

Design and Discovery of Boronic Acid Drugs.

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Abstract

Research related to boronic acids, from synthetic development to materials to drug discovery, has skyrocketed in the past 20 years. In terms of drug discovery, the incorporation of boronic acids into medicinal chemistry endeavours has seen a steady increase in recent years. In fact, the Food and Drug Administration (FDA) and Health Canada have thus far approved five boronic acid drugs, three of which were approved in the past four years, and several others are in clinical trials. Boronic acids have several desirable properties that has led to their increased use, including potentially enhancing potency of drugs and/or improving their pharmacokinetics profile. This review explores discovery processes of boronic acid drugs. It begins with a brief scope of boron in natural products and in current drugs, followed by an investigation into the various rationalizations for boronic acid incorporation and the synthetic developments that focused on facilitating their addition into organic compounds. We hope that the knowledge we have assembled in this literature review will encourage medicinal chemists to consider the potential benefits of incorporating boronic acids into their future drug discovery endeavours.

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1. Introduction

Boron is ubiquitous in nature, from being an essential component of plant structural integrity [1] and metabolism [2] to being a regulator of mammalian vitamin D levels [3] and bone health [4]. In the form of boronic acids and esters, it is considered to be safe [5] for incorporation into pharmaceutical development.

In fact, instances of boron-based studies, whether for synthetic, biological, or pharmaceutical endeavours have skyrocketed since the late 1990's. Figure 1A illustrates the PubMed literature search results including "boron" in the publication title. More specifically, boron-related drug discovery endeavours have also made increasing appearances in scientific literature. Figure 1B illustrates the PubMed literature search results of "boron" in the publication title and "drug" in the text, showing a steep escalation in usage starting in the 1990's. Boronic acids as drugs are becoming increasingly relevant. In fact, four boron-containing drugs have been approved in the past five years, (Bortezomib as approved in 2005), with several others in clinical trials.

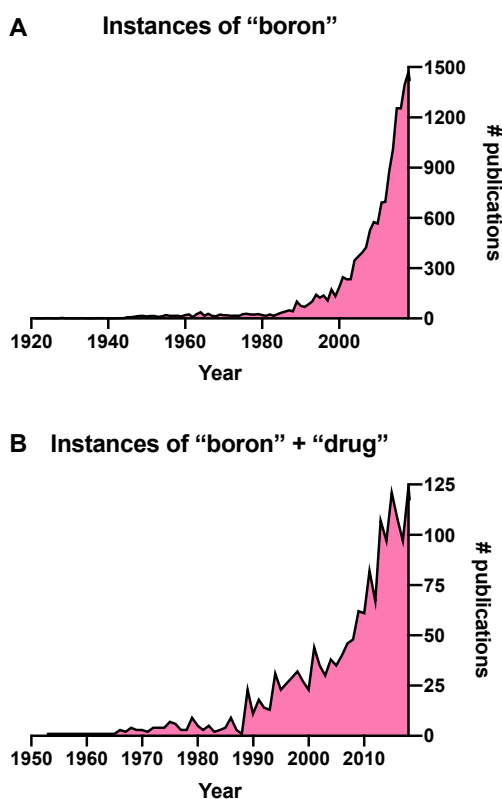


Figure 1: PubMed search results of the terms "boron" (A) and "boron" + "drug" (B).

Despite the use of boronic acids in diagnostic tools [6, 7], Boron Neutron Capture Therapy (BNCT) radiation treatment [8, 9], bioconjugation [10], materials [11], and catalysis [12], among others, our focus in this review is on small molecule drugs containing boronic acids and esters and their associated design and therapeutic application. Reasons for incorporation of boronic acids into drug discovery endeavours vary, ranging from improvement of drug activity to enhancement of pharmacokinetic properties. The discovery process of boronic acids also depends on the approach, such as substrate mimicry or peptidomimetics design, rational design via computational methods, or use as bioisosteres to substitute for certain functional groups.

This review outlines the rationalization of boronic acid use and associated discovery processes, including incorporation of boronic acid moieties into bioactive compounds. While we do include a diverse scope of boron-based drug applications, this review in no means covers the vast span of boronic drug discovery (Figure 1), but instead provides examples of progress made so far in pharmaceutical applications of boronic acids, along with a few examinations of the rationale behind inclusion of boronic acids.

2. Occurrence of boron in nature.

2.1. Boron in bacteria.

Boromycin (Figure 2), isolated from a *Streptomyces antibioticus* strain in African soil, was the first ever natural product found to contain trace amounts of boron [13]. Since then, this macrolide has been studied for its therapeutic properties. One study reported nanomolar potency against several HIV-infected cell lines [14]. Boromycin was also studied for its potent antibiotic activity against several strains, including *Mycobacterium tuberculosis* [15]. A related macrolide, Aplasmomycin, isolated from *Streptomyces griseus* and named for its anti-plasmodium activity, was discovered about 10 years later and has a structure similar to that of Boromycin [16] (Figure 2). In these two natural products, boron has a structural role, inducing the folding of the polyols into compact structures.

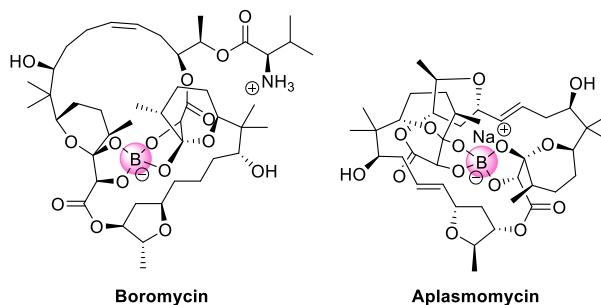


Figure 2: Boron in bacterial-derived antibiotics.

These two bacterial compounds were both recently found to inhibit a biomolecular pathway in *Helicobacter pylori*, bacteria implicated in stomach cancer [17]. Derivatives of these two antibiotics (not shown), including Aplasmomycin B and C and *N*-acetylboromycin, also occur naturally and have also exhibited antibiotic activity. For a more comprehensive review of these natural boron-containing macrolides, see Dembitsky *et al.* [18].

2.2. Boron in plants.

Boron is vital to plants and algae. In fact, boron deficiency in plants is detrimental to survival and often leads to plant death [19]. Similarly to the natural products described above, it is known to form complexes with polysaccharide moieties – through interactions with diols – in the plant cell wall membrane and is therefore essential for structural integrity [1]. The mechanism is not fully understood, but one study of tobacco plants revealed that boron-deficient plants contained more reactive oxygen species (ROS) than control plants. The researchers hypothesize the ROS levels build up as a signalling mechanism when there is a disturbance in the structural integrity of the cell wall as a result of boron loss [19]. Although cell wall assembly seems to be its major role, boron is also known to be essential in several other plant biomechanisms, such as nitrogen fixation and plant metabolism [2].

2.3. Importance in mammalian systems

Although the full extent of boron's roles in mammalian biological systems is not completely understood, several studies link boron to various mammalian biomechanisms. In fact, the World Health Organization (WHO) declares boron as a “probably essential element” for humans [20].

Boron is suggested to be important in mammalian bone health and is present in higher concentrations in bones than in other tissues [20]. A study conducted by Gorustovich *et al.* aimed to determine the effect of boron-deficient diets on dental bone modelling and remodelling. It was found that boron-deficient diets inhibited bone formation when compared to boron-supplemented control diets, although the mechanism was not elucidated. These results were consistent with earlier discoveries that boron deprivation in rats led to decreased bone volume in vertebral development [21]. Another study employing mice with diabetes-induced osteoporosis showed that boron supplements improved bone strength and overall health in not only the diabetic mice, but also in the control group. These results were consistent with other animal studies, and suggest that boron supplementation may be beneficial for bone strength [4]. However, further studies would need to be conducted to demonstrate parallel effects in humans.

Boric acid treatment even advances wound healing. A preliminary study revealed that treatment with 3% boric acid solution on intensive care patients with deep wounds resulted in transfer to normal care three times as quickly as patients receiving standard treatments [22]. Another *in vivo* study of boron delivered in the form of a boric acid solution showed upregulation of synthesis of extracellular matrix proteins responsible for tissue reparation, although further studies are needed to determine the mechanisms of these observations [23].

Several studies have shown that vitamin D deficiencies are compensated by boron supplementation. One study involved boron supplementation in the vitamin-D-adequate or -inadequate diets of chickens. It was found that addition of boron improved overall chicken health (mineral levels, body weight, food consumption, etc.) in both the vitamin-D-inadequate and control groups, though the increase was greater in the vitamin-D-inadequate group [24]. This study, along with other studies relating dietary boron to vitamin

D levels, led to one group hypothesizing that boron's potential mechanism involves inhibition of an enzyme involved in metabolism of vitamin D to an inactive form [3].

Even from the few above studies, it is clear that boron is implicated in several mammalian biomechanisms. For more comprehensive reviews on boron in biological systems, see Uluisik *et al.* [25].

3. Scope of boronic acid drugs

3.1. Approved boron-containing drugs

So far, five approved drugs exist on the market that contain boron (Figure 3). The first to be approved was Bortezomib, marketed under the name Velcade®, approved by the U.S. Food and Drug Administration (FDA) in 2005 [26] and by Health Canada in 2008 [27] for the treatment of multiple myeloma. The structure was originally discovered through the study of substrate mimics in the form of peptidic aldehydes, which, through co-crystallization with the target were found to bind covalently to the nucleophilic threonine residue. However, as aldehydes are unsuitable for further drug development studies, boronic acid analogs were tested and showed high potency [28, 29]. Although several proposed mechanisms of anti-cancer activities have been reported, its major mechanism of action involves the ubiquitination pathway of protein degradation; Bortezomib is a proteasome inhibitor, blocking the degradation of apoptotic proteins in tumour cells [30]. Through co-crystallization studies, it is suggested to act as a reversible covalent inhibitor, blocking the action of nucleophilic threonine residues in the active sites of the proteasome [31].

Ninlaro®, or Ixazomib, similarly to Bortezomib, was approved by the FDA in 2015 [32] and by Health Canada in 2016 [33] for treatment of multiple myeloma [32] and is a second generation proteasome inhibitor [34]. It is the first oral proteasome inhibitor [32], as Velcade® (Bortezomib) is currently administered as weekly injections [26]. Ixazomib was discovered from a screening of boron-containing proteasome inhibitors with improved pharmacokinetic properties over Bortezomib. Its mechanism of action was found to be nearly identical to that of Bortezomib, yet it was found to be more potent, less prone to inducing adverse side effects (higher specificity), and even to treat certain patients whose tumours have developed resistance to Bortezomib [34]. Although approved, Ixazomib is also under clinical trials as part of combination therapies to treat multiple myeloma [34, 35].

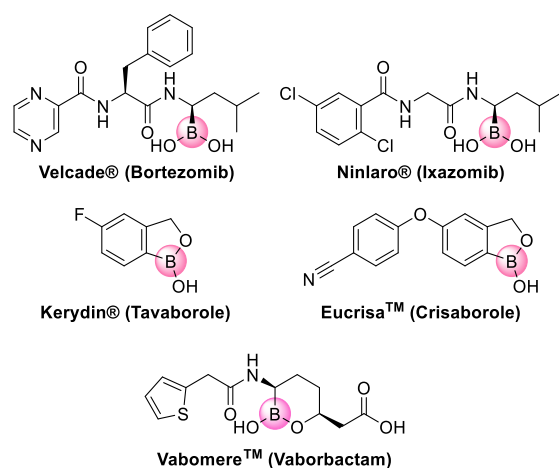


Figure 3: Approved boron-containing drugs

Kerydin®, or Tavaborole, received global approval [36] by the U.S. FDA in 2014 to treat onychomycosis, a fungal infection [37]. Its structure was originally discovered through structure-activity relationship (SAR) studies of a similar anti-bacterial borinic ester. Upon testing against several types of fungi, it was found to have broad-spectrum antifungal activity [38, 39]. Tavaborole's mechanism of action is believed to involve the inhibition of fungal Leucyl-tRNA synthetase, preventing protein synthesis and thus fungal growth, and is three orders of magnitude more selective for fungal Leucyl-tRNA synthetase than the human equivalent. The necessity of the boron-containing moiety was confirmed by 50-fold loss of inhibitory activity upon testing analogues that substituted boron for carbon [40].

