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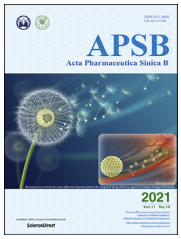

REVIEW

Recent developments in the medicinal chemistry of single boron atom-containing compounds

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KEYWORDS

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Covalent inhibitors;
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Linker components

Abstract Various boron-containing drugs have been approved for clinical use over the past two decades, and more are currently in clinical trials. The increasing interest in boron-containing compounds is due to their unique binding properties to biological targets; for example, boron substitution can be used to modulate biological activity, pharmacokinetic properties, and drug resistance. In this perspective, we aim to comprehensively review the current status of boron compounds in drug discovery, focusing especially on progress from 2015 to December 2020. We classify these compounds into groups showing anticancer, antibacterial, antiviral, antiparasitic and other activities, and discuss the biological targets associated with each activity, as well as potential future developments.

Abbreviations: ACTs, artemisinin combination therapies; ADCs, *Acinetobacter*-derived cephalosporinases; AML, acute myeloid leukemia; AMT, aminopterin; BLs, β-lactamases; BNCT, boron neutron capture therapy; BNNPs, boron nitride nanoparticles; BNNTs, boron nitride nanotubes; CEs, carboxylesterases; CIA, collagen-induced arthritis; ClpP, casein protease P; COVID-19, coronavirus disease 2019; cUTI, complex urinary tract infection; dCTPase, dCTPase pyrophosphatase; GSH, glutathione; HADC1, class I histone deacetylase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LeuRS, leucyl-tRNA synthetase; MBLs, metal β-lactamases; Mcl-1, myeloid cell leukemia 1; MDR-TB, multidrug-resistant tuberculosis; MERS, Middle East respiratory syndrome; MIDA, *N*-methyliminodiacetic acid; MM, multiple myeloma; Mtb, *Mycobacterium tuberculosis*; MTX, methotrexate; NA, neuraminidase; NS5B, non-nucleoside polymerase; OBORT, oxaborole tRNA capture; QM, quinone methide; OPs, organophosphate; PBA, phenylboronic acid; PDB, Protein Data Bank; PPI, protein−protein interaction; RA, rheumatoid arthritis; ROS, reactive oxygen species; *Sa*ClpP, *Staphylococcus aureus* caseinolytic protease P; SARS-CoV-2, syndrome coronavirus 2; SBLs, serine β-lactamases; SERD, selective estrogen receptor downregulator; SHA, salicyl hydroxamic acid; TB, tuberculosis; TTR, transthyretin; U4CR, Ugi 4-component reaction.

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1. Introduction

The application of boron in medicine dates back to the early 19th century, when boric acid ($B[OH]_3$) was used as a mild antiseptic. However, boron derivatives were long neglected thereafter as a result of largely unfounded claims that they are unstable and toxic. In recent years, there has been an upsurge of interest in the therapeutic potential of boron-based drugs[1–5] since the approval of bortezomib (**1**), a boronic acid-containing drug[6], by the U.S. Food and Drug Administration (FDA) in 2003. Since then, tavaborole (**2**) has been approved for the treatment of onychomycosis[7], ixazomib (**3**) for the treatment of multiple myeloma (MM)[8], crisaborole (**4**) for the treatment of atopic dermatitis[9] and vaborbactam (**5**) for the treatment of bacterial infections[10] (Fig. 1). Other boron-containing drug candidates are also in various stages of clinical trials[11–19](Table 1).

A key reason for the increasing research and development interest in boron-containing drugs is probably that boron compounds can easily interconvert between neutral trigonal planar sp^2 and tetrahedral sp^3 hybridization states, and thus can adopt a diverse range of binding modes during target engagement[20]. Many proteins have been co-crystallized with boron-containing compounds, and structural data are available in the Protein Data Bank (PDB). Boronic acids can form trigonal covalent[21], tetragonal covalent[22] or bidentate covalent adducts[23] with nucleophiles such as serine, lysine, tyrosine, threonine and cysteine residues in target proteins, and are thus useful as high-affinity ligands with low molecular mass. Moreover, adduct formation is reversible, and so the random covalent modification of unintended targets by covalent warheads can be minimized[24]. Furthermore, the two hydroxyl groups of boronic acids provide four lone pairs and two hydrogen bond donors, thus offering six opportunities to form direct or water-mediated hydrogen bond contacts with key amino acid residues. These hydrogen bonds can have a high degree of covalency; *i.e.*, the hydrogen bonds are short and the interaction energy between boronic acid and the target protein is large. Multiple hydrogen bonds are effective to enhance the affinity of the ligand (or inhibitor) for the target and to facilitate binding even in the presence of drug resistance-related mutations[25,26]. In addition, the boron moiety can interact with metal ions in enzymes (Fig. 2)[27]. These attributes are valuable when designing candidate drugs to work in the context of selection pressures for drug resistance.

The boronic acid pharmacophore is an established glycan-binding functional group that forms reversible complexes with diol groups commonly found in sugar molecules and glycoproteins[28–31]. Exploiting this behavior, multimeric boronic acids have been designed to target pathogens' extracellular glycans, both to overcome inherent cell envelope barriers and to prevent the development of drug resistance[32]. Boronic acid-containing prodrugs of hydroxylated drug molecules have also been developed to reduce first-pass metabolism and thus to increase bioavailability[33–35]. In addition, boronic acids, together with catechols and *cis*-alkyl diol partners, have been explored as linker components for ligands and proteins to address challenging drug targets[36–38]. The reaction between reactive oxygen species (ROS) and aromatic boronic acids has been exploited for the construction of ROS-triggered boronic acid prodrugs that are selectively activated under pathological conditions, thereby improving targeting and reducing toxicity[39–41]. Boron clusters that contain plural boron atoms are also useful in drug design[42]. For example, carborane with a high content of ^{10}B, which absorbs thermal neutrons, is stable under biological conditions and can penetrate the cell membrane, making it an excellent candidate for boron neutron capture therapy (BNCT)[43,44].

Thus, boron compounds appear to have great potential for development of next-generation target-specific drugs. In this perspective, we aim to comprehensively review the current status of boron compounds in drug discovery, focusing especially on progress from 2015 to December 2020. We will consider compounds showing anticancer, antibacterial, antiviral, antiparasitic and other activities in separate sections, and discuss the biological targets associated with each activity, as well as potential future developments.

2. Boron-containing anticancer agents

Cancer is the second leading cause of death and is the cause of one-sixth of all deaths worldwide. There were 9.6 million deaths from cancer in 2018[45]. At present, chemotherapy is still the main treatment method, but anticancer drugs typically cause toxicity, have side effects, and eventually encounter drug resistance[46,47]. Thus, there is a continuing demand for improved anticancer drugs. One design approach is to utilize specific abnormalities of the tumor microenvironment, such as overexpressed enzymes or high levels of reactive oxygen species (ROS), to target therapeutic agents. In this context, boronic acids serve as excellent covalent ligands, and have been used in ROS-triggered prodrugs. Boronic acid-based prodrugs have also been used to improve the pharmacokinetic properties of anti-estrogens based on their glycan-binding capability. BNCT is also used to treat cancer, based on the ability of boron to capture thermal neutrons. Here we will consider boron-based anticancer agents in terms of their targets.

2.1. *Agents targeting key enzymes*

Bortezomib, a break-through MM treatment, is a reversible boronic acid inhibitor, which preferentially targets the proteasomal $\beta5$ active site. The drug blocks the catalytic $\beta5$ site by binding covalently to

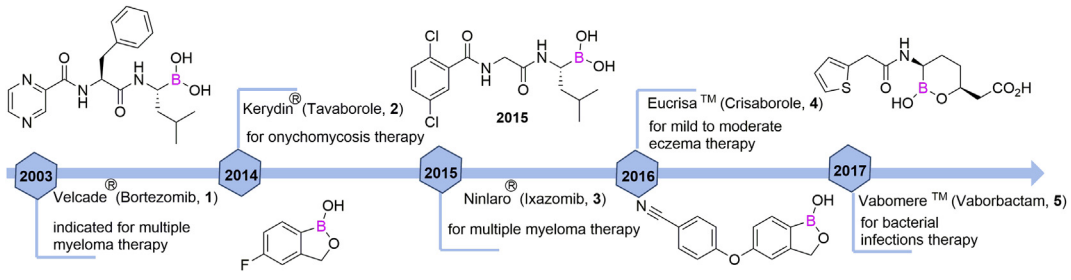
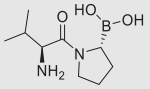
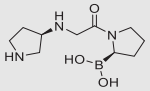
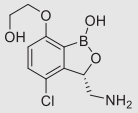
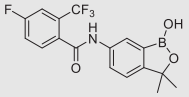
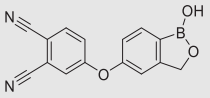
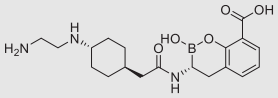
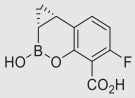
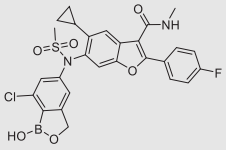


Figure 1 Timeline and structures of FDA-approved boron-containing drugs.

Table 1 Boron-containing drugs in clinical trials.

Name	Structure	Conditions or disease	Phase
Talabostat (PT100, **6**)		Advanced malignant solid neoplasm Recurrent malignant solid neoplasm	II
Dutogliptin (PHX-1149, **7**)		Acute myocardial infarction Acute myocardial ischemia STEMI-ST elevation myocardial infarction	II
GSK656, **8**		Tuberculosis	II
Acoziborole (SCYX-7158; AN5568, **9**)		Trypanosomiasis, African Gambiense trypanosomiasis Sleeping sickness	II III
AN2898, **10**		Dermatitis, atopic	II
Taniborbactam, **11**		Urinary tract infections Acute pyelonephritis	III
QPX7728, **12**		Bacterial infections	I
GSK2878175, **13**		Hepatitis C, chronic	I

the hydroxyl group of the N-terminal threonine residue. While bortezomib is a first-line therapy for multiple myeloma, most patients develop drug resistance, leading to disease recurrence[48]. Overexpression of class I histone deacetylase (HDAC1) in MM cells confers resistance to bortezomib, but importantly, HDAC1 inhibitors could reverse bortezomib resistance[49]. Zhou et al.[50] synthesized a series of peptide boronate derivatives as HDAC/proteasome dual inhibitors by substituting zinc-binding groups of HDAC inhibitors onto solvent-exposed sites of bortezomib (Fig. 3). The most promising inhibitor **14**, in which the zinc-binding moiety originates from the HDAC inhibitor entinostat (**15**) and the covalent-binding group originates from bortezomib, exhibited more potent proteasome inhibition than bortezomib (IC_{50} values of 1.1 *vs.* 19.4 nmol/L) and showed good selectivity for HDAC1 (IC_{50} = 255 nmol/L). Compound **14** showed antiproliferative activity against MM cell lines RPMI-8226, U266 and KM3 with IC_{50} values of 6.66, 4.31 and 10.1 nmol/L, respectively. Notably, it showed potent antiproliferative activity towards the bortezomib-resistant MM cell line KM3/BTZ with an IC_{50} value of 8.98 nmol/L, a level far exceeding that achieved by bortezomib (226 nmol/L) or even the combination of entinostat and bortezomib (1:1, 98.0 nmol/L). Dual proteasome/HDAC inhibitors are promising candidates for the treatment of proteasome inhibitor-resistant MM, and further developments in this area can be expected.

dCTPase pyrophosphatase 1 (dCTPase) is overexpressed in multiple carcinomas and is involved in promoting tumor cell growth and in maintenance of stemness[51]. To exploit the therapeutic potential of dCTPase inhibition, Llona-Minguez and co-workers[52] screened a proprietary compound collection of 5500 compounds *via* HTS-adapted Malachite Green assay and identified a hit compound **16** that inhibits dCTPase with an IC_{50} value of 0.057 μmol/L. Subsequent optimization led to the identification of boronic acid derivative **17** that showed enhanced cellular efficacy (EC_{50} = 0.046 μmol/L) and synergized with cytidine analogues in killing HL60 leukemia cells. But unfortunately, compound **17** showed only moderate aqueous solubility (52 μmol/L) and low plasma stability (11% remaining after 4 h). To improve the ADME properties of compound **17**, they masked the boronic acid functionality with *N*-methyliminodiacetic acid (MIDA) ester to provide derivative **18**, which displayed greater aqueous solubility (>100 μmol/L), improved plasma stability (86% left after 4 h) and enhanced cellular permeability, while exhibiting comparable dCTPase inhibitory activity (IC_{50} = 0.047 μmol/L, Fig. 4). Intriguingly, compound **18** also presented a favorable characteristic of CYP inhibition, with only modest inhibition of CYP2C.

