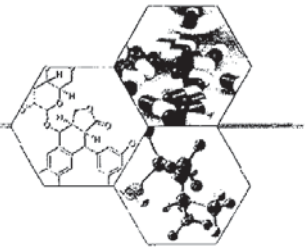
Review



Therapeutic potential of boron-containing compounds

Relative to carbon, hydrogen, nitrogen and oxygen, very little is currently known about boron in therapeutics. In addition, there are very few boron-containing natural products identified to date to serve as leads for medicinal chemists. Perceived risks of using boron and lack of synthetic methods to handle boron-containing compounds have caused the medicinal chemistry community to shy away from using the atom. However, physical, chemical and biological properties of boron offer medicinal chemists a rare opportunity to explore and pioneer new areas of drug discovery. Boron therapeutics are emerging that show different modes of inhibition against a variety of biological targets. With one boron-containing therapeutic agent on the market and several more in various stages of clinical trials, the occurrence of this class of compound is likely to grow over the next decade and boron could become widely accepted as a useful element in future drug discovery.

The physical, chemical and biological properties of boron offer medicinal chemists a rare opportunity to explore and pioneer its utility in chemotherapeutics. However, up until the last few years, boron has mostly been overlooked by medicinal chemists in their design of drug molecules. In trying to discern why boron has not been widely considered, we found a common belief within the medicinal chemistry community that boron is toxic. However, as we have investigated this claim, we have found it to be largely unfounded. The belief that boron is toxic most likely comes from the fact that boric acid ($B[OH]_3$) is an ingredient of ant poisons. However, boric acid, has an LD_{50} of 2660 mg/kg (rat, oral), which is similar to regular table salt at 3000 mg/kg (rat, oral) [101]. Another source of the toxicity concern may have arisen from the toxicity of Velcade® **(49)**, the only boron-based therapeutic currently on the market and widely prescribed by oncologists. Velcade is approved for the treatment of multiple myeloma and works through inhibition of the proteasome. Recently, research has shown that the toxicity of Velcade is due to its mechanism of action and not simply because boron is present in the molecule [1].

The overwhelming data for the safety of boron are to be noted. Boric acid is the main ingredient in 'Goop' the soft semi-solid, often brightly colored toy that children enjoy squeezing through their fingers; boric acid is used as a preservative in eye wash and in vaginal creams; it is used as a buffer in biological assay solutions; boron is also found in high concentrations in fruit, vegetables and nuts. We consume in the range of 0.3–4.2 mg of boron per day [2] and it is considered an essential plant nutrient, although its biological functions are currently unknown. Studies at Anacor Pharmaceuticals found background concentrations of boron in mouse plasma samples of approximately 200 ng/ml [Wheeler C. Unpublished Data]. Therefore, it does appear that the body is familiar with boron. The boronic acid group in Velcade has been shown to be metabolized to boric acid and the body seems to manage its metabolism and excretion [3]. Paraboronophenylalanine was used for boron neutron-capture therapy (BNCT) and found to be safe in multiple species including human. The LD_{50} values of free base paraboronophenylalanine were determined to be more than 3000 mg/kg in rat by intraperitoneal or subcutaneous administration. In repeat dose studies in rats, paraboronophenylalanine was administered subcutaneously for 28 days and the 300-mg/kg group exhibited no significant finding when compared with the control group [4]. In humans, paraboronophenylalanine was administrered via infusion as a complex with fructose, up to 900 mg/kg of bodyweight [5], and BSH ($Na_2B_{12}H_{10}$-SH), another boron therapeutic for BNCT, was administered at the 100-mg/kg bodyweight level [6]; both proved to be safe and well tolerated. From all these data, we have concluded that boron is not an inherently toxic element, such as mercury, and can be considered by medicinal chemists for use in therapeutics. The toxicology question now is not what happens to boron, but what happens to the

Stephen J Baker†, Charles Z Ding, Tsutomu Akama, Yong-Kang Zhang, Vincent Hernandez & Yi Xia
†Author for correspondence
Anacor Pharmaceuticals, Inc.,
1020 East Meadow Circle,
Palo Alto, CA 94303, USA
Tel.: +1 650 543 7500
Fax: +1 650 543 7660
E-mail: sbaker@anacor.com

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rest of the molecule should boron be eliminated from the parent molecule, a standard question for any drug candidate of nonboron origin.