Eucrisa™, or Crisaborole, was approved in 2016 by the U.S. FDA [41] and by Health Canada in 2018 to treat mild to moderate eczema [42]. It was discovered by the same research group as that who discovered Tavaborole through a screening of a boron-containing compound library against phosphodiesterase 4 (PDE4) and cytokine release factors, both implicated in anti-inflammatory response pathways [43].

Vabomere™ is a combination drug (Figure 1 includes only the boronic acid component) approved by the U.S. FDA [44] and Health Canada in 2017 [45] to treat bacterial infections [46]. It includes Vaborbactam, a β -lactamase inhibitor, and Meropenem, an inhibitor of bacterial cell-wall synthesis. Although Vaborbactam is not an antibacterial itself, it is administered in combination with the carbapenem Meropenem to prevent its hydrolysis by β -lactamases [47]. In terms of its discovery, boronic acids were already known to be potent β -lactamase inhibitors through their reversible covalent bond with catalytic serine residues [48]. Vaborbactam was therefore designed by structure-based modifications of various known active analogues. It was intended to be a reversible covalent inhibitor, and crystallography studies confirm its covalent complexation with the catalytic serine (pdb: 4XUZ) [49]. Furthermore, the researchers successfully induced selectivity over other mammalian serine proteases through incorporation of a *cyclic* borinic acid, which would not fit in the smaller active sites of native serine proteases with more flexible substrates [49].

3.2. Boron-containing drugs under investigation

Although not yet approved, there are several boronic acid drugs under investigation in clinical trials (Figure 4).

Dutogliptin [50] is a dipeptidyl peptidase 4 (DPP4) inhibitor. It failed in Phase II clinical trials for diabetes mellitus, but it is now under investigation in a combination therapy with granulocyte-colony stimulating factor (G-CSF) to treat myocardial infarctions. While implicated in diabetes, DPP4 is also responsible for degradation of factors responsible for recruiting stem cells for cardiac muscle repair. A Phase II trial of co-administration of Dutogliptin with G-CSF, a stem cell mobilizer [51], is currently underway [52].

Acoziborole, also referred to as SCYX-7158 or AN5568, is a parasite-inhibiting drug candidate to treat Human African Trypanosomiasis (HAT) [53], although neither its biological target nor its mechanism of action is known [54]. Current available HAT treatments are unfortunately quite cytotoxic and lack efficacy. Acoziborole, on the other hand, is safe, orally bioavailable, and has the potential to be administered in one sole dose [53]. It is currently in Phase III trials [55].

GSK3036656, another benzoxaborole compound, is a leucyl-tRNA synthetase inhibitor for treatment of Tuberculosis infections [56]; it was designed to be a reversibly covalent inhibitor that binds to Ade76 of tRNA and prevents RNA synthesis [57]. It's structure is a modified version of GSK2251052, or AN3365, which failed in Phase II due to development of resistance [58]. An SAR study produced GSK3036656, a potent inhibitor with favorable pharmacokinetic properties that shows selectivity for bacterial leucyl-tRNA synthetase over the human homologue [57]. This compound is currently in Phase II studies for Tuberculosis [59].

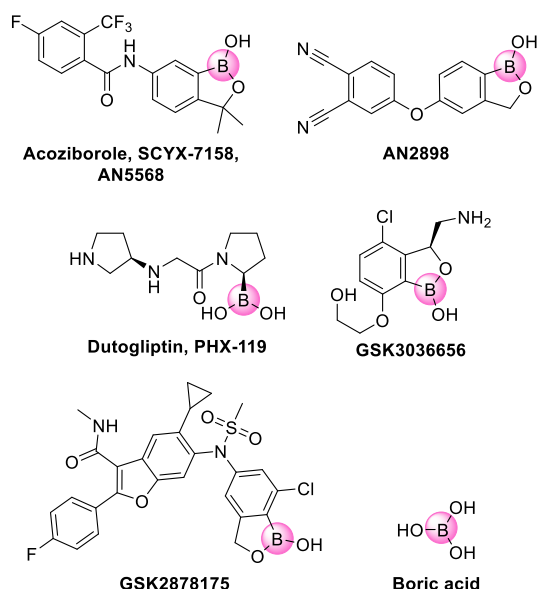


Figure 4: Boron-containing drugs in clinical trials

Similar to the already-approved Crisaborole is AN2898, another phosphodiesterase 4 inhibitor for the treatment of atopic dermatitis [60]. Clinical trials are ongoing, but in a Phase II study, it was deemed to be safe and effective for treatment [61]. As can be seen in Figure 3 and Figure 4, the structures are nearly identical, save for one extra nitrile in AN2898's side chain.

An antiviral compound, **GSK2878175**, has completed Phase 2 clinical trials [62] as a combination therapy targeting the Hepatitis C virus RNA polymerase NS5B enzyme. Its design stemmed from optimizations of the metabolic profile of a failed clinical candidate. After several rounds of structural modification, *in vitro* and *in cellulo* assays confirmed potent activity of **GSK2878175**, and *in vivo* studies confirmed its superior pharmacokinetic profile [63].

Although not a synthetic drug, boric acid itself is currently in Phase II/III clinical trials as BASIC (Boric Acid, Alternate Solution for Intravaginal Colonization), formulated as a cream to treat bacterial vaginosis (BV) [64]. Separate Phase IV clinical trials are also ongoing, testing a boric acid in combination with probiotics – as a combination capsule – to treat BV and candidiasis, or yeast infection [65].

While there are only a handful of boron-containing drugs currently in clinical trials, several have been halted for various reasons. One example is AN3365, mentioned above. Currently, however, studies of analogs are underway which have produced compounds that appear to evade this resistance [66]. Others include Talabostat (PT-100), a multi-target anti-cancer drug which failed in Phase III [67] ; PHX1766, an HCV protease inhibitor that failed in accelerated Phase I trials [68] ; and Delanzomib, a proteasome inhibitor similar to Bortezomib and Ixazomib that failed in Phase I/II trials due to limited efficacy [69].

Based on the frequency of boron drugs reaching Phase II clinical trials, it is likely there will be further developments and more approvals in coming years.

3.3. Over-the-counter boron-containing drugs and supplements

As discussed earlier, elemental boron supplements have been used in many animal studies to investigate the role of boron in mammalian systems. Although not approved by the FDA, general safety has led to the sale of boron supplements as long as they are not labeled as a treatment for any disease [70].

Although it is undergoing clinical trials as a cream to treat BV [48], boric acid solutions and powders have been available over-the-counter for many years, such as for ophthalmic [71] or vaginal [72] use, though its effectiveness may be questionable.

Calcium fructoborate (CF), sold under FruiteX-B®, is found naturally in fruits and vegetables and is a complex of fructose with boronic acids (intracellular) or esters (extracellular) [73]. It is sold as a supplement whose claims include improvement of bone and cardiovascular health. Although these claims are not fully

substantiated, a double-blind study on middle-aged adults with osteoarthritis did conclude that CF led to improvement in quality of life of patients in the short-term with a favourable prognosis for inflammation. The mechanism of action for this result is not fully known, although *in vitro* studies reveal that CF is responsible for inhibiting the release of proteins responsible for inflammation response (e.g. interleukins) [74]. The claim of improvement in cardiovascular health is still preliminary, although early clinical studies indicate that CF significantly reduces levels of low-density lipoprotein and triglycerides while raising levels of high-density lipoprotein, suggesting that CF may improve cardiovascular health [75]. Despite these results, long-term studies and larger cohorts are necessary for more conclusive results.

3.4. Boron-containing compounds in drug discovery

Though they have yet to lead to approved drugs, there have been countless drug discovery endeavours that have incorporated boron into the target molecules for a variety of therapeutic purposes. The following sections highlight some medicinal chemistry applications of boronic acids so far.

3.4.1. Anti-cancer boron-containing compounds

As described earlier, Bortezomib, or Velcade® (Figure 3), was the first boronic acid drug to be approved by the U.S. FDA for the treatment of multiple myeloma,[26] followed several years later by Ixazomib, or Ninlaro® (Figure 3) [32]. These approvals have led to a surge of boronic acids drug discovery. Furthermore, due to off-target effects and resistance development against Bortezomib,[29] research continues into proteasome inhibitors, especially after the clinical failure of Delanzomib (Figure 5) [69]. Han *et al.* recently conducted an SAR relationship study of urea-containing peptidic compounds as proteasome inhibitors. From this study, they discovered compound **1** (Figure 5). In *in vitro* assays, **1** exhibited sub-picomolar activity against the human 20S proteasome. Furthermore, its activity against eleven human cancer cells lines was consistently in the nanomolar range, and *in vivo* mice assays revealed that not only was its anti-tumour activity similar to that of Bortezomib, but it also exhibited lower toxicity and more promising pharmacokinetic properties. Based on these promising results, this compound is currently in pre-clinical studies [76]. More recently, Lei *et al.* have focused on the discovery of a proteasome inhibitor that would not only be effective for multiple myeloma, but also triple-negative breast cancer. Through an SAR study of Bortezomib and Ixazomib analogues, they discovered compound **2** (Figure 5), an unusual eight-membered ring boronic ester pro-drug, which exhibited low nanomolar activities *in vitro* and *in cellulo* similar to those of the two approved drugs. *In vivo* assays against triple negative breast cancer in mice also yielded promising results, including tumour necrosis. However, the pharmacokinetics of **2** require lead optimization, as *in vivo* bioavailability is low [77].

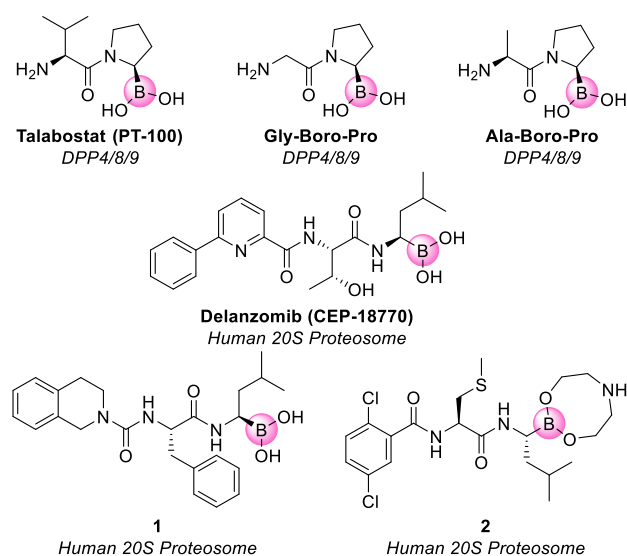


Figure 5: Examples of boronic acid compounds as anti-cancer therapeutics

In much more recent context, the dipeptidyl peptidase (DPP) family of serine proteases, including DPP8 and DPP9, have been discovered to be involved in various biomechanisms in cancer and associated immune

response [78]. Studies utilizing DPP8/9 inhibitor Talabostat (Figure 5), a drug that failed in Phase III as a non-selective DPP/FAP/POP inhibitor [67], show that inhibition leads to induction of cell death through several immune response mechanisms [79]. However, results are still inconclusive, as Talabostat is non-selective and the extent of its mechanisms of action is not fully known [78]. DPP8 and DPP9 are very structurally similar to a widely-studied homologous enzyme, DPP4, and many studies focused on inhibitors, mainly on nitriles [80] but some boronic acids (Gly-Boro-Pro and Ala-Boro-Pro, Figure 5) [81], targeting DPP4 for diabetes treatment were tested on both DPP8 and DPP9 to determine selectivity. Similarly, while there exist several FDA-approved DPP4 inhibitors (e.g. sitagliptin, saxagliptin) [82], DPP4 has more recently been discovered to be implicated in certain epithelial cancers. In fact, preliminary studies have demonstrated that in patients with diabetes and colorectal or lung cancer, DPP4 inhibition is associated with greater overall survival [83], though further studies are required. Additionally, recent accounts associate DPP4 inhibitor use with increased risk for pancreatic cancer, although results so far are inconclusive, as longer-term studies are required [84].

