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Molecular targets and their application examples for interrupting chitin biosynthesis

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ABSTRACT

Chitin is an abundant aminopolysaccharide found in insect pests and phytopathogenic microorganisms but absent in higher plants and vertebrates. It is crucial for mitigating threats posed by chitin-containing organisms to human health, food safety, and agriculture. Therefore, targeting the chitin biosynthesis-associated bioprocess holds a promise for developing human-safe and eco-friendly antifungal agents or pesticides. Chitin biosynthesis requires chitin synthase and associated factors, which are involved in the modification, regulation, organization or turnover of chitin during its biosynthesis. A number of enzymes such as chitinases, hexosaminidases, chitin deacetylases are closely related and therefore are promising targets for designing novel agrochemicals that target at chitin biosynthesis. This review summarizes the advances in understanding chitin biology over the past decade by our research group and collaborates, specifically regarding essential proteins linked to chitin biosynthesis that can be exploited as promising pesticide targets. Examples of small bioactive molecules that against the activity of these targets are given.

1. Introduction

Chitin, the most abundant aminopolysaccharide in nature, is a linear polymer of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) and serves as a crucial component in the exoskeletons of insects and crustaceans, the egg shells of nematodes, the sporangia of oomycetes, and the cell walls of fungi and some protozoa [1–3]. Many chitin-containing organisms pose threats to human health, food safety, and agricultural production. However, chitin is generally absent in higher plants and vertebrates such as mammals, amphibians, birds, and reptiles [4]. Therefore, promising prospects lie in the development of human-safe and eco-friendly antifungal drugs and pesticides directed at chitin.

Chitin biosynthesis involves a key enzyme named chitin synthase, which repeatedly transfers a GlcNAc moiety from an activated sugar donor UDP-*N*-acetylglucosamine (UDP-*N*-GlcNAc) to the nonreducing 4–hydroxyl group of a growing chitin chain [5]. In insects and fungi, several cellular enzymes have been identified to interact with chitin synthases, suggesting accessory factors are required for accomplishing chitin biosynthesis. Chitin preparations derived from natural sources, like crab and shrimp shells or insect exuviae, exhibits varying degrees of deacetylation due to enzymatic processes that convert chitin to chitosan by an enzyme named chitin deacetylase (CDA) [6,7]. During insect periodical molting, the degradation of old cuticle chitin is finely tuned and coupled to the synthesis of new cuticle chitin. Chitin biodegradation typically involves three enzymatic steps: The initial step is commonly catalyzed by lytic polysaccharide monooxygenases (LPMO), while the second step is usually catalyzed by two types of enzymes, namely endo-acting non-processive chitinases, and processive chitinases. Ultimately, β-*N*-acetyl-D-hexosaminidase (Hex) hydrolyzes oligosaccharides to GlcNAc [8]. Alongside these pivotal enzymes, various other factors, such as cuticle proteins, hormones, and interacting proteins of enzymes, play influential roles in chitin metabolism [9,10].

In the last decade, there has been significant exploration into multiple facets of chitin metabolism, greatly enhancing our understanding of chitin biology. This review focuses on the substantial contributions made by our team and collaborators with respect to essential proteins associated with chitin biosynthesis and relevance as well as small bioactive molecules developed to against these proteins. Some examples that might be used in controlling pest insects and pathogens are given.

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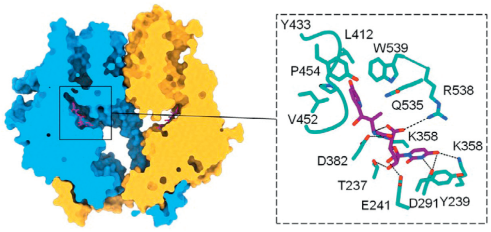

Fig. 1. Inhibition of PsChs1 by nikkomycin-Z. Sliced-surface view (left) of the nikkomycin-Z-binding site and detailed interactions between nikkomycin-Z and PsChs1 (right).

2. Chitin synthases

2.1. Molecular targets for chitin synthase and its auxiliary proteins

2.1.1. Chitin synthases

As early as 2011, Qu *et al.* identified chitin synthases *Of*ChsA [11] and *Of*ChsB [12] at the genetic level, responsible for chitin synthesis in the epidermis and midgut peritrophic membrane, respectively, of the agricultural pest *Ostrinia furnacalis*. *Of*ChsA exhibits two alternative-splicing exons, namely exons 2a and 2b, as well as exons 19a and 19b [13]. Studying chitin synthase on a protein level presents considerable challenges due to its status as a membrane-integrated protein with multiple transmembrane domains. However, Chen *et al.* successfully addressed this issue by analyzing the cryo-electron microscopy structure of *Ps*Chs1 (structures of enzymes and their complexes with inhibitors are listed in the same sequence as presented in Table S1 (Supporting information)) [14], a chitin synthase found in *Phytophthora sojae*. The structural analysis revealed the reaction chamber responsible for governing chitin synthesis, as well as its competitive inhibition by the inhibitor nikkomycin-Z (Fig. 1), providing a structural basis for inhibition of chitin synthesis.

2.1.2. Chitin synthase-interacting proteins

Our studies indicate that chitin synthase requires auxiliary proteins, like chitin synthase-interacting proteins, to fulfill its pivotal role in growth and development. Specifically, the interacting proteins of chitin synthase (krotzkopf verkehrt, kkv) in *Drosophila melanogaster* were identified, including choline transporter-like protein 2 (Ctl2) [15], sarco/endoplasmic reticulum Ca^{2+}-ATPase (Serca) [16] and fatty acid binding protein (Fabp) [17]. Knocking down them individually in the epidermis caused larval and pupal lethality, while in wings, it resulted in smaller, crinkled wings, a significant decrease in chitin deposition, and the loss of chitin lamellar structure.

2.2. Application examples for chitin synthesis

2.2.1. Inhibitors of chitin synthases

Before achieving a significant breakthrough in deciphering the structure of chitin synthase, we have to prompte the exploration of its inhibitors primarily through indirect approaches. Inspired by the diverse biological effects exhibited by many pyridazine derivatives containing oxadiazine heterocycles, as well as the intriguing bioactivity displayed by compounds incorporating hydrophobic and long alkyl chains, Ke *et al.* synthesized a series of novel 4*H*-1,3,4-oxadiazin-5(6*H*)-one derivatives [18]. Compounds **5i** (**1**) (as shown in Fig. 2, inhibitors are numbered according to the order in which they appear in the article) and **5m** (**2**) exhibited significant inhibitory activity on chitin biosynthesis. The oxadiazole heterocycles compound, DOW416, interferes with the formation of insect tissue and epicuticle. Thus, Ke *et al.* synthesized two series of 1,3,4-oxadiazoline heterocycle derivatives [19]. All synthesized compounds, particularly **C1** (**3**) and **C15** (**4**), potently inhibited chitin synthesis in yeast. Motivated by the aforementioned findings, Ke *et al.* subsequently developed a series of diacylhydrazine derivatives possessing hydrophobic alkyl chains [20]. Compounds **6b** (**5**), **6g** (**6**), **6j** (**7**), and **6q** (**8**) exhibited moderate to good inhibitory activity. Utilizing the structure of the chitin synthase donor substrate, UDP-GlcNAc, and the modeled structure of bacterial chitin synthase NodC, Chen *et al.* engineered a series of novel UDP-sugar analogues [21]. These newly synthesized molecules, exemplified by compounds **10** (**9**), **12** (**10**), and **13** (**11**), displayed high inhibitory activities against chitin synthase and demonstrated antifungal effects on plant-parasitic fungi such as *Fusarium graminearum*, *Botrytis cinerea*, and *Cochliobolus lagenarium*. In addition, Zhang *et al.* synthesized a novel series of 1,2,3,4-tetrahydroquinoline derivatives by incorporating active natural product groups into pesticide molecule structures [22]. Notably, compound **4fh** (**12**) demonstrated slightly stronger inhibitory activity towards chitin synthase compared to polyoxin D.