Working with AAA + chaperones such as ClpX, casein protease P (ClpP) maintains cellular homeostasis by degrading defective proteins. Human ClpP is highly expressed in acute myeloid leukemia (AML) cells. Therefore, hClpP inhibitors or activators could be promising candidates for therapy of AML[53,54]. α-Aminoboronic acids were considered to have the potential to covalently inhibit hClpP activity, as they are reported to be selective inhibitors of *Mycobacterium tuberculosis* (Mtb) ClpP[55]. On the basis of the Ugi 4-component reaction (U4CR), Tan et al.[56]

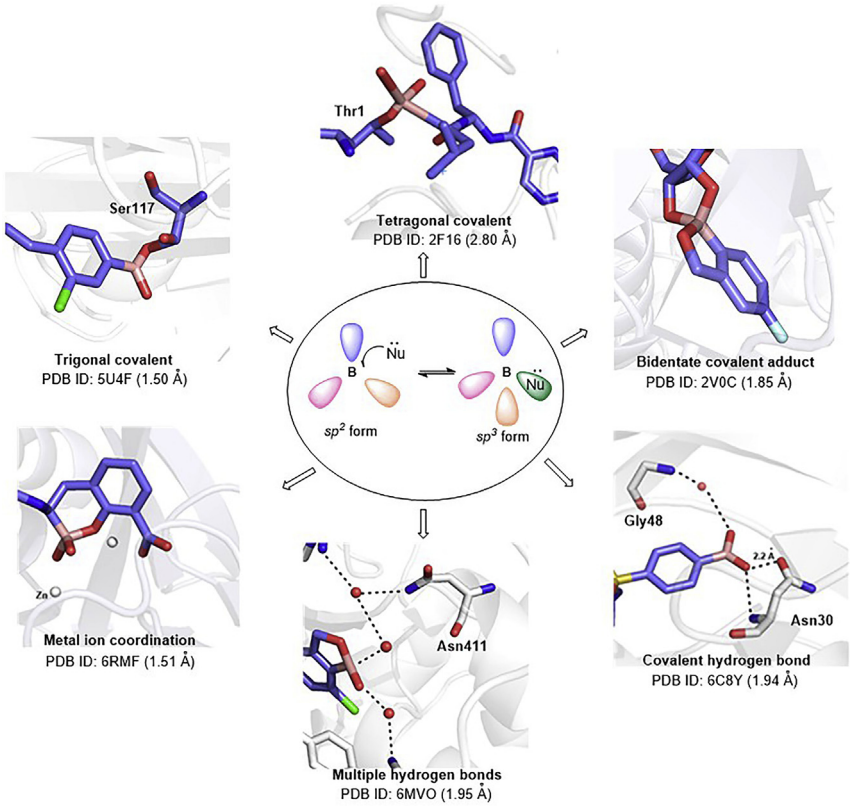


Figure 2 Diverse structures of boron compounds and their interaction modes with biological targets.

built a α-aminoboronic acid virtual library using BIOVIA Pipeline Pilot and filtered the resulting U4CR library against the active site of hClpP to find covalent inhibitors. They identified three α-aminoboronic acids represented by compound **19**, which inhibited hClpP in the low micromolar range. Importantly, in contrast to the potent hClpP inhibitor AV167 (IC_{50} = 1.54 μmol/L) previously reported by Hackl et al.[57], these compounds also inhibited hClpXP (Fig. 5). Therefore, α-aminoboronic acid should be a promising template for the development of inhibitors of hClpXP.

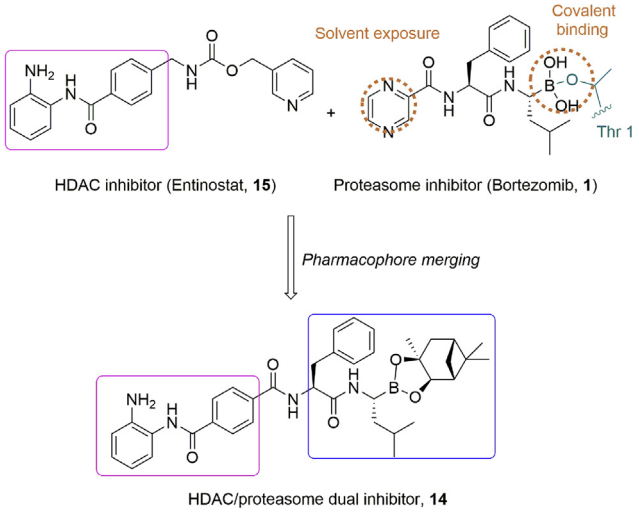


Figure 3 Design of HDAC/proteasome dual inhibitor **14**.

Myeloid cell leukemia 1 (Mcl-1) is a prosurvival protein that is overexpressed by tumor cells and is associated with resistance to apoptosis, being involved in multiple protein−protein interactions (PPIs). Akçay et al.[58] used a docking method to design the first reversible covalent inhibitors targeting Lys234 of Mcl-1 by placing a boronic acid carbonyl warhead in noncovalent Mcl-1 inhibitors and selecting those that positioned the warhead carbonyl within ~3 Å of Lys234 (Fig. 6). Biochemical and cellular examination revealed an increased activity with incorporation of the warhead; for instance, more than 24-fold enhancement of the EC_{50} value towards MOLP-8 cell line was detected between noncovalent inhibitor **20** (>11 μmol/L) and the corresponding warhead-containing **21** (0.46 μmol/L). Further alkylation of the indole nitrogen of this scaffold afforded **22** with significantly enhanced efficacy (EC_{50} = 0.075 μmol/L). These reversible covalent inhibitors induced caspase activation in a manner dependent on Mcl-1 without showing general cytotoxicity, and Lys234 was confirmed to be the residue involved in the covalent modification by means of point-mutation studies combined with mass spectrometry.

2.2. *Agents targeting reactive oxygen species*

Reactive oxygen species (ROS) are a family of redox-active small molecules, such as hydrogen peroxide (H_2O_2) and oxygen radicals, which are widely present in biological systems. Oxidative stress due to abnormal production of H_2O_2 can lead to the development and progression of serious diseases such as cancer, inflammation, obesity and diabetes[59]. Boronic acids and their

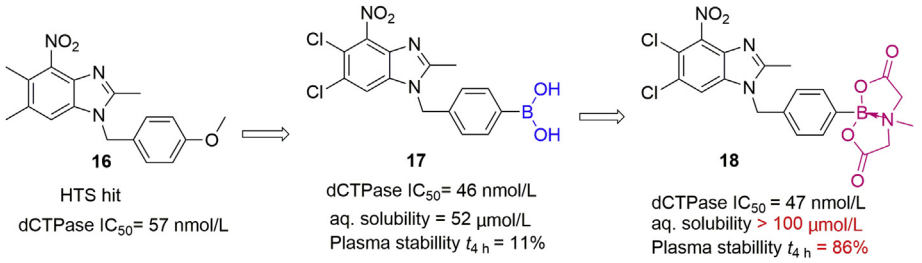


Figure 4 Design of dCTPase inhibitor **18**.

esters are cleaved by H_2O_2 and other ROS to produce the corresponding alcohol and boric acid, which is considered non-toxic to humans (Fig. 7)[60]. In drug development, boronic/boronate groups are often used to replace the hydroxyl groups of active agents in order to achieve selective release of drugs in the tumor microenvironment. The phenylboronic/boronate functionalities are connected to the active drug structure through a direct C−N/C−O bond or a self-immolative spacer (such as a carbamate linkage). Oxidation of such a spacer triggers a self-immolative cascade, leading to the release of the active drug (Fig. 7)[61].

Chen et al.[62] developed several new aromatic nitrogen mustards with boronic esters or boronic acids as ROS-based activators to improve the selectivity for tumor cells. These prodrugs are not deleterious to normal cells owing to the electron-withdrawing effect of the boronate group, but H_2O_2 in cancer cells cleaves the boronic ester or boronic acid to a hydroxyl group, which increases electron release to the nitrogen of the mustard moiety, affording a highly electrophilic aziridinium ring that induces DNA cross-linking (Fig. 8). These prodrugs caused 40%−80% cell death of primary leukemic lymphocytes but less than 25% apoptosis of normal lymphocytes. The most potent compound (**23**) displayed an IC_{50} value of around 5 μmol/L in CLL cells. In further work[63], various aromatic substituents were incorporated into compound **23** to improve the biophysical properties, and compound **24** potently induced apoptosis in CLL lymphocytes with IC_{50} values between 3 and ~778 nmol/L. In addition, **24** was more cytotoxic toward breast-cancer MDA-MB-468 cells (IC_{50} = 3.43 μmol/L) than clinically used chlorambucil (IC_{50} = 48.7 μmol/L) or melphalan (IC_{50} = 34.44 μmol/L). More importantly, compound **24** is highly selective for cancer cells with no apparent toxicity to normal lymphocytes at the highest concentration tested (10 μmol/L). It efficiently inhibited tumor growth in an MDA-MB-468-derived xenograft mouse model without apparent side effects.

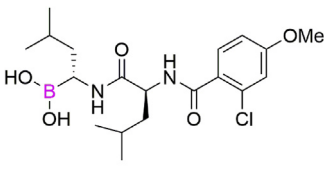


Figure 5 The structure of inhibitor **19**.

Compared with normal cells, cancer cells show increased generation of ROS and are also more susceptible to further ROS insults[64]. Cancer cells, through upregulating antioxidant systems such as glutathione (GSH), adapt to oxidative stress and thus counteract ROS insults[65]. Quinone methide (QM, **25**), an antioxidant inhibitor with anticancer activity, rapidly alkylates GSH, thus inhibiting the cellular antioxidant systems[66]. Cinnamaldehyde (**26**) induces ROS generation in cancer cells and inhibits the growth of human cancer cells with minimal cytotoxicity to healthy cells, but poor bioavailability limits its clinical application[67]. Based on these studies, Noh et al.[68] reported a novel hybrid anticancer prodrug **27**, which, under oxidative conditions, can release QM and cinnamaldehyde dependent on the activation of H_2O_2 and acidic pH, respectively, in cancer cells and thus exerts synergistic therapeutic actions (Fig. 9). Firstly, the generation of both QM and cinnamaldehyde in prostate cancer DU145 cells was confirmed. Then they demonstrated that **27** induced a significant reduction (>50%) of the GSH level in DU145 cells, while it had no effect on the GSH level in non-malignant NIH3T3 cells. The hybrid drug **27** exhibits cytotoxicity towards DU145 cells and SW620 cells with IC_{50} values of 48 and 76 μmol/L, respectively, being significantly more cytotoxic than cinnamaldehyde and a quinone methide generator. Importantly, in xenograft mouse models using SW620 and DU145 cells, **27** displayed more potent anticancer action than the combination of cinnamaldehyde and a quinone methide generator. Its efficacy was comparable to that of the anticancer drug camptotethin at the dose of 2 mg/kg.

Apart from prodrug development, selective reactions have also been applied for fluorescence detection of H_2O_2, gene expression and point-of-care assay. For example, Chang's group[69] used boronic esters for the development of H_2O_2-activated fluorescent probes to explore the physiological roles of H_2O_2 in living systems. Govan et al.[70] developed a genetically encoded gene activation system through the use of a boronate group-substituted estrone molecule. Upon oxidation of the prodrug by H_2O_2 after binding to the estrogen receptor, active estrone is released, thereby activating gene expression.

2.3. *Agents targeting estrogen receptor*

Boronic prodrugs of anti-estrogen compounds significantly reduce the first-pass metabolism of hydroxylated drug molecules, leading to increased bioavailability. It is suggested that boronic acid blocks rapid clearance by forming reversible complexes with 1,2 and 1,3 diol groups of sugar molecules and glycoproteins, thereby facilitating enrichment in plasma as well as making the boronic acid moiety inaccessible to glucuronidation. Moreover, such

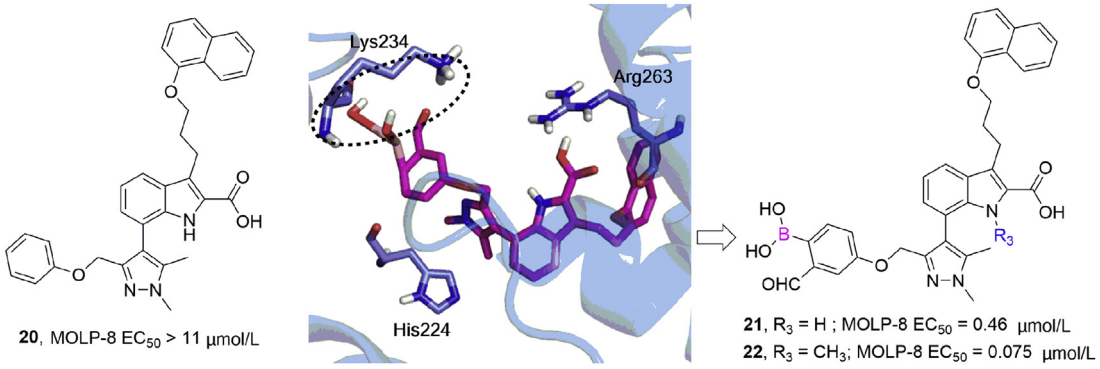


Figure 6 Structure-based design identified covalent Mcl-1 inhibitors targeting Lys234: Docked structure of inhibitor **21** (PDB ID:3WIX) and structures of compounds **20**—**22**.

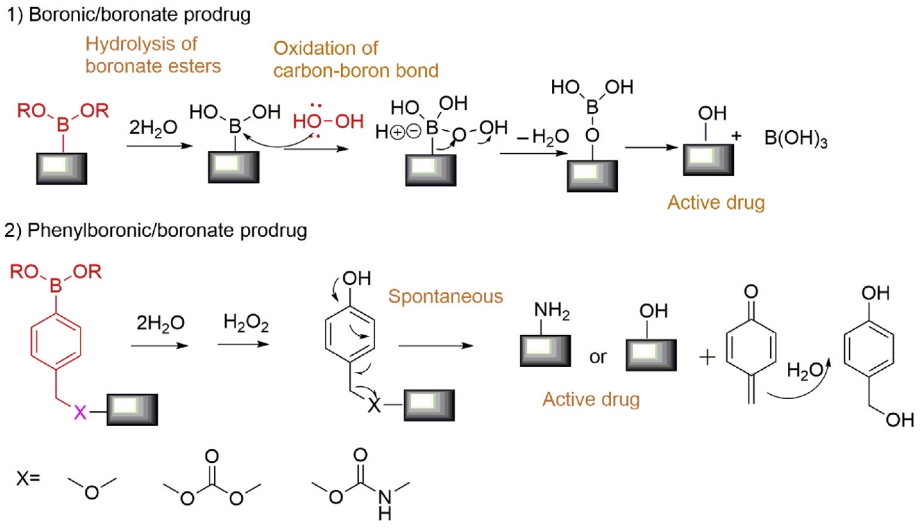


Figure 7 Oxidation of boronic prodrugs to release the active drug.

