to compound **3m**. The mechanisms of interaction of the linkers and thiazole group with *Of*Hex1 were similar to those of **3m**, and the quinoline group bound with a hydrophobic patch comprising Trp483 and Trp490 outside of subsite −1.

Although compounds **3m** (K_i = 0.34 μmol/L) and **6a** (K_i = 2.3 μmol/L) showed different inhibitory activity as **Q2** did (K_i = 1.4 μmol/L) against *Of*Hex1, the predicted binding modes of **3m** and **6a** were similar to that of **Q2** in the crystal structure of *Of*Hex1 in a complex with Q2 [19] (Fig. 2C). First, binding of the thiazole group of **3m**, **6a** and the thiadiazole group of **Q2** occurred in subsite −1 of the active pocket in the same fashion. These groups were sandwiched by Trp524 and Trp448 and their N3 atoms formed hydrogen bonds with the phenolic hydroxyl group of Tyr475. Second, the linkers of **3m**, **6a** and **Q2** were bent into a curved conformation and the secondary nitrogen atoms formed hydrogen bonds with the catalytic residue Glu368. These results suggest that the compounds discovered in this study inhibit *Of*Hex1 by a similar mechanism as **Q2**.

In summary, we designed, prepared and evaluated a series of substituted naphthalimide and quinoline derivatives as potential inhibitors of *Of*Hex1. Compound **3m** was the most potent inhibitor with a K_i value of 0.34 μmol/L. Quinoline analogs with an intramolecular N-H hydrogen bond mimic the naphthalimide configuration to maintain the inhibitory activity potency.

Acknowledgments

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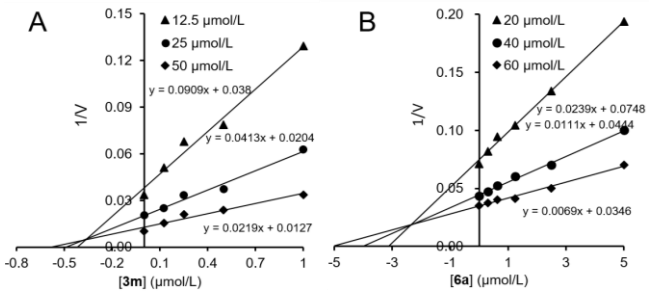


Fig. 1. Inhibitory kinetics of compounds **3m** (A), **6a** (B) against *Of*Hex1.

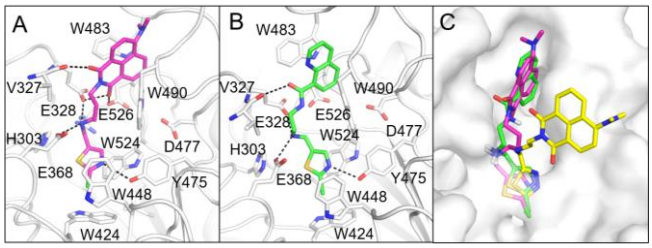
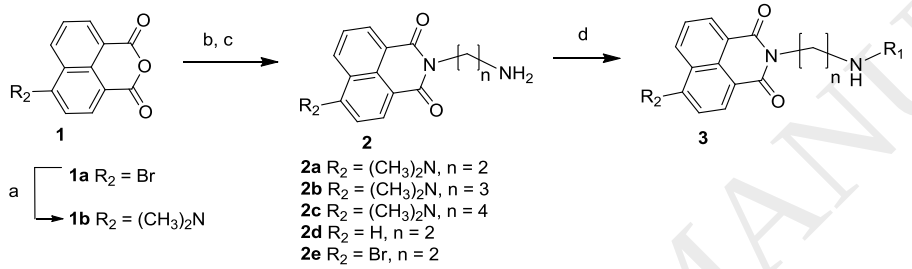
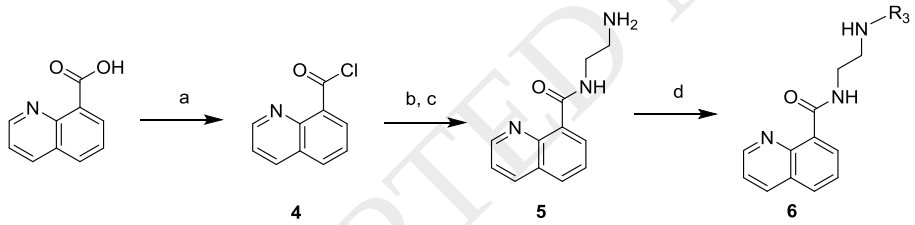