2.2.2. Nanoscale RNAi carriers

The inherent instability of dsRNA and inadequate uptake through oral administration both pose significant hurdles for its practical implementation in insect pest management. Lu *et al.* developed core-shell nanoparticles to encapsulate vulnerable dsRNA payloads using block copolymers composed of poly(ethylene glycol)-polylysine(thiol). While oral delivery of RNAi is challenging in *Locusta migratoria*, our optimized RNAi construct effectively suppressed the expression levels of both *Lm*Chs1 and *Lm*Chs2, inducing discernible phenotypic changes [23].

3. Chitin modification, regulation, and organization

3.1. Molecular targets in chitin modification, regulation, and organization

3.1.1. CDAs

Insect CDAs, belonging to the carbohydrate esterase 4 (CE4) family, are crucial for the modification, reorganization, and synthesis of chitin. Liu *et al.* were the first to report the structural and biochemical features of insect CDAs from *Bombyx mori*: *Bm*CDA1, possibly functioning in cuticle modification, and *Bm*CDA8, which may modify peritrophic membranes in the midgut [24]. Besides *Bm*CDA8, Liu *et al.* also characterized two other anterior midgut-expressed CDAs (*Bm*CDA6 and *Bm*CDA7) from *Bombyx mori* [25]. Of the three enzymes, *Bm*CDA7 and *Bm*CDA8 were observed only at the feeding stage, while *Bm*CDA6, inactive towards PM chitin, was expressed almost exclusively at the mid-molt stage.

Phytopathogenic fungi secrete CDA as a means to evade the host's immunological defense mechanisms during infection. Specifically, Liu *et al.* demonstrated that the deacetylation activity of CDA is crucial for fungal pathogenicity. They determined the crystal structures of two representative phytopathogenic fungal CDAs: *Vd*PDA1 from *Verticillium dahliae* and Pst_13661 from *Puccinia striiformis* f. sp. *tritici* [26].

3.1.2. Hormones

The nuclear receptor-mediated 20-hydroxyecdysone (20E) signaling pathway plays a vital role in insects. Zhao *et al.* identified a cDNA encoding a *Locusta migratoria* hormone receptor 39 (*Lm*HR39) based on transcriptomics data [27]. The transcription of *Lm*HR39 could be induced by 20E *in vivo*. RNAi experiments implied that the *Lm*HR39 is involved in the regulation of chitinase genes. On another note, the elaborate regulation of tissue- and stage-specific expression of chitin synthase is a prerequisite for insect development. Zhang *et al.* report a regulatory mecha-

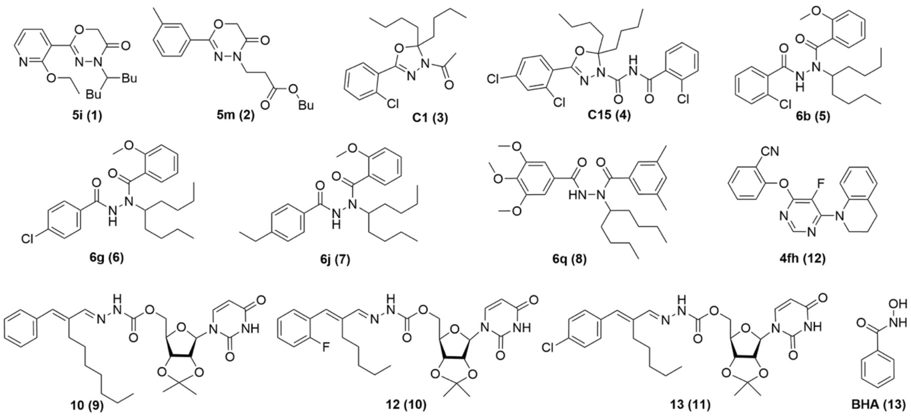

Fig. 2. Chemical structures of chitin synthases inhibitors and CDAs inhibitor. Inhibitors were numbered according to the order in which they appeared in the article.

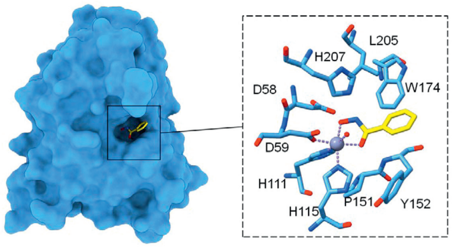

Fig. 3. Inhibition of Pst_13661 by BHA. Surface view of the BHA bound in Pst_13661 (left) and interactions between BHA and Pst_13661 (right).

nism for the pupa-specific expression of ChsA-2b in *Bombyx mori* [28].

3.1.3. Cuticle proteins

Insect cuticle is comprised of a protein matrix and chitin fibers, which exhibit varying degrees of deacetylation. Therefore, specialized proteins are presumed necessary to bind deacetylated chitin chains together. Qu *et al.* were the first to report a chitin-binding protein, *Bm*CPAP3-D1, exhibited the binding activity toward deacetylated chitin [29]. Qi *et al.* found cycloxaprid's bioactivity against lepidopteran pests to be comparatively inferior. They revealed that cuticle proteins appear to be crucial for the insensitivity to cycloxaprid by comparing the transcriptomes of cycloxaprid-treated and untreated *Ostrinia furnacalis* larvae. In line with this conjecture, pretreatment of larvae with methoprene resulted in a 1.12-fold enhancement in cycloxaprid's bioactivity [30]. To comprehend how molecular interactions between cuticle proteins and chitin govern cuticle assembly, Gong *et al.* discovered a newly identified and highly abundant cuticular protein, hypothetical-1 from *Ostrinia furnacalis* (*Of*CPH-1), capable of forming coacervates in the presence of chitosan [31].

3.2. Application examples for chitin modification

The crystal structures of *Vd*PDA1 and Pst_13661 revealed that Zn^{2+}, which is necessary for activity, was coordinated by the Asp-His-His-triad (Fig. 3). Thus, metal ion chelating agents could potentially serve as inhibitors of CDAs. In practice, Liu *et al.* discovered that benzohydroxamic acid (BHA) (**13**) and its three derivatives showed good inhibitory activities toward *Vd*PDA1 and Pst_13661 [26]. BHA demonstrated high activity against diseases in cotton, wheat, and soybean caused by *Verticillium dahliae*, as well as four fungal plant pathogens, namely *Puccinia striiformis* f. sp. *tritici*, *Fusarium oxysporum*, *Fusarium graminearum*, and *Rhizoctonia solani*.

4. Chitin degradation

4.1. Molecular targets in chitin degradation

In order to perform a comprehensive analysis of the chitinolytic enzymes engaged in chitin degradation, Qu *et al.* conducted proteomic assessments on molting fluids of *Bombyx mori*, revealing the prevalence of only four abundant ones in molting fluids. These include two members of the insect glycoside hydrolase family 18 (GH18) endochitinases (group I chitinases and group II chitinases, ChtI and ChtII), a GH18 exochitinase (group h chitinases, Chi-h), which are exclusively found in lepidopterans, and an insect glycoside hydrolase family 20 (GH20) group 1 β-*N*-acetyl-D-hexosaminidase (Hex1). The specific gene expression patterns in tissues and stages provide compelling evidence for the involvement of these four enzymes in cuticular chitin degradation [32]. In exploring factors influencing enzyme processivity within the chitinolytic enzyme system, Qu *et al.* scrutinized an insect chitinase cocktail, comprising *Of*Chi-h, *Of*ChtI, and *Of*ChtII, using high-speed atomic force microscopy [33]. Their findings underscore the significant role of the endochitinase in enhancing the processivity of the exochitinase.