Why do we not have more US FDA-approved boron-containing therapeutics to date? Our primary conclusion is that it is because the organoboron chemistry field is still in its infancy and we do not have a very large portfolio of chemical reactions to introduce boron into organic molecules, nor do we have a good understanding of the compatibility of boron-containing molecules in common synthesis. Over the last two decades, and since Suzuki–Miyaura coupling reactions have become more widespread, there has been a significant increase in organoboron chemistry, which has led to the introduction of new catalysts and new methods of incorporating boron into organic molecules and has provided more insight to the chemical compatibilities of organoboron compounds. This chemistry development is now allowing medicinal chemists to build drug-like boron-containing molecules to finally explore the usefulness of boron in chemotherapeutics.

Similar to hydrogen, carbon, nitrogen and oxygen, boron is, quite simply, another useful atom! Boron can be considered the equal and opposite of nitrogen. Nitrogen is a Lewis base, boron is a Lewis acid; nitrogen has a full *p*-orbital (lone pair), boron has an empty *p*-orbital; nitrogen is nucleophilic, boron is electrophilic; nitrogen sits to the right of carbon in the periodic table, boron sits to the left. Boron in organic molecules is most commonly present as a boronic acid group ($R\text{-}B[OH]_2$), where its pKa usually ranges from 7 to 9, considerably higher than carboxylic acids. This means that, at physiological pH, boronic acid is uncharged and in the trigonal planar sp^2 form (**Appendix, 1**). At pHs above its pKa, a hydroxy group coordinates to the empty *p*-orbital, forming a dative bond, and boron is in a tetrahedral sp^3 form (**2**).

The empty *p*-orbital can also be occupied by a lone pair from other nucleophiles, including alcohols and amines, allowing boron to form a dative bond with biological nucleophiles such as enzyme residues, including serine, and hydroxy groups from carbohydrates and nucleic acids. Boron can form a bond with a therapeutic target that is neither ionic nor an irreversible covalent bond. Also, the pKa of boron can be tuned by chemical modification of the molecule to make it a stronger or weaker electrophile. Modulation of electronic and peripheral substitutions can allow boron to improve its selectivity towards its desired target. Boronic acids can also form borate esters with alcohols. However, these are usually unstable and hydrolyze easily in water. Under certain circumstances, the stability of these borate esters can be increased through intramolecular cyclization, (e.g., forming a diester with both hydroxyl groups of a 1,2-*cis*-diol, such as ethylene glycol, to form a 5-membered dioxaborolane ring). Substituted *cis*-diols are even more stabilized, due to steric hindrance preventing water approaching the boron and subsequent hydrolysis. The properties, preparation and applications of boronic acids have been comprehensively reviewed [7].

Boron in therapeutics has been reviewed in depth [7–9]. This review is intended to describe some of the recent advances since those earlier reports, as well as to describe some older chemistry of the diazaborines and to make a judicial prediction about the potential future of boron in drug discovery.

Diazaborine

R-B(OH)-N=N-R', six-membered heterocycle ring containing a boron, two nitrogen and three carbon atoms

Therapeutic areas containing boron-based therapeutics

■ Diazaborines & enoyl reductase

Early history of diazaborines

One of the first classes of boron-containing compounds evaluated as therapeutics was the diazaborines [10]. Diazaborines were first synthesized by Dewar who was investigating the 'nonbenzenoid' aromaticity of heterocycles [11]. Dewar did not report their medicinal application, but rather that they were tool compounds to demonstrate the potential of replacing the C-C unit in aromatic compounds with the isoelectronic B-N bond. However, it was Gronowitz who saw a similarity with the hydrazone-containing nitrofuran antibiotic nitrofurantoin (**3**); diazaborines, he reasoned, contained an internal hydrazone and might also share the same antibiotic activity.

His work demonstrated the first reported antimicrobial activity for a boron-containing compound [12,13]. Starting first with structurally similar nitrothiophene (**4**), Gronowitz quickly established that the nitro substitution on the ring was not necessary and that the most dramatic impact on activity was observed when changing the hydrazine component. A strong preference for 2-*N*-sulfonyl substitution in analogs possessing antibacterial activity was noted and served as the template for future research. Several patents followed, demonstrating the apparent interest in this area and culminating in the largest such evaluation being published by Sandoz Pharmaceuticals [14]. In this study, the MIC activity of 80 different diazaborines

was consistent with previous observations that activity was confined almost exclusively to Gram-negative bacteria. It was generally established that the ranking of potency, relative to the arene ring, followed the order: thienodiazaborines, benzodiazaborines, furanodiazaborines, with pyrolodiazaborines being inactive. In the 2-*N*-sulfonylalkyl-thienodiazaborine series, the effect of homologating the alkyl chain was striking, with **5** having a high MIC against *Escherichia coli* of more than 50 µg/ml, compared with 6.25 µg/ml for **6**. Methylation of the thiophene ring gave a slight boost in potency to provide the most promising diazaborine reported (Sa 84474 **[7]**) with an MIC of 1.25 µg/ml.

