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1. Synthesis of ureido thioglycosides as novel insect β-*N*-acetylhexosaminidase OfHex1 inhibitors

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Abstract: The insect β-*N*-acetylhexosaminidase OfHex1 from *Ostrinia furnacalis* (one of the most destructive agricultural pests) has been considered as a promising pesticide target. In this study, a series of novel and readily available ureido thioglycosides were designed and synthesized based on the catalytic mechanism and the co-crystal structures of OfHex1 with substrates. After evaluation via enzyme inhibition experiments, thioglycosides **11c** and **15k** were found to have inhibitory activities against OfHex1 with the K_i values of 25.6 μM and 53.8 μM, respectively. In addition, all these ureido thioglycosides exhibited high selectivity toward OfHex1 over hOGA and HsHexB (K_i > 100 μM). Furthermore, to investigate the inhibitory mechanism, the possible binding modes of **11c** and **15k** with OfHex1 were deduced based on molecular docking analysis. This work may provide useful structural starting points for further rational design of potent inhibitors of OfHex1.

Keywords: Ureido thioglycosides, trichloroethoxycarbonyl (Troc), β-*N*-acetylhexosaminidase, OfHex1, inhibitors

1. Introduction

Chitin, the second most abundant naturally occurring polysaccharides, is the key component of the insect exoskeleton, nematode eggshell and fungal cell wall.[1] Importantly, chitin is absent from higher mammals and plants.[2] Thus, the enzymes in chitin metabolism (biosynthesis and biodegradation) are recognized as promising targets for developing green pesticides.[3-5]

The insect β-*N*-acetylhexosaminidase OfHex1 is the important component enzyme in chitin

degradation of insect.[6] OfHex1 from the agricultural pest, Asian corn borer *(Ostrinia furnacalis)*, can efficiently hydrolyze β-1,4-linked chitooligosaccharides into N-acetyl-D-glucosamine (GlcNAc).[6] Interference with this physiological process can disrupt molting and metamorphosis of *Ostrinia furnacalis*, eventually resulting in insect death. [6-7] Furthermore, the crystal structure of OfHex1 (the only insect-derived β-*N*-acetylhexosaminidase, PDB ID: 3NSM) has been reported.[8] Therefore, the development of eco-friendly pesticides with OfHex1 as the target has a good foundation and important research significance.[8-10]

To date, a number of small molecule inhibitors targeting OfHex1 have been reported, including PUGNAc, TMG-chitotriomycin[8, 11], naphthalimides[10, 12-14], NGT[15], phlegmacin B1[16], berberine[17], pyrimethamine[18] and thiazolylhydrazone derivatives[19]. Amongst these compounds, PUGNAc is a classic and broad-spectrum β-*N*-acetylhexosaminidases inhibitor, with a K_i value of 0.24 μM against OfHex1[9]. The crystal structure of OfHex1-PUGNAc (PDB: 3OZP) showed that the sugar moiety of PUGNAc could tightly bound to the -1 subsite of OfHex1 and the hydrophobic phenyl was sandwiched by Val327 and Trp490 at the +1 subsite. [9] The structure-activity relationship studies also revealed that the inhibitory potency of PUGNAc derives from the glycosyl moiety (GlcNAc), sp2-hybridized carbon at the C-1 position, and *N*-phenylcarbamate group. [20] These results indicated that glycosyl moiety and aromatic fragment were crucial in the design of potent OfHex1 inhibitors. In addition, TMG-chitotriomycin is the most potent OfHex1 inhibitor and can only inhibit β-*N*-acetylhexosaminidases from chitin-containing organisms. [8] The high inhibitory potency of TMG-chitotriomycin mainly comes from the positively charged *N*,*N*,*N*-triMe group, which can interacts with catalytic Asp367 and Glu368 at the -1 subsite of OfHex1. [8] This suggested that the 2-substituent at the glycosyl moiety could exert a critical effect on the potency against OfHex1.

Prompted by these observations, we selected glycosyl moiety and hydrophobic groups (naphthalimides or benzoyl) as the frame structures. Then, we introduced alkyl chains to connect these two moieties and converted the glycosidic bond to thioglycosidic bond (to prevent the compounds from being degraded by OfHex1). Considering the crucial roles of 2-substituent at the glycosyl moiety, we further modified the acetamido group of GlcNAc to ureido group (with more hydrogen bonding donors and hydrogen bond acceptors) at the 2-position, hoping to improve the binding affinity toward OfHex1. Accordingly, several classes of ureido thioglycosides were synthesized and their inhibitory activities against OfHex1, human β-N-acetylhexosaminidase B (HsHexB), and human O-GlcNAcase (hOGA) were evaluated (**Figure 1**).