4.1.1. GH20 β-*N*-acetyl-D-hexosaminidases

Prior to the systematic analysis of chitinolytic enzymes, Yang *et al.* isolated and characterized a Hex1 (*Of*Hex1) from *Ostrinia furnacalis*, indicating its involvement in insect chitin catabolism [34]. Subsequently, Liu *et al.* obtained the recombinant *Of*Hex1 [35] and acquired the crystal structure of *Of*Hex1, along with its complex with the substrate analogue *N*,*N*,*N*-trimethyl-D-glucosamine (TMG)-$(GlcNAc)_3$ [36]. The crystal structures of wild-type *Of*Hex1 and the mutant *Of*Hex1(V327G) in complex with the inhibitor PUGNAc highlighted structural variations that influence diverse sensitivities to PUGNAc [37]. Through molecular docking of OfHex1 with inhibitor allosamidin, showcasing the distinct size and shape of *Of*Hex1's pocket compared to human Hex (*Hs*Hex), indicated allosamidin's potential to selectively inhibit *Of*Hex1 [38]. The comprehensive structural and mutational analyses revealed the essential role of Glu328 in conjunction with Trp490 for substrate binding [39,40]. Apart from *Of*Hex1, insects harbor multiple genes encoding other Hexs that serve various physiological functions. Liu *et al.* identified two Hexs, *Of*Hex2 and *Of*Hex3, cloned from *Ostrinia furnacalis* [41]. OfHex2 is presumed to play similar roles as *Hs*HexB in insect [42]. *Of*Hex3 is involved in both molting and fertilization processes [43]. The final one, named FDL (*Of*FDL), contributes significantly to *N*-glycan modification [44].

4.1.2. GH18 chitinases

Among the three abundant chitinases in molting fluids, ChtI (*Of*ChtI) from *Ostrinia furnacali* were firstly expressed, and characterized by Wu *et al.* [45]. Further studies reported the unliganded and oligosaccharide-complexed crystal structures of *Of*ChtI [46]. Successively, Liu *et al.* investigated crystal structures of Chi-h from *Ostrinia furnacalis* (*Of*Chi-h) and its complex with chitoheptaose [47]. Chen *et al.* presented the crystal structures of catalytically active domains of *Of*ChtII [48]. *Of*ChtII was expressed earlier than *Of*ChtI and *Of*Chi-h during the molting transition and was responsible for completing the pretreatment of crystal chitin substrates [49]. Liu *et al.* investigated crystal structures of the group III chitinase (*Of*ChtIII) from *Ostrinia furnacalis* [50]. The gene-expression pattern and subcellular localization of *Of*ChtIII mirrored that of *Of*ChsA, suggesting involvement in the chitin-synthesis pathway. The group IV chitinase of *Ostrinia furnacalis* (*Of*ChtIV) is specifically expressed in the midgut. Liu *et al.* showed that *Of*ChtIV exhibited high stability and mycelial hydrolytic activity in the extreme midgut environment through hyper-*N*-glycosylation [51]. Furthermore, Chen *et al.* presented the first crystal structure of a representative nematode chitinas *Ce*Cht1 from *Caenorhabditis elegans* [52]. These chitinase crystal structures mentioned above provide a solid basis for the development of inhibitors.

4.1.3. LPMOs

LPMOs are abundantly present in various insect species and belong to the auxiliary activities family 15 (AA15) LPMOs (LPMO15). Qu *et al.* selected *Tribolium castaneum* and *Locusta migratoria* to investigate the functions of insect LPMO15 subgroup I-like proteins (LPMO15–1s) [53]. In both species, LPMO15–1-deficient animals were unable to undergo exuviation and exhibited mortality due to impaired turnover of the chitinous cuticle. Qu *et al.* also investigated the function of midgut-specific *Lm*LPMO15–3 during development in *Locusta migratoria* [54]. Knockdown of *Lm*LPMO15–3 at instar nymph stage resulted in lethal phenotypes, characterized by incomplete digestion of the peritrophic matrix.

4.2. Application examples for chitin degradation

4.2.1. Inhibitors of OfHex1

The expression and crystallization of *Of*Hex1 fifteen years ago marked a significant milestone in the study of chitinolytic enzymes. Since then, substantial progress has been made in the study of its inhibitors. Screening and optimizing known inhibitors based on structural insights represent a promising strategy for inhibitor development. TMG-(GlcNAc)$_3$ (**14**) (Fig. 4) is a natural inhibitor of insect Hexs. Our structural analysis indicated that the two GlcNAc residues at the reducing end might be unnecessary. Therefore, Yang *et al.* designed a significantly simplified skeleton molecule, TMG-(GlcNAc)$_2$ (**15**), which exhibits activity equivalent to TMG-(GlcNAc)$_3$ [55]. The symmetrical bis-naphthalimide M-31850 (**16**) was previously obtained by screening for specificity against human Hex (*Hs*Hex). Liu *et al.* designed an unsymmetrical dyad of naphthalimide and thiadiazole, **Q2** (**17**), which shifted naphthalimide specificity from *Hs*Hex to insect and bacterial Hexs [56]. NAG-thiazoline (NGT) (**18**) is a well-established inhibitor against most beta-GlcNAcases. Using co-crystallization, Liu *et al.* designed a compound NMAGT (**19**) with a bulky substituent on the thiazoline ring of NGT, exhibiting a K_i value of 0.13 µmol/L, which is 600-fold lower than the K_i value observed for NGT [57]. Through screening a library of microbial secondary metabolites, phlegmacin B$_1$ (**20**) was identified as an inhibitor of *Of*Hex1 with K_i values of 26 µmol/L. Injection and feeding experiments demonstrated that phlegmacin B$_1$ has an insecticidal effect on *Ostrinia furnacalis* larvae [58]. Inspired by the work above, Duan *et al.* noted that compounds with a large conjugated plane were highly potent inhibitors of GH20 Hex. They first revealed that berberine (**21**), a typical compound with a large conjugated plane, acted as competitive inhibitors of *Of*Hex1 [59].

The synthesis of novel inhibitors through leveraging highly active groups obtained previously presents an efficient approach for inhibitor development. In a study by Yang *et al.*, a series of thiazolylhydrazone derivatives were designed, amalgamating a thiazoline group from NGT and a naphthalimide group from **Q2**. Among these derivatives, **3k** (**22**) exhibited increased potency with a K_i of 10.2 µmol/L. Subsequent optimization led to the discovery of an even more potent inhibitor, derivative **7** (**23**), boasting a K_i value of 2.1 µmol/L [60]. Using a similar approach, Yang *et al.* synthesized a range of **Q2** derivatives. Among these derivatives, compound **3m** (**24**) displayed the most promising inhibitory activity, while compound **6a** (**25**) demonstrated superior activity among the quinoline-containing derivatives [61]. They also simultaneously designed and synthesized two additional series of thiazolylhydrazones. Derivatives **I-3d** (**26**) and **II-3d** (**27**) exhibited remarkable inhibitory activities against *Of*Hex1 [62]. In efforts to enhance the inhibitory efficiency of naphthalimide derivatives, Shen *et al.* undertook rational molecular design and optimization, culminating in the synthesis of compounds **15r** (**28**) and **15y** (**29**). These compounds showcased superior activity and selectivity toward *Of*Hex1 compared to lead compounds [63]. Furthermore, the triazole moiety demonstrates optimal structural attributes for forming hydrogen bonds and π-π stacking interactions with biological targets. Dong *et al.* designed a series of novel glycosyl triazoles. Among these, compound **17c** (**30**) displayed suitable activity and selectivity against *Of*Hex1 [64]. *C*-Glycosides are frequently employed as analogs of native *O*-glycosides. Liang *et al.* synthesized a series of novel C-glycosidic oximino carbamate derivatives. Compound **7k** (**31**) demonstrated the most potent inhibitory activity against *Of*Hex1, in addition to exhibiting remarkable larvicidal activity against *Plutella xylostella* [65].

