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Boron Therapeutics on the Horizon

Michael P. Groziak

No pharmaceutical based on boron has yet made it to market, but this may soon change. The new millennium has brought with it some unique classes of bioactive boron compounds that are sufficiently mature in development to be considered significant and timely advances in their respective chemotherapeutic areas. Because boron is seldom seen as a constituent of a bioactive agent, this review relates some of the pertinent biologic and physiologic properties of boron and then describes in detail those boron-based agents clearly visible on the therapeutic horizon. Highlighted agents include boronic acids and boron heterocycles as potent proteasome inhibitors, β-lactamase inhibitors, dipeptidyl peptidase inhibitors, inositol trisphosphate receptor modulators, antibacterials, and antiestrogens. As these new agents are welcomed into the therapeutic armamentarium, others will surely follow and the prescribing clinician will already have an awareness and appreciation of the unique benefits that these compounds have to offer.

Keywords: boron, drug development, proteasome, β-lactamase, anticoagulant, antithrombotic, dipeptidyl peptidase IV, inositol trisphosphate receptor, antibacterial, antiestrogen.

INTRODUCTION

Boron, the element immediately to the left of carbon in the periodic table, has some unique and potentially valuable properties to offer to medicine, but unfortunately it has been greatly underutilized in therapeutic agent development to date. There are two principal reasons for this. The first is that very few boron-containing natural products are available to serve as an intellectual spark for medicinal chemists in their drug-design efforts, and to make matters worse, these turn out to be rather poor models. The ionophoric macrodiolide antibiotics boromycin,[1,2] aplasmomycin,[3–5] and tartrolon B[6–8] are all carbon/oxygen-based macrocycles that tightly yet reversibly complex boric acid in its borate conjugate base form through a network of four dissociable B-O bonds. These tetrahedral borate anion complexes are such potent potassium ion carriers that they are highly toxic to both bacteria and mammalian cells. They are the only boron-containing natural products known. No boronic acid natural product has ever been found, and it may be that nature simply lacks the biosynthetic enzyme machinery needed to form a carbon-boron bond. This, in turn, suggests that nature might also lack the metabolic enzymes needed to break them. If this turns out to be true, it would be highly significant to the development of boronic acid-based therapeutic agents.

The second reason for the underutilization of boron in drug development is that very few organic chemists have explored the construction of stable boron-containing molecular platforms on which new bioactive agents could be built.[9] The deliberate engineering of boron in a hydrolysis-resistant and charge-useful manner into such a platform requires considerable thought and planning in molecular design. For example, whereas anionic tetrahedral borates like those in the macrodiolide natural products are of very limited utility as molecular design fragments because of their charge, the carbon-substituted forms of boric acid $B(OH)_3$ are considerably more useful—but primarily just in monosubstituted form. This form is typified by the very weakly acidic boronic acids $RB(OH)_2$, which are water stable and neutral compounds at physiologic pH and thus are quite well suited for pharmaceutical agent design. Disubstitution, conversely, gives the borinic acids R_2BOH, which are

Pharmaceutical Discovery Division, SRI International, Menlo Park, CA.
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Address for correspondence: Pharmaceutical Discovery Division, SRI International, Menlo Park, CA 94025–3493, USA; e-mail: michael.groziak@sri.com

acidic compounds existing largely as tetrahedral anionic borinates at physiologic pH. Trisubstitution gives the triorganoboranes R_3B, which are extremely acidic and for all practical purposes useless in drug design. Thus, it is not at all surprising to find that most of the boron-based therapeutics currently on the horizon are either boronic acids themselves or boron heterocycles that are simply internally complexed versions of boronic acids.