Apart from the proteasome or DPP family, boronic acids in anti-cancer pursuits include modifications of failed drug Combretastatin A-4 targeting tubulin assembly by Kong *et al.* [85], and design of epidermal growth factor receptor (EGFR) tyrosine kinase (TK) inhibitors by Ban *et al.*, both design rationales explored in more detail later [86].

3.4.2. Anti-viral boron-containing compounds

Viral proteases are also common biological targets of boron-based inhibitors, such as the NS3 protease of the hepatitis C virus (HCV). Though there exist approved drugs for the HCV NS3 serine protease [87], research has gone into discovery of inhibitors that replace the α -ketoamide moiety of approved drugs with boronic acid moieties, taking advantage of the catalytic serine in the active site. For example, one group based at Anacor Pharmaceuticals has studied on modifying telaprevir and boceprevir, both linear hexapeptides, with cyclic boronic acids (**3**, Figure 6) [88], but eventually improvements in structure and a few additional HCV NS3 approved drugs danoprevir and vaniprevir [87] led to studies of macrocyclic drug structures (**4**, Figure 6) [89]. More recent examples of viral NS3 protease inhibition with boronic acids include that of flaviviruses such as dengue fever virus (DV) and Zika. One group in particular discovered modified dipeptides (Phe-Arg) containing boronic acids as reversible covalent groups (**5**, Figure 6) that were over one hundred times more active than the carboxylic acid derivatives [90].

On a separate note, the human immunodeficiency virus (HIV) aspartic acid protease was recently targeted with an aromatic boronic acid exhibiting subpicomolar activity (**6**, Figure 6), two orders of magnitude more potent than its previously-published carboxylic acid derivative (Figure 11) and current HIV approved protease inhibitor, Darunavir [91]. In a follow-up SAR study, Ghosh *et al.* [92] studied a set of analogues of Darunavir and this compound. Their study design and rationale are discussed on more detail later, with similar studies.

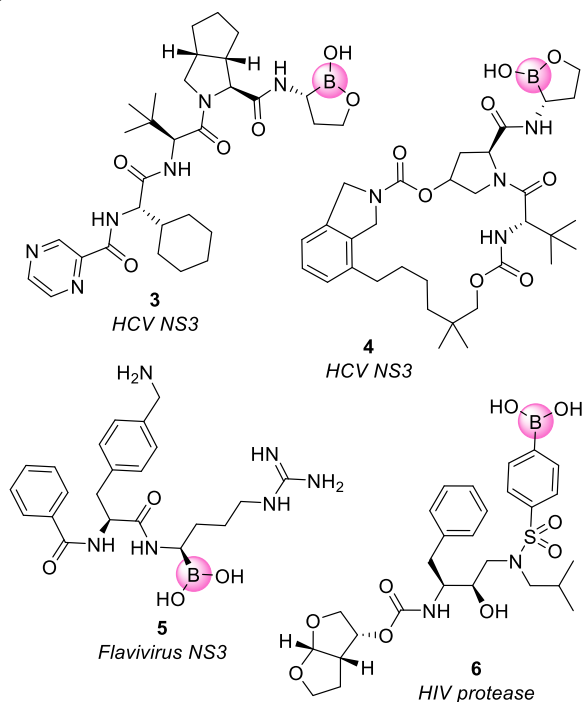


Figure 6: Examples of boronic acid compounds as antivirals

3.4.3. Other anti-infective boron-containing compounds

Boronic acids have been used quite widely in drug discovery studies targeting fungal, bacterial, and parasitic infections. As discussed earlier, some boronic acid drugs have either been approved (Figure 3) or are currently in clinical trials (Figure 4) for anti-fungal or anti-parasitic therapeutics, all of which contain the boronic acid benzoxaborole structure. This scaffold was also recently applied, again by Anacor Pharmaceuticals, to studies of the parasitic infection cryptosporidiosis, yielding compound AN7973 (Figure 7A). This compound exhibited potent *in vitro* and *in vivo* activities against infected mice and showed favourable pharmacokinetics. It is currently in pre-clinical studies.

The bacterial enzyme β -lactamase has also been a target in boronic acid drug discovery, and is the target of approved drug Vaborbactam (Figure 3) [93]. One of the most potent β -lactamase inhibitors reported to date was discovered through a fragment-guided *in silico* design (**7**, Figure 7A) and exhibited sub-nanomolar activity *in vitro* and promising results *in vivo*, though pharmacokinetics need improvement [94]. Nevertheless, drug discovery studies targeting β -lactamase have continued due to increasing need to combat anti-bacterial resistance [93].

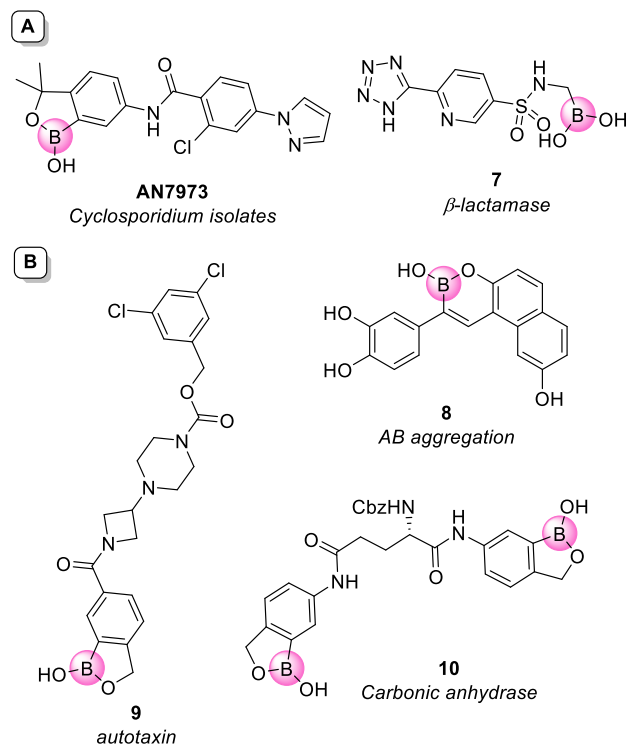


Figure 7: Examples of bioactive boronic acid compounds (A) targeting non-viral infections and (B) for other therapeutic applications

3.4.4. Other therapeutic applications of boron-containing compounds

Besides as anti-cancer and anti-infective therapeutics, boron drugs have a number of other applications. In fact, one recent study discovered anti-Alzheimer's drugs from modifying curcumin, a known amyloid-beta (AB) aggregation inhibitor, to contain a borinic acid moiety (**8**, Figure 7B). After an SAR study, compound was found to not only be a potent AB aggregation inhibitor on the same order as curcumin, but also exhibited good antioxidant activity, as oxidative stress is associated with neurodegeneration [95].

As previously discussed, the benzoxaborole scaffold has been applied over a large scope of enzymes, including the phosphodiesterase 4 (PDE4) inhibitor [43] Crisaborole, to treat mild to moderate eczema [41]. Autotaxin, a target also implicated in inflammation, as well as fibrosis and cancer, has been targeted with boronic acid drugs, including the benzoxaborole scaffold [96] and aromatic boronic acids [97, 98]. Kraljić *et al.* have designed benzoxazole analogues (**9**, Figure 7B) of recently-discovered hits that not only exhibited submicromolar potency against autotaxin, but also very favourable pharmacokinetic properties [96], though further biological studies are needed to confirm potency.

Recently, Larcher *et al.* discovered a series of *bis*-benzoxaboroles targeting carbonic anhydrase, an enzyme implicated in several diseases, though isoform selectivity is difficult.[99] They found their linked *bis*-benzoxaborole inhibitors to be potent against the disease-implicated carbonic anhydrases, while remaining selective over the human cytosolic form. Their most promising compound, **10** (Figure 7B), contains two benzoxaboroles connected by an almost-symmetrical seven-atom linker containing one stereocenter [100].

3.5. Design of boron in drugs

Reasons and rationalizations for inclusion of boron, namely in the form of boronic acids, varies, as covered in a section above highlighting approved drugs and drugs in clinical trials. This section focuses in more detail on the design and rationalization leading to incorporation of boronic acids into drug discovery endeavours.

3.5.1. Boronic acids as bioisosteres

Boronic acids are ionically stable in physiological pH [101], making them promising unionized alternatives for ionized bioisosteres (Figure 8).

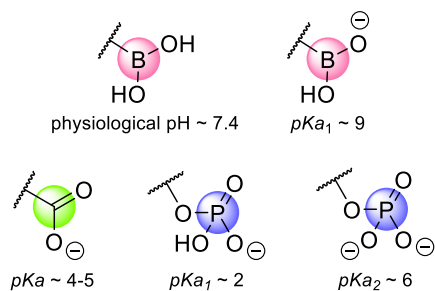


Figure 8: pKa of boronic acid [101], carboxylic acid [102], and phosphate protons [103].

As a more specific example, boronic acids have been studied as bioisosteres of carboxylic acids. Their structures are similar, but the boronic acid's higher pKa allows it to be unionized at physiological pH [102]. Examples of this bioisostere application include studies by Albers *et al.* [97, 98] and Ghosh *et al.* [92]. A discussion detailing their design rationale follows.

Phosphates, while not as similar in structure to boronic acids as carboxylic acids, have also been replaced with boronic acids to study nucleosides [103]. For example, the Vasseur research group has synthesized analogues of DNA nucleotides, replacing the phosphate group with a boronic acid (Figure 9A). Computational studies revealed that these boronucleotides were very structurally very similar to their native analogues [104]. Furthermore, reaction with native ribonucleotide uridine gave the corresponding dinucleotide through reaction of the boronic acid with diol of the RNA nucleotide (Figure 9B) [105].

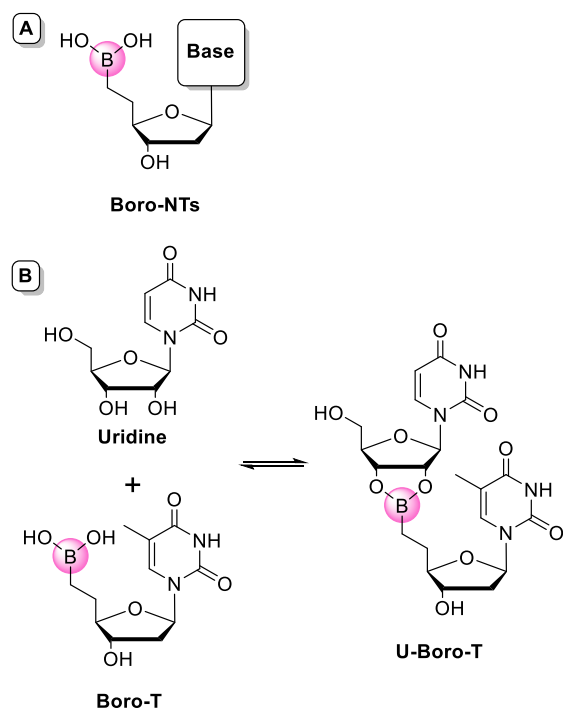


Figure 9: Vasseur's boronucleotide analogues. (A) structure of the boronucleotide, replacing the phosphate with a boronic acid [104]; (B) reversible reaction of RNA's uridine with the boronucleotide thymidine analogue [105].

For a more comprehensive review of boronic acids in nucleic acid chemistry, see a review from the Vasseur research group [106].

3.5.2. Utilizing boronic acids for improvement of pharmacokinetic properties

Addition of boron or replacement of certain functional groups with a boron-containing group affects the octanol-water partition coefficient ($\log P$) and distribution coefficient ($\log D$), which, in turn, affects several pharmacokinetic properties [107].

One example of utilizing a boronic acid as an isostere for a phenol group to improve solubility was investigated by Kong *et al.* [85]. In their study of analogs of Combretastatin A-4, an anti-cancer agent halted in Phase II clinical trials [108], they aimed to improve both the activity and solubility of the compound without the use of a phosphate prodrug. Their replacement of an aromatic phenol with an aromatic boronic acid (Figure 10) led to not only improved bioactivity, but a nearly two-fold improvement in solubility in acidic media, suggesting the enhanced stability and solubility upon oral administration [85].