The utilization of structure-based virtual screening for identifying inhibitors stands as an efficient strategy in inhibitor development. Building upon the crystal structure of *Of*Hex1, a virtual screening of approximately 200,000 small molecules from the SPECS database was conducted. Twenty-eight compounds were chosen for subsequent bioactivity evaluation, and compound **3** (**32**) exhibited potent inhibitory activity against *Of*Hex1 [66]. In a parallel study, Dong *et al.* carried out sequential virtual screenings of the ZINC library, housing 8 million compounds. They ultimately selecteed 15 molecules for further enzymatic assays. Among these, compound **5** (**33**) displayed promising inhibitory activity against *Of*Hex1, demonstrating significant selectivity [67]. More recently, a comprehensive approach utilized the SPECS and Maybridge databases, alongside our in-house library of 2393 molecules, for structure-based virtual screening. After simplification of structures, compounds featuring a biphenyl–sulfonamide skeleton emerged as potential *Of*Hex1 inhibitors. Compounds **10k** (**34**), **10u** (**35**), and **10v** (**36**) exhibited K_i values comparable to the most active nonglycosyl-based inhibitor, **Q2** [68]. What distinguishes these newly discovered compound skeletons are their simple chemical structures, ease of synthesis, and potent activity.

4.2.2. Inhibitors of other Hexs

In addition to *Of*Hex1, we have utilized the aforementioned approaches to investigate inhibitors targeting Hex across various subgroups, species, or GH families. Guo *et al.* synthesized a novel and efficient skeleton comprising naphthalimide and methoxyphenyl moieties. The most potent inhibitor, compound **7a** (**37**), exhibits high inhibitory activity with K_i values of 0.63 µmol/L against HsHex [69]. Similarly, a series of naphthalimide-scaffold were designed based on molecular modeling analysis of *Of*Hex2. The most potent inhibitor, compound **20** (**38**), displayed a K_i value of 0.37 µmol/L

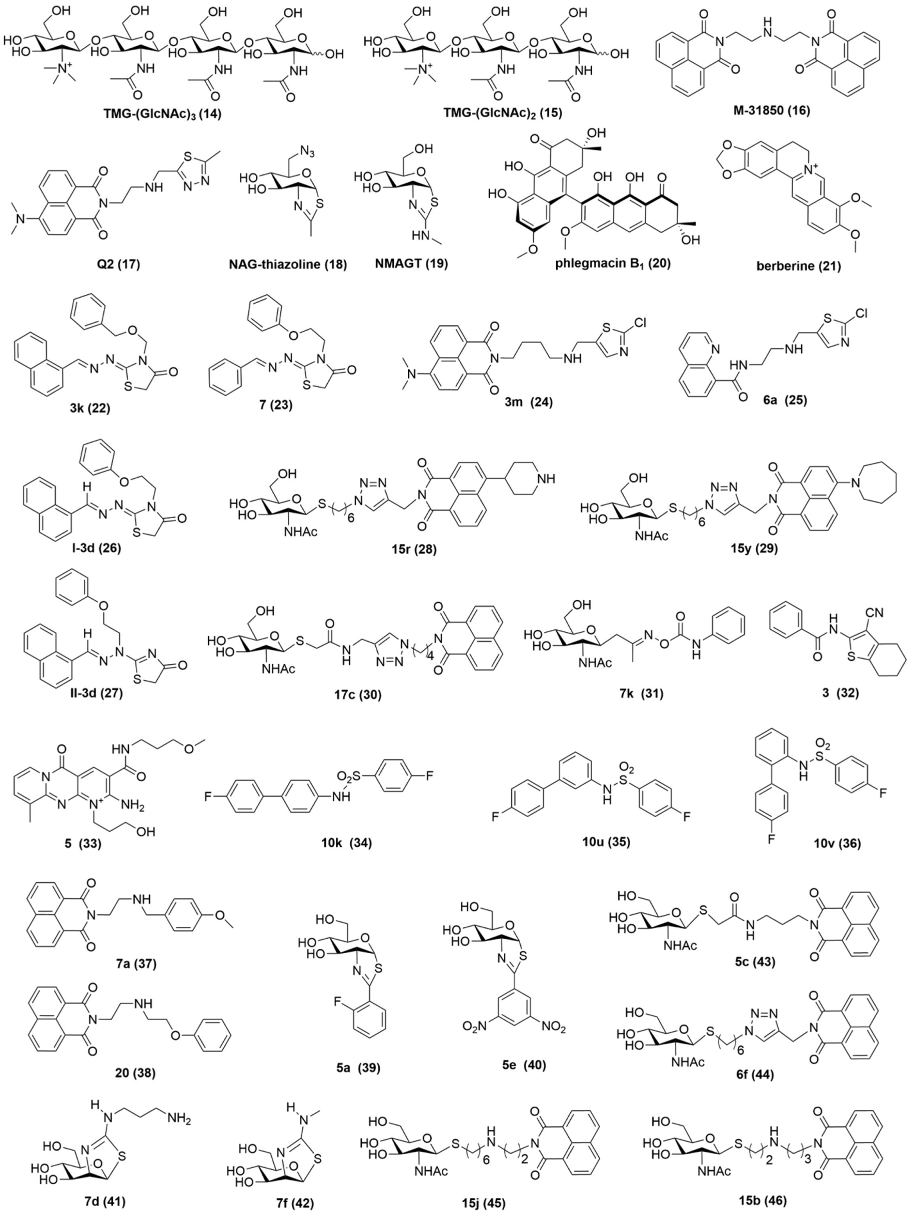

Fig. 4. Chemical structures of Hex inhibitors. Inhibitors were numbered according to the order in which they appeared in the article.

[70]. The GH84 β-*N*-acetyl-D-hexosaminidase (OGA) is responsible for removing GlcNAc from serine or threonine residues of glycoproteins. Kong *et al*. generated a total of 24 new NGT derivatives across two separate batches. Among the compounds screened, compounds **5a** (**39**) (The half maximal inhibitory concentration (IC$_{50}$) = 12.6 μmol/L, hOGA) and **5e** (**40**) (IC$_{50}$ = 12.5 μmol/L, *Of*OGA) [71], as well as compounds **7d** (**41**) (IC$_{50}$ = 6.4 μmol/L, hOGA) and **7f** (**42**) (IC$_{50}$ = 11.9 μmol/L, hOGA) [72], demonstrated their high potency as inhibitors. Moreover, Chen *et al*., using the complex structure of hOGA-PUGNAc as a basis, synthesized a series of novel thioglycosyl–naphthalimide hybrid inhibitors. Notably, compounds **5c** (**43**) (hOGA, K_i = 3.46 μmol/L; *Of*Hex1, K_i > 200 μmol/L) and **6f** (**44**) (hOGA, K_i > 200 μmol/L; *Of*Hex1, K_i = 21.81 μmol/L) exhibited high selectivity [73]. Additionally, different linkers were introduced into the naphthalimide-bearing thioglycoside derivatives to enhance interactions. The most potent compounds were **15j** (**45**) (K_i = 0.91 μmol/L, *Hs*HexB; K_i > 100 μmol/L, hOGA) and **15b** (**46**) (K_i = 3.76 μmol/L, hOGA; K_i = 30.42 μmol/L, *Hs*HexB), which displayed significant selectivity between these two enzymes [74].