BIOLOGICAL ASPECTS OF BORON

Boronic acids are fairly common and easily prepared synthetic organic compounds. Many are commercially available, and none to date has been found to be unusually toxic. They are stable under physiologic conditions but can be induced under laboratory conditions to undergo chemical deboronation via either hydrolysis or oxidation. The former generates a hydrocarbon and the latter an alcohol from the organic fragment, but both produce boric acid. It follows, then, that no matter whether a boronic acid–based therapeutic agent is actively metabolized or simply undergoes chemical degradation in vivo, the production of boric acid is not to be unanticipated. It is therefore important to be aware of some of its biologic and physiologic properties.

Boron, present solely as boric acid or its borate salts in nature, is a micronutrient soil component essential for growth in vascular plants,[10,11] which take it up by the roots.[12] It is required by diatoms, but not fungi or bacteria, and it has been shown to stimulate growth in yeast.[13] A specific biochemical function for boron in mammals has yet to be determined, but dietary supplementation with it is known to increase significantly plasma steroid hormones,[14] alter plasma lipid metabolites, and improve bone strength,[15] thus having implications in clinical conditions like arthritis.[16] Boron nutriture may be important for brain and psychological function,[17] and indeed it may be important throughout the life cycle.[18] A recent study indicates that boron metabolism in humans may be subject to genetic regulation.[19] Evidence for its role as an essential nutrient for humans steadily accumulated during the past decade,[20–22] and it now seems likely that dietary boron merits some form of guidance under a recommended dietary allowance.[23–25] Earlier this year, the US Food and Nutrition Board set a Tolerable Upper Intake Level (UL) for boron at 20 mg per day.[25A] In the United States, coffee and milk account for the largest total boron intake because of their high consumption rate, but wine, raisins, and peanuts and other nuts actually have a higher boron content.[26] Interestingly, boron in drinking water was once thought to play a beneficial role in the pathology of dental caries,[27] it has attracted some attention in alternative medicine for treating osteoarthritis,[28] and it has been used by athletes as a nutritional supplement for building muscle mass.[29]

Boric acid (LD_{50} of 3450 mg/kg orally in the mouse and 2660 mg/kg orally in the rat) and its simple borate salts like borax have been studied in great detail and pose no significant toxicity threat,[30] even though there is considerable exposure from consumer products.[31] Serious poisoning of humans with boric acid is unlikely to result from a single acute ingestion,[32] and aggressive treatment is not necessary[33] unless there has been inadequate urine flow and dehydration for several days.[34] Boric acid does not associate strongly with serum proteins but instead rapidly diffuses to the extravascular space without accumulating in tissues and is excreted efficiently via glomerular filtration.[35] Pregnancy has been shown to have little or no effect on the renal clearance of boric acid in both rats[36] and humans.[37] Additional health-related information on boric acid and simple organoboron compounds can be found in the contributions to the International Symposia on the Health Effects of Boron and Its Compounds[38,39] and in specialized reviews of the chemistry and biology of simple inorganic and organic boron compounds.[40,41]

PROTEASOME INHIBITORS

Boronic acids interconvert with ease between a neutral trigonal planar form and an anionic tetrahedral borate one, and this property enables them to potently inhibit biochemical acyl group transfer reactions, especially hydrolyses like those catalyzed by the enzymes chymotrypsin, trypsin, thrombin, and other proteases. Boronic acid–based protease inhibition first emerged three decades ago with the discovery of good inhibitors of chymotrypsin.[42–45] A few of the boronic acids effect simple competitive inhibition from their anionic tetrahedral borate form, but most undergo a reversible yet strong covalent attachment from their neutral trigonal planar form to a protease active site nucleophile—usually a serine residue.