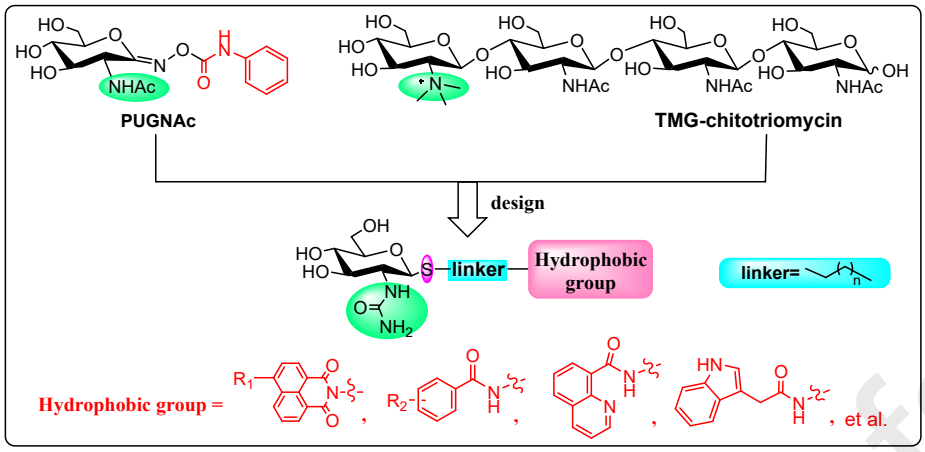
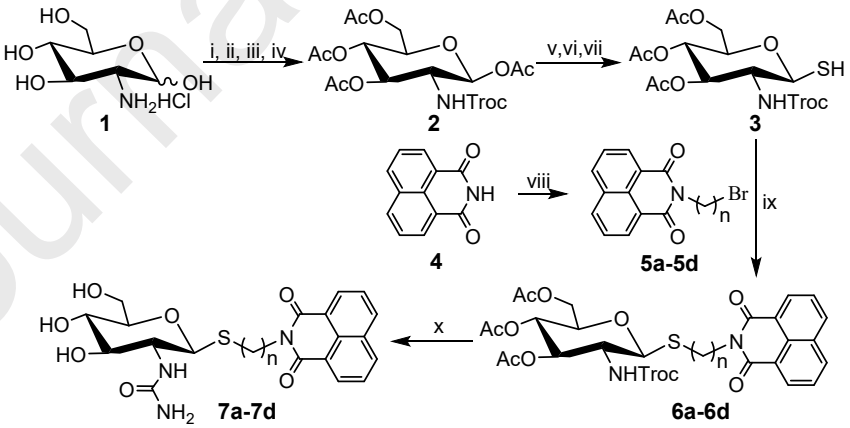


Figure 1. Design of novel ureido thioglycoside derivatives for OfHex1

2. Results and discussion

2.1. Synthesis of ureido thioglycosides 7a-7d

Inspired by our previous studies[12-13], we first selected naphthalimide moiety as the hydrophobic group of the target compound. Then, we focused on studying the influences of the length of linker on the inhibitory potency against OfHex1. The target compounds **7a-7d** are synthesized and outlined in **Scheme 1**. Briefly, compound **3** were obtained according to literature methods[21] and reacted with bromides **5a-5d**[22] to afford trichloroethyl carbamates **6a-6d**. Then refer to our recently reported synthetic method of ureido glycosides,[21] precursors **6a-6d** were reacted with ammonia (in MeOH, 7M) at room temperature for 60 h to yield target compounds **7a-7d** in one step (**Scheme S1**).



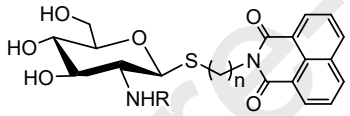
7a: n=3; **7b**: n=4; **7c**: n=5; **7d**: n=6

Scheme 1. Synthesis of ureido thioglycosides **7a-7d**. (i) *p*-anisaladehyde, NaOH, H_2O; (ii) Py, Ac_2O; (iii) acetone, HCl, H_2O; (iv) Et_3N, CH_2Cl_2, TrocCl; (v) HBr, CH_3COOH, CH_2Cl_2; (vi) thiourea, acetone; (vii) $Na_2S_2O_5$, CH_2Cl_2, H_2O; (viii) K_2CO_3, CH_3CN; (ix) K_2CO_3, acetone, H_2O; (viii) NH_3, MeOH

2.2. Inhibitory potency of ureido thioglycosides 7a-7d

The target compounds **7a-7d** were evaluated for their inhibitory potency against OfHex1, hOGA, and HsHexB at the concentration of 100 μM. As shown in **Table 1**, upon extending the linker (carbon atoms from three to six), a gradual increase in the inhibitory activity against OfHex1 was observed. Compound **7d** bearing six carbon atoms (n = 6, **Scheme 1**) showed the higher potency with an inhibition rate of 59.5% at a concentration of 100 μM. In addition, the ureido group at 2-positon of the glycosyl moiety (**7d**) could improve the inhibitory activity against OfHex1 compared to that of glycosyl moiety bearing acetyl group (**21e** [22], unpublished data). These finding also revealed that the 2-ureido group (of these target compounds) was helpful for increasing the potency against OfHex1. Furthermore, **7a-7d** showed lower potency against hOGA and HsHexB, suggesting that these compounds had suitable selectivity toward OfHex1.

Table 1. Inhibition rate of compounds **7a-7d** and **21e**[22] against OfHex1, hOGA, and HsHexB.