4.2.3. *Inhibitors of* Of*ChtI*

Based on the catalytic mechanism, Chen *et al*. discovered that the inhibitory effects of fully deacetylated chitooligosaccharides (GlcN)$_{2-7}$ (**47**) (Fig. 5) against *Of*ChtI are dependent on the degree of polymerization. The injection of mixed (GlcN)$_{2-7}$ into fifth instar larvae of *Ostrinia furnacalis* resulted in 85% larval arrest and subsequent death within 10 days [75]. Utilizing virtual screening from the ZINC database, Jiang *et al*. developed two chemical series of chitinase inhibitors, **FQ** (**48**), displaying specific inhibition against *Of*ChtI, and **TP** (**49**), exhibiting broad inhibitory activity against chitinases derived from insects, humans, fungi, and bacteria [76]. Revolving around the identical concept, Dong *et al*. found a chemical fragment and five variant compounds as inhibitors of *Of*ChtI from SPECS chemical database. Compound **3** (**50**) showed preferential inhibitory activity with a K_i value of 1.5 μmol/L against *Of*ChtI [77]. It possessed a large modification space at 6-position of compound 3 based on the predicted binding mode. Its analogue compound **8** (**51**) with 6–*tert*-pentyl showed preferential inhibitory activity with a K_i value of 0.71 μmol/L [78]. Expanding

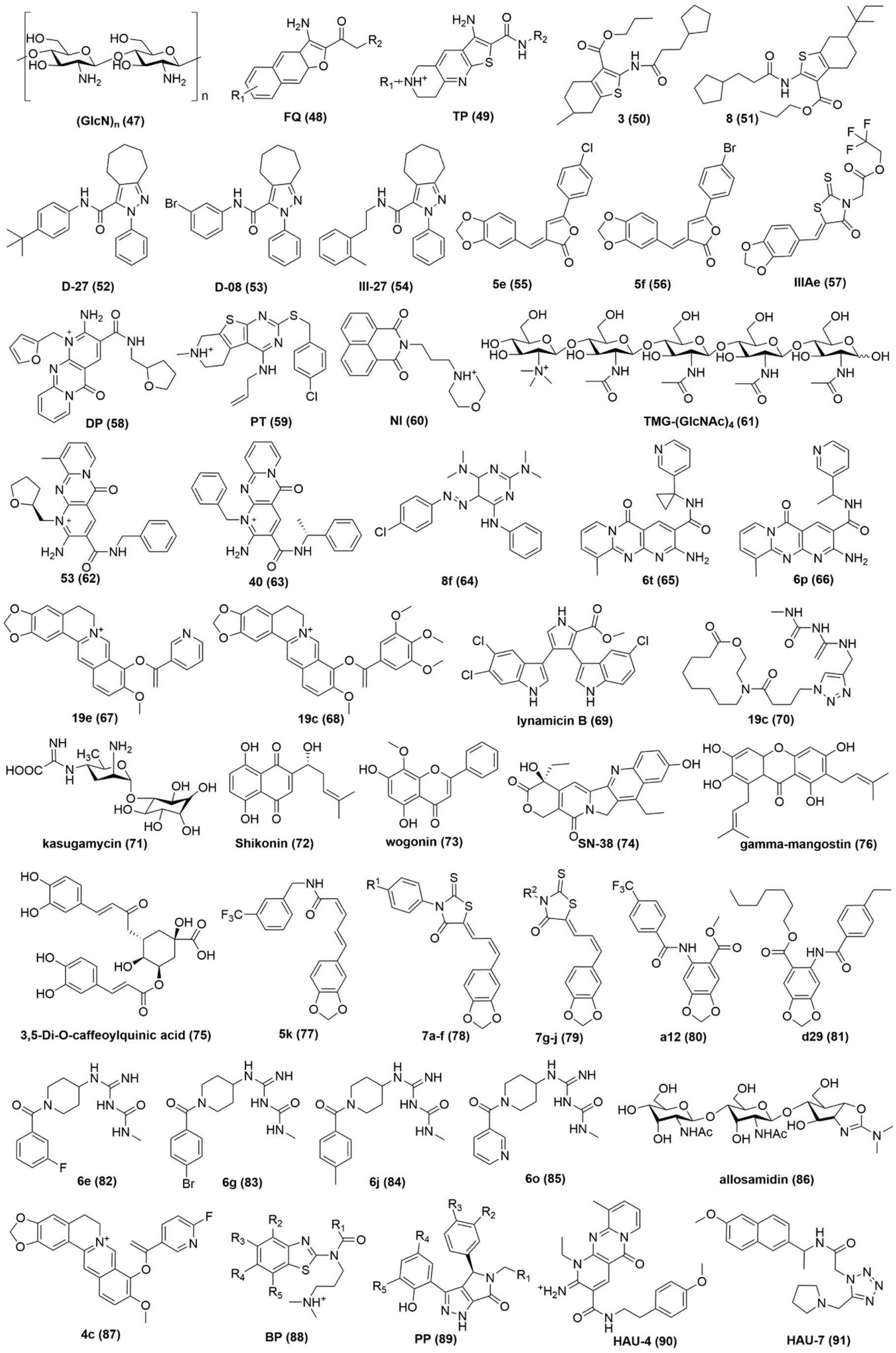

Fig. 5. Chemical structures of chitinase inhibitors. Inhibitors were numbered according to the order in which they appeared in the article.

on the active lead compound **6i**, Jiang *et al.* synthesized a series of novel heptacyclic pyrazolamide derivatives. Notably, compound **D-27** (**52**) exhibited promising activities against *Plutella xylostella* and *Mythimna separata* [79]. Additionally, compound **D-08** (**53**) induced aberrant molting in *Plutella xylostell*, while its derivative **III-27** (**54**) exhibited superior activity against *Plutella xylostella* [80]. Han *et al.* initially discovered that piperine, a natural product, exhibited partial inhibition of *Of*ChtI. Subsequently, they synthesized compounds **5a–f** by incorporating a butenolide scaffold into piperine. Compounds **5e** (**55**) and **5f** (**56**) demonstrated moderate insecticidal activity against *Ostrinia furnacalis* and displayed approximately 80-fold higher inhibitory activity against *Of*ChtI compared to piperine [81]. Recently, Han *et al.* designed a series of piperonyl-rhodanine derivatives. The optimized compound **IIIAe** (**57**) exhibited significant inhibitory activity against *Of*ChtI ($K_i = 2.4\,\mu$mol/L) and demonstrated potent insecticidal efficacy with a mortality rate of 63.33% [82].

4.2.4. *Inhibitors of* Of*ChtI*

The exploration of small molecules as inhibitors of ChtII had been limited until our report on two constructs of *Of*ChtII. Subsequently, Chen *et al.* conducted a screening of the compound library in our laboratory and identified four potent inhibitors: (GlcN)$_8$ (**47**), dipyrido-pyrimidine derivative (**DP**) (**58**), piperidine-thienopyridine derivative (**PT**) (**59**), and naphthalimide derivative (**NI**) (**60**). Injection of the inhibitors into 4th instar *Ostrinia fur-*

nacalis larvae resulted in defects in development and pupation [83].

4.2.5. Inhibitors of OfChi-h

When Chen *et al.* first investigated *Of*Chi-h and obtained its crystal structure, they simultaneously discovered that *Of*Chi-h was inhibited by TMG-(GlcNAc)$_4$ (**61**), an inhibitor of *Of*Hex1 [47]. Similarlly, Chen *et al.* identified phlegmacin B$_1$ [58] and two thiazolylhydrazones derivatives **I-3d** and **II-3d** [62], which demonstrated inhibitory activities against *Of*Hex1, as also effective inhibitors of *Of*Chi-h. Through hierarchical virtual screening of the ZINC database, Jiang *et al.* isolated dipyrido-pyrimidine derivatives compound **53** (**62**) as an insect-selective *Of*Chi-h inhibitor with a K_i value of 9 nmol/L, and compound **40** (**63**) as a novel, human Chit1-selective inhibitor with a K_i value of 49 nmol/L [84]. Subsequently, Dong *et al.* designed a series of azo-aminopyrimidine derivatives. Among these derivatives, compound **8f** (**64**) stood out as the most potent with a K_i value of 64.7 nmol/L. Notably, this compound exhibited superior insecticidal activity when compared to the control pesticide hexaflumuron [85].