The proteasome is the major nonlysosomal endoprotease in cells, where it generates antigenic peptide ligands for the major histocompatibility complex (MHC) class I proteins. Suppressing the production of antigens for cytotoxic T cells by inhibiting the proteasome, therefore, is an important approach to modifying the cytotoxic immune response. This has obvious applications in the areas of transplant rejection and autoimmune disease.[46] Inhibiting the proteasome has applications in cancer chemotherapy as well. For ex-

ample, the dipeptide boronic acids like PS-341 (Fig. 1A) represent a new class of proteasome inhibitor[47] that are entering early-phase clinical trials as anticancer drugs.[48–50] PS-341, an *N*-pyrazinylcarbonylated derivative of the dipeptide boronic acid Phe-Leu-$B(OH)_2$, is potently cytotoxic against cultured MCF-7 human breast cancer cells with an IC_{90} of 50 nmol/L on 24 hours of exposure to the drug. The combination of proteasome inhibition with conventional chemotherapy may have significant potential in overcoming the high incidence of chemotherapy resistance.[51] In addition, the ability to suppresses β-amyloid peptide (A beta) secretion from cultured cells has been found to correlate extremely well with a peptide boronic acid's potency at inhibiting the proteasome. New boron-based chemotherapeutics for Alzheimer's disease may therefore be forthcoming.[52]

ANTICOAGULANTS

In the anticoagulant field, the clinically used heparins and the vitamin K antagonist warfarin may soon be joined by new boronic acid inhibitors of the trypsin-like protease thrombin and the coagulation factor Xa. Several of these types of compounds are now moving into clinical trials.[53] The inhibition of thrombin by boronic acids has been under development for nearly a decade,[54–59] with the aim to produce a drug to complement hirudin. The development of orally bioavailable boronic acid inhibitors of coagulation factor Xa is more recent,[60] and interest is high because cell signaling by Xa contributes to pro-inflammatory responses in vivo.

β-LACTAMASE INHIBITORS

Bacterial resistance to the β-lactam antibiotics has created a pressing need for new therapeutic agents for the treatment of β-lactam–resistant infections. Many β-lactamases depend on an essential active-site serine residue to effect catalysis, making them ideal targets for potent inhibition by boronic acids. Early on, simple boronic acids and even boric acid itself were found to competitively inhibit a β-lactamase from *Bacillus cereus*.[61] Soon afterward, boronic acids active against β-lactamases from *Pseudomonas aeruginosa* and *Escherichia coli*[62] and from *Citrobacter diversus* and *P. aeruginosa*[63] were uncovered. Using a rational design approach, the simple boronic acids were structurally elaborated to resemble the drugs penicillin G and methicillin, and these new compounds were found to potently inhibit β-lactamases from *B. cereus* and *P. aeruginosa*.[64]

An x-ray crystal structure of 3-aminophenylboronic acid bound by *E. coli* AmpC β-lactamase was obtained, and this was used as a guide to screen a large number of other boronic acids for activity.[65] One of the most potent uncovered in this manner was the benzothiophene boronic acid known as BZBTH2 (Fig. 1B), inhibitory with a K_i of 27 nmol/L against this intrinsically resistant β-lactamase.[66] In a follow-up study, the x-ray crystal structure of BZBTH2, itself bound by AmpC β-lactamase, was solved to assist in the structure-based development of third-generation boronic acid inhibitors. At the same time, BZBTH2 properties that are rather un-β-lactam in nature were discovered, namely, that this agent is unaffected by two common