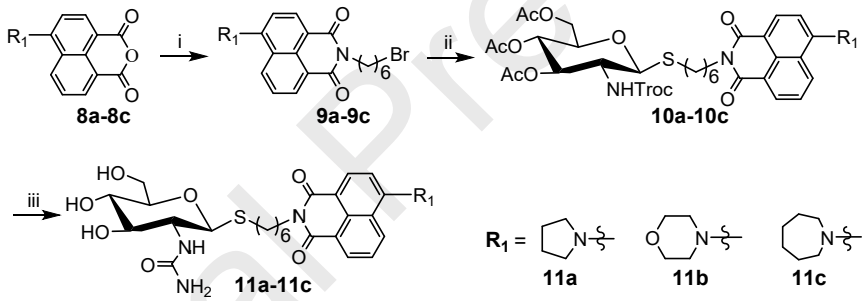
Compd	n	R	Inhibition rate at 100 μM (%)		
			OfHex1	hOGA	HsHexB
7a	3	$CONH_2$	3.8 ± 1.0	20.5 ± 1.3	34.7 ± 0.5
7b	4	$CONH_2$	16.8 ± 2.6	8.8 ± 1.8	11.9 ± 1.9
7c	5	$CONH_2$	43.5 ± 0.9	13.7 ± 2.9	38.9 ± 3.1
7d	6	$CONH_2$	59.5 ± 2.1	7.1 ± 0.9	5.1 ± 2.2
21e[a]	6	$COCH_3$	31.9 ± 2.9	25.9 ± 1.8	27.6 ± 1.3

[a] Structure of compound **21e** were taken from ref 22.

2.3. Modification of ureido thioglycoside 7d

On the basis of structure-activity relationships from the first stage, thioglycoside **7d** was selected for structural modification. Firstly, we retained the frame structure of ureido thioglycoside and fixed the linker with six carbon atoms (n = 6, **Scheme 1**). Then, we focused on structural derivatization for naphthalimide group, including introduction of 4-substituted group on the naphthalimide or replacement of naphthalimide with other hydrophobic groups (benzene ring, heterocyclic ring, and alkyl group). Accordingly, several classes of ureido thioglycosides were synthesized (**Scheme 2-4**).

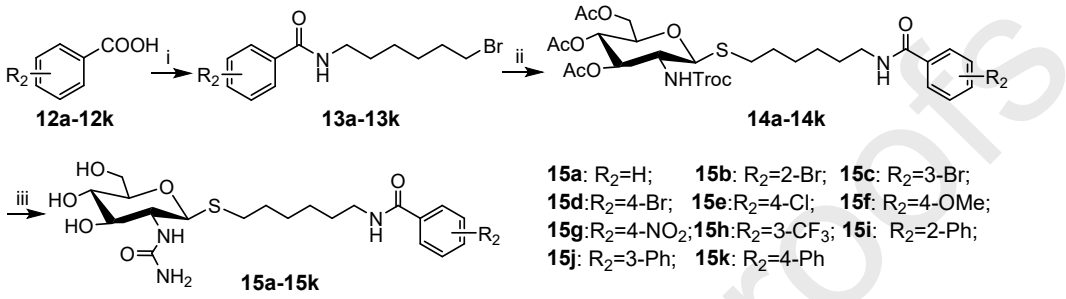
Firstly, we introduced nitrogen-containing cycloalkane groups (based on our previous work[12]) to the 4-position of naphthalimide and synthesized ureido thioglycosides **11a-11c** (**Scheme 2**). Briefly, substituted naphthalic anhydrides **8a-8c** were selected as the starting material and reacted with 6-bromohexan-1-amine to afford bromide **9a-9c**, and then stirred with thiol **3** in acetone/water (2:1, v: v) to obtain acetyl-protected precursors **10a-10c**. Finally, deprotected via methanol-ammonia catalysis resulted in the target compounds **11a–11c** (**Scheme 2** and **S2**). The analysis of compounds **11a–11c** against OfHex1 (**Table 2**) showed that the 4-substituent at the naphthalimide group could significantly affect the inhibitory potency of these compounds. Specifically, inhibitors bearing 4-pyrrolyl (**11a**) and 4-azepanyl (**11c**) groups increased the inhibitory activity against OfHex1 compared to lead compound **7d**. However, the addition of 4-morpholino (**11b**) at the naphthalimide group decreased the potency, which suggested that the oxygen atom at nitrogen-containing cycloalkane group may be detrimental to the binding affinity with OfHex1. Further IC_{50} determination showed that **11c** exhibited relatively good activity (OfHex1, IC_{50} = 28.1 μM) and selectivity (hOGA and HsHexB, IC_{50} >100 μM) against OfHex1 (**Table 3**).