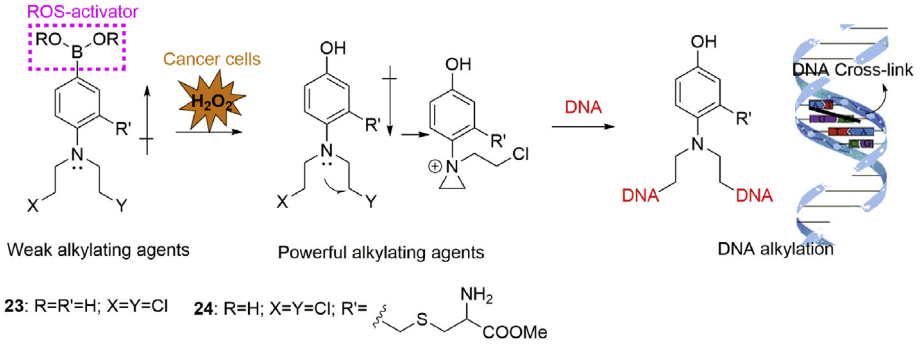


Figure 8 Targeting ROS-producing cancer cells; the structures of prodrugs **23** and **24**.

complexes may serve as a drug reservoir. Endoxifen (**28**) is a selective estrogen receptor modulator (SERM) that is in clinical trials, but it undergoes rapid first-pass metabolism through *O*-glucuronidation, leading to poor bioavailability. Zhang et al.[34] designed a boronic acid prodrug of endoxifen, ZB483 (**29**), which produced a 40-fold increase in peak endoxifen concentration and an 80-fold increase in the area under the curve (AUC) value (Fig. 10).

Fulvestrant (**31**), a selective estrogen receptor downregulator (SERD), is indicated for metastatic breast cancer therapy, but poor oral bioavailability has hampered its clinical application. However, substitution of the 3-hydroxyl group of fulvestrant with a

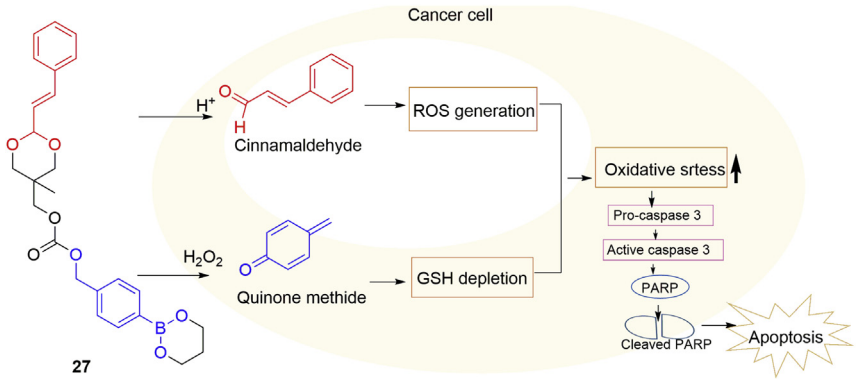


Figure 9 A dual-stimulus-responsive hybrid anticancer drug **27**.

boronic acid moiety allowed the compound to evade first-pass metabolism[33]. After a single oral dose of 8.3 mg/kg in mice, fulvestrant-3 boronic acid (ZB716, **32**) afforded far superior half-life ($t_{1/2}$ = 23.5 h), peak concentration (C_{max} = 169.8 ng/mL) and area under the curve (AUC = 2547.1 ng h/mL) in comparison to fulvestrant when given by s.c. injection to mice. At the same time, ZB716 is as potent as fulvestrant in binding ERα (IC_{50} = 4.1 nmol/L) and downregulating ERα (Fig. 11). Improvement of poor bioavailability can allow physicians to prescribe a lower dose, potentially reducing side effects, increasing patient compliance, and lowering the risk of cancer recurrence. The coordination of boronic acid with diols from sugar molecules has also been exploited to enhance insulin delivery in the treatment of diabetes[71].

2.4. *Agents with unknown targets*

Zhang et al.[72] developed a series of 7-propanamide derivatives based on an antimalarial benzoxaborole **33**, obtaining a compound **34** with submicromolar antiproliferation activity towards ovarian cancer cells. Further structure−activity relationship studies found that replacement of the phenyl group with biphenyl and introduction of a hydrogen-bond acceptor group led to a significant increase of potency. The most potent compound **35** showed an IC_{50} value of 21 nmol/L against ovarian cancer cells, with good selectivity between cancerous and normal cells (nearly 200-fold selectivity: SKOV3 *vs.* WI-38 cells). Compound **35** effectively induced cancer cell apoptosis and inhibited colony formation (Fig. 12). More importantly, compound **35** also demonstrated

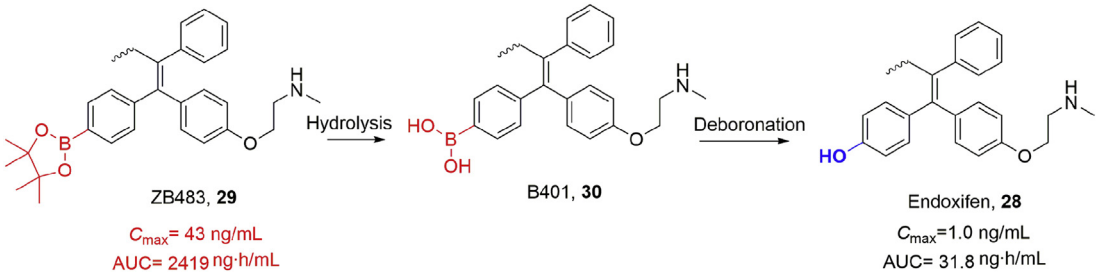


Figure 10 Metabolic conversion of ZB483 to endoxifen.

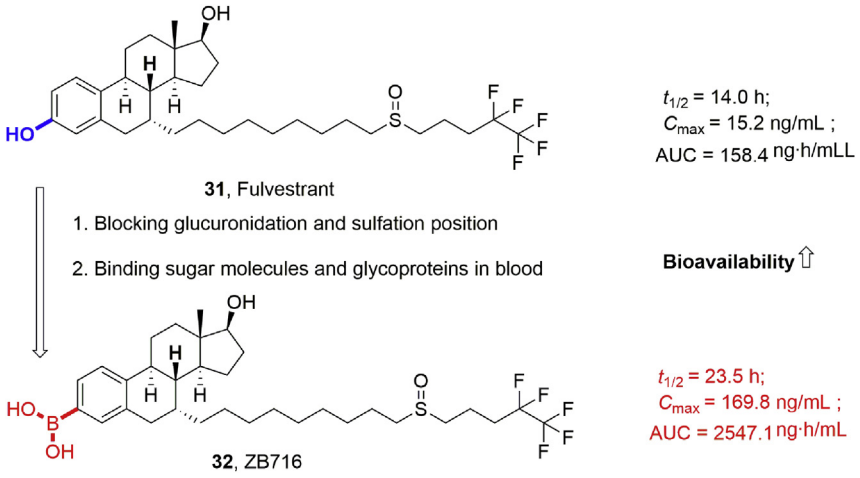


Figure 11 Design of ZB716.

in vivo efficacy and low toxicity in a tumor xenograft mouse model at dosages of 2 and 10 mg/kg. Although the cellular target of these anticancer benzoxaboroles is not clear, these structures provide a new direction for anticancer drug research.

2.5. *Boron neutron capture therapy*

BNCT is currently an important mode of tumor therapy. When ^{10}B-rich agents are selectively accumulated in malignant cells, the ^{10}B atoms absorb low-energy (<0.5 eV) neutrons (thermal neutrons) and disintegrate to afford an alpha (^{4}He) particle and a lithium nucleus (^{7}Li). The alpha particles deposit high energy along a short path length (<10 μm; approximately the diameter of a single cell), and consequently kill tumor cells containing boron without damaging surrounding normal cells[73]. Clinical studies of BNCT have focused on locally recurring malignant glioma and head and neck cancer. The clinical efficacy of two boron agents, 4-dihydroxyborylphenylalanine (BPA, **36**) and sodium mercaptoundecahydrododecaborate (BSH, $Na_2B_{12}H_{11}SH$, **37**, Fig. 13), has been confirmed in patients with the above tumors[74–77]. However, some issues remain. BSH does not show active targeting or uptake, and has undesired interactions with other biomolecules, while BPA displays poor water solubility, low boron content and lack of specificity[78]. In recent years, the advent of new in-hospital neutron accelerators has generated renewed interest in BNCT[79,80]. However, efforts to develop improved boron delivery agents, including amino acids, carbohydrates, and nanoparticles, have not yet made a breakthrough. At present, the best way to further improve the clinical efficacy of BNCT may be to optimize the administration and delivery methods of BPA and BSH[81].

2.6. *Boron nitride nanotubes and nanoparticles*

Boron nitride nanotubes (BNNTs) and boron nitride nanoparticles (BNNPs) are currently considered promising candidates for drug delivery. For example, folic acid-functionalized BNNTs are specifically taken up by glioblastoma multiforme, and therefore may be useful to deliver drugs to glioblastoma multiforme in the brain[82].

3. Boron-containing antibacterial agents

In the United States alone, more than 2.8 million people acquire an antibiotic-resistant infection every year, resulting in an annual death toll of more than 35,000[83]. Among infectious diseases in general, tuberculosis (TB) caused by Mtb is the main killer. In

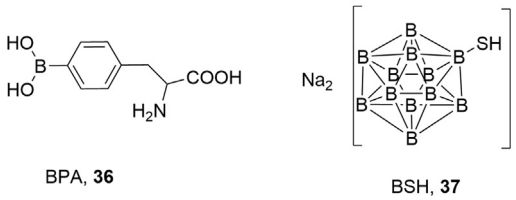


Figure 13 Structures of BPA and BSH.

2015, the worldwide incidence of multidrug-resistant tuberculosis (MDR-TB) was approximately 580,000, leading to an estimated 250,000 deaths. The latest available data show that the treatment success rate for MDR-TB is only 56%[84]. In more than 70 years, only two completely new drugs for the treatment of MDR-TB have entered the market. Therefore, there is an urgent need for new antibacterial agents. In the 1970s, boric acid was reported to reversibly inhibit serine β-lactamases produced by *Bacillus cereus*[85]. Boronic acids are regarded as transition-state analogues of β-lactamases and are currently considered promising candidates to combat antibiotic resistance. It was shown that boronic acids selectively kill Mtb through targeting Mtb leucyl-tRNA synthetase (LeuRS) based on the oxaborole tRNA capture (OBORT) mechanism or *via* chelation of its unique cell wall glycans. Boronic acids have also been developed as covalent binding inhibitors of *Staphylococcus aureus* caseinolytic protease P (*Sa*ClpP). These approaches represent a step towards pathogen-specific, next-generation antibiotics.

3.1. *Targeting β-lactamases*

β-Lactam antibiotics are currently among the most widely used drugs to treat bacterial infections, but the rapid evolution and spread of antibiotic resistance are having a dramatic impact on their efficacy[86]. The main mechanism leading to resistance is hydrolysis of the amide bond of the lactam ring by serine β-lactamases (SBLs; Ambler A, C and D) or metal β-lactamases (MBLs; Ambler B), as illustrated in Fig. 14A and B. Both β-lactamase-catalyzed hydrolysis processes involve a high-energy tetrahedral (sp^3 hybridized) intermediate[49,85,87]. One approach to this issue is combined administration of β-lactamase inhibitors with β-lactam antibiotics. Unfortunately, currently commercially available BLIs, such as sulbactam and clavulanic acid, are themselves β-lactams, and bacteria can quickly develop resistance to these chemically similar molecules. In contrast, boronic acid transition-state analogs

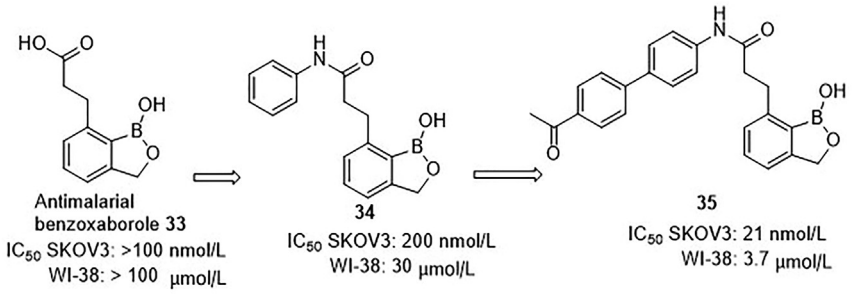


Figure 12 The structures of inhibitors **33**–**35**.

are a different class of β-lactamase inhibitors (Fig. 14C). The capacity of boron to morph between hybridisation states enables boronate-based inhibitors to mimic substrates of β-lactamases (in their sp^2 state), allowing them to bind efficiently to β-lactamases, and they can then react with the SBL nucleophilic serine or MBL-Zn(II)-bridged water to form the sp^3 state, mimicking a high-energy intermediate. Thus, boronate-based inhibitors are promising candidates for SBL/MBL inhibition.

3.1.1. *Inhibitors of SBLs*

1-Amido-2-(*m*-carboxyphenyl)ethane boronic acid derivatives, generated by replacing the carbonyl of the β-lactam ring with boron-containing groups, are potent SBL inhibitors[88]. Considering that replacement of a phenyl ring by a triazole ring would enable the installation of various R groups to explore the topology of the β-lactamase active site, Caselli et al.[89] designed and synthesized a series of highly substituted α-amido-β-triazolylethane boronic acids by utilizing click chemistry (Fig. 15). The resulting compounds showed potent activity against class C SBLs (P99 and PDC-3) with low micromolar to low nanomolar inhibition constants, which were as low as 4 and 23 nmol/L towards PDC-3 and P99, respectively, for the most potent compound **38**. These compounds significantly reduced bacterial resistance when used in combination with cefotaxime.