To better understand the requirements for activity, a nonboron analog, 4-hydroxy-3-(*p*-tolylsufononyl)isoquinoline, was prepared and found to be inactive. It is important to note that these synthetic efforts were not guided by knowledge of the mechanism of action. In fact, the target was initially believed to be lipopolysaccharide synthesis [15], which was consistent with the observation that activity was confined almost exclusively to Gram-negative bacteria. Subsequent studies would reveal the actual target to be fatty acid biosynthesis, but it took another 12 years until the crystal structure was reported [16,17].

Investigation into the structure–activity relationships of diazaborines during this time was almost exclusively limited to diazaborines containing the sulfonyl side chain. Problems with this particular class of diazaborine are evident from the literature as little progress has been made since. This is possibly due to two reported cases of toxicity.

Forbes and Davies reported toxicology studies of a furano derivative (ICI 78911 **[8]**) that was in development for the treatment of Gram-negative infections. Toxicology studies in rodents yielded no abnormal findings. However, corneal ulceration in dogs was evident following administration of three daily doses of 25 mg/kg [18] and was cited as the reason to stop further development.

Grassberger *et al.* cautioned against the potential toxicity associated with this class and openly speculated that boron could be involved [14]. However, no toxicity data were published and no proof (or testable hypothesis) that boron was the origin of toxicity was offered. A retrospective on Grassberger's work then misinterpreted these comments as proof that boron cannot be used clinically because of the 'inherent toxicity of boron-containing compounds' [19]. This has had a most unfortunate consequence as subsequent articles have referenced this review propagating the notion that boron is toxic [19]. More recent studies [20–24] with other classes of diazaborines have not mentioned any reports of toxicity.

Enoyl reductase is the target for *N*-sulfonyl diazaborines

Enoyl reductase (ENR) is an enzyme involved in fatty acid biosynthesis. ENR is a target of a front-line anti-TB drug isoniazid and the antimicrobial agent triclosan [9]. *N*-sulfonyl-substituted diazaborine inhibitors of ENR form a covalent B-O ester with the 2-hydroxyl group of the cofactor nicotinamide adenosine ribose, forming an inhibitor–substrate adduct bound in the enzyme active site. Co-crystal structures for a variety of sulfonyldiazaborines have been published that explain the preference for the sulfonyl group and confirms boron is critical to the mechanism of inhibition [25]. In addition to accepting an intramolecular hydrogen bond from the boron hydroxyl group, the electron-withdrawing nature of the sulfonyl group stabilizes the negative charge on the boron atom and induces a conformational bend into the molecule that orients the sulfonyl substituent into a cavity of the active site.

Diazaborines for TB

Renewed interest in this area followed the publication of diazaborine's mechanism of action with evaluation of new classes of diazaborines including the isosteric 2,4,1-benzo[*e*]diazaborines, targeting *Mycobacterium tuberculosis* [24]. Comparisons were made with two front-line TB drugs: isoniazid and pyrazinamide.

While none of the diazaborines tested had activity near the potency of isoniazid, two derivatives **(9 & 10)** had MIC values in the range of 8–16 µg/ml, which is superior to pyrazinamide (~200 µg/ml).

Diazaborines as steroid mimics

Another potential application of diazaborines is in the design of 'ultra-high' fidelity estrogen structural mimics **(11)**.

Crystalographic data for compound **11**, which contains an intramolecular hydrogen bond, confirm the estrogen-like conformation of these boron-containing heterocycles [23]. Screening for antiproliferative activity against MCF-7 human breast cancer cells demonstrated an IC_{50} value for **11** of approximately 5 µM [9].

■ Boronic acid hepatitis C virus serine protease inhibitors

Hepatitis C virus (HCV) infection is a major cause of human liver disease. It is estimated that over 200 million people worldwide are chronically infected with HCV. HCV was first identified by molecular cloning in 1989 [26] and is an enveloped virus containing a single-strand RNA molecule of positive polarity with approximately 9600 base pairs. The HCV serine protease NS3/4A is considered to be an essential enzyme for the replication of the virus and has been a clinically validated drug target by BILN-2061 [27].