Scheme 2. Synthesis of ureido thioglycosides **11a-11c**. (i) 6-bromohexan-1-amine, EtOH; (ii) **3**, K_2CO_3, acetone, H_2O; (iii) NH_3, MeOH

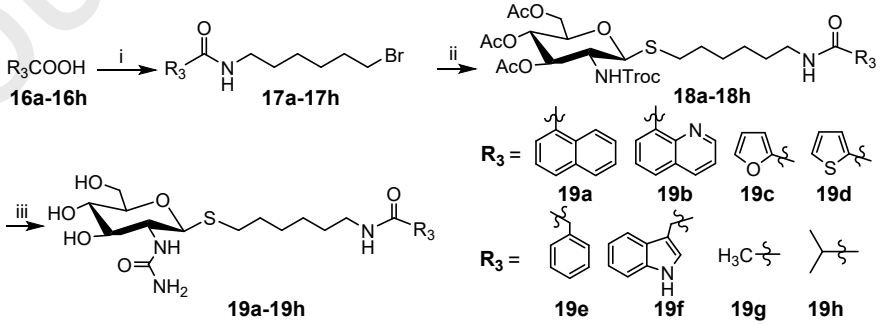
We then synthesized a series of substituted phenyl group-bearing ureido thioglycosides **15a-15k**. As shown in **Scheme 3**, benzoic acid compounds **12a-12k** were reacted with 6-bromohexan-1-amine to acquire intermediates **13a-13k**. Subsequently, the coupling of **13a-13k** with thiol **3** in acetone and H_2O resulted in acetyl-protected precursors **14a-14k**, and then stirred with ammonia (in MeOH, 7M) at room temperature to obtain target compounds **15a-15k** (**Scheme S3**). The bioassay results of compounds **15a-15k** against OfHex1, hOGA, and HsHexB were shown in **Tables 2-3**. Most of the compounds in **15a-15k** exhibited a relatively weak activity toward these three enzymes. In detail, compounds **15a-15j** displayed < 16 % inhibition rate against OfHex1, and only one compound (**15k**) exhibited the higher activity with an inhibition rate of 63.7 %. These results suggested that the size and the position of the substituent on benzene ring may be the main

factor to affect the activity toward OfHex1 rather than the electronic properties (electron-withdrawing and electron-donating). The presence of a larger phenyl group on benzene ring (**15i-15k**) led to the increased potency. Moreover, the activity order of the substituent on benzene ring was para > meta > ortho (**15k** >**15i** >**15j**, **15d** >**15b** >**15c**). Further IC_{50} assay results showed that **15k** possessed the moderate activity toward OfHex1 with the value of 55.7 μM (**Table 3**).



Scheme 3. Synthesis of ureido thioglycosides **15a-15k**. (i) 6-bromohexan-1-amine, EDCI, DMAP, Et_3N, DCM; (ii) **3**, K_2CO_3, acetone, H_2O; (iii) NH_3, MeOH

To further improve the structure-activity relationship of these ureido thioglycosides, we replaced the substituted phenyl group in **15a–15k** with the naphthyl, heterocyclyl, phenethyl, and alkyl group, respectively. Thus, compounds **19a-19h** were synthesized. The synthetic route of compounds **19a-19h** were identical to compounds **15a–15k** and outlined in **Scheme 4** and **Scheme S4**. Analysis of compounds **19a-19h** against OfHex1 (**Table 2**) showed that the naphthyl (**19a**), quinolinyl (**19b**), furyl (**19c**), thienyl (**19d**), phenethyl (**19e**), and alkyl (**19g**, **19h**) groups could not enhance the activity. A special case was ureido thioglycoside bearing an indolyl group (**19f**), which exhibited the highest inhibitory potency against OfHex1 among compounds **19a-19h**, with the IC_{50} value of 72.3 μM (**Table 3**).



Scheme 4. Synthesis of ureido thioglycosides **19a-19h**. (i) 6-bromohexan-1-amine, EDCI, DMAP, Et_3N, DCM; (ii) **3**, K_2CO_3, acetone, H_2O; (iii) NH_3, MeOH

Table 2. Inhibition rate of compounds **11a-11c**, **15a-15k**, and **19a-19h** against OfHex1, hOGA, and HsHexB.

Compd	Inhibition rate at 100 μM (%)		
	OfHex1	**hOGA**	**HsHexB**
11a	**63.7 ± 2.5**	24.6 ± 1.6	1.3 ± 0.8
11b	19.1 ± 0.4	12.0 ± 1.9	1.9 ± 1.0
11c	**94.0 ± 0.6**	43.1 ± 2.0	1.8 ± 0.5
15a	4.2 ± 1.3	0.1 ± 0.1	3.1 ± 1.6
15b	3.3 ± 1.9	2.3 ± 0.8	0.4 ± 0.1
15c	1.0 ± 1.6	1.6 ± 2.3	0.8 ± 0.2
15d	8.9 ± 2.1	13.1 ± 1.0	0.6 ± 0.1
15e	7.3 ± 2.0	6.7 ± 1.4	3.3 ± 0.9
15f	0.1 ± 0.5	4.0 ± 2.7	11.6 ± 0.4
15g	6.3 ± 3.0	16.8 ± 0.5	6.8 ± 1.6
15h	1.1 ± 0.2	48.2 ± 0.2	12.9 ± 0.8
15i	15.3 ± 0.3	10.6 ± 1.7	0.6 ± 1.7
15j	13.4 ± 1.7	0.2 ± 0.5	0.3 ± 1.9
15k	**75.3 ± 2.5**	2.7 ± 2.4	6.1 ± 2.0
19a	0.7 ± 1.8	16.8 ± 0.3	1.9 ± 1.8
19b	1.5 ± 0.6	1.1 ± 0.6	5.0 ± 0.7
19c	7.3 ± 2.6	2.8 ± 1.6	11.8 ± 1.2
19d	3.2 ± 1.4	1.3 ± 1.9	9.6 ± 0.7
19e	3.3 ± 0.9	6.7 ± 2.7	4.1 ± 1.2
19f	**76.8 ± 1.3**	0.8 ± 1.1	12.8 ± 1.3
19g	1.3 ± 2.7	3.5 ± 0.6	8.7 ± 3.0
19h	11.5 ± 1.4	5.7 ± 1.2	0.4 ± 0.2