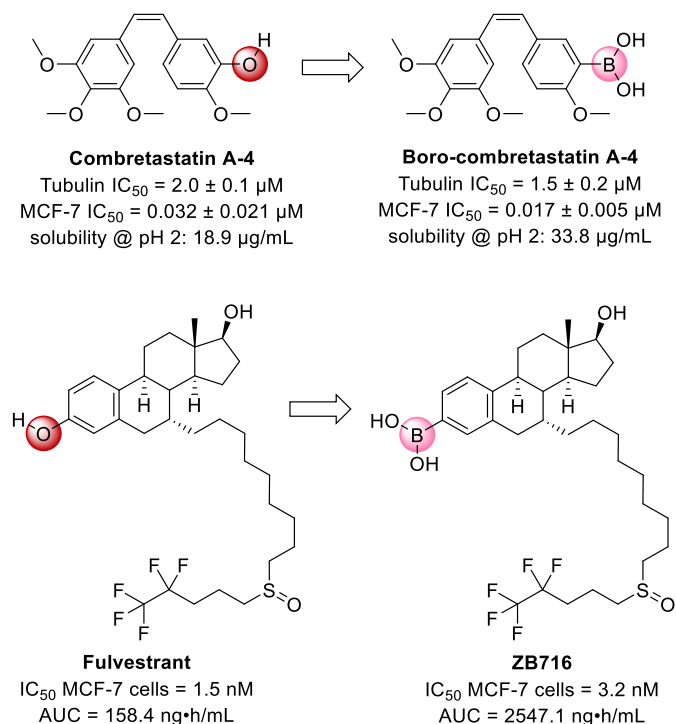


Figure 10: Improved properties of upon utilizing the boronic acid group as an isostere for (A) Combretastatin A-4 [85] and (B) Fulvestrant [109].

Another example of utilizing boron for improved bioavailability comes from Liu *et al.* and their research in the discovery of breast cancer selective estrogen receptor downregulators (SERDs). The researchers aimed to improve the compound Fulvestrant [109], the only FDA-approved SERD (Figure 10) [110]. Wanting to overcome rapid glucuronidation of its phenol, the researchers replaced the phenol group with an aromatic boronic acid. Not only did the activity of the boron analogue remain nearly equipotent as Fulvestrant *in cellulo* against breast cancer cells, but it displayed superior pharmacokinetic properties and was therefore more potent *in vivo* [109]. More specifically, incorporation of a boronic acid slowed down the clearance rate of Fulvestrant, allowing for a slow release upon slower metabolic oxidation of the boronic acid to the phenol. ZB716 is currently in pre-clinical development [111]. The same research group, continuing their studies of SERDs, has since utilized the boronic acid moiety as an orally available bioisostere for phenols in their discovery and development of other anti-breast cancer drugs [110].

3.5.3. Utilizing boronic acids for improvement of drug activity

Boronic acids have been utilized as bioisosteres for several different functional groups, including carboxylic acids as mentioned above. One example from Albers *et al.* involves a study in which the researchers replace a carboxylic acid with a boronic acid in aims to improve potency of their hit autotaxin inhibitor [97, 98]. Their rationale included knowledge of a threonine nucleophilic residue; they realized that, while the carboxylic acid moiety could hydrogen bond to the nucleophilic threonine, a boronic acid could act as an electrophile to block the enzyme's activity reversibly [97].

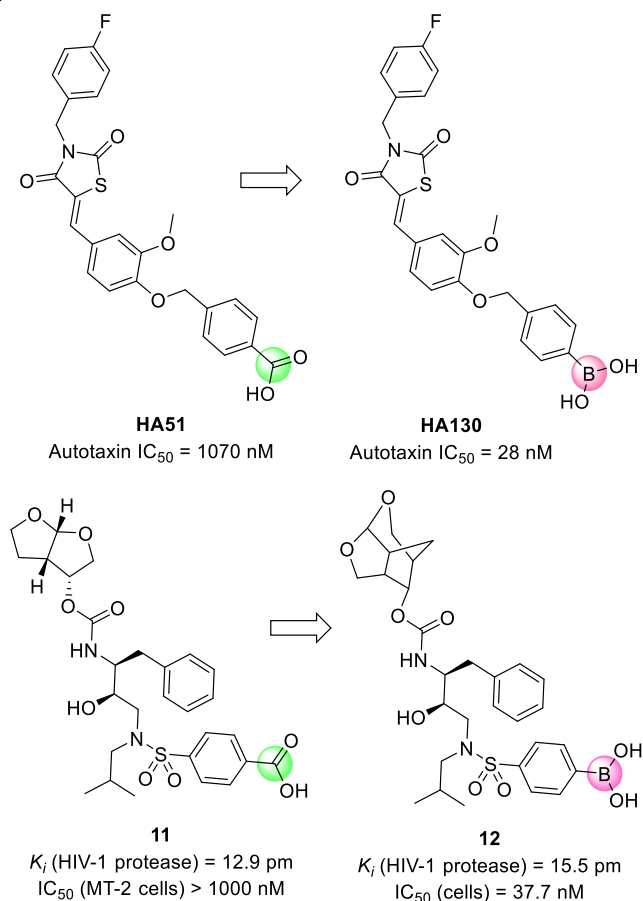


Figure 11: Use of a boronic acid as an isostere for a carboxylic acid to improve anti-autotaxin activity [97, 98] and anti-HIV activity [92].

In their studies of HIV protease inhibitors, Ghosh *et al* discovered analogues of Darunavir containing either carboxylic acids or boronic acids (Figure 11). Upon testing the inhibitors against the enzyme itself, both series of compounds exhibited low-nanomolar potencies. When tested against MT-2 cells, however, the boronic acids retained their potency, while the carboxylic acids potency decreased by at least 2 orders of magnitude. This inactivity was attributed to the inhibitors' presumed inability to cross the cell membranes, as their binding mode was very similar by X-ray crystallography (aside from their differing bicyclic side chains) [92].

3.5.4. Boronic acids as reversible covalent inhibitors

When used as an isostere for a carboxylic acid, boronic acids can be used in peptide mimics as a reversible covalent group. In fact, most drug discovery studies depend on boron's ability to react with a serine or cysteine residue in the active site of various protease enzymes. (For a much more comprehensive review of boronic acids as inhibitors of proteases, see Smoum *et al* [112]. For a review on covalent inhibitors, see De Cesco *et al* [113]) Figure 12 outlines the general reaction of three reversibly covalent warheads in a serine protease, as compared to the natural peptide substrate. Unlike the weakly electrophilic nitrile **C**, the aldehyde and boronic acid reactions **B** and **D** result in a tetrahedral intermediate that mimics that of the substrate reaction **A**, which likely explains their higher potencies and longer residence time in the active sites serine proteases [114].

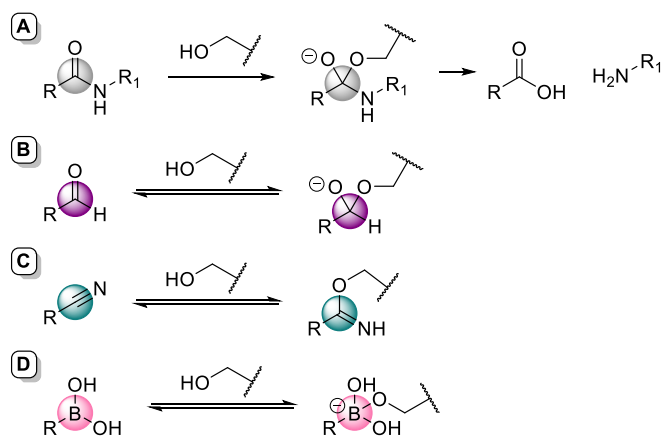


Figure 12: Comparison of various reversibly covalent warheads (B-C) to a peptidase-substrate reaction (A), using the example of serine proteases [114]. The serine proton is either transferred to the electrophile or the basic residue of the catalytic triad, depending on the enzyme's mechanism. Figure adapted from Plescia *et al.* [114].

Nevertheless, although aldehydes are ubiquitous in nature, their high reactivities cause oxidative stress in humans and lead to cytotoxic, mutagenic, and carcinogenic effects, among others [115]. Boronic acids, on the other hand, are generally considered safe [5] and are therefore preferred over aldehydes for drug development. Figure 13 shows the mechanism of the reversible covalent bond formation between a catalytic nucleophilic residue and the electrophilic boronic acid.

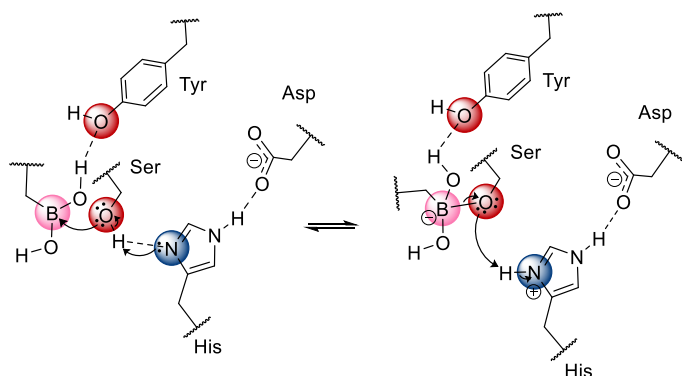


Figure 13: Generic reversible mechanism of a catalytic serine residue attacking an electrophilic boronic acid, including stabilization by a tyrosine residue.

One study takes advantage of this transition state mimic in their SAR studies to target β -lactamases. Instead of the irreversibly-binding β -lactam group, the researchers incorporated a reversibly covalent boronic acid (Figure 14), achieving nanomolar potencies [116].

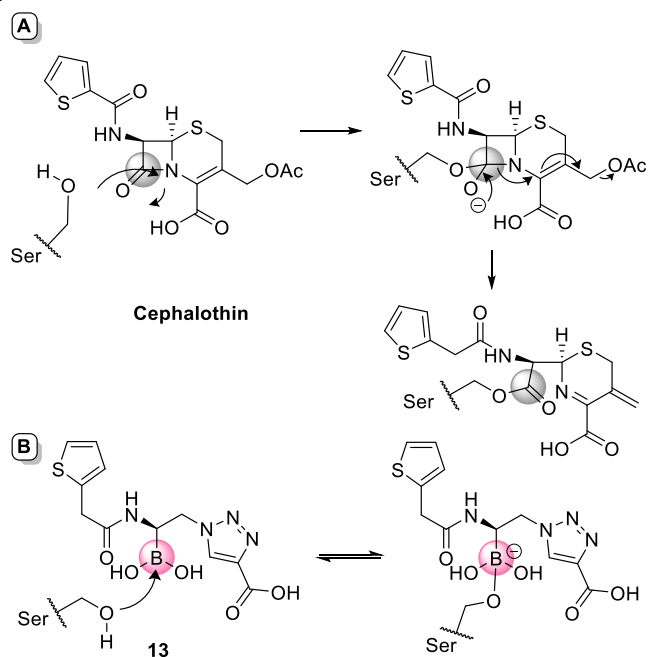


Figure 14: Transition-state mimics in the discovery of β -lactamase inhibitors. (A) irreversible reaction of β -lactamase cleaving cephalothin; (B) reversible reaction of boronic acid inhibitor **13**. Figure adapted from Rojas *et al.* [116].

Vaborbactam (Figure 3), as discussed earlier, was designed to be a serine protease inhibitor of β -lactamases to complex with the catalytic serine residue [49]. Figure 15 shows the crystal structure of β -lactamase CTX-M-15 covalently complexed with Vaborbactam.

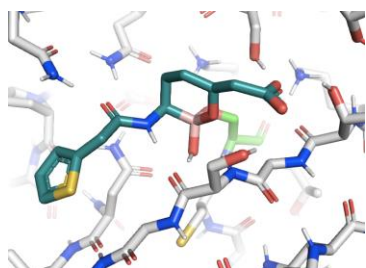


Figure 15: Vaborbactam (teal) complexed into the active site of β -lactamase CTX-M-15 (gray), nucleophilic serine highlighted (green) (pdb: 4XUZ).[49]

In another study by Ban *et al.* [86] mentioned earlier, the researchers designed several boronic acid analogues of EGFR TK inhibitors that originally contained Michael acceptors acting as irreversible inhibitors. A few are featured in Figure 16, but boronic ester analogues of each were also synthesized.