The resistance of *Acinetobacter baumannii* to all classes of β-lactam antibiotics has greatly increased over the past decade. Class C *Acinetobacter*-derived cephalosporinases (ADCs) β-lactamases, especially ADC-7, mainly mediate β-lactam resistance in *A. baumannii* and are not affected by clinically used BLIs[90]. Caselli et al.[91] continued to employ click chemistry and synthesized a new class of boronic acid transition-state inhibitors as ADC β-lactamase inhibitors, in which the amide group was replaced with triazole. These compounds showed K_i values in the range of 90 nmol/L to 38 μmol/L, with compound **39** being one of the best inhibitors of ADC-7 (Fig. 16). Crystallographic data indicated that the triazole establishes the expected hydrogen bonds with Gln120 and Asn152, maintaining the typical interactions of amidomethaneboronic acid inhibitors. In addition, multiple hydrogen bonds were observed between the O1 atom hydroxyl group of boronic acid and Ser315 and Ser64, and another O atom establishes a covalent bond with phosphate ion. These compounds restored ceftazidime or cefepime activity against classes C and A β-lactamase strains. These results demonstrate that α-triazolylmethaneboronic acid is a good scaffold for ADC β-lactamase inhibitors.

Ness et al.[92] synthesized inhibitor **40a** with greatly increased affinity for TEM-1 β-lactamases by installing a hydroxyl group on the aromatic ring. Hecker et al.[93] envisaged that cyclic boronate ester compounds might show a favorable conformation for enzyme complexation, and docked several candidates into the active site of β-lactamase *in silico*. Hit molecules were synthesized and evaluated, resulting in the discovery of vaborbactam, the first clinically approved boronic acid-containing SBLs inhibitor (Fig. 17A). In 2017, vaborbactam/meropenem (Vabomere) was approved to treat complex urinary tract infections[10]. This drug combination potently inhibits classes A and C SBLs. However, no MBLs inhibitor based on a boronic acid chemotype has yet been found.

3.1.2. *Broad-spectrum β-lactamase inhibitors (BLIs)*

Brem et al.[94] obtained cyclic boronate analogues showing nanomolar inhibition of SBLs and MBLs by applying a strategy of

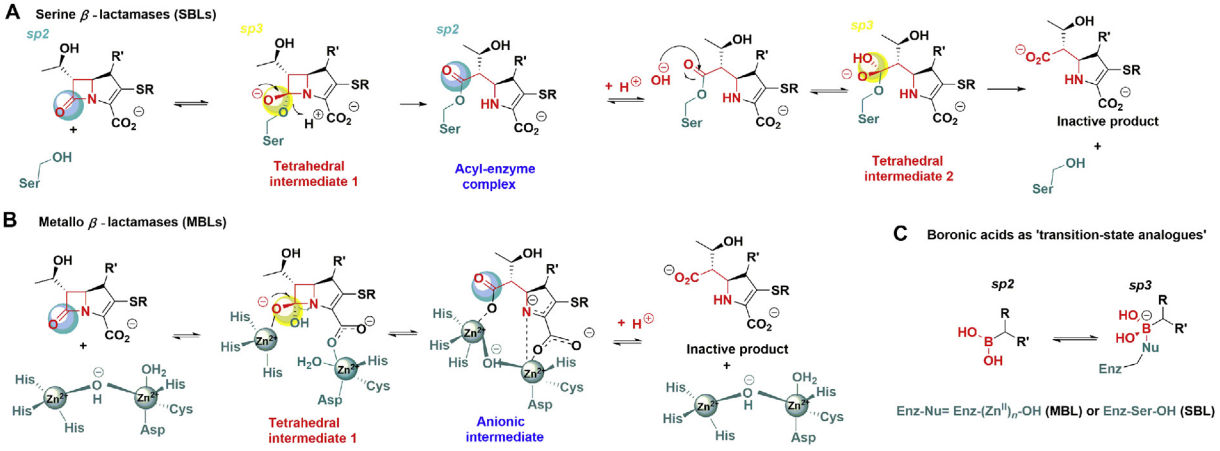


Figure 14 Mechanisms of β-lactam cleavage by (A) SBLs and (B) MBLs, exemplified by the hydrolysis of a carbapenem. (C) Mode of action of boronate inhibitors for inhibition of SBLs and MBLs.

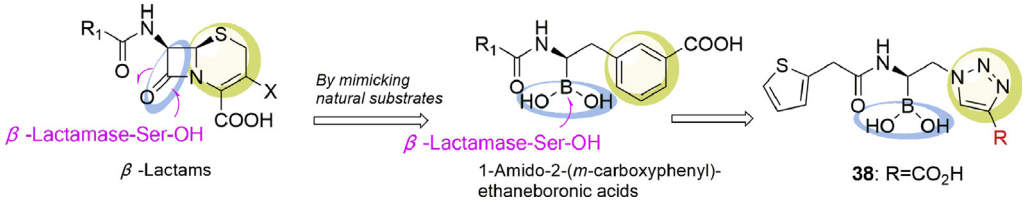


Figure 15 Design strategy for compound **38**.

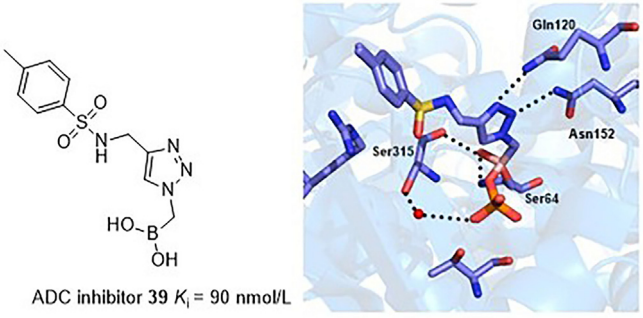


Figure 16 Planar structure of **39** and X-ray crystal structure of ADC-7/inhibitor complex (PDB code: 6TZJ).

mimicking the common high-energy tetrahedral intermediate. As shown in Fig. 17B1, the binding modes of **41** with both BcII and VIM-2 are similar to those observed for complexes of MBLs with hydrolysed β-lactams, exemplified by hydrolysed cefuroxime. For instance, in the complex obtained by co-crystallization of **41** with VIM-2, the "endocyclic" boronate ester oxygen that corresponds to the cephalosporin dihydrothiazine ring nitrogen forms a dative bond with Zn, and two boron-bound exocyclic oxygen atoms chelate another zinc atom. Furthermore, the hydrogen bonding/electrostatic interaction with Lys224 (NDM-1 and BcII) or Arg228 (VIM-2) and hydrophobic interaction with conserved Trp87 and Phe61 residues are analogous to those with hydrolysed β-lactams (Fig. 17B2). Overall, therefore, the structure of the cyclic borate complex closely matches the tetrahedral transition state in MBL catalysis, and this may be the reason why **41** shows broad-spectrum β-lactamase-inhibitory activity.

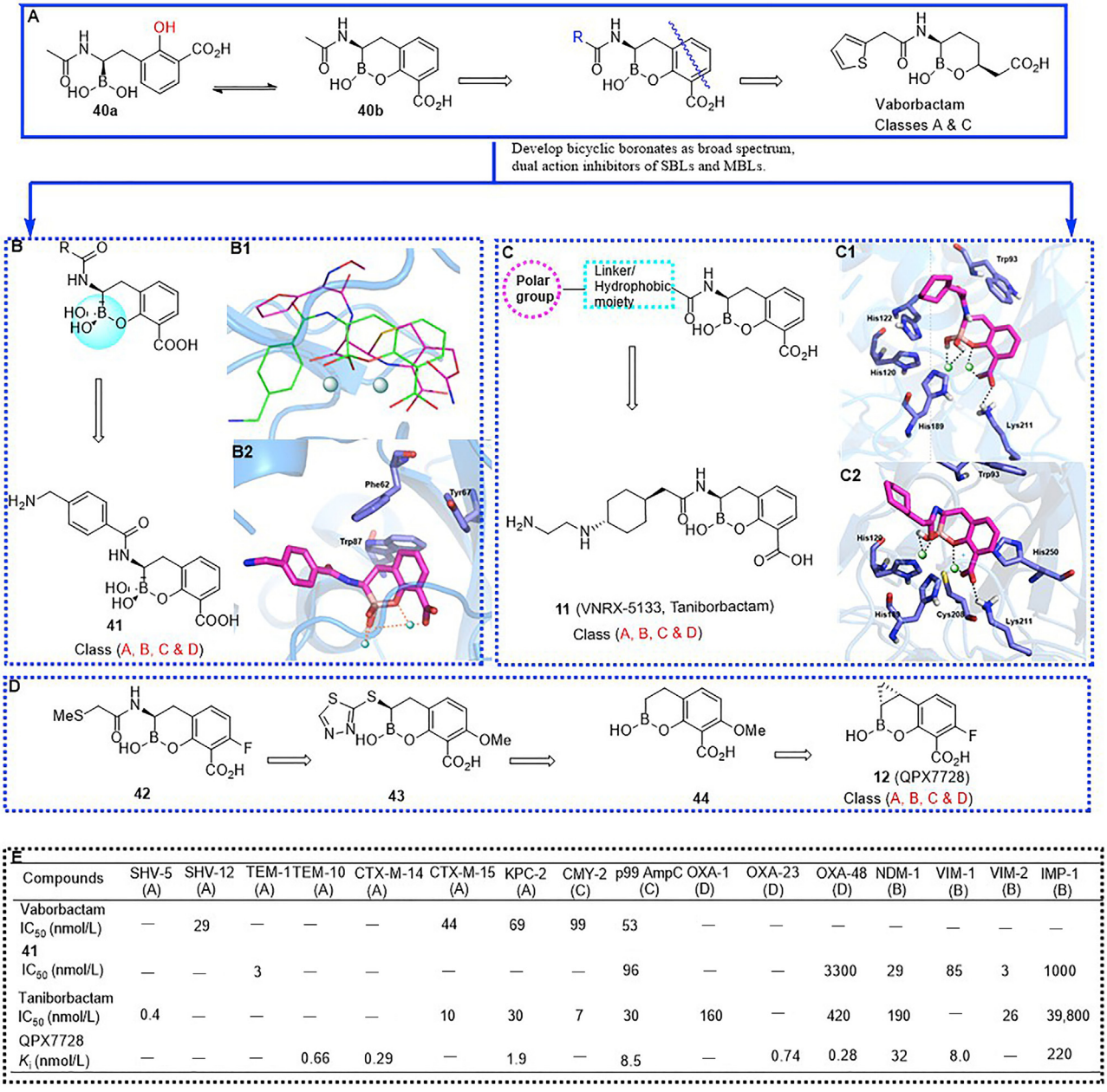
Recognizing that the cyclic boronate scaffold might serve for the development of pan-spectrum β-lactamase inhibitors, Liu et al.[27] introduced hydrophobic groups into the scaffold of **40b** to enhance van der Waals interactions with hydrophobic residues at the active sites of SBL and MBL enzymes. Next, additional distal primary amino groups were also introduced to improve Gram-negative OM permeability. These studies led to compound **11** (VNRX-5133, taniborbactam), which is a potent inhibitor of all four Ambler classes of β-lactamase and a wide range of Gram-negative bacteria (Fig. 17C). Notably, taniborbactam was selective for bacterial enzymes, being nontoxic to mammalian cells. Currently, VNRX-5133/cefepime is in phase III clinical study for patients with complicated urinary tract infections. Crystallographic analysis of VNRX-5133 complexed with NDM-1[95] revealed a similar MBL inhibition mechanism to that **41** (Fig. 17C1), though an unanticipated tricyclic structure involving cyclization of the acylamino oxygen onto the boron of the bicyclic core was also observed (Fig. 17C2). The results further support the validity of the "high-energy intermediate" strategy for the development of boron analogues as broad-spectrum β-lactamase inhibitors.

Building on the above structures, Hecker et al.[96] designed a series of methylthioacetamide analogues represented by **42** as potent inhibitors of classes A and C serine enzymes. Greatly improved potency against class B enzymes was obtained by replacing the amide linkage with a thio linkage, as exemplified by **43**. Removal of the substituent next to boron resulted in remarkable activity against class D enzymes, but unfortunately the most promising compound **44** produced a potentially toxic long-lived metabolite *via* oxidative deboronation. Structural modifications to block this reaction while retaining the desired properties culminated in the discovery of **12** (QPX7728, Fig. 17D). QPX7728 shows a remarkably broad spectrum of inhibition (classes A, B, C and D enzymes), and is well tolerated in rats, with good oral bioavailability (F%) of 24%−53%, depending upon the dose. QPX7728 has advanced to phase I clinical trials[18].

Acyclic boronates were also effective inhibitors of both SMLs and MBLs. Wang et al.[97] obtained a new MBL/SBL inhibitor **45** by fusion of (2′*S*)-3′-mercapto-2′-methylpropanamido with acyclic α-amino boronic acid. The structures of VIM-2/**45** and KPC-2/**45** complexes revealed the dual MBL/SBL inhibition mode: a thiol bridges two active site zinc ions for MBL inhibition (Fig. 18A) and the boronic acid group covalently binds with the catalytic residue Ser70 in the active site for SBL inhibition (Fig. 18B). Further examination indicated that **45** did not occupy the sub-pocket consisting of the Trp105, Ser130, and Thr235 residues, suggesting that introduction of a substituent at the 1-position of **45** might result in enhanced potency. Indeed, this approach led to compound **46**, which showed more potent inhibition of several tested MBL and SBL enzymes, including the clinically common carbapenemases VIM-2 (K_i = 0.44 μmol/L) and KPC-2 (K_i = 0.61 μmol/L).