With a meticulous analysis of existing inhibitor-chitinase interactions, Yuan *et al.* proposed that planar polycyclic compounds might establish hydrophobic stacking interactions with aromatic residues, mimicking the GlcNAc unit's stacking interactions to inhibit chitinase activity. They constructed a series of dipyridopyrimidine-3-carboxamide derivatives. Among these derivatives, compound **6t** (**65**) exhibited the most potent activity against *Of*Chi-h, with K_i values of 5.6 nmol/L. The strong stacking interaction of compound **6p** (**66**) with conserved residues found in the co-crystal structure validated the feasibility of their design [86]. Following a similar principle, Zhu *et al.* identified berberine as a moderate inhibitor of *Of*Chi-h with a K_i of 16.1 µmol/L. Their strategy to enhance its efficacy involved introducing aromatic and heterocyclic rings to the intact berberine framework. As a result, compound **19e** (**67**) emerged as a potent *Of*Chi-h inhibitor with a K_i of 0.093 µmol/L. Treatment with structurally stable compound **19c** (**68**) hindered the growth and metamorphosis of *Ostrinia furnacalis* larvae [87]. Lu *et al.* discovered lynamicin B (**69**), a natural product, as a selective inhibitor of *Of*Chi-h with a K_i value of 8.76 µmol/L. Lynamicin B demonstrated substantial insecticidal activity against various lepidopteran pests while exerting minimal effects on the ahymenopteran natural enemies of these pests [88]. Another natural product, argifin, exhibited potent inhibitory activity against chitinase. To address the challenge of its limited synthetic accessibility, Zhao *et al.* devised a strategy involving the replacement of the cyclic peptide backbone skeleton found in 12- and 16-azamacrolides. Compounds **19c** (**70**) emerged as the most potent, displaying IC$_{50}$ values of 56 nmol/L against *Of*Chi-h and inducing a 76% mortality rate for *Plutella xylostella* at a concentration of 50 mg/L [89].

4.2.6. Inhibitors of multiple chitinases

In recent years, the multitarget approach has gained traction in drug development. Qi *et al.* discovered in their initial screening of a natural products library that kasugamycin (**71**) inhibits *Of*ChtI with a K_i value of 0.47 µmol/L and *Of*Chi-h with a K_i value of 2.7 µmol/L [90]. On the basis of the same multitarget strategy, Li *et al.* conducted a high-throughput screen of the TargetMol library (1680 natural products) for inhibitors targeting *Of*ChtI, *Of*ChtII, *Of*Chi-h, and *Of*Hex1. Shikonin (**72**) and wogonin (**73**) emerged as potent inhibitors against all four enzymes, displaying significant insecticidal effects on lepidopteran agricultural pests. Additionally, 10-hydroxycamptothecin (10-HCPT) exhibited inhibitory activity against all four enzymes [91]. Later, camptothecin and its derivatives, such as **SN-38** (**74**), were identified as competitive inhibitors of *Of*ChtII and *Of*Chi-h with micromolar K_i values. Camptothecin also showed substantial insecticidal activity against *Locusta migratoria* [92]. Taking advantage of above data, Ding *et al.* devised a strategy that integrates machine learning to sift through a large natural product library from Topscience (17,600 compounds) in search of novel multitarget inhibitors. They identified 3,5-di-*O*-caffeoylquinic acid (**75**) and γ-mangostin (**76**) as inhibitors for all four enzymes, with K_i values at the µmol/L level. These compounds exhibited notable biological activities against lepidopteran pests [93]. Jiang *et al.* designed 19 novel piperine derivatives based on the interaction modes between piperine and *Of*ChtI, *Of*ChtII, and *Of*Chi-h. Among these derivatives, compound **5k** (**77**) ($K_i = 11.78$–22.82 µmol/L) was identified as the most potent multichitinase inhibitor and demonstrated superior insecticidal activity against *Ostrinia furnacalis* compared to dual- or single-chitinase inhibitors [94]. Han *et al.* synthesized a series of piperonyl-tethered rhodanine derivatives. In comparison to piperine, compounds **7a–f** (**78**) and **7g–j** (**79**) exhibited approximately 100- to 400-fold or 110- to 210-fold higher inhibitory capacity against *Of*ChtI and *Of*Chi-h, respectively. Furthermore, compounds **7a–c** demonstrated significant inhibition of the growth and development of *Ostrinia furnacalis* larvae [95]. Building upon **a12** (**80**), Jin *et al.* rationally designed a series of benzo[*d*][1,3]dioxole-6-benzamide derivatives. Among them, compound **d29** (**81**) acted simultaneously on *Of*ChtI, *Of*ChtII, and *Of*Chi-h with K_i values of 0.8, 11.9, and 2.3 µmol/L, and exhibited significant activity against two lepidopteran pests [96]. To increase the ability of the target compounds to penetrate the insect cuticle *in vivo* by increasing their lipophilicity, Zhao *et al.* synthesized a series of potent insecticides targeting *Of*ChtI and *Of*Chi-h using lipophilic piperidine as a bridge connecting the planar structures and the *N*-methylcarbamoylguanidino. Compounds **6e** (**82**), **6g** (**83**), **6j** (**84**), and **6o** (**85**) significantly affected the growth and development of *Plutella xylostell* [97].

4.2.7. Inhibitors of other chitinases

In addition to the previously mentioned chitinolytic enzymes derived from insect molting fluids, Liu *et al.* employed these approaches to investigate inhibitors targeting chitinases across different subgroups and species. Following our initial investigation of *Of*ChtIV, Liu *et al.* concurrently discovered that *Of*ChtIV exhibited inhibition by allosamidin (**86**) with a K_i value of 1.35 µmol/L [51]. Chen *et al.* synthesized a variety of berberine analogues using a hydrophobic cavity-based optimization strategy. The compound **4c** (**87**) showed an 80-fold elevated inhibitory activity against *Sm*ChiB and the human hAMCas [98]. Through hierarchical virtual screening from the ZINC database, two series of novel inhibitors against *Ce*Cht1 were successfully identified. These inhibitors featured a **BP** (**88**) scaffold [52] and a **PP** (**89**) scaffold [99]. Meanwhile, compounds **HAU-4** (**90**) and **HAU-7** (**91**) were also identified as *Ce*Cht1 inhibitors with IC$_{50}$ values of 4.2 µmol/L and 10.0 µmol/L, respectively [100]. Recently, Jin *et al.* conducted a virtual screening of a library comprising over 16,000 natural products and successfully identified lunidonine as an inhibitor of *Ce*Cht1. Subsequently, Jin *et al.* employed a pocket-based lead optimization strategy, and new compound **a12** (**80**) exhibited a strong interaction with *Ce*Cht1 and demonstrated remarkable *in vitro* nematicidal activity against *Caenorhabditis elegans* [101].

5. Conclusion and future work

Researchers have long recognized the potential of chitin biosynthesis as a target for insecticides and have maintained a dedicated focus in this area [102–104]. Correspondingly, investigations into chitin biosynthesis primarily center around insects and fungi [6,105-107]. A comprehensive book, published in 2019, delved into various aspects of chitin biology, with a specific focus on chitin remodeling enzymes and inhibitors [108]. As supplements and updates, our research advances in chitin biosynthesis have highlighted chitin-biosynthesis relevant targets for developing highly selective pesticides and anti-pathogen agents [110,111]. A variety of lead compounds with high inhibitory abilities have been reported, paving a path for a new era of agrochemicals to interrupt chitin biosynthesis [7,109-114]. The future research priorities may encompass: (1) Further in-depth biological investigations into co-factors that assist the cellular regulation and transportation of chitin synthases; (2) structural investigation into the mechanism of the co-factors; and (3) chemical modification and optimization of lead compounds to improve their biological activities.