Table 3. IC_{50} values of representative compounds for OfHex1, hOGA, and HsHexB

Compd	Inhibition rate at 20 μM (%)			IC_{50} values (μM)		
	OfHex1	hOGA	HsHexB	OfHex1	hOGA	HsHexB
11a	20.4 ± 1.5	6.1 ± 2.0	0.5 ± 0.3	68.5 ± 3.1	>100	>100
11c	54.2 ± 2.8	12.5 ± 1.7	0.1 ± 0.2	**28.1 ± 1.6**	>100	>100
15k	32.6 ± 0.3	1.8 ± 0.6	1.9 ± 1.1	**55.7 ± 2.2**	>100	>100
19f	20.8 ± 1.9	0.2 ± 0.2	3.8 ± 2.4	72.3 ± 2.9	>100	>100

2.4. Inhibitory mechanism of ureido thioglycosides 11c and 15k toward OfHex1.

Two representative inhibitors, namely **11c** and **15k**, were selected to investigate the inhibitory mechanism toward OfHex1. Firstly, the Dixon plots of inhibitors **11c** and **15k** against OfHex1 were carried out. As shown in **Figure 2**, the trendlines drawn for each concentration all intersected in quadrant two, suggesting that compounds **11c** and **15k** are competitive inhibitors of OfHex1. Moreover, the K_i values of **11c** and **15k** were determined as 25.6 ± 0.5 μM and 53.8 ± 0.3 μM, respectively.

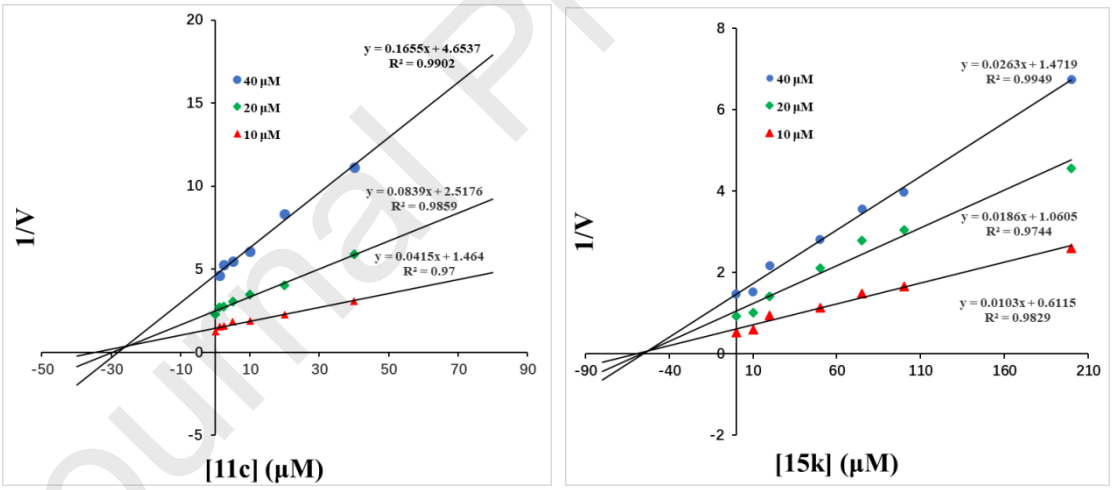


Figure 2. Dixon plots for inhibitors 11c and 15k against OfHex1

To investigate the possible binding modes of compounds **11c** and **15k** with OfHex1, the molecular docking studies were carried out. As shown in **Figure 3**, the sugar moiety of these two inhibitors was found to be tightly bound to the -1 subsite of OfHex1 and the hydrophobic groups extended out from the pocket. In detail, the hydroxyl groups (at glycosyl moiety) from **11c** could bind with residues Arg220, Asp367, Glu526 via H-bonding interactions. These interactions are coherent with those found in the complex structure of PUGNAc-OfHex1[9]. It is worth mentioning