The intention was to target the active site's cysteine residue (resultant of a tyrosine mutation) without needing an irreversible inhibitor. Upon *in vitro* and *in cellulo* testing, it was found that (1) the boronic acids were slightly more potent than their boronic ester derivatives; (2) the boronic acids **14.1** and **14.3** exhibited submicromolar activity against EGFR TK without inhibiting other human kinases (**14.2**'s linker was deemed too short upon low inhibitory activity); and (3) **14.3** continued to inhibit EGFR activity even after five-hour incubation [86]. The boronic acid therefore remained bound the cysteine residue in a pseudo-irreversible manner without the risk of a suicide inhibitor.

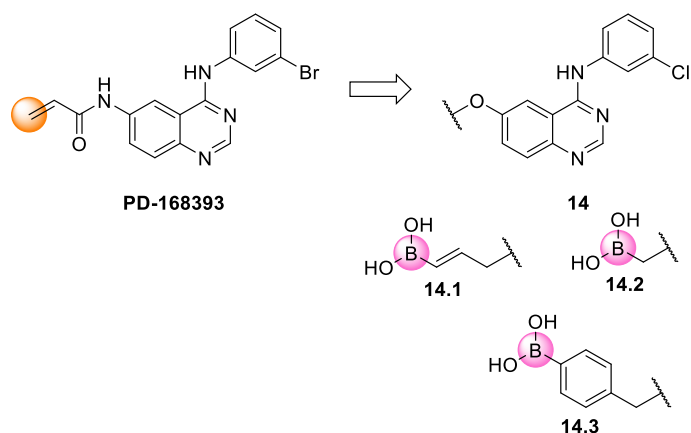


Figure 16: Modification of EGFR TK inhibitor to replace the acrylamide Michael acceptor group with the boronic acid moiety.[86]

In our own research, we have found that the replacement of nitriles in our compounds have increased both potency [117] and residence time in the active site of the enzyme prolyl oligopeptidase (POP) [114]. Figure 17A shows two of our inhibitors differing only in their electrophiles. The boronic ester derivative exhibits nanomolar activity, while the nitrile it replaced exhibited double digit micromolar activity. Figure 17B shows two other inhibitors by Jansen *et al.* [118] exhibiting potencies against POP and an homologous enzyme fibroblast activation protein α (FAP) increased by an order of magnitude upon replacement of the nitrile with the boronic acid.

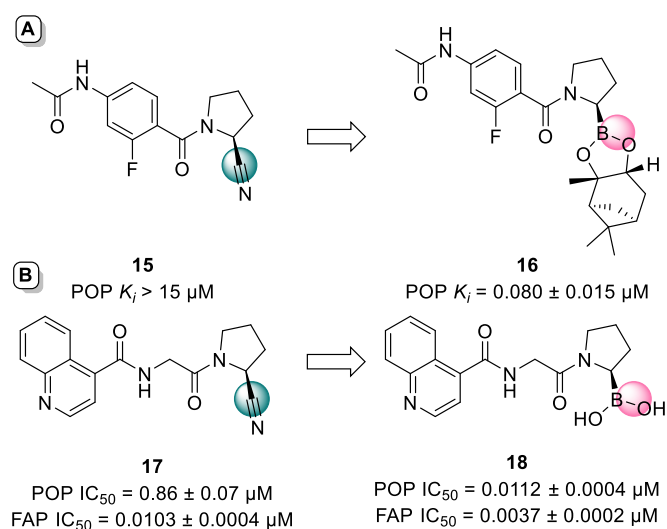


Figure 17: Inhibitors showing increased potency by replacing the nitrile electrophile with a boronic ester/acid. (A) Our group's designed POP inhibitors [117]; (B) Two POP-FAP inhibitors by Jansen *et al.* [118].

Unfortunately, as discussed in a recent review from our group, current docking programs do not account for reactivity of an electrophile nor for the kinetics of the binding/dissociation (residence time) [113]. For example, our own covalent docking program, FITTED [119-121], gives identical predicted poses for both **15** and **16** (Figure 18), yet the *in vitro* activities of these compounds differ by three orders of magnitude.

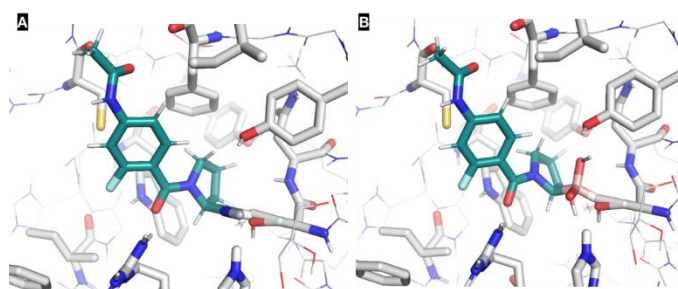


Figure 18: (A) **15** (teal) docked to POP (gray); (B) **16** (teal), hydrolyzed, docked to POP (gray). Compounds from Plescia *et al.* [117] and are docked using FITTED [119-121].

Bortezomib (Figure 3), as previously discussed, was designed to be a reversible covalent inhibitor. Several peptide analogs containing various covalent groups, including aldehydes, were probed against the tumour proteasome, though boronic acids were the only functional group that were suitable for further pre-clinical studies. Figure 19A shows Bortezomib co-crystallized to the human 20S proteasome complex (pdb code 5LF3) [122].

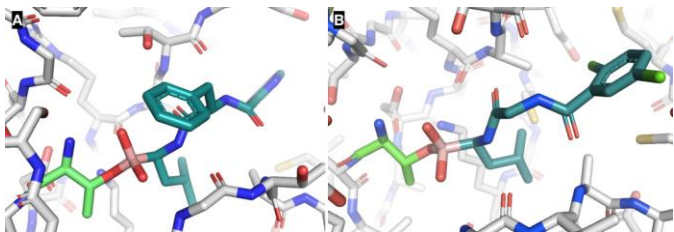


Figure 19: Bortezomib (teal) (A, pdb code 5LF3) and Ixazomib (teal) (B, pdb code 5LF7) complexed to the human 20S proteasome complex (gray), with the nucleophilic threonine highlighted (green) [122].

Ixazomib (Figure 3), whose structure is very similar to Bortezomib, was discovered several years after Bortezomib's approval, as discussed earlier. Its mechanism of action is similar: it inhibits the catalytic threonine in the active site of the 20S proteasome. Figure 19B shows Ixazomib crystallized in the active site.

Talabostat, or Val-Boro-Pro (Figure 20), is a dipeptidic boronic acid dipeptidyl peptidase (DPP) inhibitor [123]. It was marketed as a multi-target drug that inhibited cancer-implicated homologous serine proteases, FAP and DPP4, DPP8, and DPP9. Its mechanism of action involved activation of innate immune response against tumours through its dipeptidyl peptidase inhibition [124]. Talabostat was discovered during a study of DPP family inhibition. Because DPP enzymes cleave terminal dipeptides with Xaa-Pro (i.e. any amino acid adjacent to proline) sequences from their substrates, the researchers tested a number of Xaa-Pro analogs in which the proline was substituted with boroPro, or a boronic acid in place of the carboxylic acid [125] to bind to the catalytic serine [123].

Ultimately, inefficacy led to its failure at Phase III [101]. Although not confirmed, it is believed to have failed due to *in vivo* cyclization to its inactive form via the free amine reacting intramolecularly with the boronic acid moiety [126] (Figure 20).

It is speculated that Talabostat failure was also due to lack of patient tolerance at doses high enough for anti-tumoral activity, due to partial toxicity [127]. This compound continues to be studied, however, and more recently, it was used to crystallize DPP8 [128] and is the first ligand-bound crystal structure of this enzyme available on the Protein Data Bank. Figure 21 shows this crystal structure with Talabostat bound to the catalytic serine in the active site, confirming its covalent inhibition.

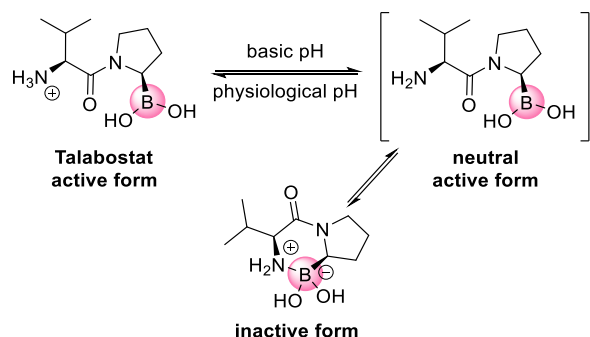


Figure 20: Cyclization of Talabostat at physiological and basic pH. Adapted from Kelly *et al.* [126].

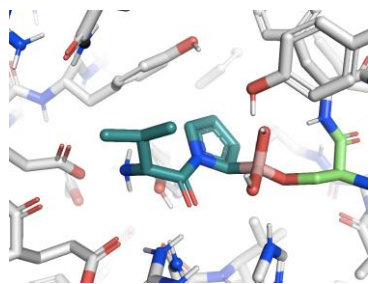


Figure 21: Talabostat (teal) co-crystallized with DPP 8 (gray) with the nucleophilic serine highlighted (green). (pdb: 6HP8) [128].

In general, reversible covalent inhibition is a promising approach in drug discovery, as outlined in a recent review out of our group [113]. Its use in medicinal chemistry endeavours is on the rise, as seen in Figure 22.

Instances of “reversible covalent”

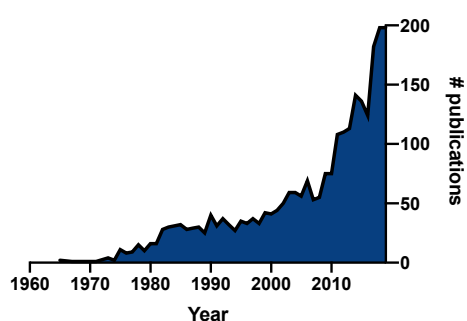


Figure 22: PubMed search results of the terms “reversible covalent.”

3.5.5. Boronic acids and esters as prodrugs

Boronic acids have been used as anti-cancer pro-drugs. Several groups have taken advantage of elevated levels of reactive oxygen species (e.g. H_2O_2) in certain cancer cells and resultant drug oxidation to synthesize prodrugs that release the active species upon oxidation. Lin *et al.* studied boronic acid substituted Camptothecin **B1** as a prodrug for neoplastic drug **SN-38** [129]. Taking advantage of elevated levels of reactive oxygen species (ROS) hydrogen peroxide in cancer cells,[130] the group used a boronic acid that would be oxidized to the hydroxylated **SN-38** (Figure 23).

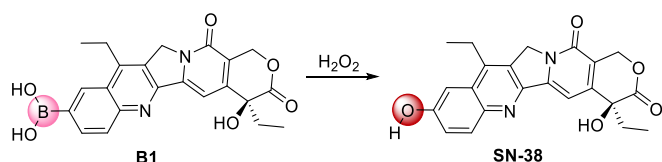


Figure 23: Oxidation of pro-drug **B1** to **SN-38** by intracellular reactive oxygen species hydrogen peroxide [129].

Upon adding to cell media, the researchers measured that nearly 60% of **B1** was converted to **SN-38** after 48h incubation. Upon testing against several cancer cell lines, they found that even with this structural change, **B1** exhibited comparable or greater cytotoxicity than **SN-38** and was actually a greater inhibitor of their target enzyme, DNA Topoisomerase I [129]. Using a boronic acid analogue, they were able to successfully design a prodrug that is not only active on its own but releases its chemotherapeutic drug *in vivo*.

Another example utilizes a boronic acid-containing extension to active drug **Belinostat** and involves a more complex prodrug release. In their quest to improve bioavailability and biocompatibility of **Belinostat**, they included boronic acid moieties, giving prodrugs **19** and **20** (Figure 24). Upon *in cellulo* testing of the active compound and both prodrugs, it was found that prodrug **19** exhibited activity three to five times less than that of **Belinostat**, but, more surprisingly, that prodrug **20** was weaker than prodrug **7** by an order of magnitude, and in one case, was 30 times less potent [131].