Peptide boronic acid derivatives, targeting the NS3/4A serine protease by trapping the catalytic Ser-139 hydroxyl functional group with its empty *p*-orbital of boron, have been investigated for more than a decade in the quest for novel agents for the treatment of HCV infection. The functional boronic acid is positioned at the peptide-1 (P1) position of the peptidomimetic. Compound **12** is an example of the class of peptide boronic acids discovered in 1996 and shows an IC_{50} of 34 nM against the NS3/4A enzyme [201].

A less-polar analog, **13**, was also made, presumably with the intention to improve cellular penetration. Afterwards, shorter peptide boronic acids and their esters with proline scaffold, such as **14**, **15** and **16**, were synthesized [202,203]. The (+)-pinanediol moiety is needed for the chiral synthesis of the P1 amino boronic acid and may also promote the cellular penetration due to its lipophilicity.

A follow-up study of **14** P1-variable analogs reveals that compound **17** has a Ki of 2 nM against NS3 protease, 1000-fold selectivity over elastase and 40-fold selectivity over chymotrypsin [28].

The enzyme potency of **17** is remarkable. The large borate ester present at the P1 site might have been hydrolyzed to expose the functional boronic acid. More recent examples have quinoline and isoindoline structures at the P2* position. Examples include compounds **18** and **19**, which have borate ester functionalities at the P1 site and quinoline and isoindoline at the P2* site, respectively. Both inhibitors exhibited increased molecular interaction with the NS3 protease, as reflected in their potency enhancement [205,206]. This also resulted in the lower peptide character of the inhibitors compared with previous compounds, such as **17**, and is a progress towards the goal of discovering inhibitors for oral use.

A further advance in this area was the successful synthesis of macrocyclic boronic acid protease inhibitors, for example **20–22** [207]. Although the biological data have not been disclosed, their enzyme potencies are likely to be better than the corresponding acyclic analogs, as observed with other nonboronic acid protease inhibitors, due to the reduced rotational freedom and the known SAR in the HCV protease inhibitor field. It is speculated that one of their derivatives might have entered clinical development [107].

Schering-Plough scientists recently published their work on boronic acid derivatives of SCH-503034 **(23)**. SCH-503034 is in advanced clinical development for the treatment of HCV [29].

As illustrated in the appendices, the replacement of P1 ethyl side chain in **24**, **26** and **28** with cyclobutylmethyl improves enzyme potencies by 50-, 68- and 260-fold, respectively, giving **25** (Ki = 10 nM), **27** (Ki = 0.5 nM) and **29** (Ki = 0.2 nM). Enzyme potencies of boronic acids are not significantly different from their pinanediol esters in comparison of **26** with **28** and **27** with **29**. Evaluation of these compounds in the cell-based replicon assay gave an EC_{90} of more than 5 μM. The poor potency suggests that these inhibitors may have very limited cell permeability.

In summary, incorporation of boronic acid into HCV serine protease inhibitors has been a successful strategy in finding novel HCV therapeutics.

■ Boronic acid as β-lactamase inhibitors

β-lactam antibiotics remain the most used antibacterial agents in clinical practice. Their mechanism of action consists of interfering with cell wall assembly by binding to penicillin-binding proteins that insert the peptidoglycan precursors into the nascent cell wall and inhibiting bacterial growth [30]. However, the continuous development of resistance represents a serious threat to the clinical utility of β-lactams, leading to an urgent requirement for new compounds [31].

β-lactamases represent the most common single cause of bacterial resistance to β-lactam antibiotics, especially in Gram-negative bacteria [32]. β-lactamases act by catalyzing the hydrolysis of the amide bond of the β-lactam ring, thus leading to biologically inactive products [33]. There are more than 450 members of the β-lactamase superfamily, divided into four classes (A, B, C and D). Classes A, C and D are serine proteases and class B is a metallo-β-lactamase. An important strategy that has been successfully utilized for overcoming β-lactamase-mediated resistance

to β-lactams has been the co-administration of the β-lactam antibiotic together with a β-lactamase inhibitor [34]. In these combinations, the β-lactamase inhibitor forms a covalent adduct with the enzyme, preventing it from hydrolyzing the β-lactam antibiotic. Three widely spread clinical β-lactamase inhibitors, clavulanic acid, tazobactam and sulbactam, are effective only against class A serine β-lactamases [35]. Therefore, there is a clear medical need for broad-spectrum inhibitors that include activity against class C and D enzymes [36].