3.2. *Targeting leucyl-tRNA synthetase (LeuRS) and extracellular glycans of Mtb*

Benzoxaboroles (also known as oxaboroles) target fungal cytoplasmic LeuRS by an oxaborole tRNA-trapping (OBORT) mechanism, in which the boron atom forms a covalent adduct with the terminal nucleotide Ade76 of tRNA to capture the 3′ end of $tRNA^{Leu}$ in the editing site, inhibiting leucylation and thus inhibiting protein synthesis (Fig. 19)[23]. Utilizing this mechanism to target *M. tuberculosis* LeuRS, Sonoiki and co-workers[98] synthesized a series of 3-aminomethyl-benzoxaboranes, in which the boron atom is bonded to the *cis*-diols of AMP, a surrogates for Ade76, as seen in the X-ray co-crystal structure of LeuRS with compound **47**. The most promising compound in this series, **48**, displayed potent inhibition of Mtb LeuRS (IC_{50} = 0.056 μmol/L), but unfortunately there were potential toxicity issues arising from its inhibition of mammalian cytoplasmic LeuRS (IC_{50} = 38.8 μmol/L). Based on the co-crystal structure, modification studies focused on the solvent-exposed C-7, C-6, and C-6/C-7 sites were performed with the aim of increasing the selectivity[99]. Efficacy studies of these derivatives identified a clinical



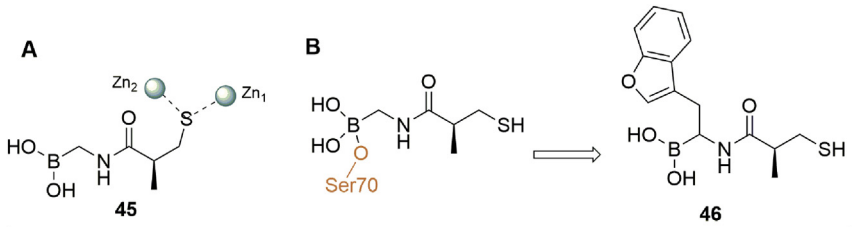
E

Compounds	SHV-5 (A)	SHV-12 (A)	TEM-1 (A)	TEM-10 (A)	CTX-M-14 (A)	CTX-M-15 (A)	KPC-2 (A)	CMY-2 (C)	p99 AmpC (C)	OXA-1 (D)	OXA-23 (D)	OXA-48 (D)	NDM-1 (B)	VIM-1 (B)	VIM-2 (B)	IMP-1 (B)
Vaborbactam IC_{50} (nmol/L)	—	29	—	—	—	44	69	99	53	—	—	—	—	—	—	—
41 IC_{50} (nmol/L)	—	—	3	—	—	—	—	—	96	—	—	3300	29	85	3	1000
Taniborbactam IC_{50} (nmol/L)	0.4	—	—	—	—	10	30	7	30	160	—	420	190	—	26	39,800
QPX7728 K_i (nmol/L)	—	—	—	0.66	0.29	—	1.9	—	8.5	—	0.74	0.28	32	8.0	—	220

Figure 17 (A)−(D) Design strategies of vaborbactam, **41**, taniborbactam and QPX7728, respectively. (B) Co-crystallization of **41** with VIM-2 (B1). The overlay compares the binding modes of **41** and hydrolysed cefuroxime complexed with NDM-1 (B2) (PDB ID: 4RL2). (C) Crystal structures of VNRX-5133 complexed with NDM-1 (PDB ID: 6RMF); chain A shows the major observed bicyclic form (C1), while chain B shows the tricyclic form (C2). (E) Activities of vaborbactam, **41**, taniborbactam and QPX7728.

candidate, GSK3036656 (**8**), which was highly selective for Mtb LeuRS over human cytoplasmic LeuRS and HepG2 protein synthesis, even though its potency towards Mtb LeuRS was not significantly improved over that of the initial analogue **48** (Fig. 19). Importantly, GSK3036656 exhibited excellent PK profiles in mouse TB infection models with high tolerability. A phase II clinical study of GSK3036656 for TB was undergoing[14].

The cell envelope of Mtb is a highly efficient permeability barrier, preventing entry of many antibiotics into cells, thus impeding anti-tubercular treatment. Boronic acids form bonds with *cis*-1,2- and 1,3-diols in carbohydrates and multiple boronic acids placed on a single scaffold provide a synergistic increase in binding affinity[28]. Guy et al.[32] designed multimeric boronic acids to selectively target structurally unique Mtb cell envelope glycans, in order to overcome the Mtb cell envelope barrier (Fig. 20). The number of, and distance between, boronic acid units greatly influenced the binding selectivity and affinity. Among the synthesized compounds, two boronic acid units with shorter linkers were selective inhibitors of mycobacteria (MICs: 780−3100 μmol/L) over other strains of bacteria, and exhibited low cytotoxicity in a panel of human cell lines. BLI data revealed that multimeric boronic acids have intense and specific interactions with isolated Mtb glycans and whole integral Mtb cells *versus* other bacterial species or mammalian cells. Whole-cell



K_i (μmol/L)	KPC-2 (A)	TEM-1 (A)	AmpC (C)	OXA-48 (D)	VIM-2 (B)	NDM-1 (B)	Shf=1 (B)	GOB-18 (B)
45	2.70	40.97	6.46	70.19	0.76	67.13	7.34	2.23
46	0.61	10.77	0.11	>300	0.44	37.95	0.26	0.13

Figure 18 X-ray structure analyses of the VIM-2/**45** (A) and KPC-2/**45** (B) complexes, leading to the development of **46**.

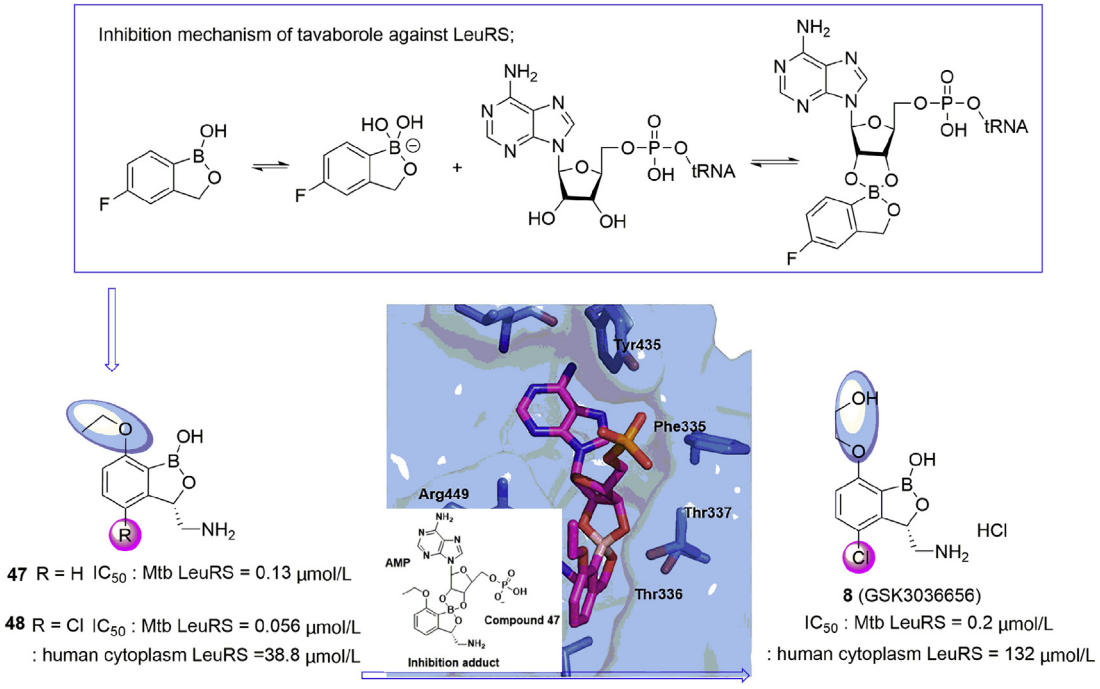


Figure 19 Mechanism of inhibition of LeuRS by tavaborole and X-ray cocrystal structure of LeuRS complexed with **47** (PDB ID: 5AGR).

proteomics identified a series of stress response instead of a single target, and this may facilitate the anti-resistance. This non-conventional approach means that the molecule does not need to cross the Mtb cell wall barrier.

3.3. *Targeting S. aureus caseinolytic protease P (SaClpP)*

Ju et al.[100] screened 2632 molecules and selected a peptidomimetic boronate, MLN9708 (**49**) with an IC_{50} value of 5.7 μmol/L

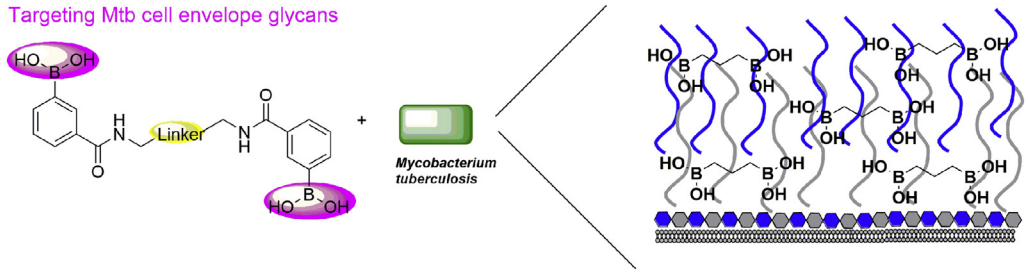


Figure 20 Design of multimeric boronic acids specifically targeting the extracellular Mtb cell-envelope glycans. The complex Mtb cell envelope and peptidoglycan layer are indicated by lines and hexagons, respectively.

against *Sa*ClpP for further optimization. Based on the idea that boron binds to residue Ser98 in the *Sa*ClpP active site, they constructed a virtual library of boron-based compounds and modeled the binding to the active site of *Sa*ClpP. Privileged substituents were selected for micro-scale combinatorial synthesis and bioassay of *Sa*ClpP-inhibitory activity. This work led to **50** (Fig. 21), which showed an IC_{50} value of 0.9 μmol/L. In addition, the crystal structure of **50**–*Sa*ClpP complex indicated that compound **50** forms a reversible covalent bond with Ser98 while stabilizing the tetradecameric structure of *Sa*ClpP. This study offers an integrated strategy for peptidomimetic boronate optimization to develop covalently binding inhibitors. However, these compounds exhibit poor selectivity between 20S proteasome and the homologous *h*ClpP and *Pa*ClpP1 from *Pseudomonas aeruginosa*, so further modification will be needed to increase the selectivity.

4. Boron-containing antiviral agents

Viral infections constitute a major threat to human health, as exemplified by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic[101−103]. Other major pathogens include influenza virus, Dengue virus, Ebola virus, Chikungunya virus, SARS-CoV, and Middle East respiratory syndrome-related coronavirus, some of which have high mortality rates and lack specific therapies. For example, the annual incidence of severe influenza is approximately 3−5 million worldwide, and there are around 290,000 to 650,000 deaths related to respiratory diseases each year[104]. The annual incidence of dengue fever worldwide is about 390 million cases[105]. Furthermore, it is very difficult to eradicate chronic infectious viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV), which may infect large numbers of people. For example, about 40 million people worldwide are infected with HIV; in 2019 the incidence of new cases was estimated to be 1.7 million and the number of deaths from HIV-caused illnesses was about 700,000[106]. Similarly, about 71 million people worldwide are infected with HCV, and approximately 399,000 people died of it in 2016[107]. In antiviral research, boronic acids are expected to be useful to facilitate drug binding to the target protein even in the presence of resistance mutations, by forming multiple hydrogen bonds or a covalent adduct. The glycan-binding property of boronic acid derivatives can also be utilized to interfere with viral binding to the cell membrane and entry into the cell.

Windsor et al.[25] inspected the interactions of darunavir (**51**) and its analogues (such as **52**−**55**) with the S2′ subsite of HIV-1 protease. Considering potential hydrogen bonds that might be formed by a boronic acid group, they replaced the aniline moiety in darunavir with phenylboronic acid (PBA), obtaining compound **56** (K_i = 0.5 ± 0.3 pmol/L), which showed 20-fold increased affinity for HIV-1 WT protease. The analogues **52**−**55** showed only less than 2-fold increases in affinity compared with darunavir (K_i = 10 pmol/L). More importantly, compound **56** retained high affinity for the drug-resistant D30N variant (K_i = 0.4 ± 0.3 pmol/L). X-Ray crystallography demonstrated that the boronic acid group participates in three hydrogen bonds (Fig. 22). In particular, the hydrogen bond between the boronic acid hydroxyl group and the Asp30 (or Asn30) carbonyl group was short (rO···O = 2.2 Å) and density functional theory analysis revealed a high degree of covalency. These data highlight the ability of boronic acids to form multiple hydrogen bonds or robust hydrogen bonds with a high degree of covalency, thereby enhancing the affinity for both wild-type and drug-resistant HIV-1 proteases.

Compound **57** is a non-nucleoside polymerase (NS5B) inhibitor of hepatitis C virus. Introduction of boronic acid moieties into compound **57** enhanced the *in vitro* potency towards wild-type HCV replicons and clinically relevant polymorphic and resistant HCV mutant replicons, resulting in a clinical candidate, GSK5852 (**58**)[108]. However, GSK5852 showed a short plasma half-life ($t_{1/2}$ = 5 h) in human volunteers as a result of facile benzylic oxidation. To overcome this issue, GSK8175 (**13**) was designed by excising the benzylic methylene group and introducing *N*-phenyl sulfonamide analogues[26]. GSK8175 displayed a 60−63 h half-life and caused a robust decrease in viral RNA levels in HCV-infected patients. The co-crystal structure of GSK8175 bound to GT 1a 316Y protein revealed that boronic acid contributes a network of six hydrogen-bonding interactions mediated by water molecules (Fig. 23). Thus, boronic acid appears to promote the binding of NS5B by establishing multiple direct and through-water H-bond interactions. GSK8175 has completed phase I clinical trial for treating HCV infection[19].

Influenza A virus (IAV) neuraminidase (NA) protein, a glycoprotein, can facilitate IAV attachment and entry into infected cells in the early period of viral life cycle[109,110].