Fig. 2. Inhibition mechanisms of compounds **3m** and **6a** against *Of*Hex1. (A) Binding mode of **3m** in the active pocket of *Of*Hex1. The compound **3m** was shown in magenta. The hydrogen bonds were shown in black dashes. (B) Binding mode of **6a** in the active pocket of *Of*Hex1. The compound **6a** was shown in green. The hydrogen bonds are shown as dashed black lines. (C) Superimposition of compound **3m**, **6a** and **Q2** in the active pocket of *Of*Hex1. **3m**, **6a** and **Q2** are shown in magenta, green and yellow respectively.

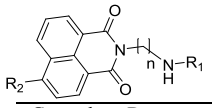


Scheme 1. Synthesis of compounds **3a-m**. Reagents and conditions: (a) 40% Dimethylamine aqueous solution, $CuSO_4 \cdot 5H_2O$, DMF, reflux, 8 h; (b) $NH_2(CH_2)_nNHBoc$, EtOH, reflux, 3 h; (c) CF_3COOH, CH_2Cl_2, r.t., 4 h; (d) R_1Cl, K_2CO_3, CH_3CN, reflux, 3 h; or R_1Cl, Et_3N, CH_2Cl_2, r.t., 5 h.



Scheme 2. Synthesis of compounds **6a-j**. Reagents and conditions: (a) $SOCl_2$, $PhCH_3$, reflux, 2 h; (b) *tert*-butyl(2-aminoethyl)carbamate, Et_3N, CH_2Cl_2, r.t., 5 h; (c) CF_3COOH, CH_2Cl_2, r.t., 4 h; (d) R_3Cl, K_2CO_3, CH_3CN, reflux, 3 h; or R_3Cl, Et_3N, CH_2Cl_2, r.t., 5 h.

Table 1
Inhibitory activities of compounds **3a**-**m** against *Of*Hex1.



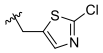
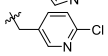
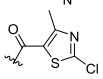
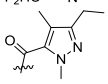
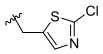
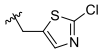
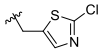
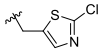
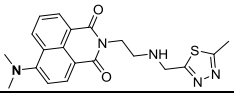
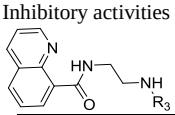
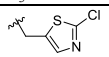
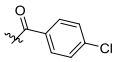
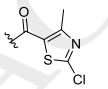
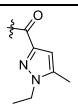
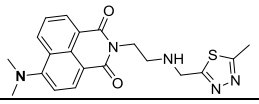
Compd.	R_1	R_2	n	Inhibitory rate (%, 10 μmol/L)
3a		$(CH_3)_2N$	2	69.1 ± 4.8
3b		$(CH_3)_2N$	2	48.0 ± 0.2
3c		$(CH_3)_2N$	2	54.0 ± 5.0
3d		$(CH_3)_2N$	2	34.0 ± 3.0
3e		$(CH_3)_2N$	2	37.0 ± 1.0
3f		$(CH_3)_2N$	2	23.0 ± 7.0
3g		$(CH_3)_2N$	2	56.0 ± 1.0
3h		$(CH_3)_2N$	2	35.0 ± 4.0
3i		$(CH_3)_2N$	2	26.0 ± 7.0
3j		H	2	25.7 ± 1.4
3k		Br	2	31.1 ± 0.9
3l		$(CH_3)_2N$	3	75.0 ± 0.0
3m		$(CH_3)_2N$	4	91.0±1.0
Q2				81.3 ± 3.2

Table 2
Inhibitory activities of compounds **6a-j** against *Of*Hex1.



Compd.	R_3	Inhibitory rate (%, 10 μmol/L)
6a		81.0 ± 0.0
6b		60.0 ± 2.0
6c		65.0 ± 3.0
6d		66.0 ± 2.0
6e		70.0 ± 1.0
6f		57.0 ± 3.0
6g		66.0 ± 2.0
6h		67.0 ± 1.0

6i		64.0 ± 1.0
6j		75.0 ± 1.0
Q2		81.3 ± 3.2