that the ureido group of **11c** formed three hydrogen bonds with Glu328, Asp477 and Trp483. As a comparison, the 2-acetyl on glycosyl moiety of inhibitor PUGNAc formed two hydrogen bonds with Asp367 and Tyr475. [9] These results suggested that 2-ureido group on glycosyl moiety might increase the binding affinity toward OfHex1. Moreover, the 4-azepanylnaphthalimide moiety of **11c** could form π-π stacking interactions with Trp448 and van der Waals interactions with Ser426, Thr427, Asn430 (**Figure 3a**). The docking mode of compound **15k** complex with OfHex1 (**Figure 3b**) showed that the glycosyl moiety was found to interact with Arg220, Asp368, Tyr475, Asp477, Trp490, Glu526 via hydrogen bonds. The linker of **15k** bound to the +1 subsite of OfHex1, and the NH (in the linker) could form a hydrogen bond with residue Val327. In addition, the biphenyl group was located at the loop314-355 region (near the entrance of the OfHex1 pocket) and interacted with Lys323, Ser324, and Cys326. The binding modes of these two systems demonstrate the importance of larger hydrophobic groups in the molecular design of OfHex1 inhibitors, which can increase the hydrophobic interactions of compounds with the +1 subsite and loop regions of OfHex1.

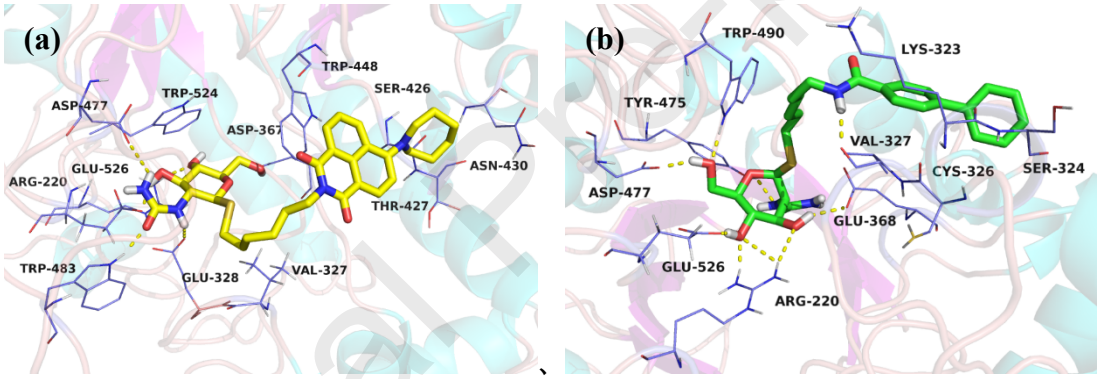


Figure 3. Predicted binding mode of compounds 11c (a) and 15k (b) with OfHex1

3. Conclusions

In summary, we present the design, synthesis, and inhibitory potencies of various ureido thioglycosides against β-*N*-acetylhexosaminidases (OfHex1, hOGA, and HsHexB). Importantly, compounds **11c** (K_i = 25.6 μM) and **15k** (K_i = 53.8 μM) showed the higher efficiency and selectivity against OfHex1. Moreover, molecular docking was carried out to allowed us to rationalize the potency of these ureido thioglycosides toward OfHex1. The structure–activity relationship as well as the molecular docking studies reveal that the 2-ureido group on glycosyl moiety and the larger hydrophobic groups (such as substituted naphthalimide or biphenyl group) are important for increasing the binding affinity of ureido thioglycosides for OfHex1. Taken in concert, the novel ureido thioglycosides reported herein may provide useful information for the further design and

development of OfHex1-related green pesticides.

4. Experimental

4.1 General methods

All chemicals, reagents and solvents were commercial sources and used without further purification. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AVANCE600 spectrometer in $CDCl_3$ or DMSO-d_6 at 25°C, and TMS was used as the internal standard. High-resolution mass spectra (HRMS) was obtained on a Bruker Daltonics Bio-TOF-Q III mass spectrometer (Bruker Co., Karlsruhe, Germany). Thin layer chromatography (TLC) was performed on silica gel GF254 plates with detection by ultraviolet (UV) light (254 nm) or by charring with 20% (v/v).

4.2 Chemical synthesis

4.2.1 Synthesis of intermediates

Detailed synthetic procedures and characterization data for all of the synthesized intermediates (**3**, **5a-5d**, **6a-6d**, **9a-9c**, **10a-10c**, **13a-13k**, **14a-14k**, **17a-17h**, **18a-18h**) are given in the **Supporting Information** (**Schemes S1-S4**).

4.2.2 Synthesis of target compounds 7a-7d, 11a-11c, 15a-15k, and 19a-19h

A solution of acetyl-protected precursors **6a-6d**, **10a-10c**, **14a-14k**, and **18a-18h** (0.5 mmol) was suspended in anhydrous MeOH (10 mL), and solution of NH_3 in MeOH (7M, 10 mL) was then added. The reaction was stirred for 60 h at room temperature, until TLC (EtOAc/MeOH/H_2O, 8:1:1 v/v/v) indicated that the reaction was complete. The mixture was concentrated *in vacuo* and recrystallized from MeOH to obtain compounds **7a-7d**, **11a-11c**, **15a-15k**, and **19a-19h**.