Boronic acid derivatives have proven to be promising selective inhibitors of the serine protease family of β-lactamases. The electrophilic boron atom acts as a mimic of the carbonyl carbon of the β-lactam ring and forms a tetrahedral adduct with the catalytic serine, which closely resembles one of the transition states of the hydrolytic mechanism [37]. Compounds **30–32** were discovered as potent inhibitors of AmpC β-lactamase. They were designed to gain interactions with highly conserved residues, such as Asn343, in addition to catalytic serine, and to bind more tightly to the enzymes. Compound **30** has a Ki value of 420 nM in AmpC. The stereocontrolled introduction of the phenyl group, mimicking the dihydrothiazine ring as well as the configuration at the C7 of cephalosporins, led to a tenfold improvement in affinity (compound **31**, Ki = 35 nM). Addition of a *m*-carboxyphenyl moiety further improved affinity against AmpC β-lactamase (**32**, Ki = 1 nM) [37].

Another series of glycylboronic acids bearing the side chains of cephalosporins and penicillins have proven to be reversible and competitive inhibitors of CTX-M β-lactamase. Compound **33**, containing the side chain of naficillin, has Ki values of 1.2 and 3.0 μM against CTX-M-9 and CTX-M-16, respectively. The 2-aminothiazole inhibitor **34**, containing the side chain from ceftazidime, has Ki values of 15 and 4 nM against CTX-M-9 and CTX-M-16, respectively. Both **33** and **34** adopted a conformation in the active site consistent with acylation transition state analogues [38].

In summary, the unique ability of the boronic acid functionality to accept an active site serine into its electophilic *p*-orbtal has provided a novel series of β-lactamase inhibitors.

■ Amino-acyl tRNA synthetase inhibitors

There is a clear need to develop new efficacious therapeutics to treat fungal infections. One of the strategies is to discover and develop novel chemotype agents. Amino-acyl t-RNA synthetases are crucial for protein synthesis, and targeting the editing domain of this enzyme is a new approach to its inhibition. A new class of boron-containing compounds, known as 1,3-dihydro-1-hydroxy-2,1-benzoxaboroles, has been identified as inhibitors of fungal leucyl t-RNA synthetase and have potent antifungal activities with MICs as low as 0.25 μg/ml against the major dermatophytes *Trichophyton rubrum* and *Trichophyton mentagrophytes* and the yeasts and molds *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* [39,208]. AN2690 **(35)** and AN2718 **(36)** are two examples of this class of compounds.

A penetration study indicated that these benzoxaborole compounds can effectively penetrate through human nail plate and reach the nail bed in sufficient concentration to inhibit fungal pathogens [40]. AN2690 **(35)** is currently in clinical development for the topical treatment of onychomycosis, a fungal infection of the nail and nail bed. AN2718 **(36)** is also in clinical trials to treat skin and other topical fungal infections.

Mechanism investigation with AN2690 **(35)** demonstrates that this compound inhibits yeast cytoplasmic leucyl-tRNA synthetase by formation of a stable AN2690–$tRNA^{Leu}$ adduct **(38)** in the editing site of the enzyme [41]. The AN2690–$tRNA^{Leu}$ adduct **(38)** is formed through the boron atom of the AN2690 **(35)** and the *cis*-diol on the 3′-terminal adenosine **(37)** of the tRNA, as proposed below.

The trapping of enzyme-bound $tRNA^{Leu}$ in the editing site prevents catalytic turnover, thus inhibiting synthesis of leucyl-$tRNA^{Leu}$ and consequentially blocking protein synthesis. This result establishes the editing site as a novel target for aminoacyl-tRNA synthetase inhibitors.

In summary, the recent discovery of boron therapeutics as amino acyl t-RNA synthetase inhibitors, acting by trapping the tRNA in the enzyme-editing domain, is expected to be a promising field for the discovery of novel antifungal therapeutics. The combination of the unique boron chemistry, molecular-level knowledge gained from crystal structure studies and rational drug design has established a powerful drug-discovery machinery to feed the development pipeline.

■ Boron-containing anticoagulants

Thrombin and Factor Xa have been promising targets for anticoagulant agents for more than a decade. A number of boro-Lys- and boro-Arg-based (boronic acid analogs of lysine and

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R-B(OH)-OR′, five-membered heterocyclic ring containing a boron, an oxygen and three carbon atoms

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