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.

CRediT authorship contribution statement

Yanwei Duan: Writing – original draft, Methodology. **Qing Yang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2024.109905.

References

[1] W. Cheng, M. Lin, M. Qiu, et al., Environ. Microbiol. 21 (2019) 4537–4547.
[2] B. Moussian, Chitin: structure, chemistry and biology, in: Q. Yang, T. Fukamizo (Eds.), Targeting Chitin-containing Organisms, Springer, Singapore, 2019, pp. 5–18.
[3] A. Schwelm, J. Fogelqvist, A. Knaust, et al., Sci. Rep. 5 (2015) 11153.
[4] E. Cohen, Chitin biochemistry: synthesis, hydrolysis and inhibition, in: J. Casas, S. Simpson (Eds.), Advances in Insect Physiology, Academic Press, San Diego, 2010, pp. 5–74.
[5] H. Merzendorfer, J. Comp. Physiol. B 176 (2006) 1–15.
[6] K. Zhu, H. Merzendorfer, W. Zhang, J. Zhang, S. Muthukrishnan, Annu. Rev. Entomol. 61 (2016) 177–196.
[7] Y. Li, L. Liu, J. Yang, Q. Yang, J. Pestic. Sci. 46 (2021) 43–52.
[8] G. Courtade, F.L. Aachmann, Chitin-active lytic polysaccharide monooxygenases, in: Q. Yang, T. Fukamizo (Eds.), Targeting Chitin-containing Organisms, Springer, Singapore, 2019, pp. 115–129.
[9] B. Moussian, Insect Biochem. Mol. Biol. 40 (2010) 363–375.
[10] H. Merzendorfer, Eur. J. Cell. Biol. 90 (2011) 759–769.
[11] M. Qu, Q. Yang, Insect Biochem. Mol. Biol. 41 (2011) 923–931.
[12] M. Qu, T. Liu, J. Yang, Q. Yang, Biochem. Biophys. Res. Commun. 404 (2011) 302–307.
[13] M. Qu, Q. Yang, Insect. Mol. Biol. 21 (2012) 395–404.
[14] W. Chen, P. Cao, Y. Liu, et al., Nature 610 (2022) 402–408.
[15] Y. Duan, W. Zhu, X. Zhao, et al., Insect Biochem. Mol. Biol. 141 (2022) 103718.
[16] W. Zhu, Y. Duan, J. Chen, et al., Insect Biochem. Mol. Biol. 145 (2022) 103783.
[17] J. Chen, X. Zou, W. Zhu, et al., Insect Biochem. Mol. Biol. 149 (2022) 103845.
[18] S. Ke, X. Qian, F. Liu, et al., Eur. J. Med. Chem. 44 (2009) 2113–2121.
[19] S. Ke, F. Liu, N. Wang, Q. Yang, X. Qian, Bioorg. Med. Chem. Lett. 19 (2009) 332–335.
[20] S. Ke, X. Qian, F. Liu, et al., Eur. J. Med. Chem. 44 (2009) 2985–2993.
[21] Q. Chen, J. Zhang, L. Chen, et al., Chin. Chem. Lett. 28 (2017) 1232–1237.
[22] X. Zhang, Z. Yang, H. Xu, et al., J. Agric. Food Chem. 70 (2022) 9262–9275.
[23] Q. Lu, H. Cui, W. Li, et al., J. Agric. Food Chem. 70 (2022) 10762–10770.
[24] L. Liu, Y. Zhou, M. Qu, et al., J. Biol. Chem. 294 (2019) 5774–5783.
[25] L. Liu, M. Qu, T. Liu, et al., J. Insect. Physiol. 113 (2019) 42–48.
[26] L. Liu, Y. Xia, Y. Li, et al., Nat. Commun. 14 (2023) 3857.
[27] X. Zhao, Z. Qin, J. Zhang, et al., Insect Mol. Biol. 28 (2019) 537–549.
[28] J. Zhang, G. Xu, B. Qiu, et al., Insect Biochem. Mol. Biol. 116 (2020) 103264.
[29] M. Qu, Y. Ren, Y. Liu, Q. Yang, Insect Mol. Biol. 26 (2017) 432–439.
[30] H. Qi, T. Liu, Q. Lu, Q. Yang, J. Agric. Food Chem. 68 (2020) 982–988.
[31] Q. Gong, L. Chen, J. Wang, et al., Biomacromolecules 23 (2022) 2562–2571.
[32] M. Qu, L. Ma, P. Chen, Q. Yang, J. Proteome Res. 13 (2014) 2931–2940.
[33] M. Qu, T. Watanabe-Nakayama, S. Sun, et al., ACS Catal. 10 (2020) 13606–13615.
[34] Q. Yang, T. Liu, F. Liu, M. Qu, X. Qian, FEBS J 275 (2008) 5690–5702.
[35] T. Liu, F. Liu, Q. Yang, J. Yang, Protein Expr. Purif. 68 (2009) 99–103.
[36] T. Liu, H. Zhang, F. Liu, et al., J. Biol. Chem. 286 (2011) 4049–4058.
[37] T. Liu, H. Zhang, F. Liu, et al., Biochem. J. 438 (2011) 467–474.
[38] Y. Wang, T. Liu, Q. Yang, Z. Li, X. Qian, Chem. Biol. Drug Des. 79 (2012) 572–582.
[39] T. Liu, Y. Zhou, L. Chen, et al., PLoS One 7 (2012) e52225.
[40] T. Liu, Q. Wu, L. Liu, Q. Yang, Process. Biochem. 48 (2013) 103–108.
[41] T. Liu, M. Qu, Q. Yang, J. Yang, X. Qian, J. Biotechnol. 136 (2008) S109.
[42] F. Liu, T. Liu, M. Qu, Q. Yang, Int. J. Biol. Sci. 8 (2012) 1085–1096.
[43] M. Qu, T. Liu, P. Chen, Q. Yang, PLoS One 8 (2013) e71738.
[44] Y. Huo, L. Chen, M. Qu, Q. Chen, Q. Yang, Arch. Insect Biochem. Physiol. 83 (2013) 115–126.
[45] Q. Wu, T. Liu, Q. Yang, Insect Sci. 20 (2013) 147–157.
[46] L. Chen, T. Liu, Y. Zhou, et al., Acta Crystallogr. D: Biol. Crystallogr. 70 (2014) 932–942.
[47] T. Liu, L. Chen, Y. Zhou, et al., J. Biol. Chem. 292 (2017) 2080–2088.
[48] W. Chen, M. Qu, Y. Zhou, Q. Yang, J. Biol. Chem. 293 (2018) 2652–2660.
[49] M. Qu, S. Sun, Y. Liu, et al., Insect Sci. 28 (2021) 692–704.
[50] T. Liu, W. Zhu, J. Wang, et al., Acta Crystallogr. D: Struct. Biol. 74 (2018) 30–40.
[51] T. Liu, X. Guo, Y. Bu, et al., Insect Biochem. Mol. Biol. 119 (2020) 103326.
[52] Q. Chen, W. Chen, A. Kumar, et al., J. Agric. Food Chem. 69 (2021) 3519–3526.
[53] M. Qu, X. Guo, S. Tian, et al., Commun. Biol. 5 (2022) 518.
[54] M. Qu, X. Guo, L. Kong, L. Hou, Q. Yang, Insect Sci. 29 (2022) 1287–1298.
[55] Y. Yang, T. Liu, Y. Yang, et al., ChemBioChem 12 (2011) 457–467.
[56] T. Liu, P. Guo, Y. Zhou, et al., Sci. Rep. 4 (2014) 6188.
[57] T. Liu, M. Xia, H. Zhang, et al., FEBS Lett. 589 (2015) 110–116.
[58] L. Chen, T. Liu, Y. Duan, X. Lu, Q. Yang, J. Agric. Food Chem. 65 (2017) 3851–3857.
[59] Y. Duan, T. Liu, Y. Zhou, T. Dou, Q. Yang, J. Biol. Chem. 293 (2018) 15429–15438.
[60] H. Yang, T. Liu, H. Qi, et al., Bioorg. Med. Chem. 26 (2018) 5420–5426.
[61] H. Yang, H. Qi, T. Liu, et al., Chin. Chem. Lett. 30 (2019) 977–980.
[62] H. Yang, H. Qi, Z. Hao, et al., Chin. Chem. Lett. 31 (2020) 1271–1275.
[63] S. Shen, L. Dong, W. Chen, et al., J. Agric. Food Chem. 67 (2019) 6387–6396.
[64] L. Dong, S. Shen, W. Chen, et al., Bioorg. Med. Chem. 27 (2019) 2315–2322.
[65] P. Liang, Q. Xu, R. Chen, et al., Carbohydr. Res. 520 (2022) 108629.
[66] Y. Dong, S. Hu, X. Zhao, et al., Pest Manag. Sci. 76 (2020) 3030–3037.
[67] L. Dong, S. Shen, Y. Xu, et al., J. Biomol. Struct. Dyn. 39 (2021) 1735–1743.
[68] T. Chen, W. Li, Z. Liu, et al., J. Agric. Food Chem. 69 (2021) 12039–12047.
[69] P. Guo, Q. Chen, T. Liu, et al., ACS Med. Chem. Lett. 4 (2013) 527–531.
[70] Q. Chen, P. Guo, L. Xu, et al., Biochimie 97 (2014) 152–162.
[71] H. Kong, W. Chen, H. Lu, et al., Carbohydr. Res. 413 (2015) 135–144.
[72] H. Kong, W. Chen, T. Liu, et al., Carbohydr. Res. 429 (2016) 54–61.
[73] W. Chen, S. Shen, L. Dong, J. Zhang, Q. Yang, Bioorg. Med. Chem. 26 (2018) 394–400.
[74] S. Shen, W. Chen, L. Dong, et al., J. Enzyme Inhib. Med. Chem. 33 (2018) 445–452.
[75] L. Chen, Y. Zhou, M. Qu, Y. Zhao, Q. Yang, J. Biol. Chem. 289 (2014) 17932–17940.
[76] X. Jiang, A. Kumar, T. Liu, K. Zhang, Q. Yang, J. Chem. Inf. Model. 56 (2016) 2413–2420.
[77] Y. Dong, X. Jiang, T. Liu, et al., J. Agric. Food Chem. 66 (2018) 3351–3357.
[78] Y. Dong, S. Hu, X. Jiang, et al., J. Agric. Food Chem. 67 (2019) 3575–3582.
[79] B. Jiang, X. Jin, Y. Dong, et al., J. Agric. Food Chem. 68 (2020) 6347–6354.
[80] B. Jiang, B. Guo, J. Cui, et al., Bioorg. Med. Chem. Lett. 30 (2020) 127500.
[81] Q. Han, N. Wu, H. Li, et al., J. Agric. Food Chem. 69 (2021) 7534–7544.
[82] Q. Han, N. Wu, J. Zhang, et al., J. Agric. Food Chem. 71 (2023) 18685–18695.
[83] W. Chen, Y. Zhou, Q. Yang, J. Biol. Chem. 294 (2019) 9358–9364.
[84] X. Jiang, A. Kumar, Y. Motomura, et al., J. Med. Chem. 63 (2020) 987–1001.
[85] L. Dong, S. Shen, X. Jiang, et al., J. Agric. Food Chem. 70 (2022) 12203–12210.
[86] P. Yuan, X. Jiang, S. Wang, et al., J. Agric. Food Chem. 68 (2020) 13584–13593.
[87] L. Zhu, L. Chen, X. Shao, et al., J. Agric. Food Chem. 69 (2021) 7526–7533.
[88] Q. Lu, L. Xu, L. Liu, et al., J. Agric. Food Chem. 69 (2021) 14086–14091.
[89] Z. Zhao, Q. Xu, W. Chen, et al., J. Agric. Food Chem. 70 (2022) 4889–4898.
[90] H. Qi, X. Jiang, Y. Ding, T. Liu, Q. Yang, Front Mol. Biosci. 8 (2021) 640356.
[91] W. Li, Y. Ding, H. Qi, T. Liu, Q. Yang, J. Agric. Food Chem. 69 (2021) 10830–10837.
[92] Y. Ding, S. Chen, F. Zhang, et al., J. Agric. Food Chem. 71 (2023) 1845–1851.
[93] Y. Ding, S. Chen, H. Liu, T. Liu, Q. Yang, J. Agric. Food Chem. 71 (2023) 8769–8777.
[94] Z. Jiang, D. Shi, H. Li, et al., J. Agric. Food Chem. 70 (2022) 10326–10336.
[95] Q. Han, N. Wu, Y. Liu, et al., J. Agric. Food Chem. 70 (2022) 7387–7399.
[96] X. Jin, T. Sun, B. Guo, et al., J. Agric. Food Chem. 71 (2023) 8345–8355.
[97] Z. Zhao, W. Chen, Y. Dong, et al., J. Agric. Food Chem. 71 (2023) 12431–12439.