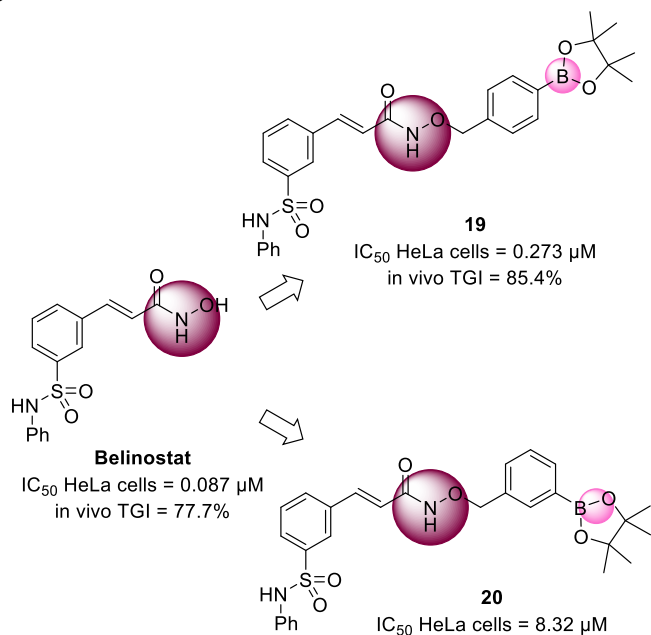


Figure 24: Comparison of drug and boronic prodrug activities. Figure adapted from Zheng *et al.* [131].

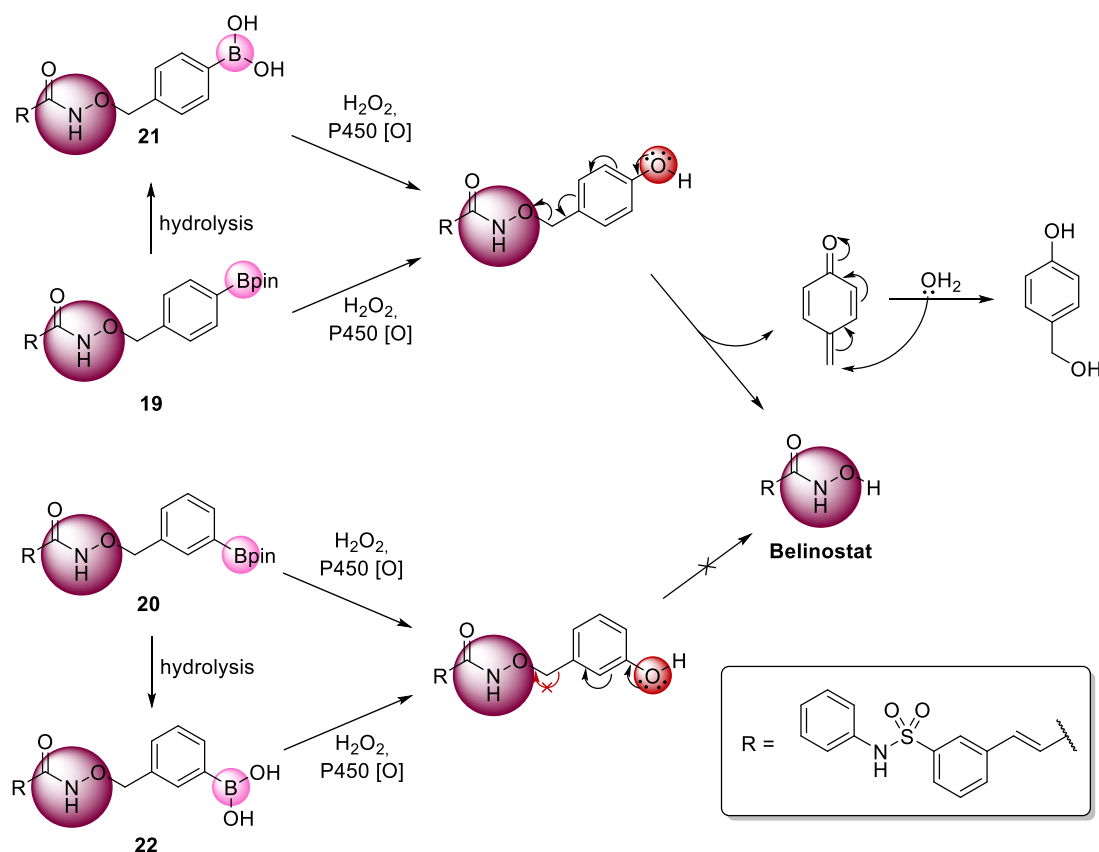


Figure 25: Proposed mechanism of oxidation of prodrug and subsequent release of drug. Figure from Zheng *et al.* [131]. pin refers to the pinacol protecting group

To explain this large difference in activity between the prodrugs **19** and **20**, the authors proposed a mechanism to determine the means by which the active compound **Belinostat** is released (Figure 25). Upon assumed oxidation by H_2O_2 present in the cells, only prodrug **19** is able to undergo a mechanism to release the resultant phenolic moiety and a *para*-quinone; prodrug **20** rests as a phenolic intermediate. The inability of prodrug **20** to release the active form of the drug likely explains its decreased activity *in cellulo*. When **Belinostat** and prodrug **19** were tested in mice, however, the *in vivo* efficacy of tumour growth inhibition

(TGI) of **19** was significantly greater, contradicting *in cellulo* data. Tissue analysis found that **19** released **Belinostat**, but amounts of boronic acid **21** remained, potentially contributing to slower release and therefore higher efficacy [131].

Our own group has used boronic esters as prodrugs for their corresponding boronic acids. The basic buffer used in our assays hydrolyzed the (+)-pinanediol-protected boronic ester **23** (Figure 26A) to their respective free boronic acids within 20 minutes after mass spectroscopy analysis (Figure 26B) [114, 117]. This study's results allow for a much more diverse scope of potential drugs in future medicinal chemistry endeavours: harsh conditions normally used to cleave boronic esters to free boronic acids (strong acid, BBr₃/BCl₃, fluoride, etc.) normally affect other sensitive functional groups, such as methoxy esters or Boc- or Cbz-protected amines. Using a buffer-sensitive boronic ester allows for inclusion of many more functional groups that contribute to potency of potential drugs.

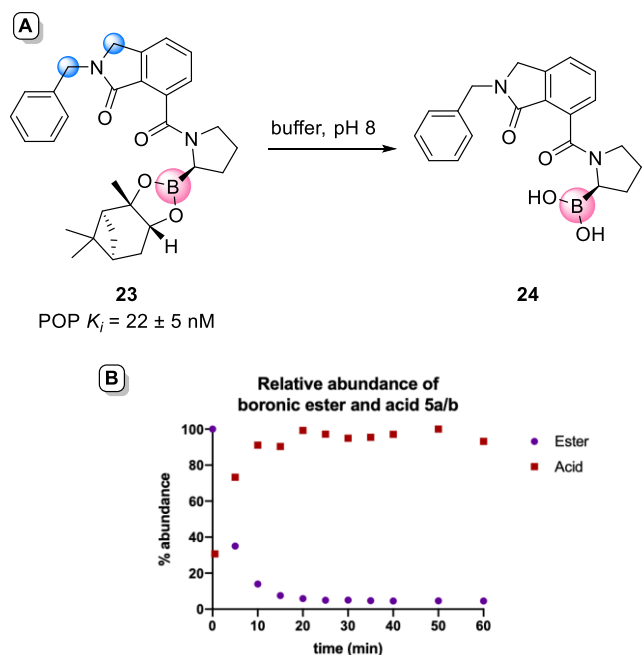


Figure 26: (A) Buffer-mediated hydrolysis of compound **23** to the active species **24**; BCl₃-sensitive carbons highlighted in blue; (B) Mass spectroscopy study of the boronic ester hydrolysis. Figure adapted from Plescia *et al.* [114].

In fact, in our own synthetic efforts, the harsh conditions attempted to hydrolyze **23** resulted in (1) premature precipitation of the starting material in strongly acidic media during attempted transesterification of the (+)-pinanediol auxiliary and (2) mixtures of debenzylated products upon utilizing BCl₃ to remove the (+)-pinanediol protecting group. *In vitro* testing the boronic ester directly allowed us to obtain a very potent compound without having to sacrifice the study of a boron-based drug [114].

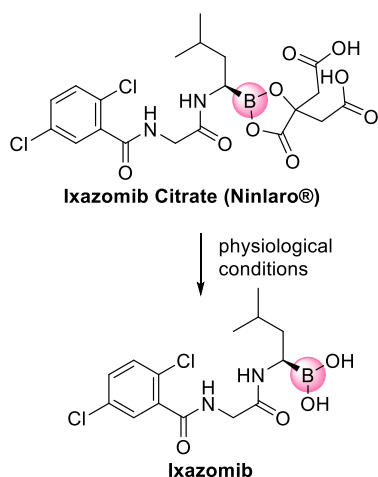


Figure 27: Hydrolysis of prodrug Ixazomib Citrate to Ixazomib.

Ninlaro®, or Ixazomib, is formulated as a prodrug so as to ensure oral bioavailability. The boronic acid is complexed with a citrate molecule to form a citrate ester, which is cleaved under aqueous physiological conditions to give the active form Ixazomib (Figure 27) [34].

From these examples, it is clear that not only can a boronic acid or ester be a prodrug to release the desired chemical species *in vivo*, but a boronic ester can also be utilized to release the desired boronic acid drug. For a more comprehensive review of boronic acids and esters as prodrugs, see Cadahía *et al.* [132].

3.5.6. Using computational methods

Certain discoveries of bioactive boronic acid compounds originated with predictive computational methods. One case from the Shoichet research group designed a virtual screening protocol to discover boronic acid inhibitors of AmpC β -lactamase. The researchers screened a virtual library of 23,000 commercially available boronic acids. They then selected several ligands that scored in the top two percent and tested them both *in vitro* and against several cell lines. Several hits were obtained, and one in particular (**7**, Figure 28A, B) exhibited *in vitro* potency of 10 nM and was potent against cells when administered with Cefotaxime [133].

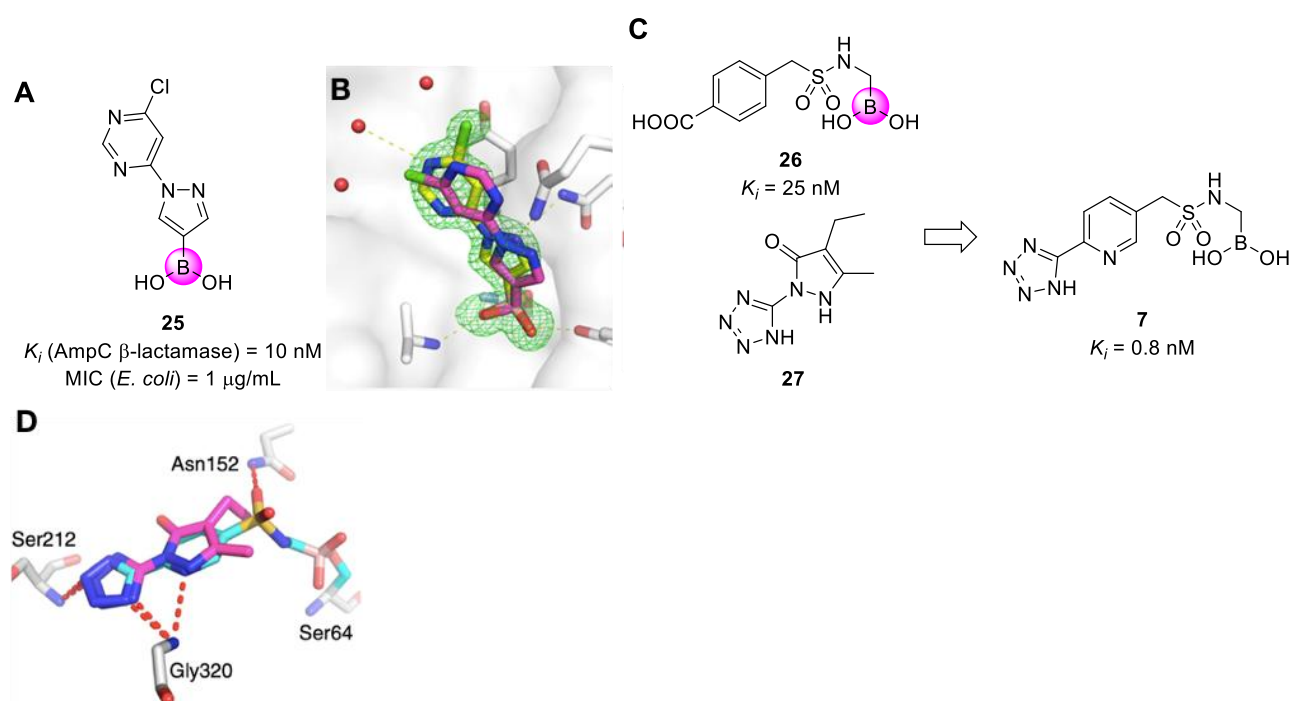


Figure 28: Computational methods for boronic acid drug discovery. (A) boronic acid hit **25** discovered by virtual screening; (B) crystal structure of hit **25** (yellow, green electron map) superposed over its predicted binding pose (magenta) to AmpC β -lactamase; (A and B from London *et al.* [133]) (C) hit **26** for optimization with one fragment **27** used in the *in silico* design, giving resultant hit **7** [94]; (D) docking prediction of fragment **27** superposed to docking prediction of **7** (from Eidam *et al.* [94])