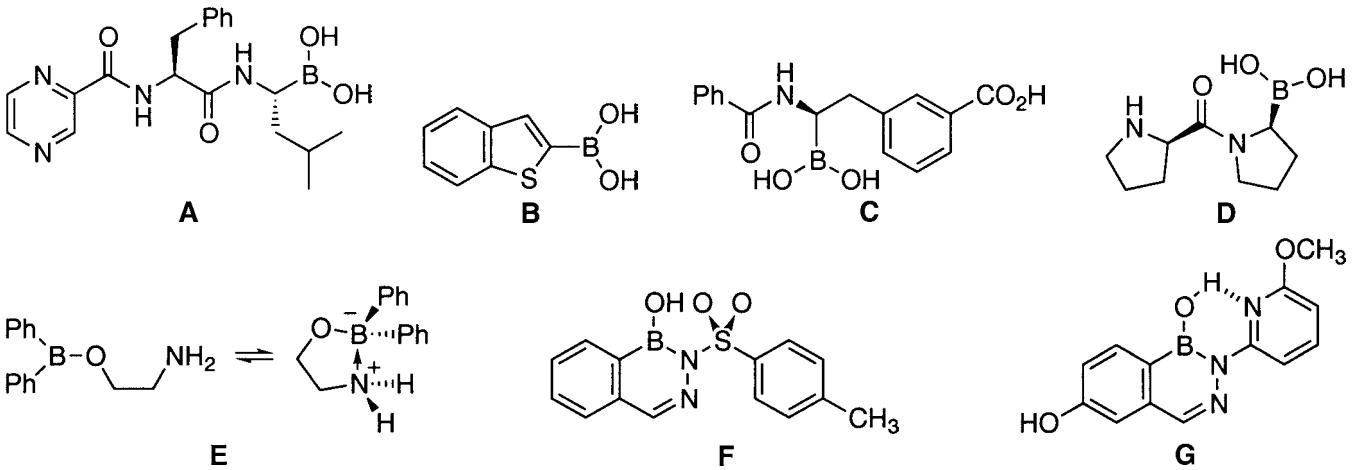

Fig. 1. Chemical structures of the proteasome inhibitor PS-341 **(A)**, the β-lactamase inhibitor BZBTH2 **(B)**, the β-lactamase inhibitor (1*R*)-1-benzamido-2-(3-carboxy-2-hydroxyphenyl)ethylboronic acid **(C)**, the dipeptidyl peptidase IV inhibitor ProboroPro **(D)**, the inositol 1,4,5-trisphosphate receptor modulator 2-APB **(E)**, the enoyl acyl carrier protein reductase inhibitor benzodiazaborine **(F)**, and a boron estrogen mimic **(G)**.

resistance mechanisms, and, furthermore, it does not induce the expression of AmpC.[67]

One of the first rationally designed boronic acid inhibitors of the TEM-1 β-lactamase from *E. coli* was a very potent compound, with a K_i of 110 nmol/L.[68] An x-ray crystal structure of the bound inhibitor was obtained, and even more potent compounds were designed based on that structure.[69] This effort produced (1*R*)-1-phenylacetamido-2-(3-carboxyphenyl)ethyl boronic acid (Fig. 1C), a compound closely resembling the best known TEM-1 β-lactamase substrate, benzylpenicillin (penicillin G).[70] This boronic acid is inhibitory with a K_i of 5.9 nmol/L, an amazing potency supporting the widespread contention that boronic acids are superior to all other kinds of inhibitor species, as was demonstrated for the class C β-lactamase of *Enterobacter cloacae* P99.[71]

DIPEPTIDYL PEPTIDASE IV INHIBITORS

The serine protease dipeptidyl peptidase IV (DP-IV, also known as CD26 and Tp103) is a 103-kd activating molecule expressed on the surface of human T lymphocytes. It is inhibited quite effectively by a boron compound known as ProboroPro, the dipeptide boronic acid (D)Pro-(D)Pro-$B(OH)_2$ (Fig. 1D).[72–76] In contrast to other serine proteases, DP-IV hydrolyzes the normally inhibitory *N*-peptidyl-O-acyl hydroxylamines, thus hinting at a catalytic mechanism for this proline-specific enzyme that is somehow different from the others. Proline-containing peptide boronic acids are usually somewhat unstable, but the acyl pyrrolidides like ProboroPro have a good stability and typically inhibit DP-IV in the micromolar and sometimes even nanomolar range. DP-IV is virtually absent on resting T cells, but after activation, it is strongly expressed and becomes involved in signal transduction during the immune response. Some of the newly developed boronic acid DP-IV inhibitors potently suppress T-cell proliferation.