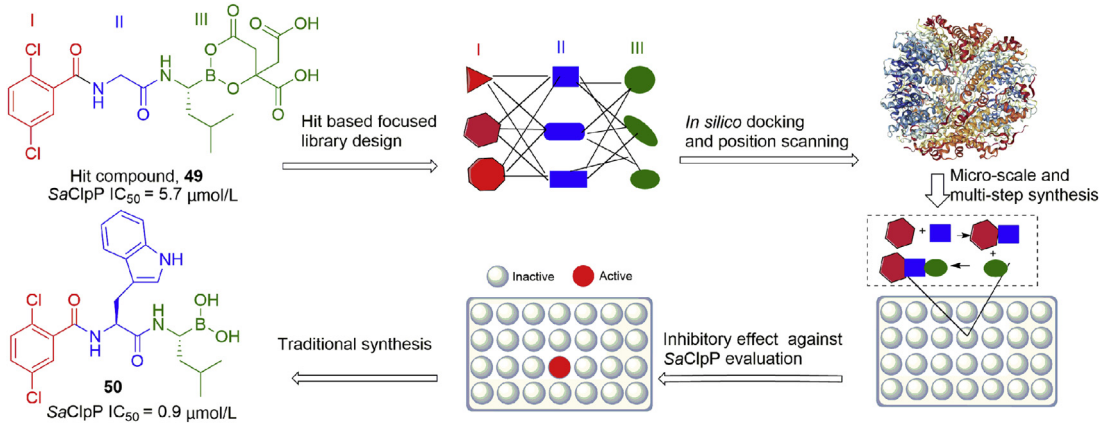


Figure 21 Integrated strategies to optimize hit compound **49**.

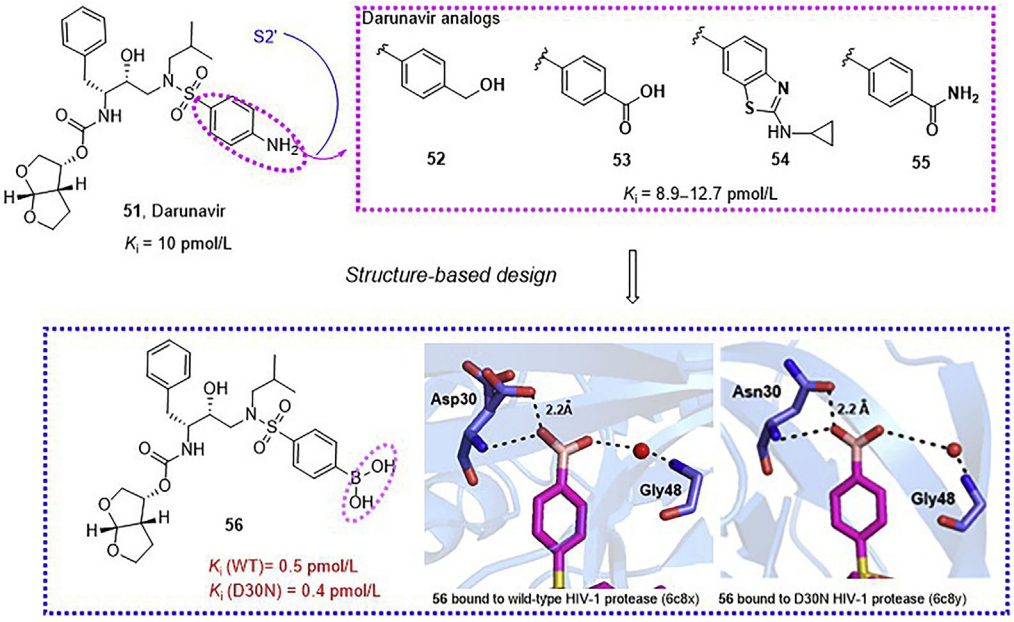


Figure 22 Structures of compounds **51**–**56** and interactions between **56** and the S2′ subsite of HIV-1 protease.

Boronic acid derivatives possess good glycan-binding ability and reduce the cytotoxicity of some quindoline derivatives[111], and Wang et al.[112] utilized these features to design a novel series of novel boronic acid-modified quindoline derivatives as IAV inhibitors to interfere with viral binding and cell entry. Boronic acid-modified compounds, exemplified by **59**, effectively prevented the entry of viral RNP into the nucleus and reduced the virus titer in IAV-infected cells. For example, in virus yield reduction assay, compound **59** possessed a broad antiviral spectrum against all strains tested including H1N1 strains

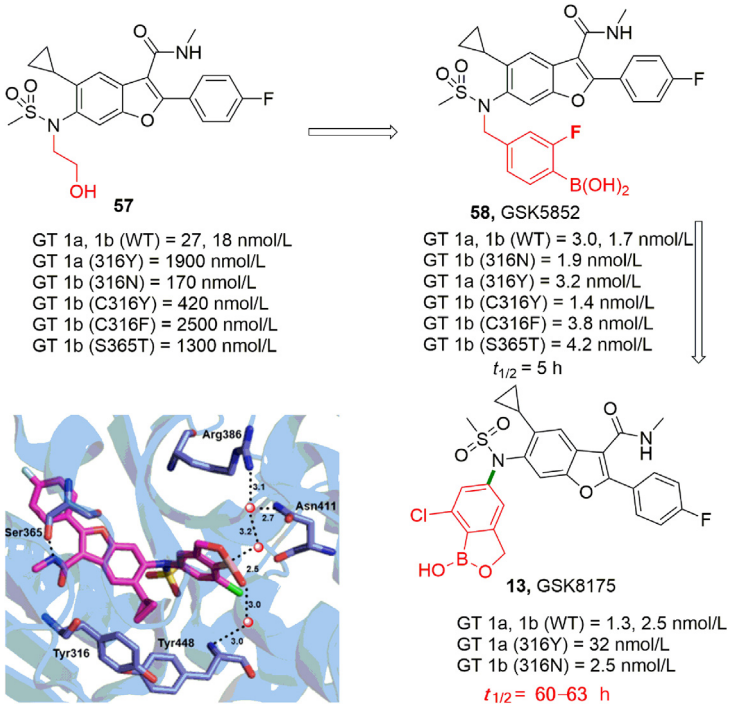


Figure 23 The design of GSK8175 and the cocrystal structure of GSK8175 bound to GT 1a 316Y protein (PDB ID: 6MVO).

A/California/04/2009 (Cal09) and A/Puerto Rico/8/34 (PR/8) and H3N2 strain A/swine/Minnesota/02719/2009 (Minnesota) with IC_{50} values of 4.3 ± 0.7, 2.5 ± 0.4, and 3.0 ± 0.4 μmol/L, respectively, being superior to boronic acid-free compound **60** (25.5 μmol/L, Fig. 24). More importantly, IAV-infected mice treated orally with compound **59** showed better survival rates than oseltamivir-treated mice. The results of NA inhibition assay and molecular docking indicated that boronic acid modification may enhance the interaction between NA protein and quindoline derivatives to block viral infection.

Introduction of a boronic acid moiety into C-terminal of dipeptidic inhibitors against Zika, West Nile, and dengue viral proteases achieved a thousand-fold affinity gain compared to the amide or carboxylic acid analogues (**61** and **62**)[113]. The most active compound **63** had K_i values of 0.051, 0.082 and 0.040 μmol/L, respectively. Crystallographic study showed that boronic acid can form a five- or six-membered ring with glycerol, suggesting that it would be able to take a variety of three-dimensional shapes to interact with surrounding amino acid residues in the target protein. Indeed, in the crystal structure of **63** in complex with ZIKV NS2B-NS3 protease[114], a six-membered boronate structure was observed, in which the boron atom forms a covalent bond with Ser135 and two oxygen atoms are linked to Gly133 and His51 through hydrogen bonds (Fig. 25B). In the case of the West Nile virus[113], the NS2B-NS3 protease recognizes a five-membered boronate structure with similar interactions (Fig. 25C). But, although the boronic acid moiety significantly contributes to the target affinity, the antiviral effect in cells is relatively weak. This is attributable to the high polarity of the boronic acid moiety and the basic side chain, which hinders passage through the cell membrane. To overcome this issue, the boronic acid was modified by esterification with diols to obtain prodrugs.

5. Boron-containing antiparasitic agents

Malaria is a life-threatening disease, and *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest threat among the five parasitic species that cause malaria in humans. In 2018, the incidence of malaria worldwide was about 228 million cases, and the estimated death toll was 405,000. Artemisinin combination therapies (ACTs) have become an indispensable tool in the control of malaria. However, the emergence of resistance to both artemisinin and partner drugs is threatening the efficacy of ACTs, especially in Southeast Asia[115]. New agents are needed urgently, preferably with novel mechanisms of action, and boron-containing compounds, especially benzoxaborole, seem to be promising candidates. Benzoxaborole exhibits good antimalarial activity against a variety of plasmodial species including *P. falciparum* strains, as well as high bioavailability. Further, it is easy to synthesize, with a low cost of production. Importantly, some boron-containing compounds appear to have a novel antimalarial target and new mechanism of action, and therefore may have potential

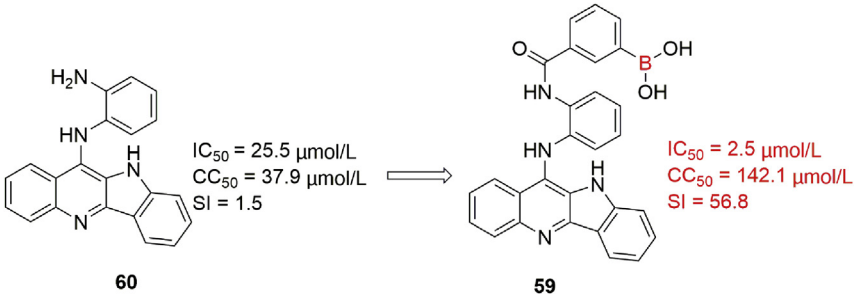


Figure 24 Structures of compounds **59** and **60**.

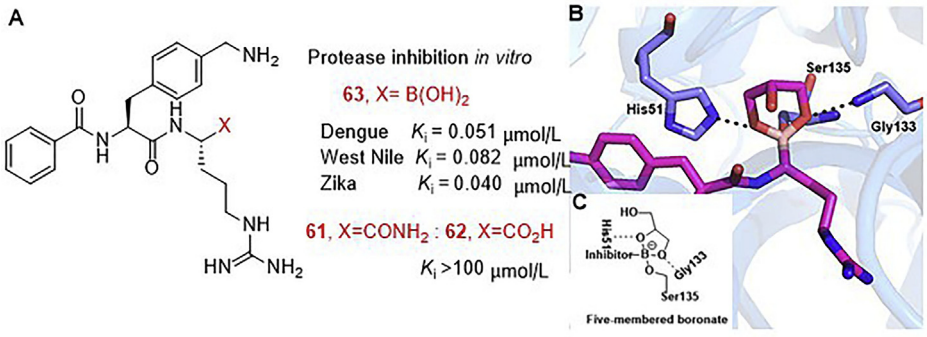


Figure 25 (A) Structures of compounds **61**−**63**. (B) Crystal structure of the ZIKV NS2B-NS3 in complex with compound **63** (PDB ID: 5LC0). (C) Schematic illustration of **63** in the substrate-binding site of WNV NS2B-NS3 protease.

for treating multidrug-resistant malaria in combination with other drugs.

In a search for new antimalarial compounds, Sonoiki et al.[98] screened a benzoxaborole library rich in LeuRS inhibitors for potency against cultured *P. falciparum*. Two 3-aminomethyl compounds, AN6426 (**64**) and AN8432 (**65**), showed activity against cultured multidrug-resistant (W2 strain) *P. falciparum* (IC_{50} = 310 and 490 nmol/L, respectively) and efficacy against murine *Plasmodium berghei* infection when administered orally once daily for 4 days (ED_{90} = 7.4 and 16.2 mg/kg). Genetic and biochemical studies suggested that AN6426 and AN8432 have a novel antimalarial mechanism of action, *i.e.*, inhibition of *P. falciparum* LeuRS. In the same year, Sonoiki et al.[116] discovered AN3661 (compound **66** in Fig. 26) by screening a benzoxaborole library against cultured *P. falciparum* asexual blood stage parasites. AN3661 was effective against multiple plasmodial species, including *P. falciparum* strains (mean IC_{50}: 32 nmol/L), Uganda field isolates (mean IC_{50}: 64 nmol/L), and *P. berghei* and *P. falciparum* infections (ED_{90}: 0.34 and 0.57 mg/kg, respectively). It was also highly effective when administered orally in mouse models of *P. falciparum* and *P. berghei* infection. Genetic and biochemical studies disclosed that AN3661 targets a homologue of mammalian cleavage and polyadenylation specificity factor subunit 3 (CPSF3).

Recent studies[117] have identified the proteasome of *P. falciparum* as a promising drug target, and combinations of proteasome inhibitors with other drugs appear to have potential for treating multidrug-resistant malaria. Xie et al.[118] screened a library of human proteasome inhibitors (peptidyl boronic acids) and identified selective anti-*Plasmodium* proteasome inhibitors. They obtained four hits showing excellent inhibitory activity and selectivity, as well as significant activity against both artemisinin-sensitive and -resistant parasites. Among them, **67** (Fig. 26) was 56 times more effective than HepG2 against *P. falciparum* cultures, showing 112-fold improvement in selectivity relative to bortezomib. Moreover, substrate profiling revealed that the *P. falciparum* 20S β2 site is more hydrophobic than the human 20S site, offering a path for researchers to design even more selective inhibitors.

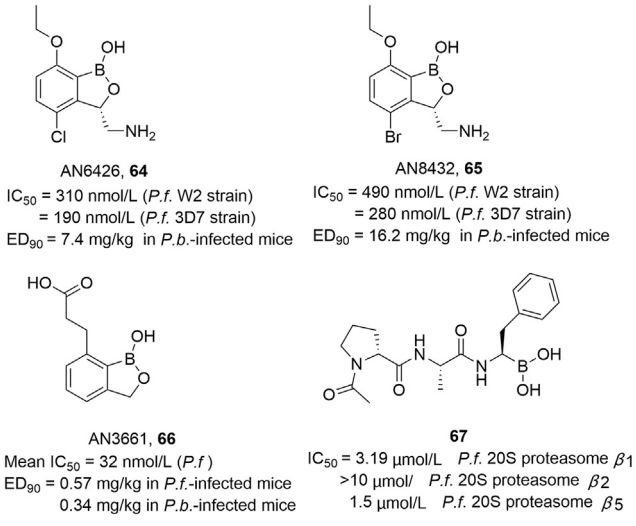


Figure 26 Structures of antiparasitic agents **64**–**67**.