2-[3-[(2-Ureido-β-D-glucopyranosyl) thio] propyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7a**): white solid; (0.19 g, 79%) yield; $[\alpha]_D^{25}$ -28.7(c=0.1, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (dd, *J* = 9.9, 7.7 Hz, 4H, ArH), 7.92 – 7.78 (m, 2H, ArH), 5.92 (d, *J* = 8.7 Hz, 1H, NH), 5.46 (s, 2H, NH_2), 5.05 – 4.93 (m, 2H, 2 OH), 4.52 – 4.38 (m, 2H, OH, H-1), 4.10 (t, *J* = 7.2 Hz, 2H, H-3, H-4), 3.65 (dd, *J* = 11.5, 6.0 Hz, 1H, H-6b), 3.53 – 3.42 (m, 1H, H-6a), 3.36 – 3.19 (m, 2H, H-2, H-5), 3.16 – 3.07 (m, 2H, NCH_2), 2.72 (t, *J* = 7.4 Hz, 2H, SCH_2), 2.02 – 1.82 (m, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.54, 158.78, 134.37, 131.36, 130.79, 127.45, 127.27, 122.14, 84.82, 81.07, 76.61, 70.89, 61.32, 55.53, 39.40, 28.01, 27.07; HRMS (ESI) calcd for $C_{22}H_{26}N_3O_7S$ ($M+H^+$) 476.1491, found 476.1498.

2-[6-[(2-Ureido-β-D-glucopyranosyl)thio]hexyl]-6-(pyrrolidin-1-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**11a**): yellow solid; (0.22 g, 76%) yield; $[\alpha]_D^{25}$ -19.4 (c=0.1, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (d, *J* = 8.4 Hz, 1H, ArH), 8.34 (d, *J* = 7.4 Hz, 1H, ArH), 8.12 (d, *J* = 8.7

Hz, 1H, ArH), 7.52 (t, *J* = 7.9 Hz, 1H, ArH), 6.75 (d, *J* = 8.8 Hz, 1H, ArH), 5.89 (d, *J* = 8.4 Hz, 1H, NH), 5.44 (s, 1H, NH), 5.15 – 4.88 (m, 2H, 2 OH), 4.58 – 4.44 (m, 1H, OH), 4.35 (d, *J* = 9.7 Hz, 1H, H-1), 4.07 – 3.85 (m, 2H, H-3, H-4), 3.78 – 3.57 (m, 5H, H-6b, 2 CH_2), 3.56 – 3.40 (m, 1H, H-6a), 3.34 – 3.19 (m, 2H, H-2, H-5), 3.13 – 2.98 (m, 2H, $ArCH_2$), 2.73 – 2.51 (m, 2H, SCH_2), 2.13 – 1.87 (m, 4H, 2 CH_2), 1.65 – 1.40 (m, 4H, 2 CH_2), 1.40 – 1.20 (m, 4H, 2 CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.82, 162.85, 158.76, 154.43, 152.17, 132.91, 132.58, 130.56, 123.17, 121.76, 121.58, 108.87, 108.44, 96.39, 84.67, 81.07, 76.63, 70.94, 61.37, 55.56, 52.92, 48.73, 29.10, 28.28, 27.61, 26.32, 25.67; HRMS (ESI) calcd for $C_{29}H_{39}N_4O_7S$ ($M+H^+$) 587.2539, found 587.2531.

N-[6-[(2-ureido-β-D-glucopyranosyl) thio] hexyl] benzamide (**15a**): white solid; (0.17 g, 77%) yield; $[\alpha]_D^{25}$ -22.3 (c=0.1, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (t, *J* = 5.5 Hz, 1H, $CH_2N\underline{H}$), 7.90 – 7.74 (m, 2H, ArH), 7.59 – 7.30 (m, 3H, ArH), 5.90 (d, *J* = 8.6 Hz, 1H, $CHN\underline{H}$), 5.44 (s, 2H, NH_2), 5.03 – 4.91 (m, 2H, 2 OH), 4.49 (t, *J* = 5.8 Hz, 1H, OH), 4.34 (d, *J* = 9.8 Hz, 1H, H-1), 3.67 (dd, *J* = 11.2, 5.8 Hz, 1H, H-6b), 3.50 – 3.39 (m, 1H, H-6a), 3.35 – 3.15 (m, 4H, H-3, H-4, H-2, H-5), 3.14 – 3.00 (m, 2H, NCH_2), 2.59 (t, *J* = 7.2 Hz, 2H, SCH_2), 1.70 – 1.42 (m, 4H, 2 CH_2), 1.41 – 1.19 (m, 4H, 2 CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.26, 158.78, 134.85, 131.07, 128.33, 127.23, 84.72, 81.08, 76.59, 70.97, 61.41, 55.58, 39.29, 29.17, 29.11, 28.28, 26.21; HRMS (ESI) calcd for $C_{20}H_{32}N_3O_6S$ ($M+H^+$) 442.2012, found 442.2002.