INS(1,4,5)P3 RECEPTOR MODULATORS

The simple borinic acid 2-aminoethyl diphenyl borinate (2-APB, Fig. 1E) was discovered to be a novel membrane-permeable modulator of the inositol 1,4,5-trisphosphate receptor.[77] 2-APB inhibits the $Ins^{(1,4,5)}P3$-induced release of calcium[78–80] and appears to inhibit the store operated calcium channels (SOCs) in hepatocytes, but curiously by a mechanism that does not involve the $Ins^{(1,4,5)}P3$ receptor.[81] 2-APB is an unusually stable borinic acid compound because of the ethanolamine side chain. This side chain intramolecularly complexes to the acidic boron center and forms a five-membered ring zwitterion (see also Fig. 1E), which is quite stable. It is also nonpolar because, with no net charge, it is electrically neutral. Together with the presence of the two lipophilic phenyl groups, this explains why this molecule so readily penetrates membranes. At present, 2-APB is a valuable pharmacologic tool in sleep research since the $Ins^{(1,4,5)}P3$-induced release of calcium appears to play a role in the resetting of the mammalian circadian clock in the suprachiasmatic nucleus.[82] Clearly, the class of 2-APB-like compounds has the potential of producing valuable therapeutics for the treatment of sleep disorders.

ENOYL ACYL CARRIER PROTEIN REDUCTASE INHIBITORS

For more than two decades, it has been known that benzodiazaborine (Fig. 1F) and other ring-fused sulfonylated diazaborine heterocycles possess antibacterial properties, particularly against gram-negative organisms.[83] These endocyclic boron-containing compounds are simply aromatic boronic acids in which the boron is additionally held clamped by a covalent bond (the B-N) to a side chain. Early indications were that these compounds affected lipopolysaccharide biosynthesis,[84–92] and recent structural studies have established that the biomacromolecular target is in fact enoyl acyl carrier protein reductase (ENR), the NAD(P)H-dependent enzyme responsible for catalyzing a late step on the fatty acid biosynthetic pathway.[93–97] Diazaborine inhibitors of ENR form a covalent B-O bond with the 2′-hydroxyl group of the cofactor NAD's ribose unit, thus assembling a tightly yet noncovalently bound borate-based bisubstrate analog species at the active site. Interestingly, ENR is the very same biomacromolecular target of the commonly used broad-spectrum (bacteria, fungi, viruses) bacteriostatic germicide triclosan[98–101] and even, oddly enough, the well-known antituberculosis drug isoniazid.

ESTROGEN MIMICS

Our own laboratory has investigated the chemical and structural properties of a variety of benzodiazaborines formed via intramolecular dehydration in 2-acylamino- and amidino-substituted benzeneboronic acids[102] and in oximes and hydrazones of 2-formylbenzeneboronic acid.[103,104] By examining the structural

features of the latter compounds, we recognized the opportunity to design new variants of these boron heterocycles that would be ultra-high fidelity estrogen structural mimics. In particular, we saw that careful molecular engineering could produce very close structural mimics of A-ring aromatic estrogens like estradiol and estrone and AB-ring aromatic equilenins like some of the components of Premarin, the conjugated equine estrogens used extensively for estrogen replacement therapy and prevention of osteoporosis and cardiovascular disease in postmenopausal women. Our first prototype (Fig. 1G) was a boron heterocycle mimic of estradiol or dihydroequilenin O17-methyl ether featuring a strong intramolecular hydrogen bond that assembles a "virtual," flexible steroid-like C-ring. An x-ray crystal structure confirmed the remarkable estrogen-like shape adopted by this compound.[105] This and some of the other early prototypes that had a biorecognition-critical estrogen A-ring phenolic hydroxyl group were examined for their antiproliferative activity against cultured MCF-7 human breast cancer cells. The IC_{50} values near 5 μmol/L found for all these compounds provides a strong impetus for our current ongoing efforts to develop these unique boron-based compounds into therapeutics for treating breast cancer, likely as antiestrogens.