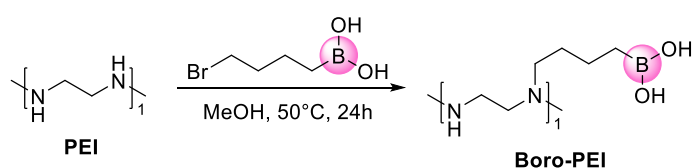
A different approach to the discovery of AmpC β -lactamase inhibitors was taken by Eidam *et al.* and used fragment-based *in silico* hit optimization [94]. Following previous studies of the Shoichet lab which used molecular docking to determine the best fragments for the enzyme active site [134], Eidam *et al.* superposed docked fragments with their hit molecule to determine the most promising side chain modifications. Through several rounds, they were able to modify their hit to improve the *in vitro* activity by two orders of magnitude into sub-nanomolar potency (Figure 28C, D) and achieve potency *in cellulo* and *in vivo* upon testing in combination with Ceftazidime [94].

Although many research groups do not use computational methods to design their target compounds, countless studies involve using molecular docking to rationalize differences in inhibitor activity to improve their compounds for future work.

4. Boronic acids in delivery systems

Apart from inclusion of boronic acids in bioactive compounds, boronic acids' ability to bind to diols (e.g. sugars) on the extracellular domain has been exploited in studies aiming to improve cellular uptake of liposomes and macromolecules; boronic and borinic acids have been conjugated to more complex molecular systems for the purpose of macromolecule delivery, such as increased uptake of gene-delivery complexes [135], transport of proteins [136], and cellular uptake of liposomes [137].

In one example, Yadav *et al.* observed uptake issues of genetic material containing terminal polyethylenimines (PEIs). Reaction of these PEIs with 4-bromobutylboronic acid yielded tertiary amines with terminal boronic acids (**Error! Reference source not found.**). These modifications increased uptake of the plasmids without compromising structural integrity of the carrier nor cell viability [135], as the boronic acids' high pKa allows for unionized interaction with the membrane.



Scheme 1: Reaction of polyethylenimines with 4-bromobutylboronic acid, from Yadav *et al.*[135]

Another research group aimed to facilitate cell entry of liposomes by incorporating boronic acids on the surface. They began with the design of an aminoglycerolipid conjugated to an aromatic boronic acid (Figure 29A and B). Through a series of fluorescent experiments using rhodamine-labeled phosphatidylethanolamine (Rd-PE), they determined that liposomes with 10% boronic acid-conjugated lipid content entered the cells, while control liposomes did not [137].

One group has incorporated benzoxaboroles into delivery vehicles for the transport of proteins over mammalian bilayers (Figure 29, C and D). They designed a delivery vehicle with benzoxaborole conjugated to *o*-hydroxydihydrocinnamic acid derivative trimethyl lock (TML), which would in turn be conjugated to green fluorescent protein (GFP), a fluorescent protein unable to traverse the lipid bilayer. A series of experiments and control experiments led to the conclusions that (1) benzoxaborole was aiding in the uptake of the GFP; (2) the uptake was proceeding through an endosomal pathway; and (3) the labeling was stable but ultimately reversible, leading to the release of the delivered target protein in the cells [136].

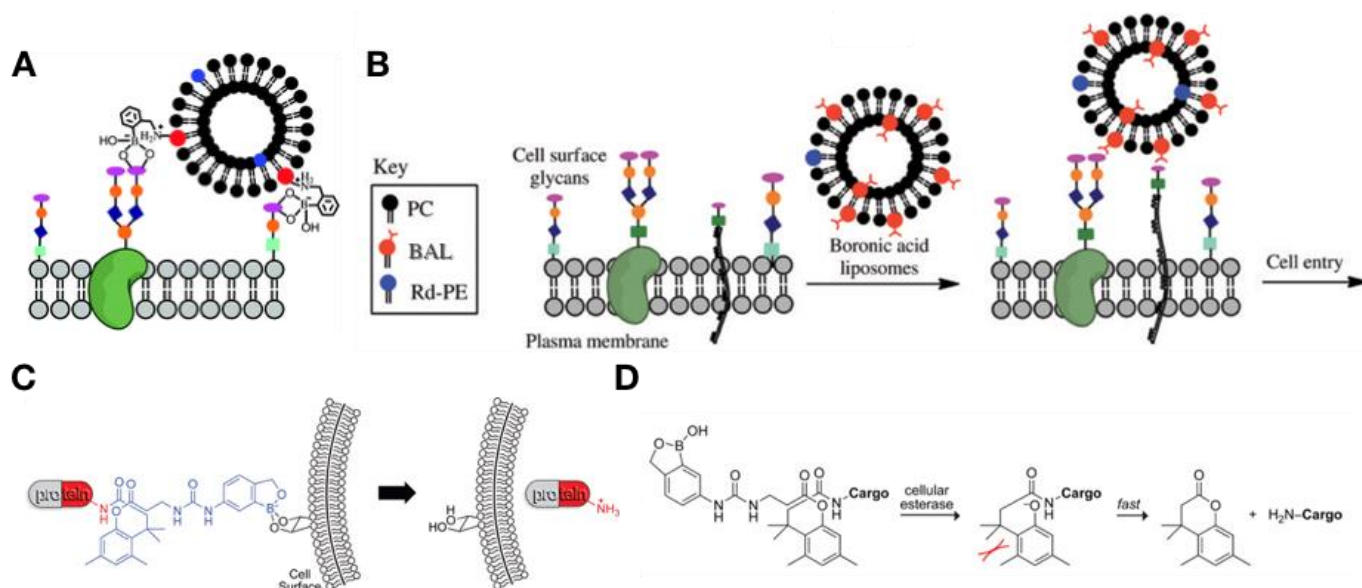


Figure 29: (A) Close-up schematic diagram of a liposome's terminal boronic acids interacting with carbohydrates on the extracellular domain of the cellular membrane lipid bilayer; (B) Schematic diagram depicting uptake of boronic acid-coated liposomes; PC = phosphatidylcholine, BAL = boronic acid-coated liposomes, Rd-PE = rhodamine-labeled phosphatidylethanolamine; (C) (D); Figures A and B from Zhang *et al.* [137]; Figures C and D from Andersen *et al.* [136].

Although there exist many other applications of boron in delivery systems, such as advanced nanomaterials and usage in radiation therapy, they are beyond the scope of this review.

5. Synthesis of boronic acid drugs

Access to all of these drugs and potential drugs would not be possible without efficient synthetic methodologies. To obtain the final boronic acids, prodrugs are usually synthesized first, as boronic acids are difficult to purify and to carry through multiple steps. Boronic acid synthesis varies depending on surrounding functional groups, whether it is to be aromatic or aliphatic, and if applicable, the desired stereochemistry of the final product. Furthermore, in the synthesis of boropeptides, such as Delanzomib, the process synthesis is not so different from the discovery synthesis. Similar methodologies allow for more efficient development [138].

5.1. Synthesis of α -aminoboronates

One of the more popular α -aminoboronic ester derivatives is the proline-derived analog. As discussed earlier, medicinal chemistry endeavours targeting certain families of peptidases have relied heavily on the use of this chiral boronic analogue of proline (Figure 30, **29**).

The increased use of α -amino boronic acids in the discovery of inhibitors of this family of serine proteases has led to the increased commercial availability of prepared and enantiopure (+)-pinanediol-protected α -amino boronic ester analogs of many amino acids, such as the very commonly used isoleucine, used in the synthesis of Bortezomib [139] and Ixazomib, and proline, used in the synthesis of Talabostat. [140]

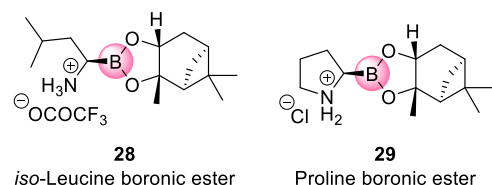


Figure 30: Commercially available enantiopure boronic (+)-pinanediol esters. [140]

The Ellman group at Yale has developed syntheses of highly enantiopure α -amino boronic esters using their own Ellman chiral auxiliary to synthesize diastereopure (*R*)- or (*S*)-*tert*-butyl-sulfinylimines [141, 142].

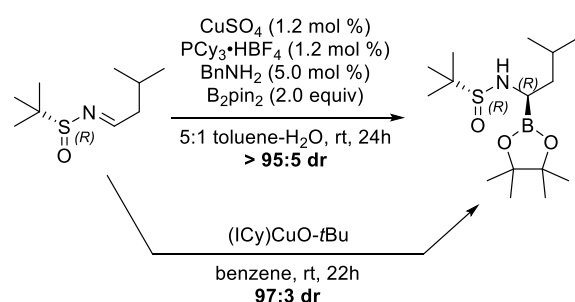
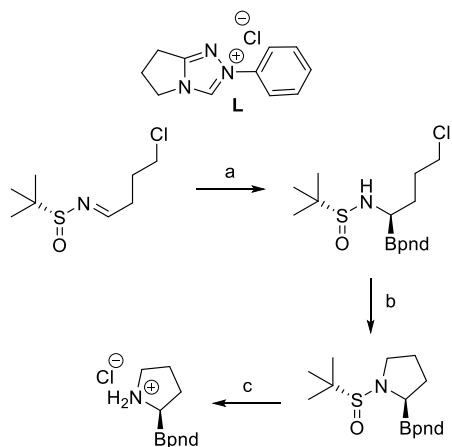


Figure 31: Ellman syntheses of diastereopure sulfinylimines [141, 142].

In fact, the Ellman group's research sparked interest in synthetic development of chiral **29** for the synthesis of boro-peptide inhibitors, as performed by Chen. *et al.* [143].



Scheme 2: Synthesis of enantiopure CB-2 via the Ellman borylation. a) CuI (10 mol%), Cs₂CO₃ (10 mol%), L (10 mol%), B₂pnd₂* (1 eq), benzene, rt, 48h; b) NaOtBu, DMF, rt, 6h; c) HCl, dioxane-MeOH, rt, 30 mins. *pnd refers to (+)-pinanediol protecting group[143]

In our own chemistry, we have found that the α -amino boronic pinacol esters are very difficult to purify and consequently difficult to carry through multiple steps, as they react with SiO₂ in flash chromatography columns and therefore require used of H₂O-deactivated SiO₂, as reported by the Ellman group [141]. However, application of a transesterification with (+)- or (-)-pinanediol as reported by the Matteson group [144] (Figure 32) gives diastereopure boronic esters that are more easily purified on a silica gel column.