Zhang et al.[119,120] identified a hit compound (**68**) with an IC_{50} of 120 nmol/L against *P. falciparum* from a library screen. To improve the antimalarial activity, they designed and synthesized a series of 6-hetaryloxy benzoxaborole derivatives. Among them, compound **69** showed IC_{50} values of 1.4 and 1.9 nmol/L against *P. falciparum* W2 and 3D7 strains in a mouse model of infection. It also showed excellent *in vivo* efficacy against *P. berghei* (ED_{90} = 7.0 mg/kg). However, it had a poor oral PK profile ($t_{1/2}$ = 1 h, F = 23%) in mice, which was attributed to facile hydrolysis of the methyl ester. Changing the ester to an amide and introducing a 7-Me substituent on the benzoxaborane core improved the PK properties, and the 7-methyl benzoxaborole pyrazine carboxamide scaffold was optimized to give compound **70**. This compound exhibited a longer half-life ($t_{1/2}$ = 5.2 h) and higher bioavailability (F = 96%) in mice. In addition, **70** demonstrated excellent *in vivo* efficacies against *P. falciparum* (ED_{90} = 0.85 mg/kg) and *P. berghei* (ED_{90} = 1.92 mg/kg) in infected mice (Fig. 27). Ames and genetic toxicity test results indicated that **70** is not mutagenic and does not damage micronuclei.

6. Other boron-containing agents

Other activities of boron-containing compounds have also been reported. For example, boronic acid covalently inhibits transthyretin (TTR) to attenuate TTR amyloidosis and covalently inhibits carboxylesterase to block resistance to organophosphate (OPs) insecticides. A new approach to rheumatoid arthritis (RA) therapy has also been reported based on the use of PBA-containing prodrugs that are activated by H_2O_2 in an inflammatory environment. In addition, the coordination properties of boron with diols have been utilized to develop self-assembling dimer agents and have been applied for the purification and modification of proteins.

6.1. *Boron moieties as functional groups for covalent complexation*

The dissociation of TTR, a homotetrameric protein, into monomers is related to the pathogenic formation of fibrils in human amyloidogenic diseases. The binding of a ligand can enhance the conformational stability of TTR, so ligands that stabilize the TTR tetramer are candidates for the treatment of TTR amyloidosis. Smith et al.[21] considered that TTR ligands binding in a covalent but reversible manner would be preferable, and developed boronic acid-based ligands for the T4-binding site of the TTR tetramer. These diboronic acids inhibit fibril formation by both wild-type TTR and a common disease-related variant, V30M TTR, in fibril formation assay. Compound **71** inhibited fibril formation as effectively as the clinically used agent tafamidis, both for wild-type TTR (3% *versus* 0% at 7.2 µmol/L) and for the V30M variant (8% *versus* 8% at 7.2 µmol/L). Co-crystallographic data of TTR with diboronic acid **71** revealed that the boronic acid moiety forms a boronic ester with Ser117 through reversible covalent bond formation (see Fig. 28).

Pesticides and insecticides such as organophosphates (OPs) and carbamates are vital tools for increasing agricultural productivity. The most common resistance mechanism of insects to OPs and carbamates involves carboxylesterases (CEs). Therefore, CEs such as αE7 from the agricultural pest *Lucilia cuprina* are targets for inhibitors designed to eliminate resistance to insecticide.

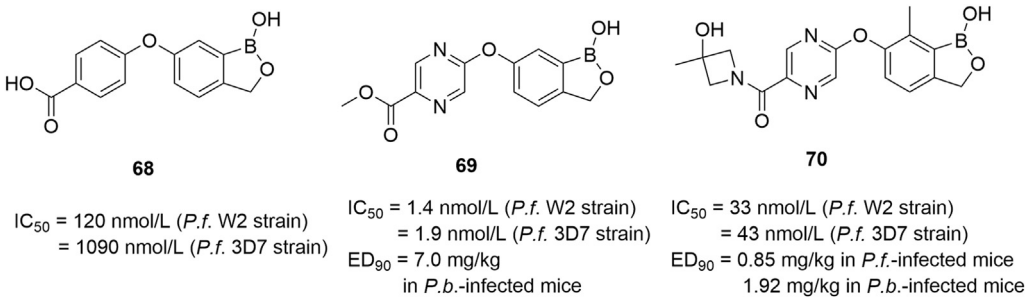


Figure 27 Structures of antiparasitic agents **68**–**70**.

Correy et al.[121] screened a virtual library of ~23,000 boronic acid derivatives against the crystal structure of αE7 using DOCKovalent, an algorithm for screening covalent inhibitors. They identified covalent inhibitors of WT αE7 with IC_{50} values ranging 520 pmol/L to 25 nmol/L. Co-crystal structures revealed that boronic acids form both trigonal planar and tetrahedral adducts with Ser218 of *Lc*αE7 (Fig. 29). Further structure–activity relationship analysis led to compound **72**, which is a potent inhibitor of WT *Lc*αE7 (IC_{50} = 0.9 nmol/L) and a common Gly137Asp variant (IC_{50} = 35 nmol/L). Compound **72** is highly selective for *Lc*αE7 over other *L. cuprina* serine hydrolases and human AChE, and shows little or no toxicity to human cells and mice. These inhibitors act synergistically with the OPs diazinon and malathion, achieving an EC_{50} reduction by up to 16-fold and restoring the sensitivity of resistant strains to OPs. Moreover, the compounds significantly increase the efficacy of chlorpyrifos, another OP, against *Myzus persicae* (a common pest), indicating broad-spectrum potential for boronic acid compounds as a class of insecticide synergists to overcome OP resistance.

6.2. *Boronic acids as hydrogen peroxide-sensitive prodrugs*

RA is an autoimmune disease whose pathogenesis is associated with oxidative stress. Under pathological inflammatory conditions, the extracellular concentration of H_2O_2 can reach 1.0 mmol/L, which is 100-fold higher than in healthy tissue[122]. In order to reduce the side effects of methotrexate (MTX, **73**) and aminopterin (AMT, **74**) RA therapy, Peiró Cadahía et al.[123] designed and synthesized hydrogen peroxide-sensitive prodrugs of MTX and AMT based on a PBA group linked to MTX or AMT *via* a carbamate linkage or a direct C–N bond (Fig. 30). Pathological concentrations of H_2O_2 activate the prodrugs **75** and **76** to release the parent drug. Compared to the parent drugs, the prodrugs exhibited moderate to good solubility, high chemical and enzymatic stability, and comparable efficacy with lower toxicity in a collagen-induced arthritis (CIA) mouse model of RA.

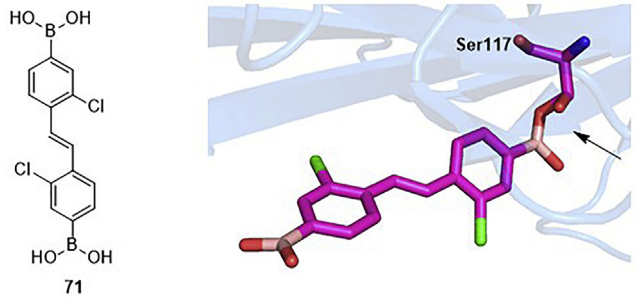


Figure 28 Planar structure of diboronic acid **71**, and structure of the wild-type transthyretin complexed with **71** (PDB ID: 5u4f).

6.3. *Boronic acids as linker components*

One approach to bypass the poor membrane permeability and poor pharmacokinetics of many high-molecular-weight compounds is to utilize bioorthogonal reactions, which proceed under physiological conditions, and are unaffected by biological small molecules or proteins[124]. For example, monomers consisting of ligands and paired bioorthogonal linkers can be delivered to the cell, pass separately through the plasma membrane, and then spontaneously dimerize to form an active inhibitor (Fig. 31). This strategy is easily adaptable to a wide range of targets, especially challenging drug targets.

Many bioorthogonal linkers can be utilized. For example, both copper-free click chemistry and Staudinger ligation exhibit bioorthogonality. However, the usefulness of the Staudinger ligation is limited by the slow reaction rate and the oxidation of reactants, while copper-free click chemistry also has limitations, though the reaction shows better kinetics (9.0×10^{-2} L/mol·S) than the Staudinger ligation[125,126]. In contrast, aryl boronic acids react reversibly with vicinal hydroxyl groups to produce boronate esters in a rapid equilibrium, and the reaction between PBA and salicyl hydroxamic acid (SHA) shows extremely fast kinetics [$(7.01 \pm 2.04) \times 10^6$ $L^2/mol^2 \cdot S$]. Multivalent bioactive inhibitors can be easily produced by using linkers containing multiple SHA moieties. Compared with the interaction between vicinal diol and

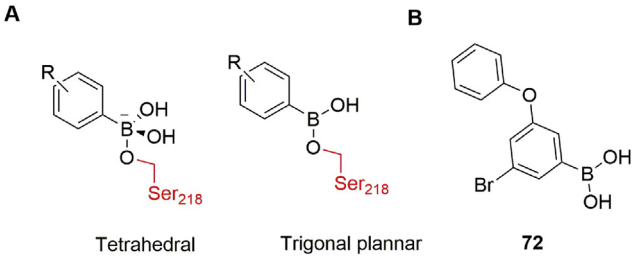


Figure 29 Structures of tetrahedral and trigonal planar boronic acid adducts (A) and compound **72** (B).

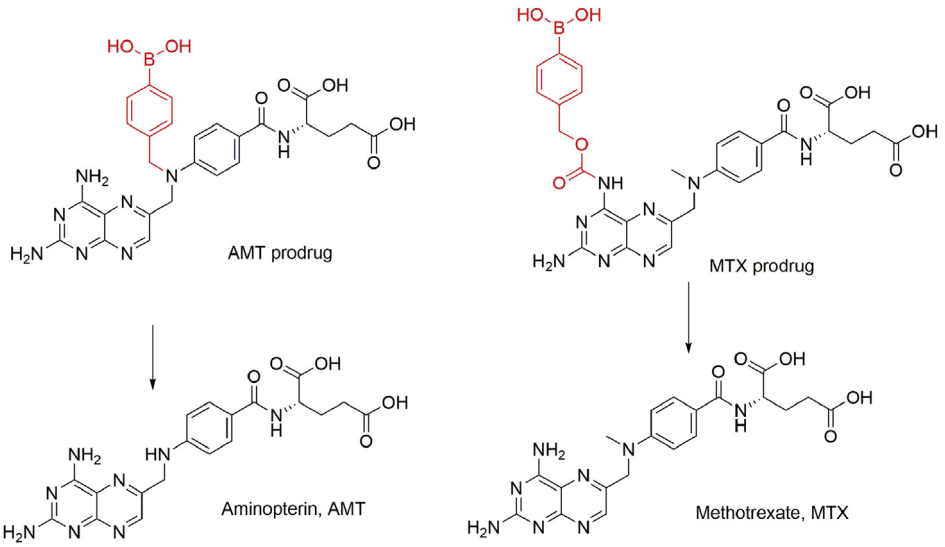


Figure 30 Chemical structures of prodrugs of AMT and MTX.

boronic acid, SHA interacts with boronic acid through oxygen and nitrogen, which increase the resistance of the borate esters to hydrolysis (Fig. 32). Importantly, these boronic acid esters show pH-dependent hydrolysis, and are expected to be cleavable under cellular conditions. According to Shin et al.[36,127], this reaction is not affected by common molecules such as amino acids, D-glucose, and ions in biological solutions. Thus, aryl boronic acids and paired diols are attractive bioorthogonal linkers that can be used to connect ligands in the intracellular environment, enabling the efficient delivery of potent and highly selective bivalent drugs in the form of small-molecular monomers.

β-Tryptase, a homotetrameric serine protease with four identical active sites, is the most abundant protein in human mast cells, and its release is related to many inflammatory and allergic responses. Thus, it is an attractive target molecule for drug development[128]. Giardina et al.[129] developed self-assembling dimeric inhibitors of tryptase by using boronic acid linkers and complementary catechols or *cis*-alkyl hydroxy partners to reversibly join two tryptase ligands. Co-dosing of successful combinations resulted in marked potency improvements over the monomers alone, and co-crystal structure analysis confirmed the presence of the dimer. For example, **77** and **78** formed a single stereoisomeric covalent boronate diester dimer (**79**), spanning approximately 27.3 Å and binding two adjacent subunits (Fig. 33), which exhibited an IC_{50} of 37 nmol/L, a 10-fold improvement over the more potent monomer. Further the heterodimer **82** formed from **80** and **81** exhibited a 35-fold improvement in IC_{50} in comparison to the more potent of the two monomers (22.1 nmol/L), and formed a phenolic-hydroxamate/boronate complex. The heterodimers showed more than 600-fold selectivity against related trypsin-family proteases. Plasma components had little effect on the synergy or efficacy of the compounds, and in the presence of the biological target, the boronic acids interact selectively with the target instead of naturally occurring diols.

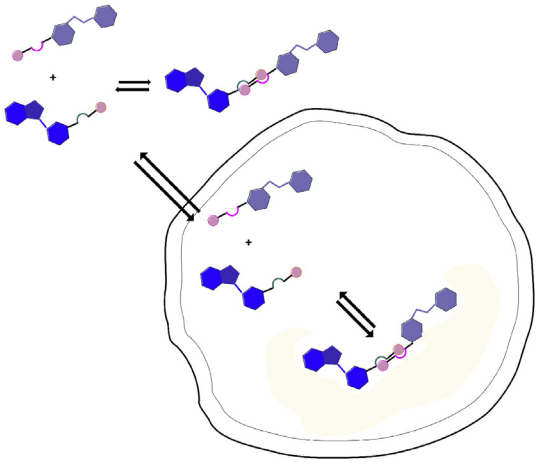


Figure 31 Schematic representation of the self-assembling dimer approach.