OTHERS

Other boron-based compounds exhibit intriguing in vitro activity and thus show some promise of evolving into useful therapeutics in the future. Examples include variants of benzodiazaborines found active against *Mycobacterium tuberculosis* $H_{37}R_v$,[106] acyclic nucleoside boronic acid derivatives targeted at HIV,[107] tetrapeptide boronic acids found to inhibit HIV-1 protease,[108] benzothiazoline boron complexes with antimicrobial properties,[109] and 3-aminophenylboronic acid (APBA), found to inhibit both the *Streptomyces griseus* NAD^+-glycohydrolase and ADP-ribosyltransferase enzymes.[110] The ongoing development of these bioactive agents will be well worth monitoring along side those that are further along in their development as useful therapeutics.

CONCLUSION

The novel classes of boron-based compounds described above are rapidly maturing into potent therapeutics in their own right, and prescribing clinicians need to be aware that they are coming on line. The fact that they are based on the unusual element boron is no cause for concern because it is only toward the end of the past millennium that organic chemists have learned to construct useful platforms with it and medicinal chemists have learned to appreciate its value.

REFERENCES

1. Hütter R, Keller-Schierlein W, Knüsel F, et al: Stoffwechselprodukte von Mikroorganismen. Boromycin. Helv Chim Acta 1967;50:1533–1539.
2. Kohno J, Kawahata T, Otake T, et al: Boromycin, an anti-HIV antibiotic. Biosci Biotechnol Biochem 1996;60: 1036–1037.
3. Okami Y, Okazaki T, Umezawa H: Studies on marine microorganisms. V. A new antibiotic, aplasmomycin, produced by a streptomycete isolated from shallow sea mud. J Antibiot (Tokyo) 1976;29:1019–1025.
4. Nakamura H, Iitaka Y, Kitahara T, et al: Structure of aplasmomycin. J Antibiot (Tokyo) 1977;30:714–719.
5. Chen TS, Chang CJ, Floss HG: Biosynthesis of the boron-containing antibiotic aplasmomycin. Nuclear magnetic resonance analysis of aplasmomycin and desboroaplasmomycin. J Antibiot (Tokyo) 1980;33:1316–1322.
6. Schummer D, Irschik H, Reichenbach H, et al: Antibiotics from gliding bacteria, LVII. Tartrolons: new boron-containing macrodiolides from *Sorangium cellulosum*. Liebigs Ann Chem 1994;3:283–289.
7. Irschik H, Schummer D, Gerth K, et al: The tartrolons, new boron-containing antibiotics from a myxobacterium, *Sorangium cellulosum*. J Antibiot (Tokyo) 1995;48: 26–30.
8. Berger M, Mulzer J: Total synthesis of tartrolon B. J Am Chem Soc 1999;121:8393–8394.
9. Groziak MP: Boron heterocycles as platforms for building new bioactive agents. In: *Progress in Heterocyclic Chemistry*. (Eds. Gribble GW, Gilchrist TL). Elsevier Science, Oxford, 2000, pp1–21.
10. Marschner H: Function of mineral nutrients: micronutrients. 9.7 Boron. In: *The Mineral Nutrition of Higher Plants*, 2nd ed. Academic Press, San Diego, 1995, pp379–396.
11. Agulhon H: Annales de l'Institut Pasteur, volume 34, 1910: The presence and utility of boron in plants. Nutr Rev 1988;46:353–355.
12. Dordas C, Chrispeels MJ, Brown PH: Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiol 2000;124:1349–1362.
13. Bennett A, Rowe RI, Soch N, et al: Boron stimulates yeast (*Saccharomyces cerevisiae*) growth. J Nutr 1999;129: 2236–2238.
14. Samman S, Naghii MR, Lyons Wall PM, et al: The nutritional and metabolic effects of boron in humans and animals. Biol Trace Elem Res 1998;66:227–235.
15. Armstrong TA, Spears JW, Crenshaw TD, et al: Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength char-

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