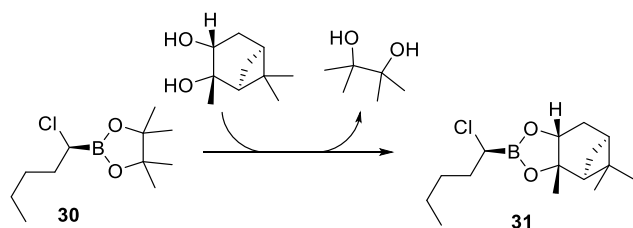


Figure 32: Matteson conversion of boronic pinacol ester to (+)-pinanediol ester via transesterification

5.2. Aromatic boronic esters and acids

The synthesis of aryl boronic esters and acids is well-established, such as in synthesis of starting material for the Suzuki cross-coupling reaction, one of the most widely used coupling reactions in medicinal chemistry. The Miyaura reaction allows for the facile synthesis of these boronic esters (Figure 33) [145]. Furthermore, purification is much simpler than that of the α -aminoboronic ester derivatives, as they can be purified by flash chromatography on normal phase silica.

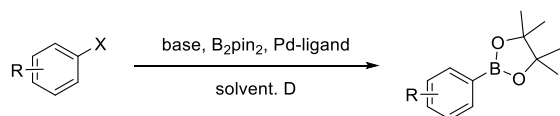


Figure 33: The Miyaura reaction [145].

Many aromatic boronic acids are available commercially (e.g. from Sigma-Aldrich [146], BoroChem [147], or Combi-Blocks [140]) either as building blocks or as known bioactive compounds for testing.

With this chemistry, the discovery of drugs containing this aromatic boronic ester group can be facilitated with *in silico* combinatorial chemistry studies; aryl halides can be virtually converted to aryl boronic acids to generate large libraries for virtual screening [133].

5.3. Other aliphatic boronic acids

Already well known and studied is the addition of bis(pinacolato)diboron, or B₂pin₂ to α,β -unsaturated compounds (Figure 34). Usually, reactions involve a metal catalyst for activation of the boron and a base for assisting in heterolytic cleavage of the B–B bond. For a comprehensive review of various conditions and associated mechanisms of addition, see Lillo *et al.* [148].

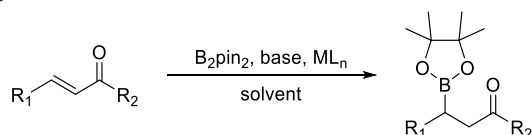


Figure 34: Addition of boron to α,β -unsaturated compounds

The Baran lab recently discovered a nickel-catalyzed decarboxylative borylation method applicable to a variety of aliphatic carboxylic acids. Their method involves simple preparation of metal- and ligand-containing suspensions, quick reaction times, and high yields. Although not completely stereo-selective, diastereoselectivity can be improved with lower reaction temperatures and steric control [149]. Their research sparked several other decarboxylative-borylation procedures, including a modified, transition metal-free procedure using blue light as a radical initiator [150], an iridium- and visible light catalyzed procedure [151], and another Baran procedure copper-catalyzed reaction [152]. A summary of these decarboxylative borylations can be found in Figure 35.

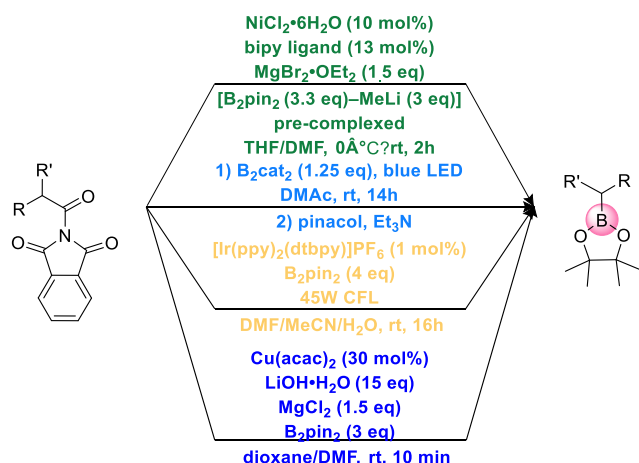


Figure 35: Decarboxylative borylations [149-152].

Baran's nickel chloride method was applied to a small library of various aliphatic carboxylic acids, including several natural products and known bioactive compounds. In fact, decarboxylative borylation was conducted to obtain known compounds **32** and **33**, bioactive against human neutrophil elastase (HNE) implicated in cystic fibrosis (CF) (Figure 36). This new synthesis, including a deprotection step, allowed for efficient preparation and gave a single diastereomer. Furthermore, the boronic acid analog of the original trifluoromethyl ketone hit exhibited a potency increase of three orders of magnitude [149].

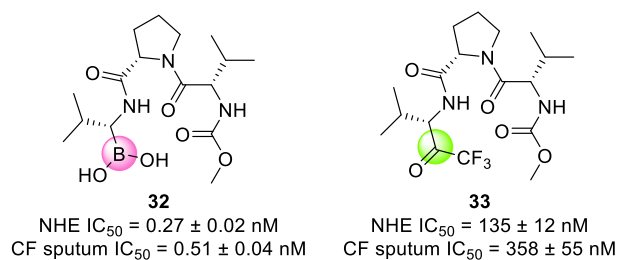


Figure 36: Compounds active against human neutrophil elastase (HNE) implicated in cystic fibrosis (CF); Compound **32** synthesized with Baran's decarboxylative deborylation [149].

While we have highlighted here only a few of the more common synthetic procedures for boronic acid incorporation, many others have been developed over the years. For a more comprehensive review of synthesis of bioactive boronic acids, see Yang *et al.* [153].

5.4. Deprotection of boronic ester pro-drugs

Boronic ester prodrugs are often deprotected before initial *in vitro* testing to their bioactive boronic acid analogues. Various methods exist for this deprotection step. The choice of conditions depends on stability of the starting compound and compatibility of the comprising functional groups.

Some research groups opt to perform simultaneous cleavage of *tert*-Butyloxycarbonyl (Boc) or carboxybenzyl (Cbz) protecting groups and boronic ester using BCl_3 or BBr_3 [154, 155]. Unfortunately, these highly reactive Lewis acids affect certain other functional groups, such as benzyl ethers/amines or methoxy groups. In the synthesis of Bortezomib, the final boronic ester is deprotected via a transesterification with isobutylboronic acid [156]. Again, with reaction conditions that require strong acid, certain functional groups are not compatible. Other methods for deprotection include a telescoped method reacting the boronic ester first with potassium hydrogen difluoride (KHF_2) to give the boron trifluoride potassium salt, followed by hydrolysis in the presence of TMSCl or LiOH to give the free boronic acid [157], or oxidative cleavage via sodium periodate [158, 159]. A summary of these methods is provided in Figure 37. Furthermore, boronic acids at the β -position of an electron withdrawing group were discovered to be susceptible to deprotection by forming an ionic bicyclic structure with diethanolamine, followed by acidic hydrolysis (Figure 37) [160]. Interestingly, this cage-like bicyclic intermediate was used in the process chemistry synthesis of a Delanzomib pro-drug for clinical trials, as it improved purity and stability [138].

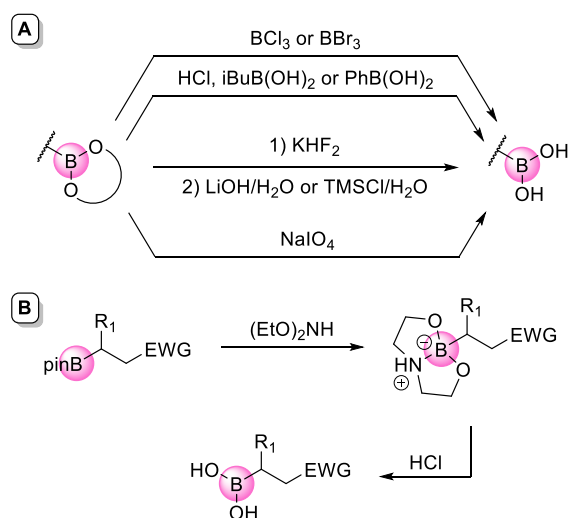


Figure 37: (A) Summary of boronic ester deprotection methods [154-159]; (B) conversion of β boronic esters to boronic acids via diethanolamine cages [160]. pin refers to the pinacol protecting group.

In our own studies, we have found that the deprotection of our boronic esters was not necessary, as our basic buffer mediated the hydrolysis of (+)-pinanediol boronic esters. Figure 38 displays two compounds and mass spectroscopy studies monitoring the relative abundance of the ester and acid species. Even for two compounds with different expected intrinsic reactivities (di-*ortho*-fluoro vs. unsubstituted), the hydrolysis rate was very similar; both esters were cleaved within twenty minutes, i.e. during the sample preparation step before the enzyme was even added.

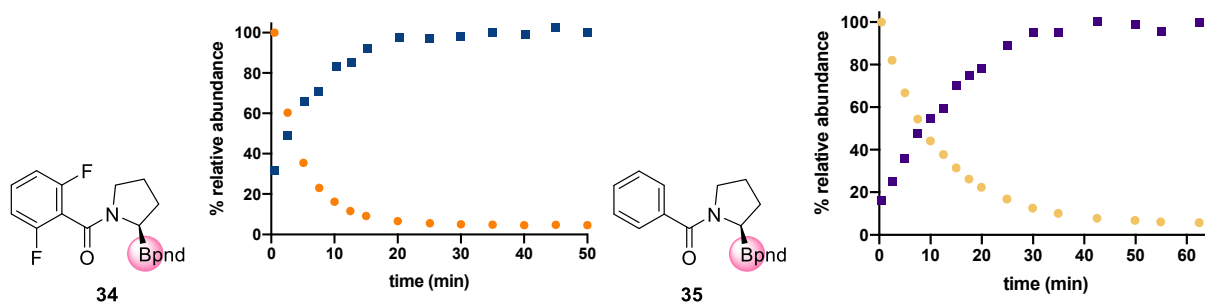


Figure 38. Hydrolysis studies of boronic esters **34** and **35** in POP assay buffer. The graphs display relative abundance of each ionic species at intervals over 50-63 minutes. Figure from Plescia *et al.* [117]. pnd refers to the (+)-pinanediol protecting group.

In the case of the protecting group not being labile enough to hydrolyze in buffer, they would need to be deprotected under the discussed conditions. Purification of the resultant boronic acids is unfortunately not so

straight-forward, as they are not suited for normal phase silica gel column chromatography, and require reverse-phase conditions, often by semi-preparative or preparative HPLC [118, 154].

6. Conclusion and Perspectives

The abundance of boron in natural products and its general safety as a mineral make it an attractive synthetic target in drug discovery endeavours. Following the approval of Bortezomib, there have been many medicinal chemistry endeavours aimed at discovering boronic acid drugs.

As we have seen in this review, several approaches have been taken in the discovery of boron-containing drugs. Because of its reversible electrophilicity, it is commonly used as a reversible covalent group to incorporate into peptides to inhibit proteases, whether that was the original intention, or the result of medicinal chemistry hit optimizations. We have also seen that boronic acids or esters, stable and unionized at physiological pH, have been incorporated as bioisosteres of ionized groups, such as carboxylic esters or phosphate groups, for either activity or pharmacokinetic improvement or for structural purposes. Boron-containing groups have additionally been used as prodrugs, either boronic esters for their corresponding acids, such as FDA-approved Ixazomib, or boronic acids and esters for their ability to be oxidized *in vivo* to their active analogue by tumour environments abundant in reactive oxygen species. Finally, we have described boron-based drugs designed by computational methods, including virtual screening and *de novo* design.

Along with drug design came the associated synthetic efforts aimed at synthesizing boronic acids. Since the approval of Bortezomib, much focus has been on the design of diastereopure aliphatic boronic esters/acids, especially α -amino boronic esters/acids. In turn, many groups have taken advantage of these discoveries to incorporate boronic acids into their drug discovery programs.

Lastly, we saw several examples of boronic acids facilitating drug and macromolecule delivery, either through incorporation into lipid bilayer for entry via liposomes or through reversible conjugation to a protein.

These explorations into boron-based drug discovery will hopefully shed light on the benefits of boron incorporation and encourage medicinal and pharmaceutical chemists to consider boronic acids and esters as possibilities and solutions in their drug discovery programs.

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