[98] L. Chen, L. Zhu, J. Chen, et al., J. Enzyme Inhib. Med. Chem. 35 (2020) 1937–1943.
[99] W. Chen, Q. Chen, A. Kumar, et al., J. Enzyme Inhib. Med. Chem. 36 (2021) 1198–1204.
[100] S. Shen, B. Ding, X. Jiang, et al., Front Chem. 10 (2022) 1021295.
[101] X. Jin, T. Sun, X. Zhang, et al., J. Agric. Food Chem. 71 (2023) 244–254.
[102] E. Cohen, Pest Manag. Sci. 57 (2001) 946–950.
[103] F. Matsumura, Pestic. Biochem. Phys. 97 (2010) 133–139.
[104] H. Merzendorfer, Insect Sci. 20 (2013) 121–138.
[105] H. Merzendorfer, L. Zimoch, J. Exp. Biol. 206 (2003) 4393–4412.
[106] S. Muthukrishnan, H. Merzendorfer, Y. Arakane, K.J. Kramer, Chitin metabolism in insects, in: L. Gilbert (Ed.), Insect Molecular Biology and Biochemistry, Academic Press, San Diego, 2012, pp. 193–235.
[107] S. Muthukrishnan, H. Merzendorfer, Y. Arakane, Q. Yang, Chitin metabolic pathways in insects and their regulation, in: E. Cohen, B. Moussian (Eds.), Extracellular Composite Matrices in Arthropods, Springer, Cham, 2016, pp. 31–65.
[108] Q. Yang, T. Fukamizo, Targeting Chitin-containing Organisms, 1st. ed, Springer, Singapore, 2019.
[109] W. Chen, X. Jiang, Q. Yang, Biotechnol. Adv. 43 (2020) 107553.
[110] A. Yu, M. Beck, H. Merzendorfer, Q. Yang, Insect Biochem. Mol. Biol. 164 (2023) 104058.
[111] T. Liu, Y. Duan, Q. Yang, Biotechnol. Adv. 36 (2018) 1127–1138.
[112] W. Chen, Q. Yang, J. Agric. Food Chem. 68 (2020) 4559–4565.
[113] Y. Ding, Q. Lu, T. Liu, Q. Yang, Adv. Agrochem. 2 (2023) 306–312.
[114] Q. Lu, H. Xie, M. Qu, T. Liu, Q. Yang, J. Agric. Food Chem. 71 (2023) 5944–5952.