Recently, Pieszka et al.[130] designed a peptide sequence that undergoes a multi-stage transformation, ultimately leading to the programmed death of cancer cells (Fig. 34). The first stage, based on dynamic formation of the PBA−SHA complex, involves linking the boronic acid-modified pro-assembling sequence to a transporter TAT (trans-activator of transcription) sequence that induces cellular uptake. After entering the cell, the boronic acid−salicylhydroxamate complex is cleaved under the acidic conditions, releasing the pro-assembling sequence. Endogenous or pathological hydrogen peroxide in cancer cells cleaves the boronic acid masking group to generate the isoleucine-serine-alanine (ISA) self-assembling motif that promotes the generation of fibrillar architectures. The formation of these superstructures, which can be imaged by fluorescence and electron microscopy, leads to programmed cell death.

Boronic acids are also promising candidates for modifying and purifying proteins due to their small size, high biocompatibility, bioorthogonality, stability and reversibility. Zegota et al.[131] reported the purification and reversible assembly of protein

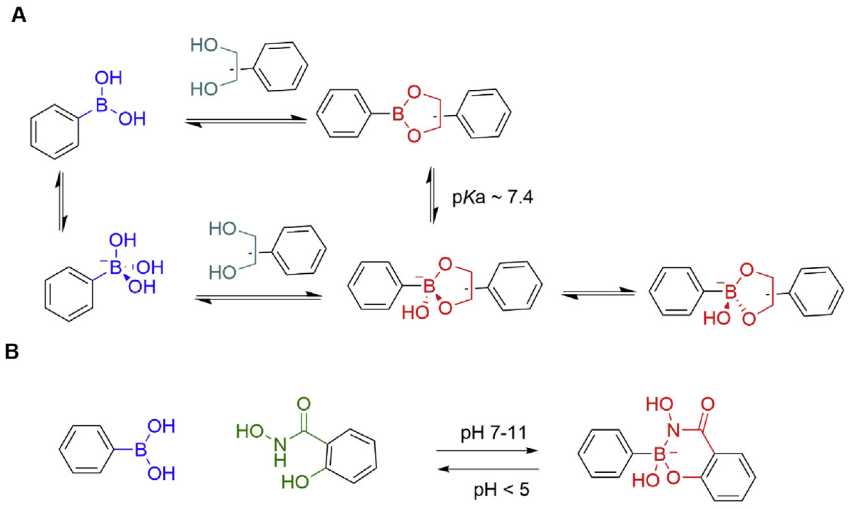


Figure 32 Schematic representation of the equilibria of boronic acid and diol/salicylhydroxamic acid that are utilized for dimer formation.

conjugates based on dynamic formation of the PBA-diol and PBA−SHA complexes (Fig. 35). Incorporation of the boronic acid tag into the protease (lysozyme) by reaction between disulfide bond of lysozyme and vinyl sulfone rebridging reagent bearing a PBA moiety was accomplished, and allowed the straightforward purification of BA-lysozyme by carbohydrate-based column chromatography. SHA-modified fluorescent dye (BODIPY FL) can be used for fast and efficient reversible functionalization of BA-lysozyme to obtain a stable biological conjugate using the interaction between boronic acid and SHA. The conjugate retains the original enzyme activity and remains intact in the cell, but can be cleaved by slight pH changes to allow further modification.

7. Conclusions and perspectives

This article illustrates the diverse applications of boronic acids in the construction of therapeutically useful bioactive molecules. Briefly, boronic acid group is unique in several ways as follows. First, boron-containing compounds bind to biological targets through reversible covalent bonds, multiple hydrogen bonds or metal ion coordination bonds, facilitating biological activity as well as overcoming drug resistance. Second, such compounds can serve as prodrugs to improve drug targeting. Third, the boronic acid moiety is inaccessible to glucuronidation, and therefore can enhance bioavailability by blocking this metabolic pathway. Fourth, the boronic acid moiety can be designed to target unique bacterial glycoproteins or extracellular glycans. Fifth, bio-orthogonal reactions involving boronic acid can be used to generate larger molecules intracellularly in order to overcome permeability issues. Sixth, boron nitride nanotubes and nanoparticles can be utilized for drug delivery. Seventh, boron cluster compounds can be used for boron neutron capture therapy.

In the past years, boron had always been ignored by medicinal chemists due to toxicity concerns in drug design. The investigation by Li et al.[2] revealed that the toxicity concerns were derived from boric acid, a component of ant poisons and the toxicity of

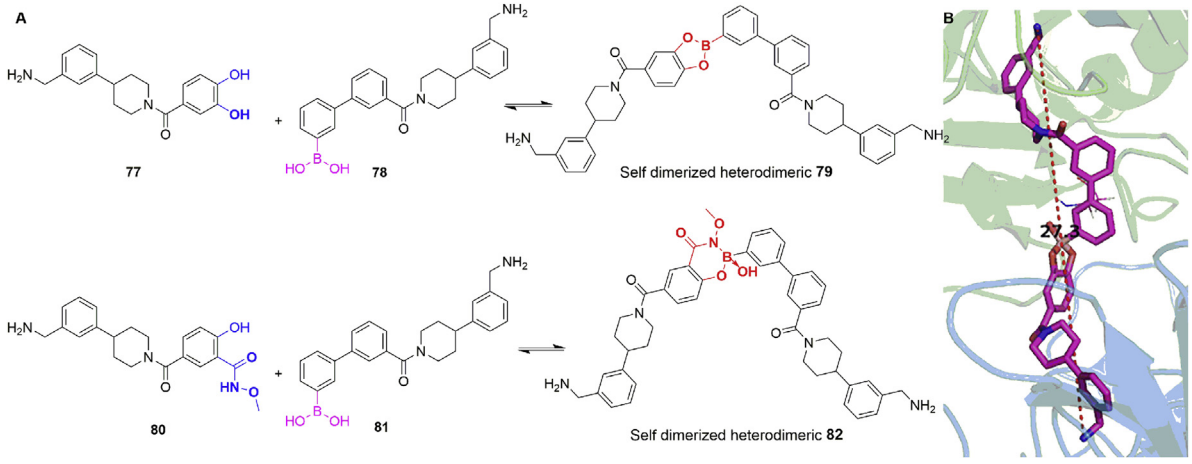


Figure 33 (A) Structures of boronic acid and partner molecules, and the self-assembled dimers. (B) X-ray crystallographic structure of the dimer of **77** and **78** bound to tryptase (PDB ID: 6P0P).

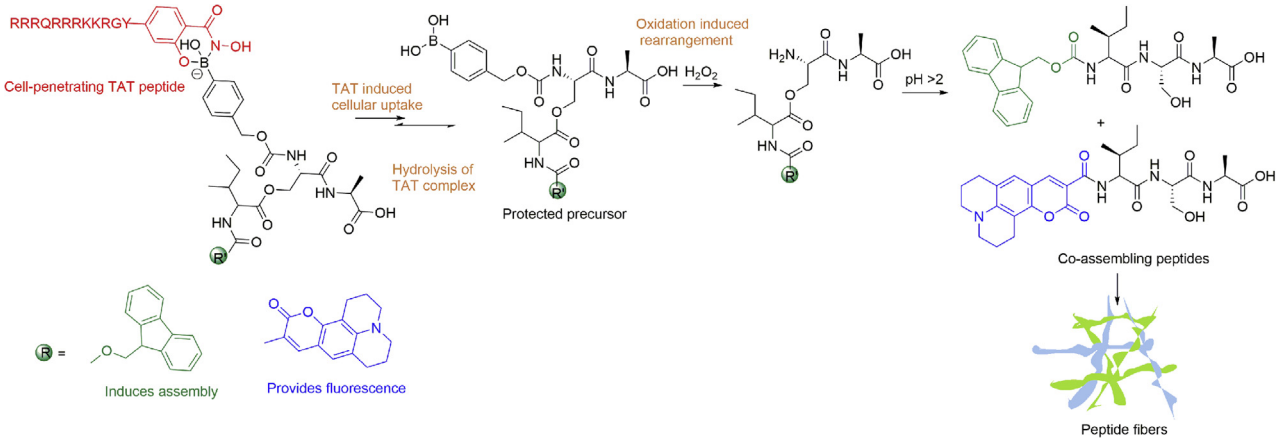


Figure 34 Intracellular co-assembly of peptides.

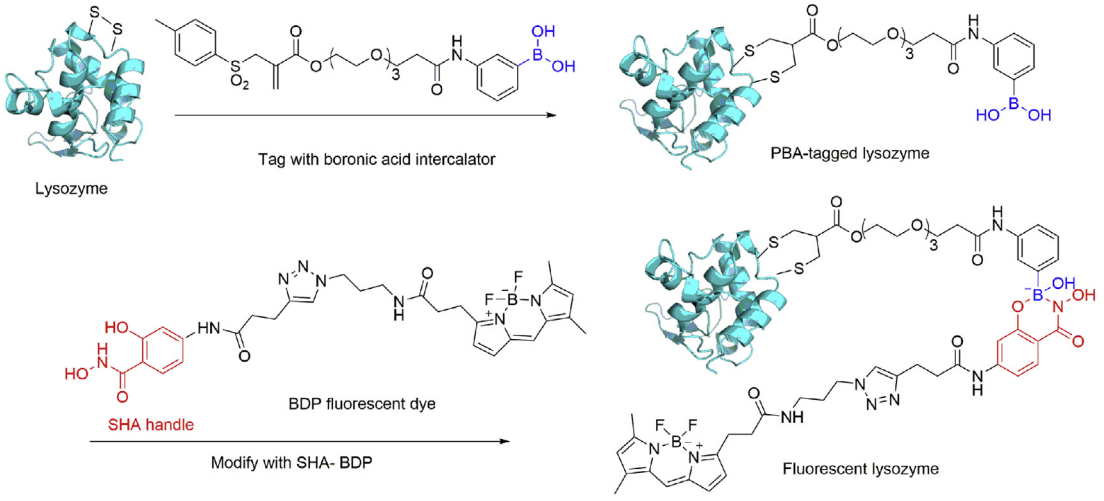


Figure 35 "Tag and modify" protein conjugation with dynamic covalent chemistry.

bortezomib. However, the LD of boric acid is 2660 mg/kg (rats, oral), which is equivalent to the LD value of table salt (3000 mg/kg rats, oral). And the toxicity of bortezomib is due to the mechanism of action rather than the presence of boron in the molecule. Moreover, recent research results show that the intake of boron is beneficial for bone growth and maintenance, brain function, and lower cancer risk[132]. Nevertheless, many challenges remain in extending the applicability of boron compounds. One significant challenge is the technical limitations of virtual screening, which has become an important tool in drug discovery. At present, most covalent docking methods are still too slow to conveniently screen thousands of compounds. Furthermore, during the calculation process, the side chain conformation of the reactive residue is usually assumed to be static, so that the result of the covalent docking depends greatly on the initially selected conformation. This assumption may be particularly problematic for boronic acid derivatives. Some covalent docking programs, such as the web server DOCKovalent developed by the Shoichet lab[133] in 2014, are suitable for screening libraries of commercially available compounds, but no software is currently available for screening large user-defined libraries of covalent ligands.

Another problem is that boronic acids are less stable than aldehydes and acrylates, which are widely used as electrophiles, and thus may be degraded before binding to the target. They also show nonspecific reactivity with endogenous nucleophiles, limiting their specificity for nucleophilic residues at the active site of target enzymes and raising the possibility of off-target effects. It is also relevant that boronic acids are relatively difficult to prepare, especially when they contain C (sp^3)–B bonds[134,135]. To solve some of these problems, boronate esters and boron heterocycles such as benzoborazole and MIDA boronate have been developed. Compared with PBA, the boron−oxygen bond of benzoborazole is not easily hydrolyzed, and the boron−carbon bond (B−C) is stronger. Consequently, under reflux in 10% HCl or 15% NaOH,

benzoborazole is more stable than PBA. Benzoborazole compounds also have higher water solubility than PBA[136]. Furthermore, the tertiary amine group in MIDA coordinates with the empty *p* orbital of boron and prevents unwanted reactions with external nucleophiles; therefore, the MIDA group contributes to increased stability and acts as a slow-release element in a biological milieu[134]. Indeed, the MIDA group has been found to improve the pharmacokinetic profile of dCTPase inhibitors.

The glycan-binding ability of boronic acid has also attracted increasing attention, but boronic acid has a wide specificity for glycoproteins. Therefore, it will be important to develop strategies to adjust the affinity of boronic acid for specific types of glycoproteins. For example, Lu et al.[31] reported a pH adjustment strategy to fine-tune the boronate affinity in order to achieve specificity for two subclasses of glycoproteins.

Better characterization of the targets of boronic acids will be useful to guide the rational design of inhibitors that incorporate additional interactions in order to improve selectivity and to reduce the propensity for development of resistance. At present, the targets of some active compounds, especially antiparasitic and anti-inflammatory agents, are still unknown. For example, the cellular targets and mechanism of action of the anticancer and anti-malarial benzoxaborate remain unclear.

Lastly, boronic acids are commonly used to target serine residues. Therefore, there is considerable scope to expand their applicability to other nucleophiles such as arginine, tyrosine and threonine. In this context, it is interesting that multivalent boronic acids targeting the extracellular glycans of Mtb have a different mechanism of action from monomeric boronic acid. We believe there is still enormous scope to extend the role of boron in drug discovery and development to obtain new agents for treating a range of diseases.

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Author contributions

Xinyong Liu and Peng Zhan were responsible for the conception and design of the review. Shu Song wrote the paper and made all the figures. Peng Zhan, Ping Gao, Lin Sun, Dongwei Kang, Jacob Kongsted and Vasanthanathan Poongavanam were in charge of checking and revision.

Conflicts of interest

The authors declare no conflict of interest.

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