N-[6-[(2-ureido-β-D-glucopyranosyl) thio] hexyl]-1-naphthamide (**19a**): white solid; (0.18 g, 73%) yield; $[\alpha]_D^{25}$ -15.8 (c=0.1, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (t, *J* = 5.6 Hz, 1H, $CH_2N\underline{H}$), 8.24 – 8.11 (m, 1H, ArH), 8.04 – 7.91 (m, 2H, ArH), 7.63 – 7.50 (m, 4H, ArH), 5.91 (d, *J* = 8.6 Hz, 1H, $CHN\underline{H}$), 5.45 (s, 2H, NH_2), 5.05 – 4.93 (m, 2H, 2 OH), 4.50 (t, *J* = 5.8 Hz, 1H, OH), 4.37 (d, *J* = 9.9 Hz, 1H, H-1), 3.69 (dd, *J* = 11.1, 5.9 Hz, 1H, H-6b), 3.51 – 3.40 (m, 1H, H-6a), 3.36 – 3.16 (m, 4H, H-3, H-4, H-2, H-5), 3.15 – 3.02 (m, 2H, NCH_2), 2.63 (t, *J* = 7.2 Hz, 2H, SCH_2), 1.67 – 1.48 (m, 4H, 2 CH_2), 1.47 – 1.29 (m, 4H, 2 CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.61, 158.78, 135.33, 133.25, 129.89, 129.65, 128.29, 126.74, 126.29, 125.50, 125.12, 125.06, 84.76, 81.11, 76.62, 70.99, 61.43, 55.61, 39.39, 29.23, 29.14, 29.09, 28.28, 26.23; HRMS (ESI) calcd for $C_{24}H_{34}N_3O_6S$ ($M+H^+$) 492.2168, found 492.2171.

4.2.3 Data for target compounds 7b-7d, 11b-11c, 15b-15k, and 19b-19h

Data for compounds **7b-7d, 11b-11c, 15b-15k, and 19b-19h** can be found in **Supporting Information**.

4.3 Enzyme inhibitory activity assays

OfHex1 and HsHexB were overexpressed in *Pichia pastoris* and purified according to previous methods.[8] hOGA was overexpressed in Escherichia coli BL21(DE3) and purified as described previously.[23]

The enzymatic activities of OfHex1, hOGA, and HsHexB measured at 30 °C using 4-

methylumbelliferyl N-acetyl-β-D-glucosaminide (4-MU-GlcNAc) as the substrate. HsHexB was assayed in 20 mM sodium citrate buffer (pH 4.5), OfHex1 and hOGA were assayed in 20 mM sodium phosphate buffer (pH 6.5). Firstly, the enzyme was pre-incubated with inhibitors in buffer for 10 min, then 4-MU-GlcNAc was added. After incubation for a further 20 min at 30 °C, the reaction mixture was terminated by the addition of 100 μL of 0.5 M sodium carbonate solution. The fluorescence was quantified (excitation at 366 nm, emission at 445 nm) on a Varioskan Flash microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The inhibition constant (K_i) was acquired using Dixon plots by linear fitting of data in Dixon plots.

4.4 Molecular docking

The Sybyl Software (Version7.3) was used for molecular docking studies. The crystal structure of OfHex1-PUGNAc (PDB code: 3OZP) [8] was used as the docking model. Prior to molecular docking, the structures of inhibitors were optimized using the MMFF94 force field. Then, the ligand protomol was created based on the Hammerhead scoring function with the molecular similarity algorithm in the active domain of receptor.[24-25] Finally, molecular docking between the ligands and the receptors were performed using the Surflex-Dock algorithm in Sybyl 7.3.

Conflicts of interest

None.

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Supporting Information

Experimental procedures, ^{1}H NMR and ^{13}C NMR spectrum.

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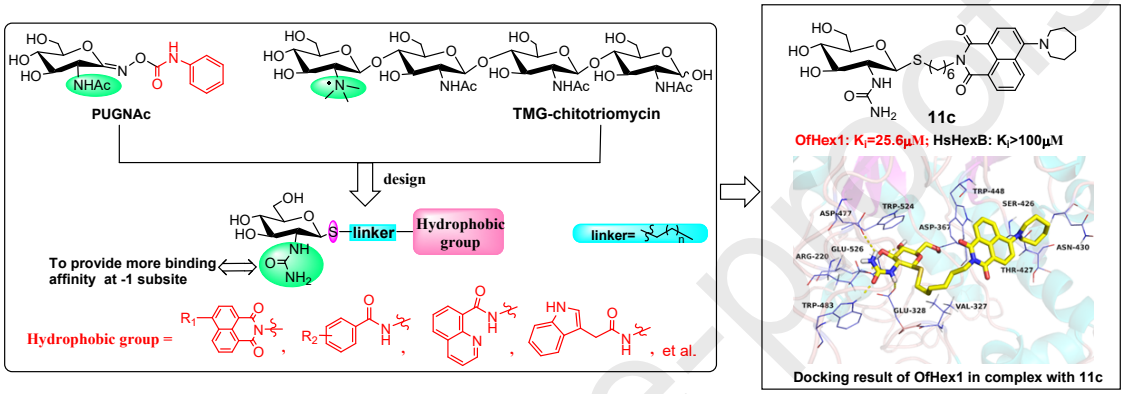

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: