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Natural and Synthetic Small Boron-Containing Molecules as Potential Inhibitors of Bacterial and Fungal Quorum Sensing

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1. Introduction

Chemical communication is a phenomenon exhibited by many different organisms and nowadays is one of the most prominent research areas at the chemistry−microbiology interface.[1–6] For more than 20 years, science has recognized the ability of different bacteria to coordinate phenotype expression using signaling substances, which is a crucial process for successful environment colonization in plants, other animal hosts, and also for human beings[7–10]

Bacteria communicate with one another via production, detection, and response to secreted chemical signal molecules called autoinducers. This communication process is called quorum sensing (QS), and it allows bacteria to synchronize behavior on a population-wide scale. Bacterial QS is mediated by low-molecular-weight molecules signals and plays a critical role in both the pathogenesis of infectious disease and beneficial symbioses. QS controls many kinds of life activities of bacteria and has important significance in medicine, industry, and agriculture. It also can be inhibited by decreasing the activity of R protein, inhibiting production of signal molecules, and by degrading the signal molecule.[11–16] The finding of QS signal molecules of microorganisms and signal transduction mechanism is beneficial for designing a signal interference method to disrupt QS signal transduction, thus to prevent and treat microorganism infection. The discovery that many bacteria use QS systems to coordinate virulence and biofilm development has pointed out a new, promising target for antimicrobial drugs. QS has been implicated in the control of bacterial behaviors such as the secretion of virulence factors,[17,18] biofilm formation,[19,20] bioluminescence production,[21,22] conjugation,[23] sporulation,[24] swarming motility,[25] and the exchange of DNA.[26] There is significant interest in the development of synthetic ligands that can intercept bacterial QS signals and modulate these outcomes.[27]

Valery M. Dembitsky received his M.S. in Organic Synthesis from the Far East State University, Vladivostok, USSR, in 1973. He holds a Ph.D. degree in Biological Chemistry from USSR Academy of Sciences, Leningrad, in 1981, and received his D.Sc. degree in Bioorganic Chemistry and Chemistry of Natural Products from M. V. Lomonosov Moscow State Academy of Fine Chemical Technology in 1997. From 1989 to 1991, he was Associate Professor at the Organic Chemistry and Biochemistry Department, Samara State University. He also was a visiting Professor at the Department of Scientific and Industrial Research, The Massey University, Palmerston North, New Zealand, 1990; Department of Organic and Biological Chemistry, Auckland University, Auckland, New Zealand, 1990; Department of Plant Chemistry, Institute of Organic Chemistry with Phytocentre, Bulgarian Academy of Science, 1990, 2006; Department of Natural Biogenesis, Institute of Microbiology, Czechoslovakia Academy of Science, Prague, 1989, 1990, 2002; Department of Marine Chemistry, Institute of Oceanology, Polish Academy of Science, Sopot, Poland, 1989, Nantes University, France, 2004. During 1991−1992, he held guest Professorship at the School of Chemistry, Melbourne University, Australia, and from 1993, he started on collaboration with Professor Raphael Ikan, Department of Organic Chemistry, Hebrew University, Jerusalem. Since 2000, he joined the Institute for Drug Research, Hebrew University. He was awarded the 1st Prize of the Russian Government in Nanotechnology and Metalonomics, 2009. He has published four books, 23 chapters in books, 80 review articles, and more than 150 research papers. His research interests are focused in the areas of organometallic chemistry, bioorganic chemistry, chemistry of natural products, and biological imaging.

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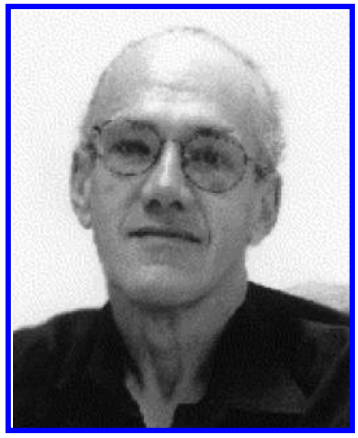


Morris Srebnik received his Ph.D. in 1984 from the Hebrew University in Jerusalem under Professor Raphael Mechoulam. On a Lady Davis Fellowship, he joined Professor H. C. Brown's group at Purdue University, where he studied the applications of organoboranes to synthesis until 1986. After a short stint at the Sigma-Aldrich Corporation, he returned to Professor Brown's group. In 1990, he accepted a position at the Department of Chemistry, University of Toledo, USA. Since 1996, he is a Professor at the School of Pharmacy, Hebrew University. His areas of interest include developing organometallic methodologies in synthesis centred around boron and zirconium, and lately also titanium, and investigating the potential uses of organoboranes in medicine. He also has an interest in isolating new sunscreen agents from natural sources such as cyanobacteria. He is the author and coauthor of more than 200 publications, one book, eight chapters in books, and 43 review articles.

Gram-positive and Gram-negative bacteria can produce species specific signal to communicate within species. The functions controlled by QS are varied and reflect the needs of a particular species of bacteria to inhabit a given niche. Many QS circuits have been described: one used primarily by Gram-negative bacteria, another used primarily by Gram-positive bacteria, a third one, that was proposed to be universal, and control of the expression of target gene. Involvements of bacterial QS in regulations of diverse responses are common in bacteria. Bacteria can from aggregates on interfaces, called biofilms, where they are much more protected against toxic agents such as antibiotics or antibodies. It is also organized in biofilms, and therefore they are very difficult to control and often even high dosages of antibiotics cannot clear infectious biofilms. Recently, it has become apparent that fungi are similar to bacteria in using specific QS molecules to regulate some population-level behaviors.[28–30a]

Organic chemists have long been interested in natural products, and their investigations have contributed to many advances in chemistry, especially the total synthesis of complex boron-containing molecules with many chiral centers. Many of these bioactive molecules are vital in the treatment of human diseases up today.[30b–e,45,54,126,136]

The large class of antibiotics (or quorum sensing inhibitors), small molecules, have a similar wide range of activities but operate by different mechanisms. The natural products used as antibiotics act by binding to specific receptors that are embedded in the cell macromolecules involved in replication, transcription, translation, or cell envelope formation. Each of these complex structures possesses many potential receptor sites for bioactive small molecules. Evidence for discrete effects of these interactions comes from transcription studies with subinhibitory concentrations of different ligands, showing a range of responses due to binding at subinhibitory concentrations to different receptors within the structure.[31–33]

This review describes boron-containing quorum sensing inhibitors (QSI) isolated from natural sources or synthesized. Many boron-containing compounds having structure similar to AI-2, and others are also reviewed. The need to discover novel small molecules that kill bacterial cells or prevent their growth, without affecting the human host, has been an ongoing challenge that has reached critical dimensions as increasing numbers of pathogens develop antibiotic resistance. Most existing antibiotics have been derived from natural products and are thus already associated with naturally occurring resistance genes in the antibiotic producing microbe. As resistance has spread, antibiotic potency and effectiveness has been maintained by continuous chemical modification.

2. Boron-Containing Compounds As Potential Quorum Sensing Inhibitors

After the discovery in 1910[34,35] that boron is one of the essential microelements for higher plants,[36] its biological role has been the subject of a number of studies.[37–41] Boron, an orphan of the periodic table of the elements, is unique not only in its chemical properties but also in its roles in biology. Biological and physiological functions for boron-containing compounds are well established, nevertheless, many questions still remain to be answered.[35,42,43]

Since the discovery of the first boron biomolecule, boromycin, in 1967,[44] several other similar biomolecules are now well-characterized.[35,40] More recently, it was shown that the boromycin is natural anti-HIV antibiotic which is produced by *Streptomyces* species and *Sorangium cellulosum*.[45]

Most recently, a bacterial cell-to-cell communication signal that requires boron was described.[33,35,41] Besides, a new feature of the role of boron comes from signaling mechanisms for communication among bacteria and among legumes and rhizobia leading to N_2-fixing symbiosis, and it is possible that new roles for B, based on its special chemistry and its interaction with Ca^{2+}, would appear in the world of signal transduction pathways.[35b] Many boron-containing natural as well as synthetic compounds with antibacterial,

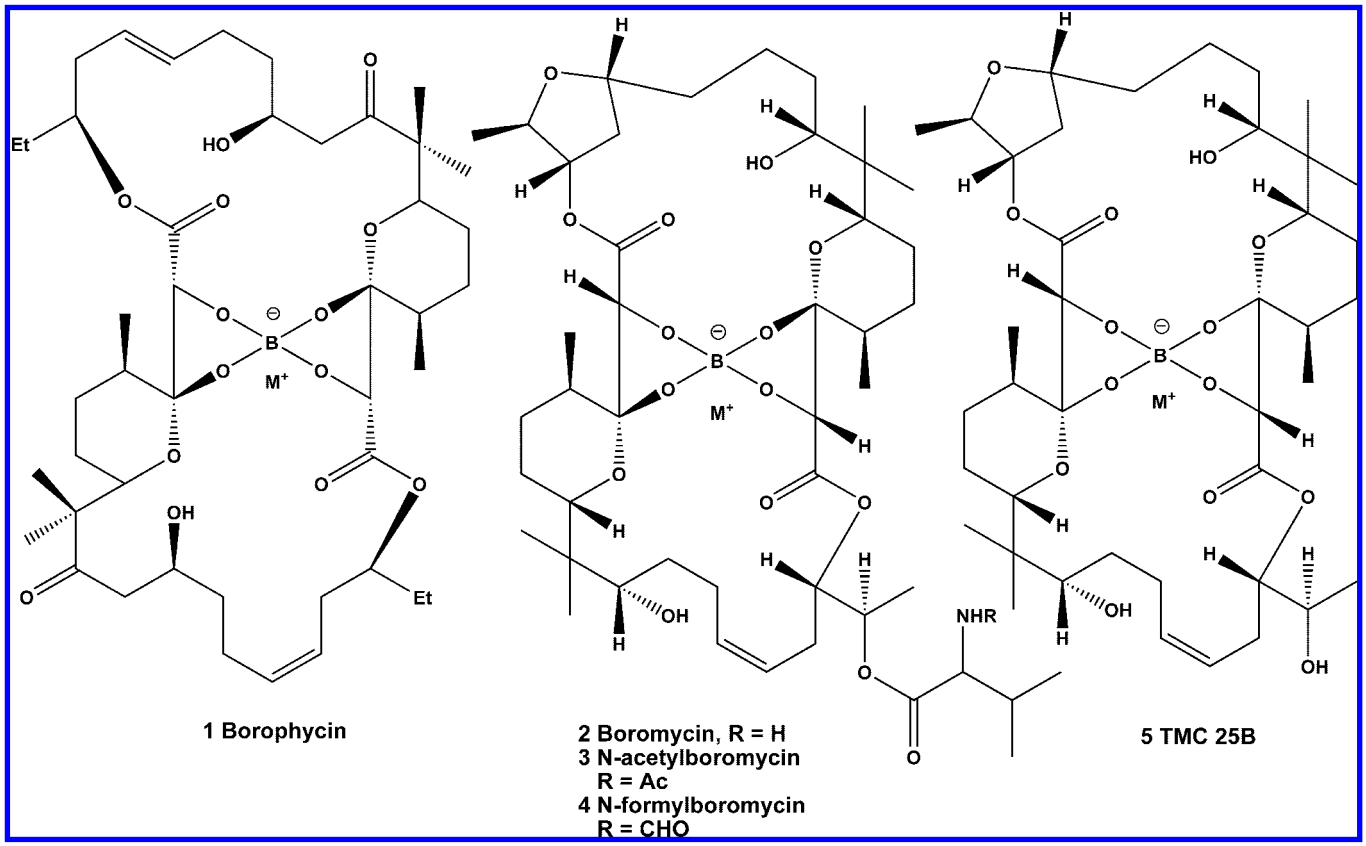


Figure 1. Chemical structure of compounds **1**−**5**.

antifunga,l and/or antiviral activities usually are autoinducers which are regulated and induced by bacterial QS. In bacteria, boron is one of the an essential parts of a signal molecules required for QS.[33,35a]

2.1. Natural Boron-Containing Compounds As Antibiotics

Four boron-containing metabolites were isolated along with the known antibiotic, borophycin **1** (Figure 1) from litophytic cyanobacterium *Nostoc spongiaeforme* var. *tenue* (Nostocaceae).[46] The potent cytotoxin, borophycin, was also isolated from lipophilic extract of marine cyanobacterium *Nostoc linckia*.[47] Boromycin **2** was first isolated from an African soil sample containing *Streptomyces antibioticus*.[44,48] Also it was isolated as a potential antihuman immunodeficiency virus (HIV) antibiotic from a fermentation broth of *Streptomyces* sp. A-3376.[49] In addition, boromycin at 0.05 μg/mL inhibited the synthesis of protein, RNA, and DNA in *Bacillus subtilis*.[50] Two antibacterial (*Staphylococcus aureus*) and antifungal (*Botrytis cinera*) compounds, *N*-acetylboromycin **3** and *N*-formylboromycin **4**, were isolated as minor components from the mycelia of the boromycin-producing *Streptomyces antibioticus*.[51] Boromycin derivative such as 027-de(2-amino-3-methyl-1-oxobutyl)-boromycin (known as desvalinoboromycin or TMC 25B) **5**, with anti-HIV activity was isolated from soil microorganisms (*Streptomyces* spp.).[52] Sodium boromycin was found to be effective against *Eimeria acervulina* and *Eimeria tenella* (Coccidia, phylum Apicomplexa, kingdom Protozoa) infestation in chickens.[53] Synthesis, biosynthesis, and biological activities of boromycin derivatives have been described and reviewed.[35a,54]

The antibiotic aplasmomycin (which is especially effective in controlling *Plasmodium berghei*) was originally isolated from *Streptomyces griseus* SS-20, found in a shallow sea sediment.[55,56] It differs from boromycin in having two identical chemical subunits surrounding the borate complex. *S. griseus* produces several variations of aplasmomycin, and the series has been designated as aplasmomycins A, B, and C (**6**−**8**) (Figure 2). Cultured *Streptomyces griseus* NCIB 11371 is also used to produce aplasmomycin, boromycin, and monoacetyl-aplasmomycin.[45a] These natural antibiotics are unique because they are the only known metabolic products containing the element boron. These seminal investigations are due mainly to the Floss group, who contributed much to the field of the biosynthetic origins of these molecules.[57,58] Aplasmomycin **6** was first isolated from a broth cultivated with a marine isolate from actinomycete *Plasmodium berghei* in which its structure was determined by Nakamura and co-workers.[59] Aplasmomycins B **7** and C **8** are produced by a strain belongs to *Streptomyces griseus* NCIB 11371.[60] A novel boron-containing antibiotic, given the name tartrolon B **9**, was isolated from Gram-negative eubacteria which live in soil and related habitats (Figure 2),[61] *Sorangium cellulosum*.[62] Absolute configuration and biosynthesis of tartrolon B were determined and investigated by Schummer and co-workers.[62b] The biosynthesis of tartrolon B **9** is closely related to boromycin **1** and aplasmomycin **6**. But with respect to the origin of the starting unit, it is similar to that of borophycin **1**. These new boron-containing complexes tartrolon B **9**, boromycin **2**, aplasmomycin **6**, and borophycin **1** are polyketides and have the same boron-binding substructure in each half of the symmetric molecules. The antibiotics, tartrolones A and B, were active against Gram-positive bacteria and mammalian cells. Tartrolone C inhibited the HIF-1 transcriptional activity under hypoxic conditions with an IC_{50} of 0.17 μg/mL.[63] Because of a favorable conformation of all macrocycle boron-containing compounds (**1**−**9**), four hydroxy groups of these substruc-

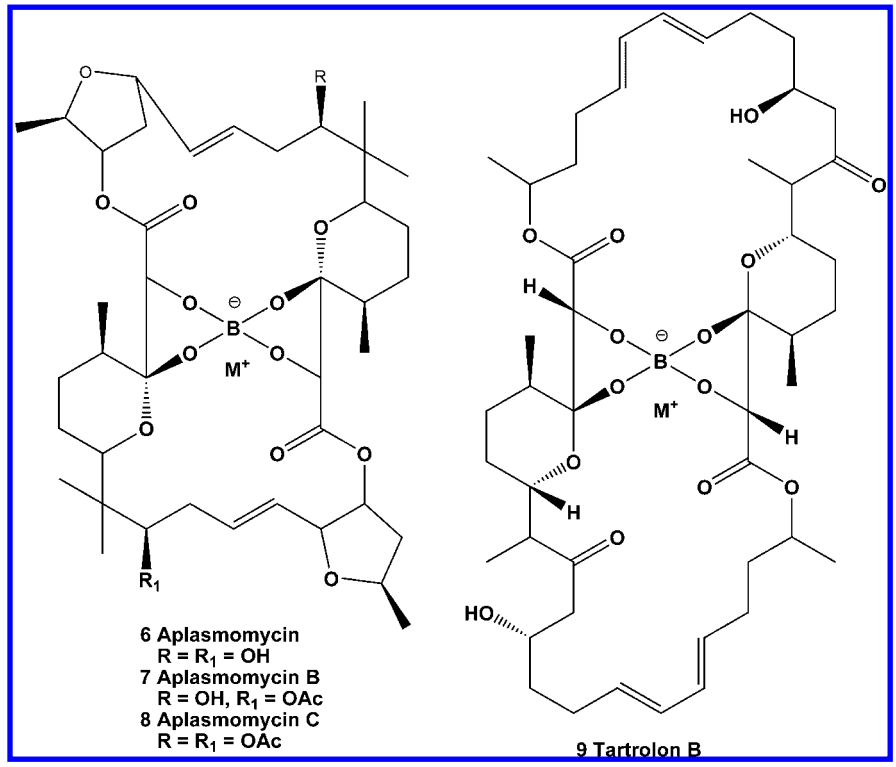


Figure 2. Chemical structure of compounds **6**−**9**.

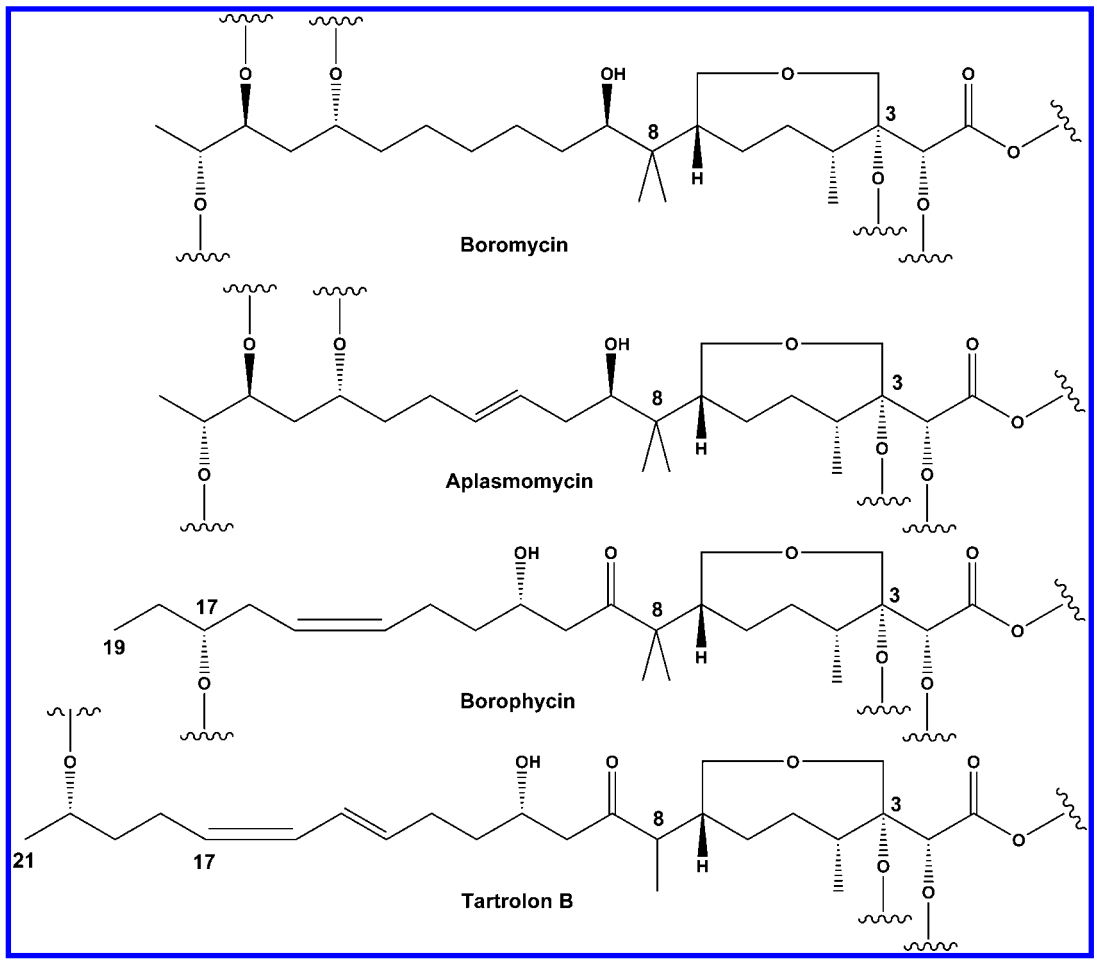
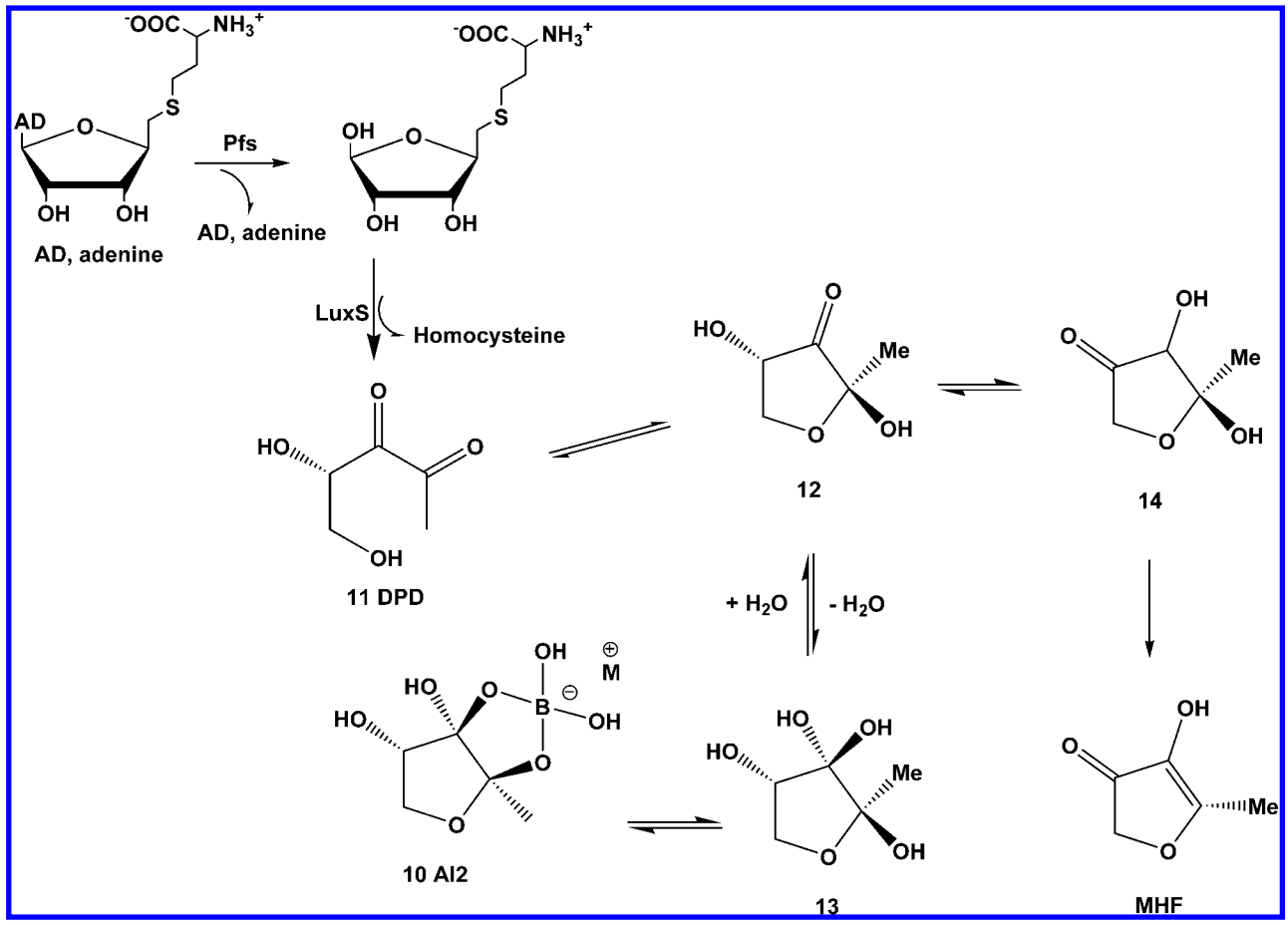


Figure 3. Comparative carbon skeleton of polyketide chains included in structures of boron containing complexes isolated from streptomycetes, cyanobacteria, and myxobacteria: borophycin, boromycin, aplasmomycin, and tartolon B.

Scheme 1



tures are ideally positioned to form a Boeseken complex[64] with boron.[62c] Berger and Muzler were the first who described a total synthesis of tartrolon B **9** (Figure 3).[65]

Vibrio cholerae, the causative agent of the human disease cholera, uses cell-to-cell communication to control pathogenicity and biofilm formation. At low cell death, *V. cholerae* activates the expression of virulence factors and forms biofilms. At high cell death, the accumulation of two quorum-sensing autoinducers depresses these traits. Two autoinducers, cholerae autoinducer-1 (CAI-1) and autoinducer-2 (AI-2) function synergistically to control gene regulation although CAI-1 is the stronger inducer. *V. cholerae* AI-2 **10** (Scheme 1) is a furanosyl borate diester, which was identified and fully characterized in *Vibrio harveyi*. Originally, AI-2 furanosyl borate diester complex **10** was identified in the bioluminescent marine bacterium *Vibrio harveyi* as one of two autoinducers that regulate light production in response to cell density.[66] Many groups of Gram-positive and Gram-negative bacteria contain synthase enzyme for the synthesis of AI-2.[67] AI-2 is produced from *S*-adenosylmethionine in at least three enzymatic steps.[68] In the final stage, 4,5-dihydroxy-2,3-pentanedione (DPD) **11** is converted to the cyclic structure of DPD, which is named pro-AI-2 **13**, and can react with borate to form a cyclic borate diester AI-2 **10**. The boric acid required for this reaction is widely available in the biosphere. AI-2 has been proposed to serve as a "universal" bacterial QS signal containing boron for interbacteria community communication.[33,69]

Chen and co-workers[33a] have shown that AI-2 represents a new class of signaling molecule, made even more interesting by the unexpected finding that it contains boron. More than 40 species of diverse bacterial genera contain the highly conserved LuxS gene. Similar to the pathway for the synthesis of pro-AI-2, *S*-ribosylhomocysteine is a produced in the *S*-adenosylmethionine utilization pathway and homocysteine is recycled back to methionine. Thus, LuxS has an important general role in bacterial metabolism and pro-AI-2 might simply be a metabolite. *V. harveyi* has apparently "hijacked" this pathway to use AI-2 as a signaling molecule involved in the quorum sensing-mediated control of bioluminescence. Biosynthesis of DPD requires the enzyme LuxS, which is present in over 60 species of bacteria.[70] DPD undergoes further rearrangements to yield molecules generically termed "AI-2" (autoinducer-2) that are active in signaling.[33] The widespread nature of LuxS and DPD production has led to the proposal that AI-2 functions in interspecies communication.[71] The AI-2 signaling cascade has been elaborated for *V. harveyi.* From X-ray studies of the receptor protein LuxP, the structure of the signaling molecule was shown to be a hydrated furan diulose to which a cyclic borate group has been attached AI-2 (**10**).

The observation of borate in a signaling role in bacteria is unusual and has generated considerable interest. Obvious questions arose concerning the role of enzymes in the conversion of DPD and its cyclization form **12** to the hydrate **13** and boron complex AI-2 (**10**) and whether the receptor protein LuxP (Figure 4) is required to provide a driving force for hydration and to stabilize the borate complex. While borate binding to *cis*-cyclopentane-1,2-diols and furanoses is well established.[69] The cyclic structure **12** is proposed to be unstable toward isomerization to **14** and then irreversible elimination to give methylhydroxyfuranone (MHF). In ad-

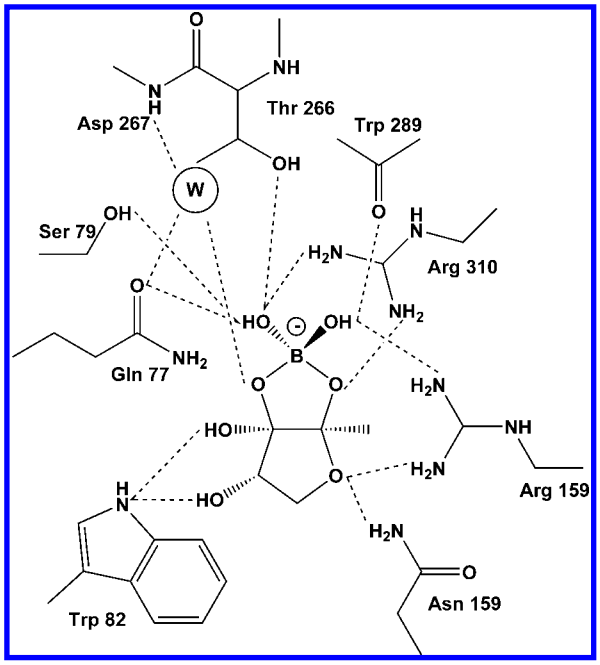


Figure 4. AI-2 and hydrogen bond network that stabilizes boron complex in the sensor protein LuxP binding site.

dition, simple α-hydroxycyclopentanones have a tendency to oligomerize through acetal formation.

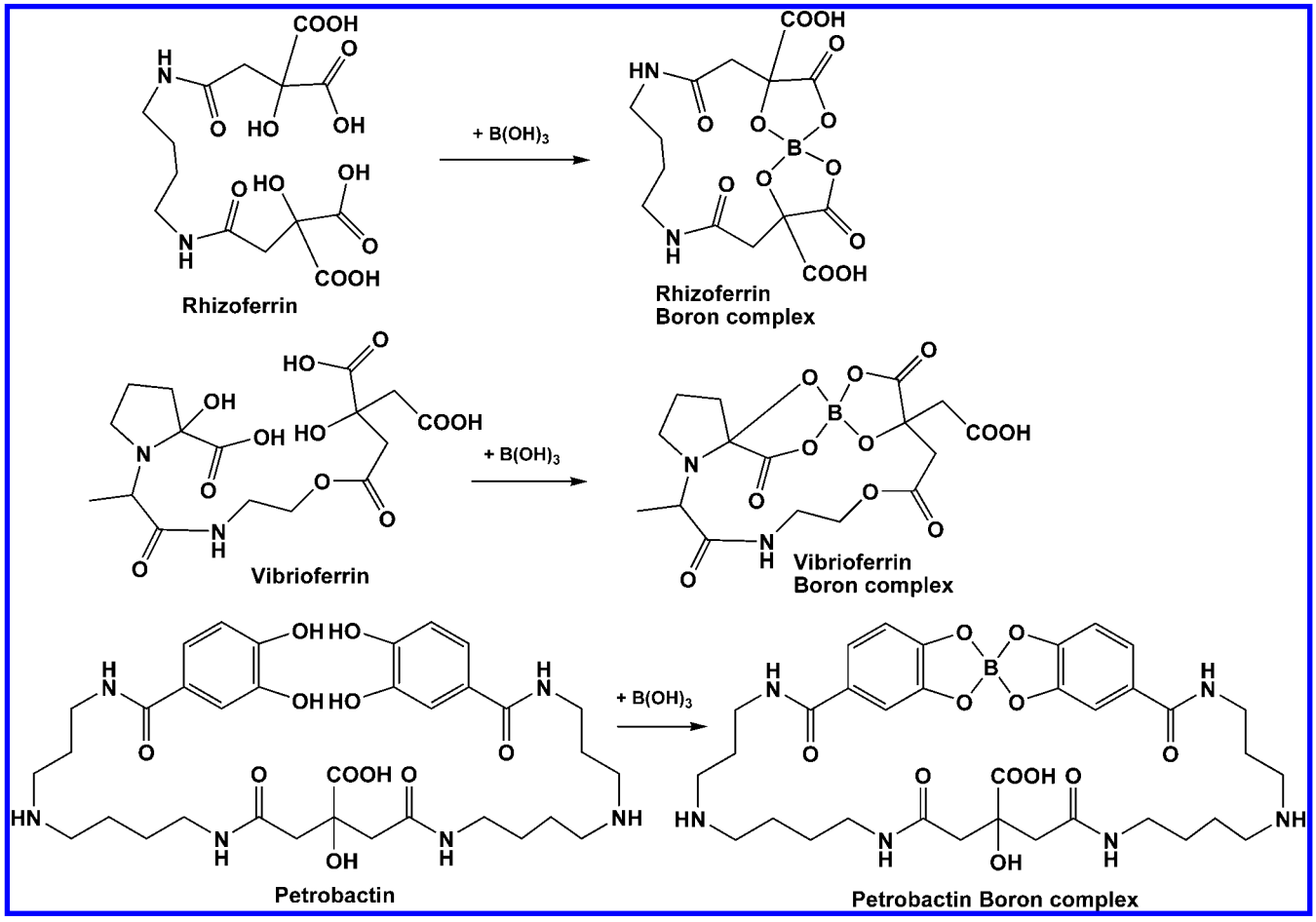
Winans[72a] has termed this "*Bacterial Esperanto*", a novel language between cell−cell communications in bacteria. At least one cyanobacterium also communicates via oligopeptides.[72b] In contrast, most signaling in proteobacteria is accomplished using *N*-acyl-homoserine lactones.[72c] However, earlier studies of AI-2, first discovered in the bioluminescent marine bacterium *V. harveyi*, suggested that it was unlikely to resemble any of these molecules.[33,66]

3. Boron Siderophore Complexes

Siderophores are small, high-affinity metal chelating compounds secreted by bacteria, fungi, and grasses (Poaceae). Siderophores are among the strongest soluble iron binding agents ever known. Several hundred compounds of terrestrial siderophores are known, although only few metabolites of marine siderophores were identified.[73a–c]Also siderophores are known to have as their primary function the binding and transport of iron from the environment into microbial cells, increasing evidence suggesting that they may also play another significant role as signaling molecules. Quorum sensing bacteria excrete low-molecular-weight chemical "messenger" molecules into the environment, which when a critical concentration is reached, trigger a signal transduction cascade. This signal cascade results in an alteration of gene expression, ostensibly in a population-dependent manner. Siderophore production and other iron transport genes are among those long reported to be under "quorum sensing" control.[73]

Siderophores are low-molecular-wight molecules that have a high specificity for chelating or binding iron or other metals, for example, aluminum, cadmium, chromium, copper, gallium, indium, lead, manganese, plutonium, uranium, vanadium, and zinc.[74a–c] It has been shown that some, but not all, siderophore classes have an unexpected binding

Scheme 2



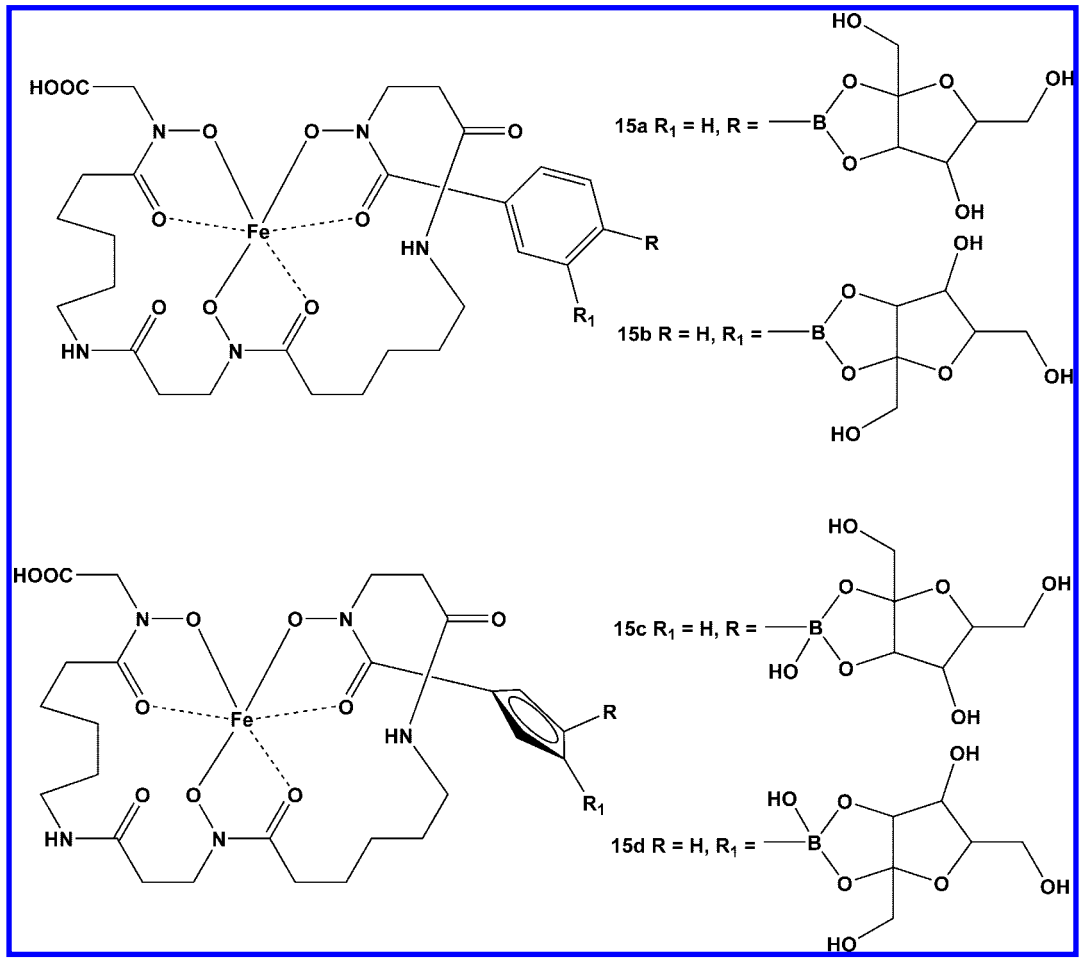


Figure 5. Linear- (**15a** and **15b**) and tripodal-trihydroxamate (**15c** and **15d**) siderophore mimics, suspending phenylboronic acid as the sugar-binding site.

affinity for boron.[74] Recently, Harris and co-workers[75] obtained boron complexes for vibrioferrin, rhizoferrin, and petrobactin (Scheme 2). Vibrioferrin is a member of the carboxylate class of siderophores originally isolated from *Vibrio parahemolyticus*, an enteropathogenic estuarine bacterium often associated with seafood-borne gastroenteritis. Recently, this siderophore was also isolated from several species of *Marinobacter*, which are closely associated or symbiotic with toxic, bloom-forming dinoflagellates such as *Gymnodinium catenatum*. Rhizoferrin is a novel carboxylate-type siderophore which has recently been isolated from *Rhizopus microsporus* and other fungi of the Mucorales (Zygomycetes), and petrobactin, a bis-catecholate, α-hydroxy acid siderophore produced by the oil-degrading marine bacterium *Marinobacter hydrocarbonoclasticus*.[76a,b]

Two linear- (**15a** and **15b**) and two tripodal-trihydroxamate (**15c** and **15d**) siderophore mimics, suspending phenylboronic acid as the sugar-binding site, were prepared.[76c] These siderophore mimics strongly bind Fe^{3+} ions, giving rise to the ligand−Fe^{3+} 1:1 complexes over a wide range of pH 2−11. The configuration of the bound sugars, implying that the phenylboronate−sugar covalent interactions are capable of inducing a chirality around the metal center (Figure 5).

4. Oxazaborolidine Derivatives

Oxazaborolidines are compounds possessing a B−N bond and are readily obtained from an amino alcohol and boronic acid.[77a] Nevertheless, despite their ubiquity in organic synthesis,[77b] the effect of oxazaborolidines on bacterial adhesion, biofilm formation, or any other pharmacological activity has been just recently known. Oxazaborolidines contain a five-member boron heterocycle; they may possess other biological activities in addition to their effect on bacterial viability in the plankton environment, as reported recently.[77c,d,78]

Dental diseases, including tooth decay, gingivitis, and periodontitis, are among the most prevalent afflictions of humankind. Oral biofilms harboring pathogenic bacteria are the major contributing virulence factors associated with these diseases.[79a,b] Adhesion of oral bacteria to the tooth surface is facilitated by physical, chemical, and biological mechanisms.[79c,d] *Streptococcus mutans* is one of bacterial species that plays a key role in dental biofilm formation and dental caries.[80] Controlling the dental biofilm is one of the major approaches to reduce dental caries and periodontal diseases.[81] Antibacterial agents are the most common means of affecting the viability of bacteria in biofilms.[82] Although effective, this approach has many clinical disadvantages, primarily the development of secondary infections and the emergence of resistant bacteria.[77a,b,78] Possible alternative means of antibacterial therapy for controlling infectious diseases have recently focused on affecting biofilm formation and bacterial accumulation.[83]

Several representative oxazaborolidines have been synthesized and evaluated against *S. mutans* for antibacterial activity.[77a,b] Minimal inhibitory concentration (MIC) values were used to determine the antibacterial efficacy of ox-

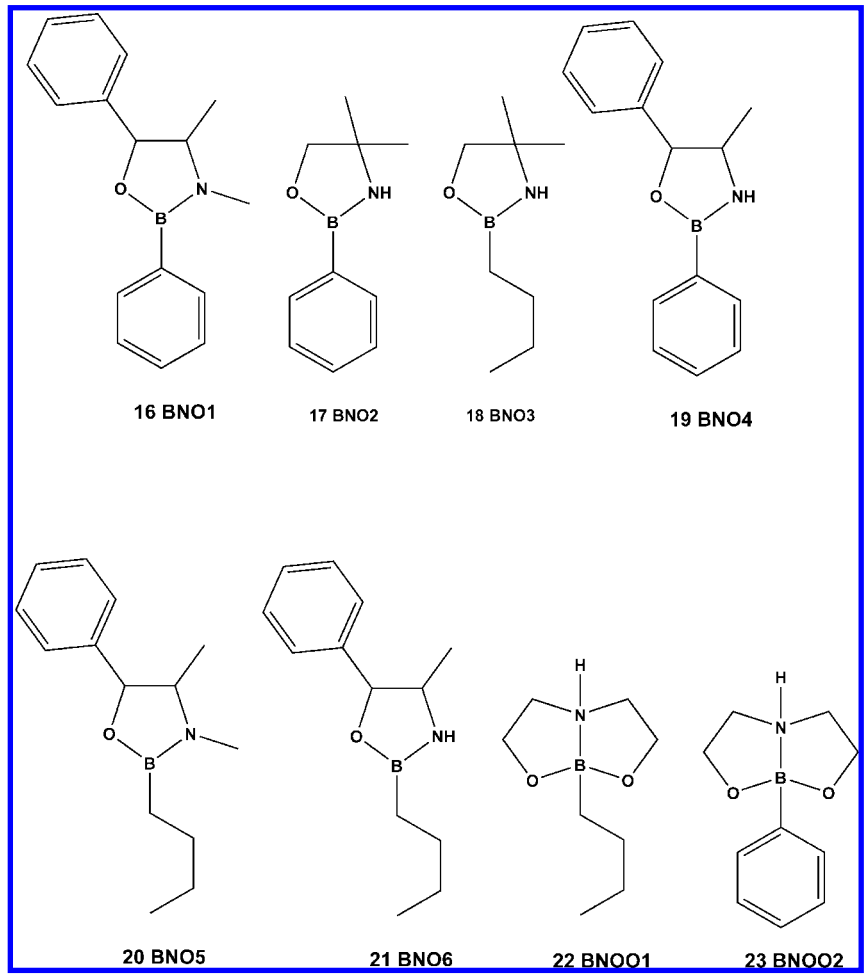


Figure 6. Chemical structure of BNO compounds **16**−**23**.

Table 1. Inhibitory Activity of Oxazaborolidine Derivatives against *S. mutans*

inhibitor	activity MIC (μM)
16	1.55
17	6.00
18	3.38
19	1.33
20	0.53
21	2.83
22	6.75
23	6.75

azaborolidines (**16**−**23**) against *S. mutans*, which is the one of the predominant bacteria in the etiology of dental caries. Although the mechanism of antibacterial action is not known at present, the most active compound in the series is **20**, which contains both an N-Me group and B-Bu group. Consequently, **16**−**19** and **21**, which do not contain either an N-Me or a B-Bu group, were less active. Compounds **22** and **23**, which are formally charged, showed the weakest activity. While boronic acids demonstrate no classic MIC at their maximal solubility in water (10 mM), all the tested oxazaborolidines demonstrated antibacterial activity at much lower concentrations (Figure 6, Table 1).

Oxazaborolidine derivatives that chemically resemble the structure of AI-2 have recently been synthesized in our laboratory.[78a,b,d,84] Five oxazaborolidine derivatives (BNO-1 to BNO-5) were tested, however, only BNO-1 (3,4-dimethyl-2,5-diphenyl-1,3,2-oxazaborolidine) and BNO-5 (2-butyl-3,4-dimethyl-5-phenyl-1,3,2-oxazaborolidine) strongly induced *V. harveyi* bioluminescence in *V. harveyi* mutant (BB170)

Table 2. Minimal Inhibitory Concentration (MIC, μM) of the Different Oxazaborolidines for *V. harveyi* BB170, BB886, and MM77

compounds	*V. harveyi* BB170	*V. harveyi* BB886	*V. harveyi* MM77
BNO-1	20−40	20−40	20−40
BNO-2	150	5−10	10−20
BNO-3	120−150	120−150	120−150
BNO-4	10−25	10−25	10−25
BNO-5	150	10−30	10−30

lacking sensor 1. A dose-dependent relationship between those oxazaborolidine derivatives and bioluminescence induction was observed with this *V. harveyi* strain (BB170). BNO-1 and BNO-5 did not affect *V. harveyi* BB886 lacking sensor 2 (Table 2).

Using a mutant strain which produces neither AI-1 nor AI-2 (*V. harveyi* MM77) showed that the presence of spent medium containing AI-2 is essential for BNO-1 and BNO-5 activity. This effect was similar when introducing the spent medium and the BNOs together or at a 3 h interval. A comparable induction of bioluminescence was observed when using synthetic DPD (pre-AI-2) in the presence of BNO-1 or BNO-5. The mode of action of BNO-1 and BNO-5 on bioluminescence of *V. harveyi* is of a coagonist category. BNO-1 and BNO-5 enhanced AI-2 signal transduction only in the presence of AI-2 and only via sensor 2 cascade. BNO-1 and BNO-5 are the first oxazaborolidines reported to affect AI-2 activity. Those derivatives represent a new class of borates which may become prototypes of novel agonists of quorum sensing mediated by AI-2 in *V. harveyi*. Therefore,

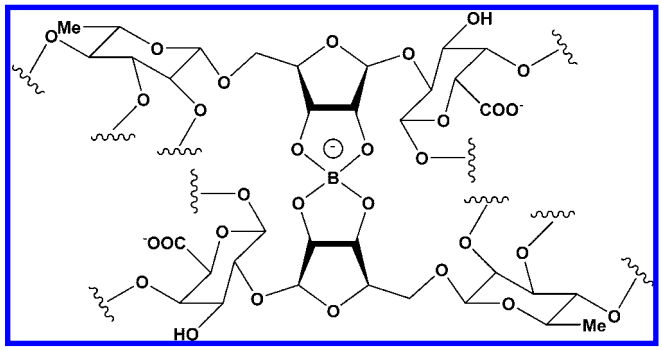


Figure 7. The central part of boron containing dimeric polysaccharide complex known as RG-II with missing links. It is present in the cell walls of all higher plants.

it appeared to us that those molecules might be suitable candidates for studying quorum sensing via the AI-2 receptors of *V. harveyi*. On the basis of the chemical structure of oxazaborolidines and the biological activity displayed (Table 2) by other boron compounds with B−N bonds, oxazaborolidines might selectively bind to a receptor like sensor-2 (AI-2 receptor of *V. harveyi*), thereby triggering an agonistic response. Only BNO-1 and BNO-5 showed the strongest effect on *V. harveyi* bioluminescence. The induction effect of BNO-1 on the bioluminescence of *V. harveyi* BB170 (sensor-1^-, sensor-2^+) was positively dependent on BNO-1 concentrations in the range of 0−600 μM. At higher concentrations, the bioluminescence response was stable. Under the same conditions, the effect of BNO-1 on *V. harveyi* strain BB886 (sensor-1^+, sensor-2^-) was much lower. BNO-5 was less effective than BNO-1 in induction of bioluminescence in *V. harveyi* BB170. Similar to BNO-1, also BNO-5 had relatively little effect on the mutant strain lacking sensor 2.

At higher concentrations of oxazaborolidines (between 6 and 120 mM), a dose−response and structure−activity relationship between the different derivatives of the synthesized oxazaborolidines and their effect on bacterial adhesion was observed. In general, compounds that contain a B-butyl group (BNO3, BNO5, BNO6 and BNOO1) showed a significant ($P < 0.05$) antiadhesion effect of 21−73% at their maximum tested concentration. Replacing the butyl group by a phenyl group (BNO1, BNO2, BNO4, BNOO2) created an adverse effect of increased adhesion of 18−62%. Thus, oxazaborolidines have the ability in vitro to act as novel agents in affecting biofilm formation and represent a step in preventing biofilm-associated diseases.[102] Because the use of oxazaborolidines as described here is novel, it offers further elucidation of the mechanism of antiadhesion.

5. Boron Sugar Alcohol Complexes

Boron alcohol complexes were discovered more than 30 years ago by Alan Darvill and co-workers, who described one of the most complex carbohydrates found in nature.[85a] Rhamnogalacturan II, or RG-II, is found in plant cell walls.[85] The carbohydrate is found in all higher plants and requires a host of different proteins to manufacture.[86]

In a normal plant, boron binds to RG-II and forms a bridge that holds everything together (Figure 7). In the mutant, a little bit of the structure of the RGII has been changed, and because of the change in shape, it cannot hold the boron quite as well. Fertilizing mutants with high levels of boron also reversed dwarfing because the high amount of available boron effectively forced RG-II to cross-link.[85,87]

Biological and physiological functions for boron-containing compounds are well established, nevertheless, many questions still remain to be answered.[35a,88] It is known that boron is absorbed from soil solution by roots mainly as the undissociated boric acid (H_3BO_3, $pKa_1 = 5.8 \times 10^{-10}$ at 25 °C) and accumulated in stalks, roots, shoots, and also cell walls of many plants.[89] Dissociation of boric acid in water is indicated by:

$$H_3BO_3 + H_2O = H_4BO_4^{-1} + H^{+1} \ (pK = 9.23)$$

In addition, boron may be of importance for maintaining the structural integrity of plasma plant cell membranes. This function is likely related to stabilization of cell membranes by boron association with some membrane constituents.[88] The formation of boron-containing complexes with *cis*-diol configurations in certain plant species plays an important role in boron transport.[90] Thus boric acid reacts with alcohols, forming boron esters and/or neutral *cis*-diol monoborate esters or monoborate complexes with sugars.[91] Also, boric acid can form borate complexes with organic acids such as malic acid neutral borate complex, monomalic acid borate complex, and the bis(malic acid) borate complex. These boron-containing compounds were found in apple juice and wine.[92]

Complexes of boron with sugars or/or sugar alcohols are utilized as nutritional supplements, with the carbohydrate portion being selected to provide a relatively high boron−sugar association constant of at least 250 and preferably 500 or more. In one class of preferred embodiments, boron is complexed with a saccharide (**24**−**42**) having coplanar *cis*-OH groups capable of forming five- or six-membered rings through ester bonding with boric acid.[93] Such complexes may advantageously comprise fructose, mannose, xylose, or sorbose (Figures 8 and 9). In another aspect of the invention, a carbohydrate−boric acid complex may exist charged or neutralized with calcium, magnesium, or other cation(s) in which calcium fructoborate is the preferential form.

6. Boronic Acids As Inhibitors of Bacterial Enzymes

Many bacteria use QS signaling systems to synchronize target gene expression and coordinate biological activities among a local population. *N*-acylhomoserine lactones (AHLs) are one family of the well-characterized QS signals in Gram-negative bacteria, which regulate a range of important biological functions, including virulence and biofilm formation.[19,20] Several groups of AHL-degradation enzymes have recently been identified in a range of living organisms, including bacteria and eukaryotes. Expression of these enzymes in AHL-dependent pathogens and transgenic plants efficiently quenches the microbial QS signaling and blocks pathogenic infections. A range of bacterial species which use AHL molecules as QS signals to regulate different biological functions, including production of virulence factors and biofilm formation of human pathogens, have been discovered.[8−10,19,20] Several groups of AHL-degrading enzymes have recently been identified in a range of living organisms, including bacteria and eukaryotes. Expression of these enzymes in AHL-dependent pathogens and transgenic plants efficiently quenches the acceleration of the QS signal and blocks pathogenic infection.[8−10]

Proteins capable of degrading these autoinducers have been called "quorum-quenching" enzymes, can block many QS

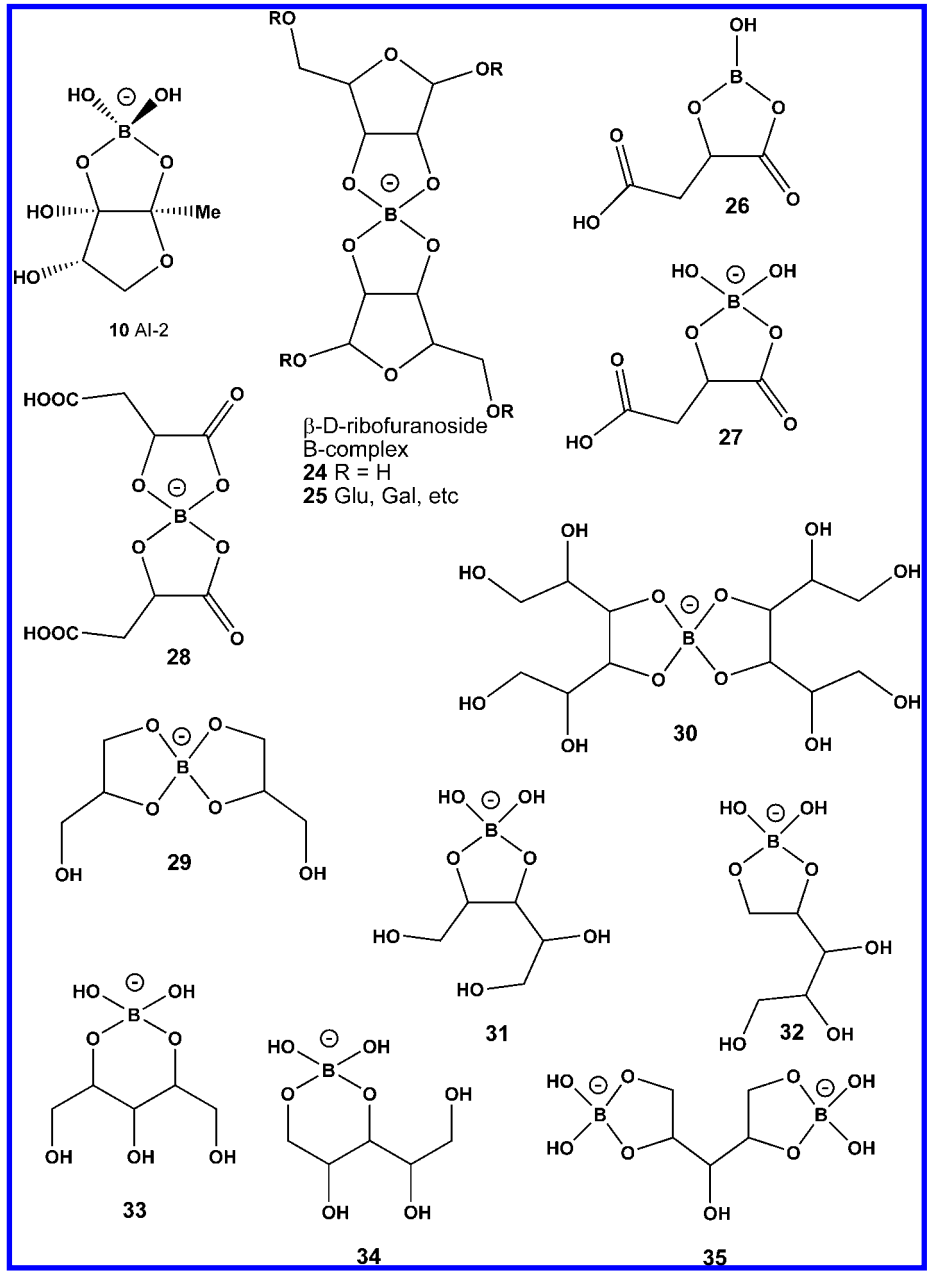


Figure 8. Boron-containing Al-2 autoinducer (**10**) and analogous compounds isolated from natural sources.

dependent phenotypes, and represent potentially useful reagents for clinic, agricultural, and industrial applications. The most characterized quorum-quenching enzymes to date are the AHL lactonases, which are metalloproteins that belong to the metallo-β-lactamase superfamily.[94] The finding that many pathogens rely on cell-to-cell communication mechanisms, known as quorum sensing, to synchronize microbial activities essential for infection and survival in the host suggests a promising disease control strategy, i.e. quenching microbial quorum sensing or in short, quorum quenching. Results obtained over the past few years have demonstrated that quorum-quenching mechanisms are widely conserved in many prokaryotic and eukaryotic organisms.[95] These naturally occurring quorum-quenching mechanisms appear to play important roles in microbe−microbe and pathogen−host interactions and have been used, or served as lead compounds, in developing and formulating a new generation of antimicrobial agents.

6.1. Boronic Acids As Selective Inhibitors of β-Lactamases

Great interest is focused on inhibition of serine-amidases, a class of enzymes that mediate several pathological conditions such as thrombosis (thrombin, factor Xa, factor VIIa), inflammation and emphysema (elastase), hepatitis C (proteases involved in replication), and bacterial resistance against β-lactam antibiotics (β-lactamases). The structure of many of these enzymes has been exhaustively mapped, and their mechanism of action was carefully investigated.[96] β-Lactam antibiotics inhibit bacterial cell wall synthesis and therefore the proliferation of microorganisms. The overexpression of β-lactamases is one of the most common and well-studied mechanisms of β-lactam antibiotic resistance.[97] β-Lactamases compete with penicillin binding proteins (PBP) in binding β-lactam antibiotics. β-Lactamases deactivate the β-lactam molecules by hydrolyzing the β-lactam ring, thus preventing the interaction of the drug with the PBPs. Different classes

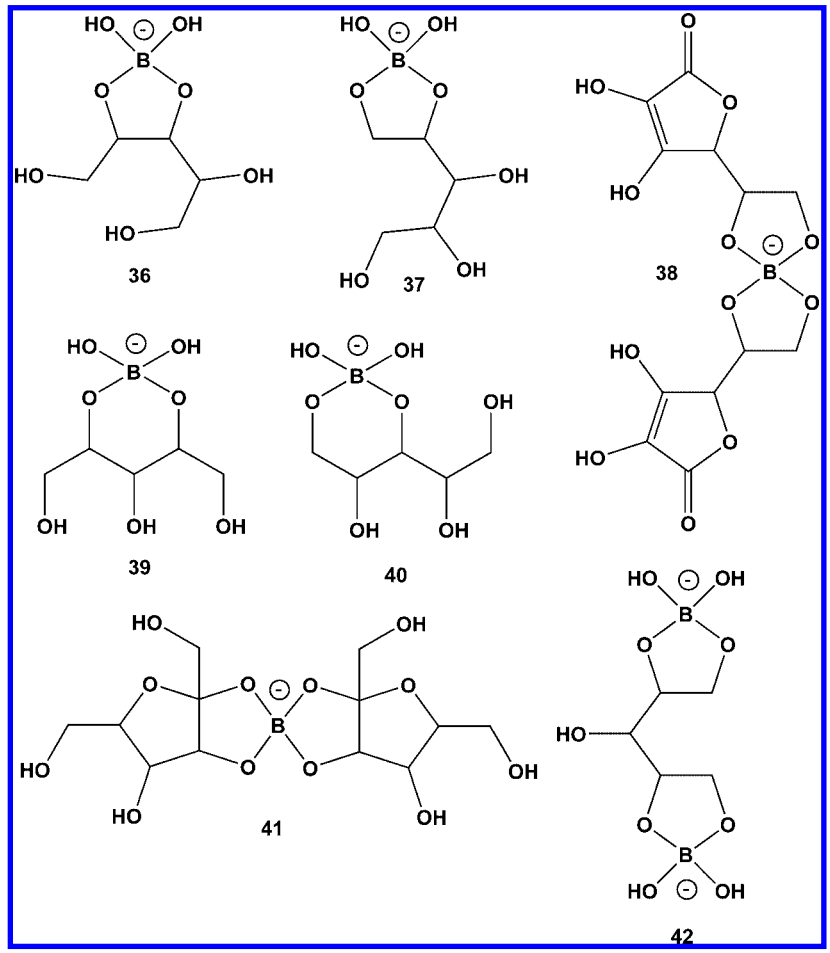


Figure 9. Common structures for arabinitol, ribitol, and xylitol boron−sugar complexes as potential inhibitors of bacterial quorum sensing.

of β-lactamases are known.[98] The most clinically important are class A β-lactamases, which include the plasmid-based TEM penicillinase, and class C β-lactamases, represented by cephalosporinases, such as AmpC-β-lactamase. To overcome the action of these enzymes, medicinal chemists have introduced "β-lactamase resistant" β-lactams (e.g., aztreonam) or β-lactam-based β-lactamase inhibitors (e.g., clavulanic acid and sulbactam). Among the β-lactamase inhibitors, clavulanic acid and sulbactam can inhibit class A β-lactamases but are mostly ineffective against class C β-lactamases. Class C β-lactamases are present in Gram-negative microorganisms, such as *Enterobacter cloacae* and *Pseudomonas aeruginosa*, which cause serious health troubles.[99]

Boronic acids have proved to be promising selective inhibitors of β-lactamase, acting as transition state analogues in order to avoid their resistance to β-lactam antibiotics like cephalosporins, cephamycins, and carbapenems and/or penicillins. These antibiotics are common in their molecular four-membered ring structure known as a β-lactam. The lactamase enzyme cleaves the ring, deactivating the molecule's antibacterial properties. β-Lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. β-Lactamases produced by Gram-positive organisms are usually secreted.[100] Many β-lactamases have active-site serine residues and are competitively inhibited by boronic acids.[100,101]

The β-lactamases produced by the two strains of *Citrobacter diversus* were inhibited by both borates and boronates, using cephazolin as substrate. The enzyme from *Pseudomonas aeruginosa* was inhibited only by boronates, using benzylpenicillin as substrate. Boric and boronic acids **43**,**44** were found to be potent as inhibitors of β-lactamases produced by two strains of *Citrobacter diversus* and by one strain of *Pseudomonas aeruginosa*. These inhibitors were also used in combination with selected β-lactams to detection if a synergism of antimicrobial activity occurred.[102]

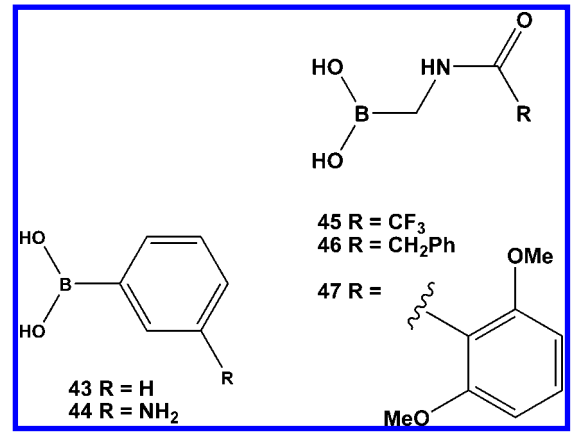


Figure 10. Chemical structures of compounds **43**−**47**.

Three boronic acids, trifluoroacetamidomethaneboronic (**45**), phenyl-acetamidomethane-boronic (**46**), and 2,6-dimethoxybenzamido-methaneboronic (**47**) acids, were prepared by Crompton et al. (Figure 10).[103] The first of these contains the side-chain moiety of penicillin G, and the last that of methicillin. The pH dependence of binding of the inhibitor (**46**) to the active-site groups in the enzyme

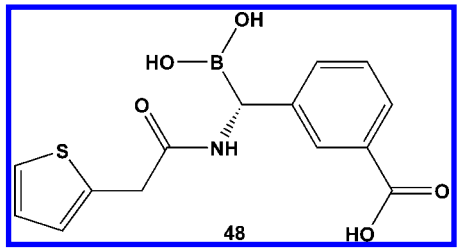


Figure 11. Chemical structure of −[(*R*)-(borono)(2-thienylacetylamino)methyl]benzoic acid **48**.

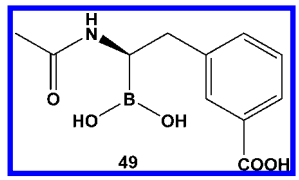


Figure 12. Chemical structure of (1*R*)-1-acetamido-2-(3-carboxyphenyl)ethane boronic acid **49**.

β-lactamase I from *Bacillus cereus* revealed that the optimum p*K* values of 4.7 and 8.2. The kinetics of inhibition provided evidence for a two-step mechanism for the binding of the boronic acids (**45**) to β-lactamase I and for benzeneboronic acid to a β-lactamase from *Pseudomonas aeruginosa*. The rate determining step is probably associated with a change in enzyme conformation as well as the formation of an O−B bond between the active-site serine OH group and the boronic acid.[103]

Recently, α-boronated *N*-acyl-3-aminomethylbenzoates and *N*-benzylamides as β-lactamase inhibitors active in nanomolar concentrations were synthesized.[104] Synthesized 3-[(*R*)-(borono)(2-thienylacetylamino)methyl]benzoic acid **48** exhibited binding constant with AmpC β-lactamase of 0.001 μM and synergic inhibiting effect on *Escherichia coli* growth at (MIC) of 1 μg/mL in combination with antibiotic ceftazidime; in the absence of **48**, the MIC of ceftazidime was 32 μg/mL (Figure 11).

Also, the synthesis of (1*R*)-1-acetamido-2-(3-carboxyphenyl)ethane boronic acid **49**, a rationally designed transition state analogue competitive inhibitor of the RTEM-1 β-lactamase from *Escherichia coli*, was reported.[105] Kinetic measurements showed that, as expected, it is a highly effective reversible inhibitor of the β-lactamase, with an inhibition constant of 110 nM (Figure 12).

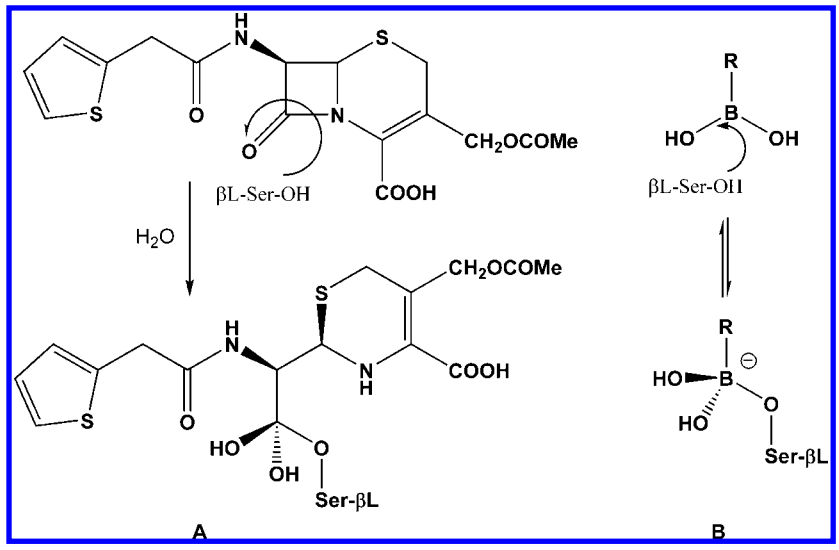
Table 3. K_i Values (nM) for Compounds 54 and 55 against Various β-Lactamases

Inhibitor	AmpC	N289A	TEM-1	TEM-30
54	1.0	17.0	64.0	7800
55	1.0	2.8	106.0	3800

Morandi and co-worker studied the inhibition of the class C β-lactamase AmpC by boronic acids, based on a biomimetic approach, highlighted that the closer the boronic acid resembles the natural substrate in its interaction with the enzyme, the higher is its inhibition (Scheme 3) .[106] Thus, moving from **50**−**54**, an increasing mimesis of the β-lactam cephalothin is displayed and higher inhibition of the β-lactamase was observed. In fact, whereas methaneboronic acid **50** (K_i 1000 μM) offers to the β-lactamase the sole interaction of the boron with the serine residue, compound **51** (K_i 30 μM), characterized by the presence of the acetamide moiety, gains the additional hydrogen bond with Asn152, Gln120, and Ala318, as also displayed by the amide at C7 of the natural substrate. A further improvement in inhibition was observed by insertion of more complex amide side chains on the boronic acid, and among these cephalothin was selected as a model, being a good compromise between complexity and inhibition (compound **52**, K_i 0.32 μM). In addition, the stereocontrolled introduction of a phenyl group, mimicking the dihydrothiazine ring as well as the configuration at the C7 of cephalosporins, led to identification of a hydrophobic binding pocket in the active site of AmpC β-lactamase, formed by Leu119 and Leu293, which accounts for 10-fold improvement in affinity (inhibitor **53**, K_i 0.035 μM). Finally, the insertion of a *m*-carboxyphenyl moiety increased the interaction of the carboxy group at C4 and further improved affinity led to the discovery of the most potent boronic inhibitor of AmpC β-lactamase ever tested (inhibitor **54**, K_i 0.001 μM).

Compound **55** inhibited AmpC with a K_i of 1 nM. While this inhibition is potent, it is not higher than that of the analogue compound **54** from which it was derived (Table 3). Comparison of **55** with compound **54** suggests that the *m*-vinylcarboxylate improves the binding energy by 2.1 kcal/Mol, as did the *m*-carboxylic acid moiety of compound **54**. Whereas this substitution reduces affinity of compound **54** by 17-fold (1.7 kcal/mol), it led to only a 2.8-fold (0.6 kcal/mol) decrease in affinity for compound **55**), consistent with

Scheme 3



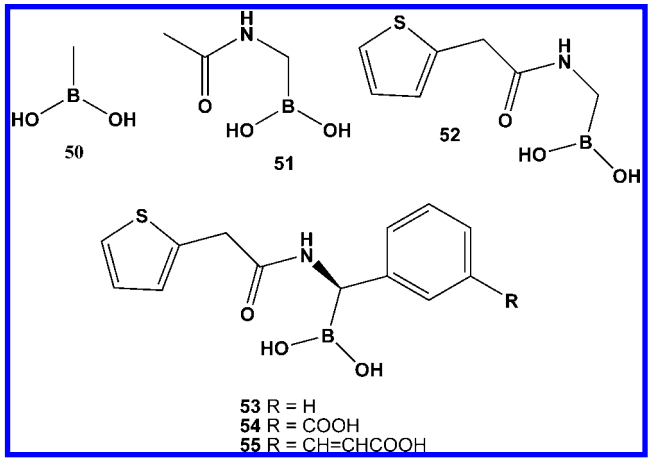


Figure 13. Chemical structures of compounds **50**−**55**.

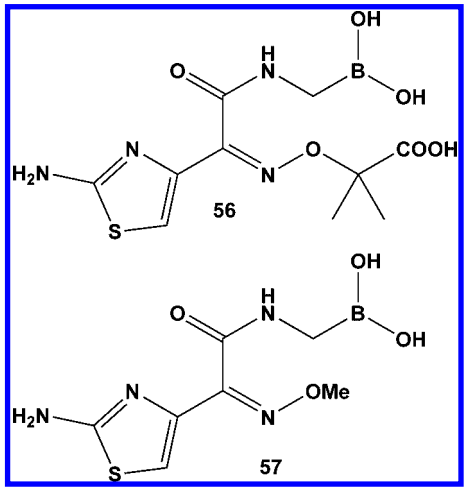


Figure 14. Chemical structure of boronic acids **56**, **57**.

the expectations, even though it retains the affinity of the parent structure, no longer does so via an important hydrogen bond with the nonconserved Asn289 (Figure 13, Table 3).

Boronic acid transition state inhibitors (BATSIs) with R1 side chains of cefotaxime and ceftazidime were assayed against SHV-1, SHV-2, SHV-5, D104K, and D104K G238S β-lactamases.[107] The D104K variant was the most susceptible to inhibition by the ceftazidime BATSI (**56**, K_i, 730 nM), while the D104K G238S variant was the most susceptible to the cefotaxime BATSI (**57**, K_i, 1.1 μM) (Figure 14).

Walsh and co-workers,[108] using (1-aminoethyl)boronic acid supplied as its silylated diisopropyl ester, found that this amino boronic acid is an inhibitor of *Bacillus stearothermophilus* alanine racemase and *Salmonella typhimurium* D-alanine:D-alanine ligase (ADP-forming). As noted above, α-amino boronic acids decompose on standing, and solutions of this compound lost much of their activity within a day. The boronic acids **58** and **59** mimic the structures and interactions of good penicillin substrates for the TEM-1 β-lactamase of *Escherichia coli* and are among the most effective inhibitors, for **58** $K_i = 5.9$ nM, and for **59** $K_i = 13$ nM (Figure 15).[109]

Aromatic boronic acids **60**−**65**, including *o*-, *m*-, and *p*-methyl-, hydroxymethyl-, and formylphenylboronic acids (Figure 16), were shown to be reversible inhibitors of class C β-lactamases, both chromosomally encoded enzymes, one from *Pseudomonas aeruginosa* and the other specified by the ampC gene of *Escherichia coli*. This inhibition may be due to the fact that both the β-lactamases are serine enzymes,

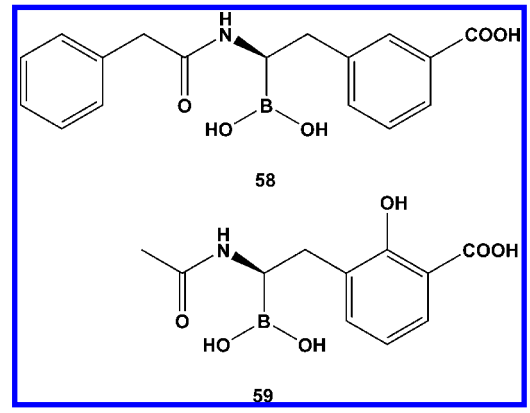


Figure 15. Boronic acids **58** and **59**.

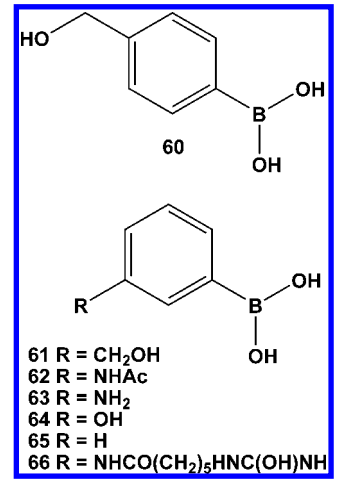


Figure 16. Aromatic boronic acids **60**−**65**.

i.e. their function entails the hydroxyl group of a serine residue acting as a nucleophile.[110] Boric and boronic acids were used as inhibitors of β-lactamases produced by two *Citrobacter diversus* strains and by one strain of *Pseudomonas aeruginosa*; all strains were clinic isolates. *Diversus* strains were inhibited by both borates and boronates, using cephazolin as substrate. The enzyme from *P. aeruginosa* was inhibited only by boronates, using benzylpenicillin as substrate. Obtained data indicated that the MICs were lowered in the presence of these inhibitors for the 2 *C. diversus* strains. In the *P. aeruginosa* strains, the MIC values were not significantly altered, thus indicating the presence of a permeability barrier for 3-aminophenylboronic acid.[111]

Several β-lactamases were purified by affinity chromatography on boronic acid **66** gels. This boronic acid **66** column was prepared with the more hydrophobic one being reserved for those β-lactamases that bind boronic acids relatively weakly. β-Lactamase I from *Bacillus cereus,* β-lactamase of *Enterobacter cloacae* P99, and K1 β-lactamase of *Klebsiella aerogenes* were among the best known β-lactamases that were purified. The procedure was also used to purify a novel β-lactamase from *Pseudomonas maltophilia* in high yield; the enzyme had an exceptionally broad substrate profile and hydrolyzed monocyclic β-lactams such as azthreonam and desthiobenzylpenicillin (Figure 16).[112]

Ni and co-workers[113] were the first to report the discovery of several boronic acid inhibitors of bacterial quorum sensing in *Vibrio harveyi* with IC_{50} values in the low to submicromolar range in whole cell assays. It is clear that none of these boronic acids **67**−**82** exhibited significant inhibition of bacterial growth when compared with the control group

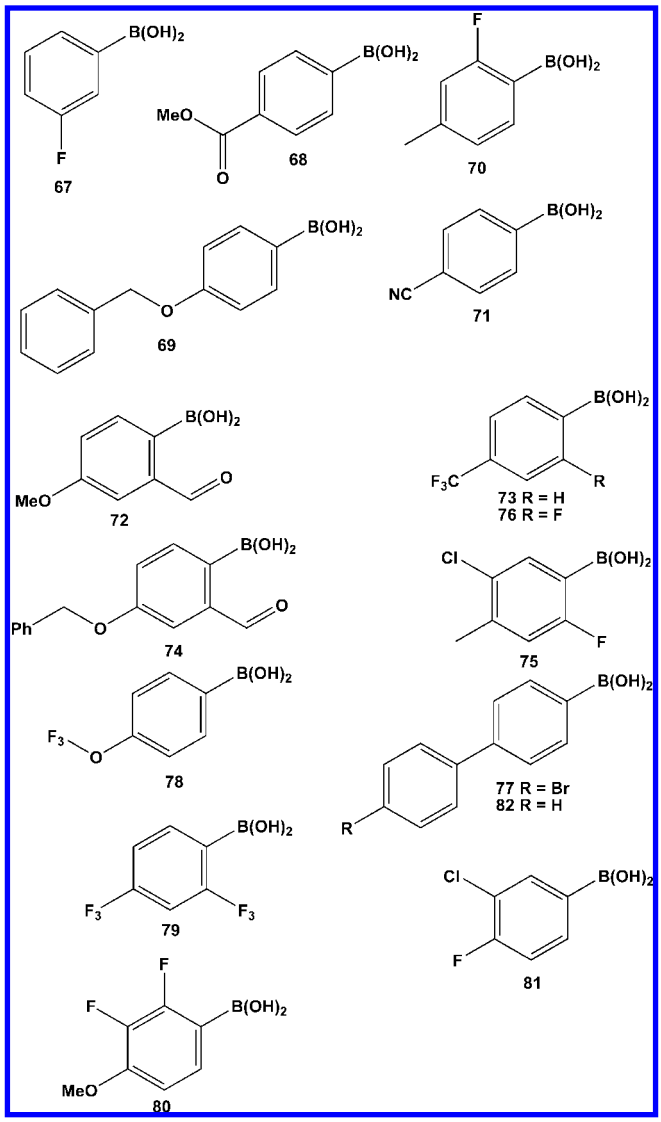


Figure 17. Boronic acids **67**−**82**.

(no boronic acid) by calculating the doubling time (Figure 17). For example, the doubling times for bacteria with all the compounds tested at or above twice the IC_{50} concentrations were about 80 min, which was the same as that of the control. The only exceptions were boronic acids **77**−**79** and **82**, which resulted in a doubling time of about 100 min, which is still qualitatively the same as the control. Therefore, no general cytotoxicity was observed at boronic acid concentrations that showed significant quorum sensing inhibition. All the compounds were tested using MM32, which was used for the study of AI-2 inhibition. To gain insight of the inhibition mechanism, it also tested the inhibitory effect of these boronic acids on the AI-1 pathway. Therefore, strain BB886 (responding to AI-1 not AI-2) was chosen for further evaluation of these boronic acids. Table 4 shows the IC_{50} values (μm) of these boronic acids against different strains of *V. harveyi*.

Inhibition of the RTEM-1 β-lactamase by boronic acids has been reported by Martin and co-workers.[114] All the phenylethyl boronic acids **83**−**94** are potent inhibitors (Table 5), with the best being the parent member of the series, phenylethyl boronic acid itself (Figure 18). Competitive inhibition was observed for all the compounds in Table 5 with the exception of 2-(4-trifluoro-methylphenyl)ethyl boronic acid **85** in which slow-binding kinetics were manifest.

Table 4. The IC_{50} Values (μM) of Boronic Acids 67−82 against MM32 and BB886 Strains of *V. harveyi*

inhibitor	MM32 (AI-2)	BB886 (AI-1)
67	9 ± 5	21 ± 7
68	5 ± 2	11 ± 4
69	4 ± 1	12 ± 2
70	4 ± 1	22 ± 11
71	6 ± 4	16 ± 6
72	6 ± 2	6 ± 1
73	5 ± 2	20 ± 4
74	0.7 ± 0.1	1.2 ± 0.1
75	4 ± 1	11 ± 2
76	2 ± 0.3	8 ± 2
77	3 ± 1	15 ± 6
78	9 ± 2	18 ± 3
79	5 ± 2	45 ± 18
80	10 ± 1	23 ± 11
81	10 ± 1	22 ± 2
82	9 ± 4	53 ± 15

Table 5. Inhibition Constants for Phenylethyl Boronic Acids 83−94

inhibitor	K_i (μM)
83	29.8 ± 0.7
84	41 ± 3
85	43 ± 3
86	44 ± 3
87	49 ± 1
88	50 ± 3
89	58 ± 3
90	83 ± 4
91	101 ± 6
92	106 ± 7
93	141 ± 7
94	276 ± 13

For substituted phenylethyl boronic acids, general trend in the potencies of inhibition is that for a given function, the K_I increases for para, meta, and ortho substitution, respectively. This is most clearly seen for the methyl-substituted inhibitors **88**−**89**, and **93**.[114]

Commercially available boronic acid derivatives of 3-aminophenyl-boronic acid **44** showed a K_i of 7.3 μM against *Escherichia coli* AmpC-β-lactamase (βL). This molecule was recognized to be a potential scaffold for new inhibitors:

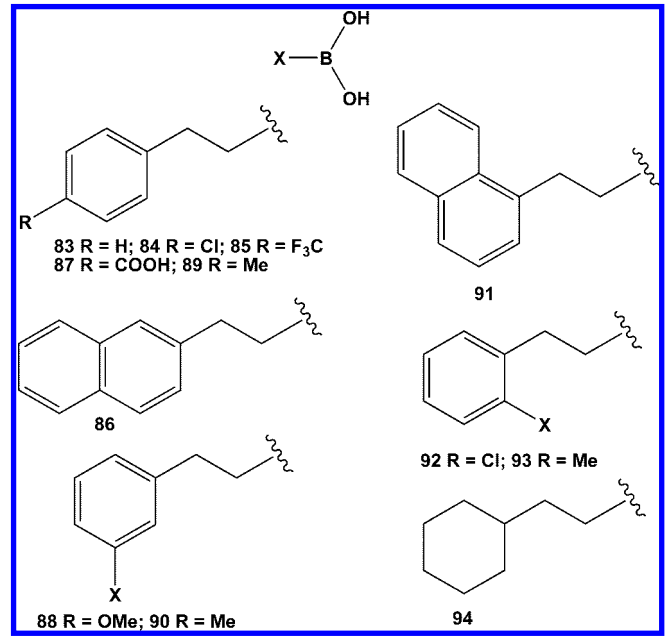


Figure 18. Boronic acids **83**−**94**.

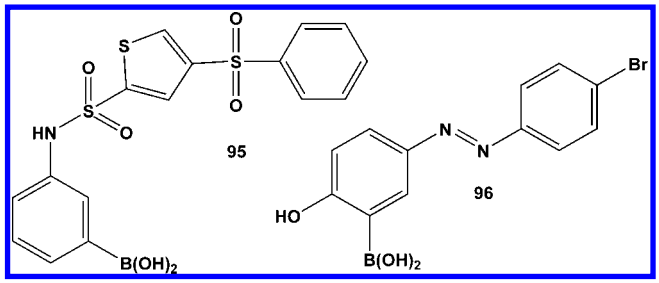


Figure 19. Boronic acids **95**, **96**.

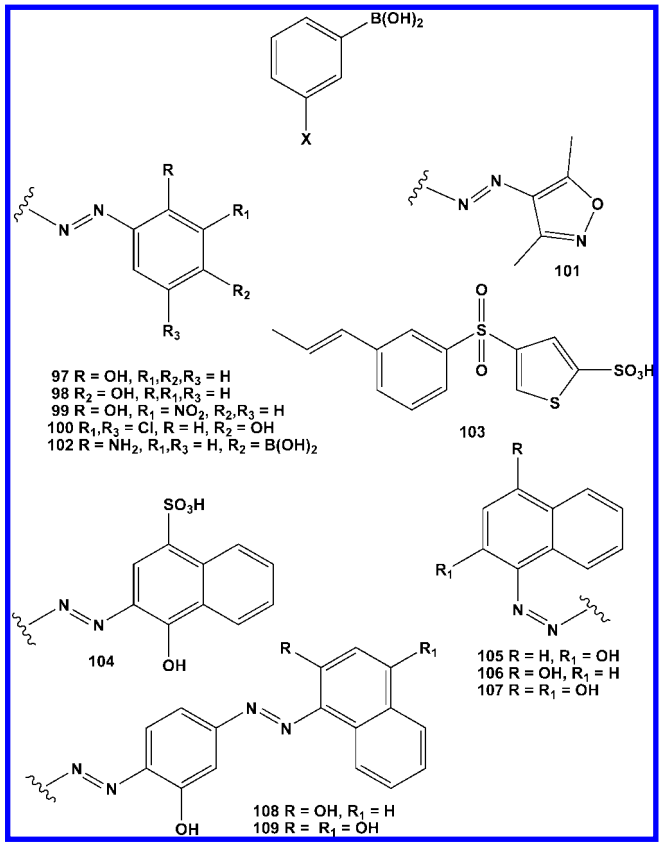


Figure 20. Aromatic boronic acids **97**−**109**.

starting from this scaffold and using structure-based drug design approaches based on the *E. coli* AmpC-βL crystal structure.[115] The most active compound, 3-(4-benzensulfonyl-thiophene-*e*-sulfonylamino)-phenylboronic acid **95**, showed a K_i of 0.08 μM, which is about 100-fold less than the K_i of the initial compound, **44**, and some phenyl-aza derivatives, such as 5-(4-bromophenylazo)-2-hydroxy-phenyl-boronic acid **96**, which inhibited *E. coli* AmpC-βL, with a K_i value of 3 μM (Figure 19).[116]

All of the newly synthesized compounds were tested against *E. coli* AmpC-βL, and apparent inhibition constant (K_i) values were documented. The K_i range was 0.3−5 μM, specifically compound **99**, which was active in the submicromolar range (0.3−1 μM).[116] The naphthol derivatives, compounds **105** and **107** showed the best inhibitory activity, with K_i values of 0.3 and 0.45 μM, respectively. Compound **106** showed an affinity that is 3-fold lower than ortho-substituted compound **105** (K_i = 0.9 and 0.3 μM, respectively). Among the phenol derivatives, compound **98**, which is 5-fold more potent compared to ortho-substituted **97** (0.7 μM, compared to 3.5 μM). Addition of a nitro group to ortho position of the hydroxyl of compound **99** did not greatly modify the affinity of the molecule; on the contrary, two chlorine substituents on compound **100** decrease the affinity by 4-fold. The introduction of an acidic group, such as a sulfonylic group, was attempted in order to improve solubility in water, but only **104** was obtained. But the activity of compound **104** was about 10 times lower than that of the other naphthalene derivatives **105**−**107** (Figure 20). By combining the phenol and the naphthol moieties that proved to be the most potent, compounds **108** and **109** were obtained and showed K_i values less than 2 μM. Thus, the affinity compared to the starting molecule **97** (K_i = 3.5 μM) is not seriously improved. Compound **103** showed the same affinity for the enzyme as the monophenol derivatives. These results demonstrate that the active site is accessible to bulky groups, so a three-ring system can be allocated to the enzyme pocket, but the compounds are not as active as expected based on simple synergistic considerations.[116]

6.2. Boron-Containing Compounds As Inhibitors of Serine Proteases and Other Enzymes

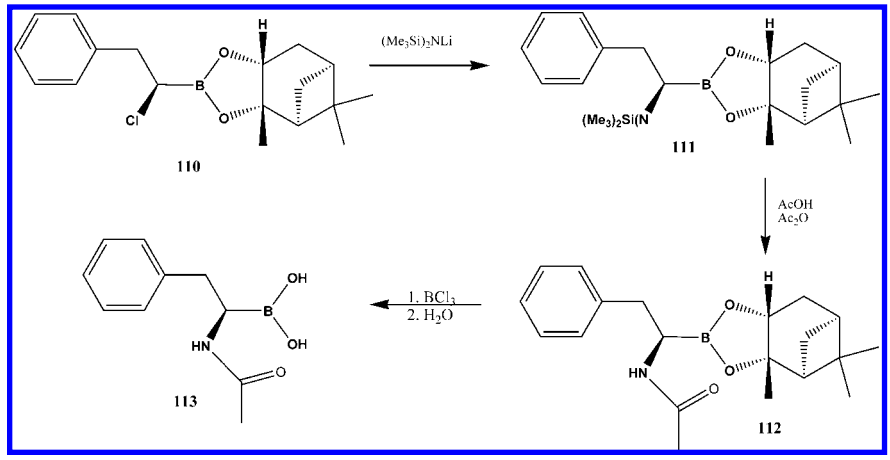
Serine proteases, a large and functionally diverse class of proteolytic enzymes, are prominent therapeutic targets because of their involvement in a host of physiological processes.[117] They catalyze peptide bond cleavage by acylation and deacylation of the active site serine residue in a sequence that involves two tetrahedral intermediates.[118] Most small molecule inhibitors of these enzymes form covalent adducts with the active site serine that mimic to some degree these tetrahedral intermediates. Peptide derivatives with electron-deficient ketones, aldehydes, boronic acids, and phosphonylating agents have been devised as analogues of the second tetrahedral intermediate,[117,119] with their selectivity among the various proteases related to the substrate specificity these enzymes manifest at the S1, S2, and higher binding sites.[120] The expression of many of these proteases is believed to be linked with pathogenicity of Gram negative bacteria, which necessitates their further study with a view to obtain more profound concepts. These enzymes have been shown to facilitate the bacterial colonization of the skin and mucous membranes. They are believed to be linked with the resistance of microorganisms to lysosomal proteolysis by phagocytes and their ensuing dissemination in the course of the infectious process. Serine proteases split coagulating factor V and enhance the permeability of blood vessels, thus inducing the hemorrhagic syndrome. The detailed study of serine proteases is closely linked with the prospects of the development of protease inhibiting preparations aimed at the suppression of the pathogenetic activity of proteases by their blocking or by affecting the mechanisms of their secretion.[121] Boronic acids are a very appealing class of serine proteases inhibitors whose rational design suffers, in spite of their therapeutic potential, from the lack of boron-related parameters in force fields commonly used for proteins.[117]

7. α-Amidoboronic Acid Derivatives

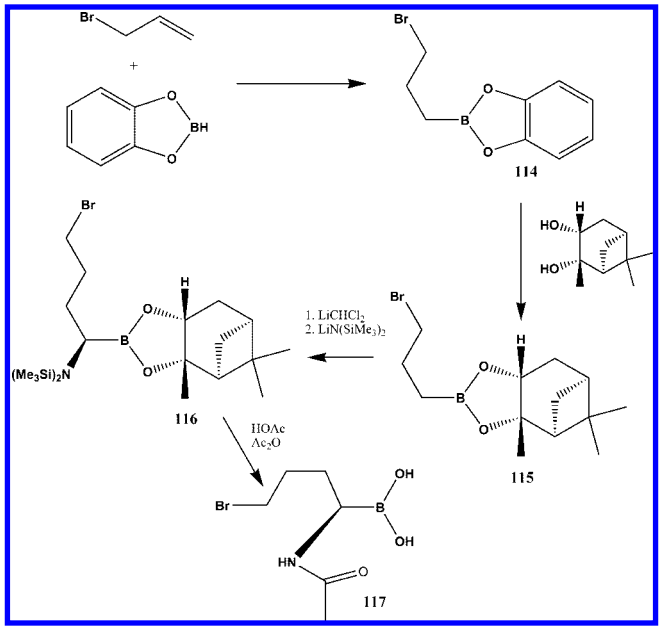
α-Haloboronic esters are usually useful for the synthesis of α-amidoboronic acid derivatives.[117,122] Nucleophilic reactions of α-haloboronic esters with carbon nucleophiles are utilized in asymmetric synthesis with displacements of the halide atom. The asymmetric conversion of a CHCl group into a boron−carbon bond can be controlled with very high precision using chiral ligands on the boron atom.

The first synthesis of the unnatural α-amidoboronic ester **113** was studied by Matteson et al.[123] (*S*)-Pinanediol (*S*)-(1-

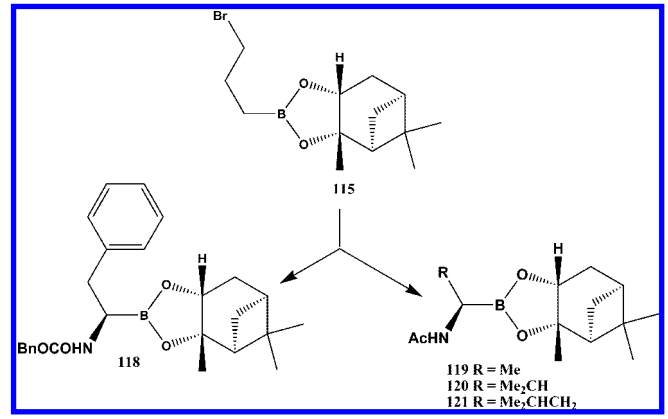
Scheme 4



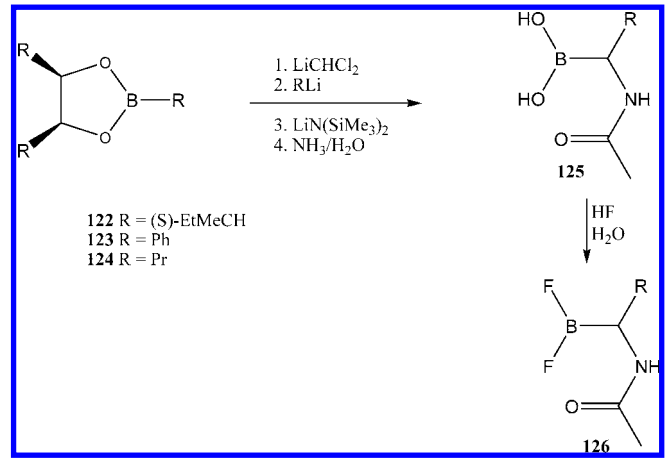
Scheme 5



Scheme 6



Scheme 7



chloro-2-phenylethyl)boronate **110** was prepared from the reaction of (*S*)-pinanediol benzylboronate with (dichloromethyl)Li,[124] followed by displacement of the chloride ion from with lithiohexamethyldisilazane, to provide **111**. Treatment of **111** with AcOH/Ac_2O produced the acetoamidoboronic ester **112**, which was cleaved with BCl_3 to yielded (*S*)-*N*-acetylboraphenylalanine **113** as shown in Scheme 4. Compound **113** was a potent inhibitor of serine proteases.[117,125] Other routes for the synthesis of **113** have been described.[122]

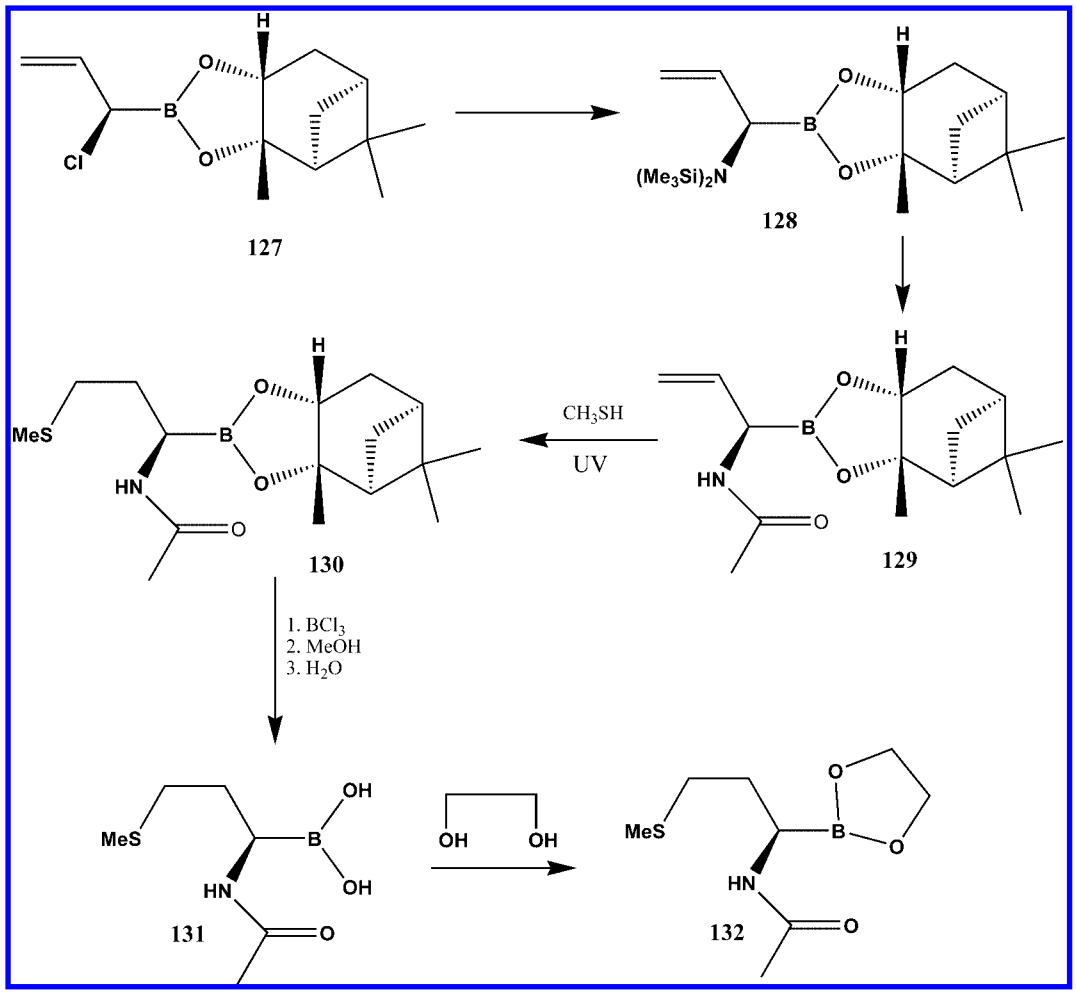
The synthesis of (*S*)-pinanediol (*R*)-(1-acetamido-4-bromobutyl)boronate **117** was achieved by multistep reaction, starting from allyl bromide reaction with catecholborane via **114**, which was transesterified with (*S*)-pinanediol to **115**. By chain elongation and amination using dichloromethyl lithium and lithiohexamethyldisilazane, it was converted to the silylated aminoboronic ester **116**. Then, it was transformed to the more stable product **117** by treatment with acetic anhydride and acetic acid (Scheme 5).[126]

In addition, the pinane amidoboronic esters **118**−**121** could be synthesized by utilizing similar chemistry (Scheme 6).[126] Enzyme inhibition studies have shown that the D-amino acid analogues **120**, **121** were active inhibitors of *Bacillus cereus* β-lactamase, with K_i = 44 and 49 μM at pH 7, respectively.[127]

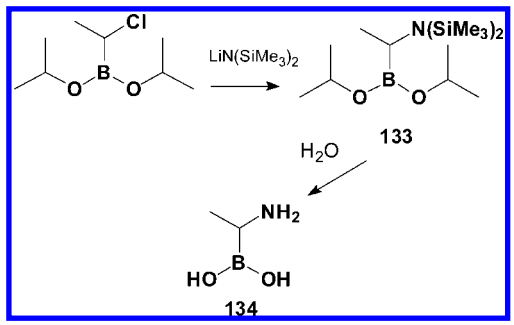
The racemic α-acetamidoboronic acids **125** have been obtained using similar chemistry. This reaction was used as the starting point for the corresponding *meso*-butanediol esters **122**−**124** and **125**, which were found to inhibit elastase and chymotrypsin.[128] The fluoro-derivatives **126** could be obtained by the treatment of **125** with aqueous hydrofluoric acid (Scheme 7).[128]

Amination of (*S*)-Pinanediol (*S*)-(1-chloroallyl)boronate **127** with lithiohexamethyldisilazane gave compound **128**, which after desilylation/acetylation gave (*S*)-pinanediol (*R*)-(1-acetamidoallyl)-boronate **129** (Scheme 8). Addition of methyl mercaptan to the unsaturated bond of **129** under UV light yielded the crystalline boronic ester **130**.[129] Treatment

Scheme 8



Scheme 9



of **130** with BCl_3 led to **131**, which was esterified by ethylene glycol to give the crystalline product **132**.

Free α-aminoboronic acids were synthesized and tested as potential enzyme inhibitors. The racemic boraalanine **134** was obtained in solution by hydrolysis of the boronic ester **133** (Scheme 9). It was found to be effective inhibitor of alanine racemase from *Bacillus stearothermophilus* with K_i = 20 mM (it was slow binding at K_i = 0.15−0.35 min^{-1}). In D-alanine/D-alanine ligase of *Salmonella typhimurium*, two binding constants for different enzyme sites were found: K_i = 35 μM and K_i = 18 μM, respectively.[130]

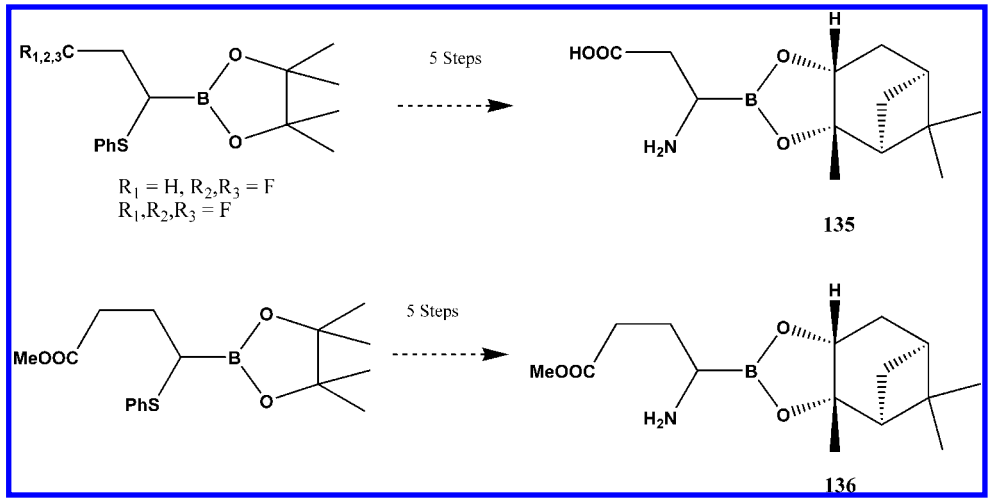
α-Aminoboronic acid esters **135** and **136** were prepared and found to be useful as inhibitors of the serine proteases, leukocyte and pancreatic elastases, cathepsin G, chymotrypsin, and hepatitis C Virus protease (Scheme 10).[131]

Initial synthetic efforts to obtain such amino acid analogues inhibitors were based on *N*-acylated analogue of glycine. For instance, dibutyl iodomethane-boronate **137** was alkylated with the sodium salt of benzamide to give **138**, which was shown to be a potent inhibitor of α-chymotrypsin. Hydrolysis of **138** gave two bioactive isomers **139a** and **139b** (Scheme 11).[132]

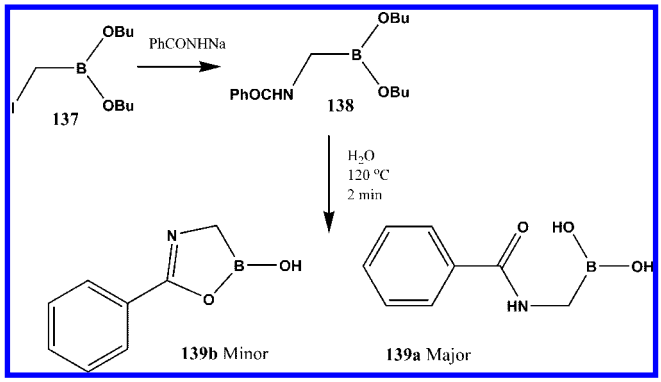
Enantiomeric 1-acetamidoboronic acids, which are *N*-acetyl transition-state inhibitor analogue of the L- and D-forms of the amino acids alanine, phenylalanine, *p*-fluoro-phenylalanine, *p*-chlorophenylalanine, and 1-naphthylalanine were synthesized (Scheme 10) and tested as inhibitors of the serine proteases subtilisin Carlsberg and α-chymotrypsin.[133] All L-(*R*)- and D-(*S*)-1-acetamidoboronic acids were prepared according to the basic strategy developed by Matteson et al.[117a,122,134] The pinanediol esters **140** gave the α-chloroboronic acids **141** in 75−95% yields with diastereoselectivites >98%. Treatment the α-chloroboronic acid **141** with lithium-hexamethyldisilazane afforded the corresponding silylated aminoboronic esters, which when heated with Ac_2O and AcOH formed the 1-acetamidoboronic esters **142**. Hydrolysis of **142** with boron trichloride gave the 1-acetamidoboronic acids **143**. Both the anhydride forms of **143** and the diethanolamine derivatives **144** were hydrolyzed to the corresponding free boronic acids **145a**−**e**. All of the boronic acids **145a**−**e** were effective inhibitors of both enzymes (Scheme 12).

The asymmetric syntheses of (*R*)-1,4-diaminobutane-1-boronic acid dihydrochloride **153** and the aminoboronic acid analogue of L-ornithine have been described[135] (Scheme 13). The 3-azidopropaneboronic ester **146** was obtained from allyl

Scheme 10



Scheme 11



bromide and converted to the optically active (+)-pinanediol derivative **147**, which could be transformed to compounds **148** and **149** or **150**. Attempts to obtain the (*R*)-1,4-diaminobutane-1-boronic acid **108** from **150** were unsuccessful. The *N*-protective group in **149** was desilylated by treatment with benzyl chloroformate to give the acetamido derivative **152**, which might be a valuable precursor of the arginine boronic acid analogues. Peptides containing C-terminal boronic acid derivatives of ornithine, lysine, arginine, or homoarginine and corresponding isothiuronium analogues are reversible inhibitors of trypsin-like serine proteases such as thrombin, plasma kallikrein, and plasmin, in addition of being useful in treatment of blood coagulation disorders and inflammation.[136]

8. Other Boron Derivatives As Antibacterial and Antifungal Agents

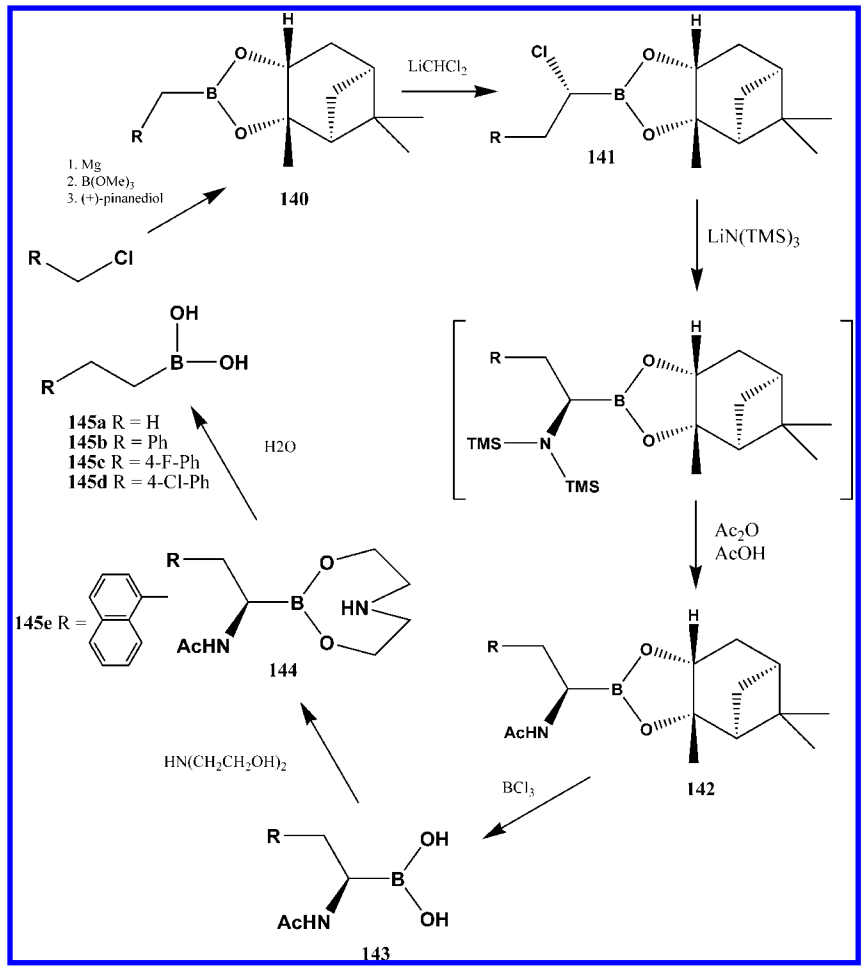
Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections by boron-containing compounds is very important field for drug research. Antipathogenic drugs target key regulatory bacterial systems that govern the expression of virulence factors.[137] Boron compounds display considerable biological properties.[38,39,117a,125a,138] For instance, it has been recently discovered that 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690) is a efficient broad spectrum antifungal agent.[139] AN2690 is a member of a new class of broad-spectrum antifungals, the benzoxaboroles, which have an unusual chemical attribute: a boron atom.[140] The molecule's potency is believed to arise from the boron atom's ability to form a stable adduct with the oxygen atoms of the leucyl-tRNA synthetase, effectively inhibiting the enzyme.[139,140] After the discovery of the excellent antifungal activity of the 5-fluoro substituted benzoxaborole (AN2690) against onychomycosis,[141] a systematic investigation of the medical applications of benzoxaboroles is being conducted. Some of them are currently in preclinical and clinical trials.[141]

Benzoxaboroles **154**−**164** were first synthesized and characterized as early as in 1957 (Figure 21).[142] They were found to have a very stable oxaborole ring and a high hydrolytic resistance of the boron−carbon bond in comparison with the corresponding boronic acids.[143] Benzoxaborole with cyanophenoxy substituents in the 5-position revealed anti-inflammatory activity against psoriasis, common skin disease, which is characterized by chronic inflammation. On the basis of structure−activity relationship studies, it was found that the 5-phenoxy group bearing an electron-withdrawing group at the para position was important for the activity, while the regioisomers of the cyano group were less active. Replacement of the cyano group retains the activity, but compounds with carboxy groups are less potent. The most active was the compound with 4-cyanophenoxy substituents (AN2728, in clinical trials) and with a 3,4-dicyanophenoxy substituent (AN2898). Halo-substituents in the benzene ring increase activity, and compounds with substituents in the position 5 showed the highest activity. 5-Chloro-substituted benzoxaborole (AN2718) is being developed for the topical treatment of tinea pedis, including the difficult to treat mocassin-type, which at present is only treatable with an oral antifungal (Table 6).

Several boron-containing molecules as antifungal agents **165**−**181** were design and synthesized (Figure 22).[144] Compound **181** and/or combination with other compounds **165**−**180** were active against fungal infections such as *Aspergilus fumigates* ATCC 13073, *Candida albicans* ATCC 90028, *C. albicans* F56, *Candida neoformans* F285, *Trichophyton mentagrophytes* F311, *Saccharomyces cerevisiae* ANA309, and *Trichophyton rubrum* F296. They also can be therapeutically effective agents to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), cryptococcal meningitis, and others.[144]

A class of boron-containing compounds termed borinic esters that showed broad spectrum antibacterial activity with minimum inhibitory concentrations (MIC) with low μ/mL range has been designed and synthesized.[145] These compounds demonstrated potential inhibition against *Caulobacter crescentus* CcrM, an essential DNA methyltransferase from Gram negative α-proteobacteria. Also, these synthetic borinic

Scheme 12



esters inhibit menaquinone methyltransferase in Gram positive bacteria.

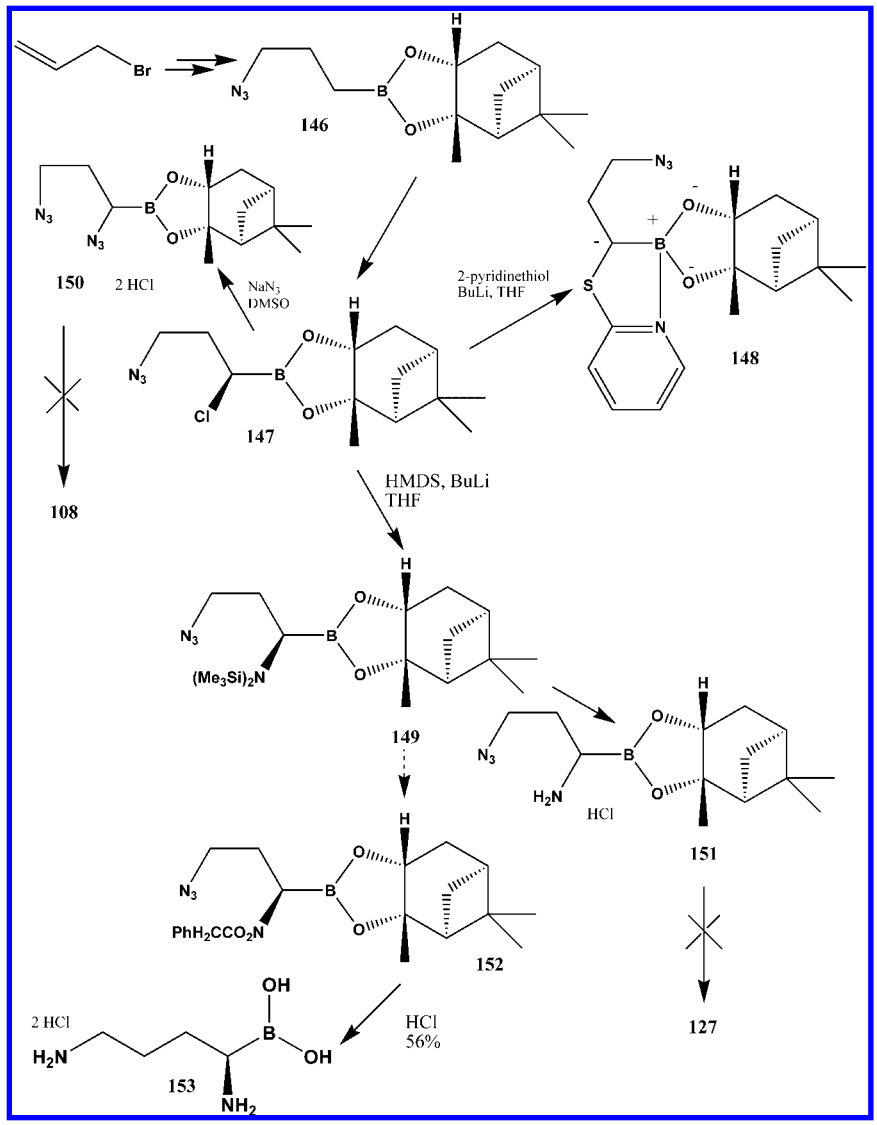
Diphenylborinic acid quinoline esters **182**−**187** were synthesized as shown in Scheme 14http://pubs.acs.org/doi/full/10.1021/jm050676a - jm050676ah00001. Arylmetal reagents were treated with boron trichloride at −78 °C in THF overnight at room temperature and after workup gave the diphenylborinic acid. Then, diphenylborinic acid was treated with 8-hydroxyquinoline derivatives in ethanol at reflux, giving the diphenylborinic acid quinoline esters **182**−**187**. Activity of diphenylborinic acid quinoline esters are shown in Table 7.

Compounds **182**−**187** were in vitro screened against CcrM and two other DNA methyltransferases, Dam, a bacterial adenine DNA methyltransferase, and *Hha*I, a bacterial cytosine methyltransferase. The results showed the remaining enzyme activity when screened at 100 μM. Compounds bearing a chloro group on the borinic acid moiety (**183**, **184**, **185**, and **187**) showed potent inhibitory activity against CcrM, whereas compounds **182** and **185** did not show significant inhibition. Furthermore, compounds **184**, **186**, and **187** showed certain selectivity for the adenine methyltransferases, CcrM and Dam, over the cytosine methyltransferase, *Hha*I.[145]

Compounds **188**−**194**, in which one of the two aryl groups was replaced with a less sterically hindered vinyl group, were designed (Scheme 15). When aryl boronic acid ethylene glycol esters reacted with vinylmagnesium bromide at −78 °C under anhydrous conditions and allowed to warm to room temperature, they yield the asymmetrical vinyl-aryl borinic acids after hydrolysis with HCl solution. The borinic acid was treated with 8-hydroxyquinoline in ethanol at reflux to afford the final products **188**−**194**. Biological activities are shown in Table 7.

Borinic acid picolinate esters were synthesized and their minimum inhibitory concentration (MIC) against Gram-positive and negative bacteria was evaluated.[146] 3-Hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl)borane was identified in having the dominant combination of antibacterial and anti-inflammatory activities. The results are given in Table 8. Two initial lead compounds possessed a symmetrical borinic acid moiety containing a 3- or 4-chloro-substituent on each ring and a 3-hydroxy group on the picolinic acid unit, giving **195** or **196**, respectively. These had reasonable activity against all pathogens. The bis(4-chlorophenyl) derivative was slightly more active against *Staphylococcus epidermidis*, *P. acnes*, and *Bacillus subtilis*, however, the bis(3-chloro-phenyl) derivative was more active against *S. aureus* (Table 8). Substitution of one chlorophenyl group, either **195** or **196**, with a pyridin-3-yl group gave **197** and **198**, respectively, essentially eliminated all activity against Gram positive bacteria even when the chloro group was introduced back in the same place on the pyridine ring **199**. Interestingly, when R^1 was thiophen-3-yl **200**, activity was lost and it was active only against *S. aureus* and *S. epidermidis*. When methyl groups were added to the 4-position of **195**, giving **201**, activity was increased against most strains except *Haemophilus influenzae*, where activity was lost (Table 8). The effect of adding alkyl groups at R^1 and synthesized the methyl **202** and phenethyl **203** derivatives

Scheme 13



of **201** was also reported. The methyl derivative **202** was less active but showed the similar activity profile to the thiophene derivative **200**. The phenethyl derivative showed remarkably similar activity to **201**, however, stability studies showed that these alkyl derivatives were not as stable as the diphenyl borinic acid picolinate ester **201**. Accordingly, this substitution was not considered for further development, and authors concluded that a phenyl group at R^1 would be the best for activity.

The influence of the substituents on the pyridine ring of picolinic acid on the activity is shown in Table 9. The 3-carboxy derivative **217** showed higher activity than **213** against *S. aureus* and also showed surprisingly potent activity against *H. influenzae*, however, it was not so effective against *S. epidermidis* and *P. acnes*. Moving the carboxy group to 4- or 5-positions, giving **218** and **219**, respectively, gave approximately equivalent activity to **217**. It was concluded that the 3-hydroxy group was optimal for activity against the major cutaneous bacterial pathogens *S. aureus* and *P. acnes*.

Anti-inflammatory activity compounds **213**, **214**, **211**, and **219** were evaluated for their ability to inhibit release of inflammatory cytokines from human peripheral blood mono-

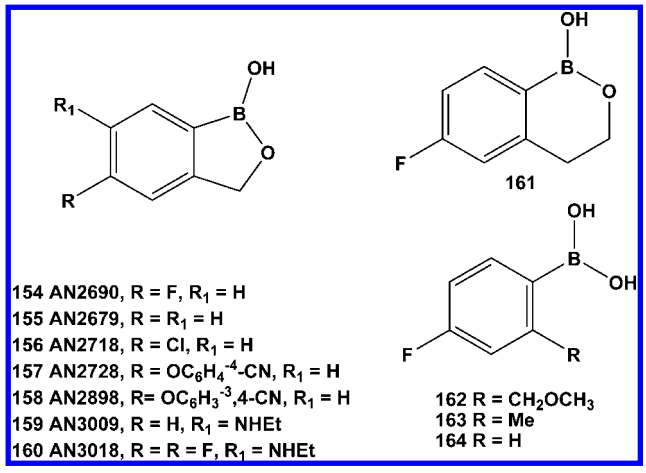


Figure 21. Benzoxaboroles **154**−**164**.

Table 6. Antifungal Activity of Some Benzoxaboroles (IC_{50} μM)

inhibitor	activity
154	2.1
161	96.0
162	>100
163	>100
164	>100

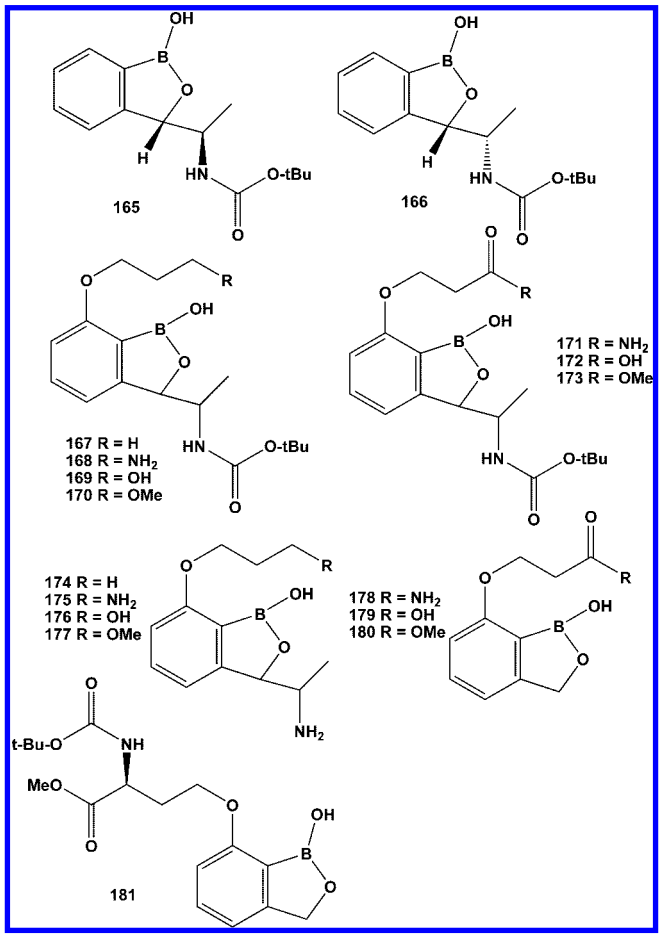
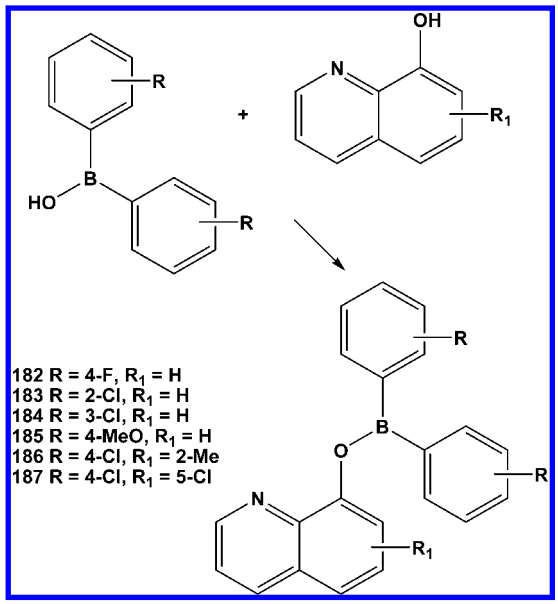


Figure 22. Chemical structure of compounds **165**−**181**.

Scheme 14



nuclear cells (PBMCs). Either lipopolysaccharide (LPS, 1 μg/mL), Concanavalin A (1 μg/mL), or phyto-hemagglutinin (PHA, 2 μg/mL) was used to induce the release of cytokines from the PBMCs. These compounds were screened at a concentration of 10 μM. ELISA kits were used to measure two pro-inflammatory cytokines, TNF-α and IL-1β, a Th1 cytokine, IFN-γ, and a Th2 cytokine, IL-4. The inhibition of cytokine release for each compound was recorded as a percent of untreated control, and the results are shown in

Table 7. Inhibitory Activity of Borinic Acid Quinoline Esters (MIC, μg/mL) against Gram Positive and Gram Negative Bacteria

inhibitor	R	R1	*S. aureus* ATCC 29213[a]	*S. epidermidis* ATCC 12228[a]	*E. faecalis* ATCC 29212[a]	*E. faecium* CT-26[a]	*B. subtilis* ATCC 23857[a]	*B. anthracis*[a]	*M. tuberculosis* H37Rv[a]	*C. crescentus*[b]	*M. catarrhalis*[b]	*F. tularensis*[b]	*Y. pseudotuberculosis* ATCC 29833[b]	*H. influenzae* ATCC 4976[b]
182	4-F	H	4.0	2.0	32.0	32.0			0.62	12.5	2.0			4.0
183	4-Cl	H	2.0	2.0	4.0	4.0	2.0	6.25	0.31	4.0	2.0	0.03	64.0	8.0
184	3-Cl	H	1.0	2.0	32.0	4.0	2.0	6.25	0.62		2.0	0.01	32.0	8.0
186	4-Cl	2-Me	16.0	8.0	32.0	16.0				2.0		6.0		
187	4-Cl	5-Cl	0.5	0.25	32.0	2.0	32.0	5.3	0.62	2.6	0.125	0.01	8.0	0.25
188	3-Cl	H	2.0	1.0	64.0	4.0	4.0	8.0	0.62		4.0		16.0	4.0
189	2-Cl	H	2.0	1.0	64.0	4.0	4.0		0.31		1.0	16.0	32.0	2.0
190	4-Cl	H	2.0	1.0	>64	4.0	4.0		0.62		1.0		32.0	2.0
191	H	H	2.0	1.0	>64	4.0					2.0		32.0	16.0
192	3-F	H	2.0	1.0	>64	2.0	2.0	8.0	0.31		1.0	16.0	32.0	2.0
193	3-Cl, 4-F	H	2.0	1.0	32.0	2.0	4.0		0.62		2.0		32.0	4.0
194	3-CN	H	1.0	1.0	8.0	2.0	4.0	8.0	0.62		2.0	8.0	32.0	2.0

[a] *Gram positive bacteria: S. aureus* ATCC 29213; *S. epidermidis* ATCC 12228; *E. faecalis* ATCC 29212; *E. faecium* CT-26; *B. subtilis* ATCC 23857; *B. anthracis*; *M. tuberculosis* H37Rv. [b] Gram negative bacteria: *C. crescentus*; *M. catarrhalis*; *F. tularensis*; *Y. pseudotuberculosis* ATCC 29833; *H. influenzae* ATCC 4976.

Scheme 15

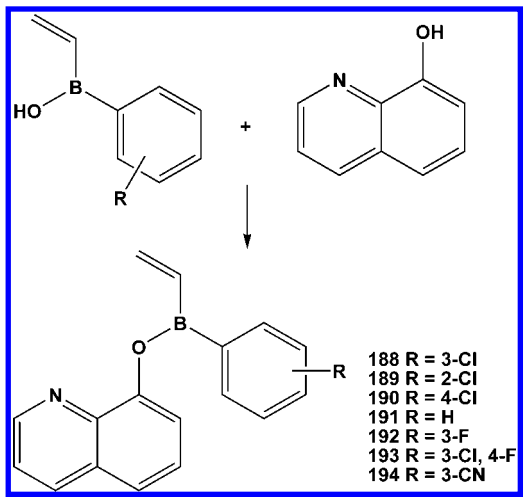
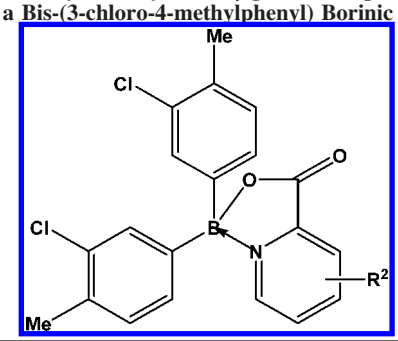


Table 10. Compound **213** showed strong inhibition of the release of pro-inflammatory cytokines but no inhibition of IFN-γ or IL-4 release. Compound **214** showed no inhibition of IL-1β release, so this was not tested for inhibition of IFN-γ or IL-4 release. Compounds **211** and **219** showed a similar activity to that of compound **213** (Table 10). By comparison, the antibiotic erythromycin showed no inhibition of pro-inflammatory cytokines. From these studies, compound **213** was shown to have the best combination of antibacterial and anti-inflammatory activities.

Diphenylborinic acid picolinate esters primarily showed activity against Gram-positive bacteria and SAR, and the diphenyl borinic acid moiety was essential for activity. Potency was increased with the positioning of methyl- and chloro-substituents on the diphenyl borinic acid moiety in combination with a 3-hydroxyl group on the pyridine ring. The most potent derivative from this effort was **213**, which was also found to inhibit the production of pro-inflammatory cytokines. As a result, **213**, AN0128 (3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl)-borane), was selected as a clinical candidate and is currently in clinical development for the treatment of dermatological diseases including acne and atopic dermatitis. AN0128 is a novel borinic acid ester with combined antimicrobial and anti-inflammatory activity.[146]

The carbonic anhydrases (CAs) enzymes belong to the β-CA genetic family, is widespread in bacteria, fungi, and archaea among others.[147] Finding selective inhibitors of the β-CAs may thus constitute a novel means of obtaining anti-infective agents (antibacterial or antifungal) possessing a different mechanism of action compared to the pharmacological agents in clinical use to which significant resistance has emerged.[148] Some aromatic/heterocyclic sulfonamides

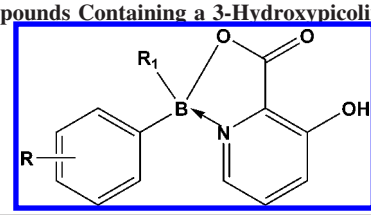
Table 9. Inhibitory Activity (MIC, μg/mL) Compounds Containing a Bis-(3-chloro-4-methylphenyl) Borinic Acid Moiety



inhibitor	R^2	*S. aureus*	*S. epidermidis*	*P. acnes*	*B. subtilis*	*H. influenzae*
212	H	0.5	>64	NT[a]	NT[a]	NT[a]
213	3-OH	1	0.5	0.3	1	>64
214	3-OAc	2	1	1	0.5	>64
215	3-COPh	0.5	32	30	64	>64
216	3-NH_2	>64	>64	1	2	>64
217	3-CO_2H	0.125	4	3	8	8
218	4-CO_2H	2	4	3	NT[a]	NT[a]
219	5-CO_2H	0.5	8	3	8	8

[a] NT, not tested.

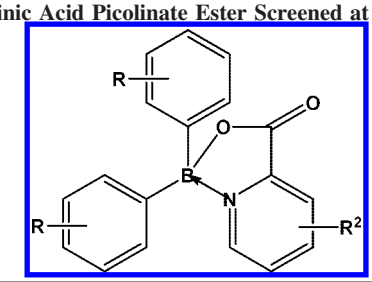
Table 8. Inhibitory Activity (MIC, μg/mL) Compounds Containing a 3-Hydroxypicolinic Acid Moiety



inhibitor	R	R1	*S. aureus*	*S. epidermidis*	*P. acnes*	*B. subtilis*	*H. influenzae*
erythromycin			0.5	0.15	0.1	0.1	4
195	3-Cl	3-Cl-Ph	0.125	8	10	16	16
196	4-Cl	4-Cl-Ph	4	1	1	1	16
197	3-Cl	pyridin-3-yl	16	32	NT[a]	NT[a]	32
198	4-Cl	pyridin-3-yl	64	32	NT[a]	NT[a]	16
199	4-Cl	2-Cl-pyridin-5-yl	32	32	NT[a]	NT[a]	32
200	3-Cl	thiophen-3-yl	32	32	10	16	32
201	3-Cl-4-Me	3-Cl-4-Me-Ph	1	0.5	0.3	1	>64
202	3-Cl-4-Me	4-Me	32	32	10	16	32
203	3-Cl-4-Me	phenethyl	0.5	1	1	1	>64
204	3-F	3-F-Ph	>64	>64	>100	>64	>64
205	3-Cl	3-SMe-Ph	8	8	3	4	>64
206	3-Cl	2-Me-Ph	8	8	3	4	>64
207	3-Cl-4-F	3-Cl-4-F-Ph	1	8	3	8	4
208	3-Cl-4-OEt	3-Cl-4-OEt-Ph	2	2	1	2	>64
209	3-Cl-4-NMe2	3-Cl-4-NMe_2-Ph	32	32	NT[a]	64	>64
210	3-Cl-4-Me	4-Me-Ph	4	2	3	2	>64
211	4-Cl-2-Me	4-Cl-2-Me-Ph	4	2	0.3	0.5	16

[a] NT, not tested.

Table 10. Percent Inhibition of Cytokine Release from PBMCs, Stimulated with Either LPS, Concanavalin A, or PHA, by selected Borinic Acid Picolinate Ester Screened at 10 μM



inhibitor	R	R^2	TNF-α	IL-1β (%)	IFN-γ (%)	IL-4 (%)
	erythromycin		22	−32	[a]NT	[a]NT
213	3-Cl-4-Me−	3-OH	100	99	−20	−21
214	3-Cl-4-Me−	3-OAc	101	−49	[a]NT	[a]NT
211	4-Cl-2-Me−	3-OH	101	103	15	57
219	3-Cl-4-Me−	5-CO_2H	100	80	24	9

[a] NT, not tested.

and several carboxylates with low micromolar activity against the two enzymes from the above-mentioned fungal pathogens.[149]

Inhibition of the β-carbonic anhydrases (CAs, EC 4.2.1.1) from the pathogenic fungi *Cryptococcus neoformans* (Can2) and *Candida albicans* (Nce103) with a series of aromatic, aryl-alkenyl-, and aryl-alkyl-boronic acids was reported.[150] Aromatic, 4-phenyl substituted-, and 2-naphthylboronic acids were the best Can2 inhibitors, with inhibition constants in the range of 8.5−11.5 μM, whereas aryl-alkenyl and aryl-alkyl-boronic acids showed K_is in the range of 428−3040 μM. Nce103 showed a similar inhibition profile, with the 4-phenylsubstituted- and 2-naphthylboronic acids possessing K_is in the range of 7.8−42.3 μM, whereas the aryl-alkenyl and aryl-alkylboronic acids were weaker inhibitors (K_is of 412−5210 μM). The host human enzymes CA I and II were also effectively inhibited by these boronic acids. The $B(OH)_2$ moiety is thus a new zinc-binding group for designing effective inhibitors of the α- and β-CAs. Phenylboronic acid **220** acts as a very weak hCA I inhibitor, with an inhibition constant of 1.56 mM. However, the presence of various substituents in the para position to the $B(OH)_2$ moiety in the aromatic boronic acids **221**−**226** leads to a drastic increase of enzyme inhibitory activity. Thus, a 4-methyl group leads to a compound with a K_I of 278 μM **221**, whereas an *n*-Bu such group to a more efficient inhibitor, with a *K*I of 7.9 μM **222**. Beneficial substitution patterns for hCA I inhibition are also those present in compounds **223**−**226** (methoxy-, bromine-, phenyl- and phenoxy-), with the biphenylboronic acid **225** being the best hCA I inhibitor detected in this study, with a Ki of 3.7 μM (an increase of potency compared to the lead **220** of 421.6 times). The β-naphthylboronic acid **227** as well as the arylalkenyl/alkyl derivatives **228**−**233** also show effective hCA I inhibitory properties, with inhibition constants in the range of 6.5−12.5 μM. It is interesting to note that for the elongated derivatives **228** and **229**, the introduction of the methyl group only slightly increased the inhibitory properties, whereas for the lead **220** and its 4-methyl-substituted derivative, the difference in inhibition is very important, with the methyl derivative **221** being 5.6 times higher hCA I inhibitor compared to **220** (Figure 23, Table 11).

A series of novel 2,3-dihydro-4-pyridones boronate esters using the aza Diels−Alder reaction with Danishefsky's diene and imines derived from formyl-phenylboronic acids were

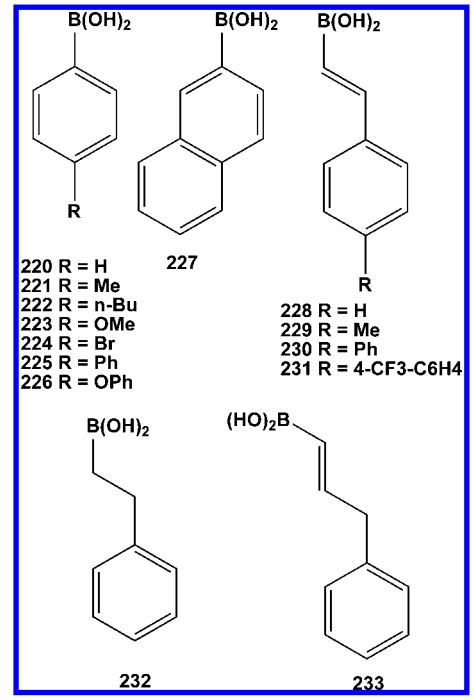


Figure 23. Boronic acids **229**−**233**.

Table 11. Inhibitory Activity of Boron-Containing Compounds of Human CA Isozymes I, II (cytosolic) and Fungal β-CAs Can2 and Nce103

		*K*I (μM)			
inhibitor	R	hCA I	hCA II	Can2	Nce103
220	H	1560	1050	810	30850
221	Me	278	10.8	8.9	9.0
222	*n*-Bu	7.9	8.7	10.9	8.0
223	MeO	10.9	7.9	11.4	15.6
224	Br	11.7	7.0	9.6	15.9
225	Ph	3.7	4.5	9.9	7.8
226	PhO	6.0	11.5	11.5	8.6
227		6.5	6.0	11.0	9.3
228	H	12.5	534	490	779
229	Me	12.1	617	521	460
230	Ph	10.7	373	8.5	42.3
231	4-CF_3−C_6H_4	9.5	27.6	3040	5210
232		11.4	17.9	428	412
233		8.6	18.1	506	633
AZA[a]		0.25	0.012	0.010	0.132
DCP[a]		1.20	0.038	1.20	0.91
EZA[a]		0.025	0.008	0.087	1.07

[a] Standard sulfonamide inhibitors (acetazolamide AZA, dichlorophenamide DCP, ethoxzolamide EZA).

prepared.[151] Two new boron-containing compounds, **234** and **235**, exhibited moderate antifungal activity against four fungi, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, and *Saccharomyces cerevisiae* (Figure 24).

There has been considerable interest in thiosemicarbazones and other urea derivatives, for their various bioactivities, especially for the treatment of parasitic diseases such as malaria.[152] Thiosemicarbazones are known to kill several species of protozoan parasites through the inhibition of cysteine proteases as well as through action against other targets.[153] Commercially available phenylboronic acids **236**−**238** showed inhibition of the R39 d,d-peptidase from *Actinomadura* sp. strain and of PBP2xR6 and PBP2 × 5204 (penicillin resistant) from *Streptococcus pneumoniae* (Figure 25).[154a]

Organoboron semicarbazone **238a**,**b** and thiosemicarbazone complexes **238c**,**d** were prepared by treating mixed

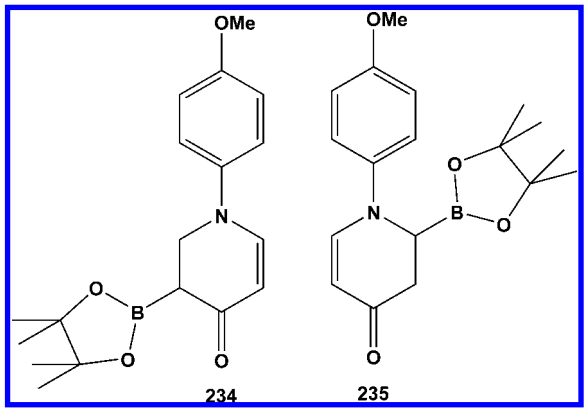


Figure 24. 2,3-Dihydro-4-pyridones boronate esters **234**, **235**.

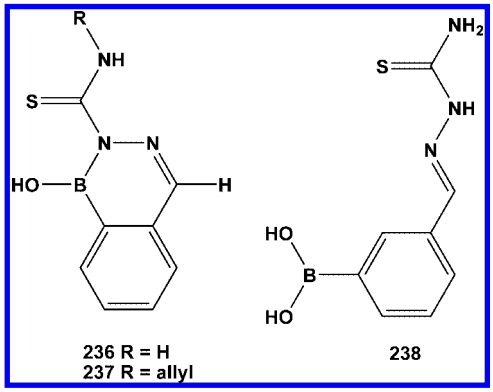


Figure 25. Phenylboronic acids **236**−**238**.

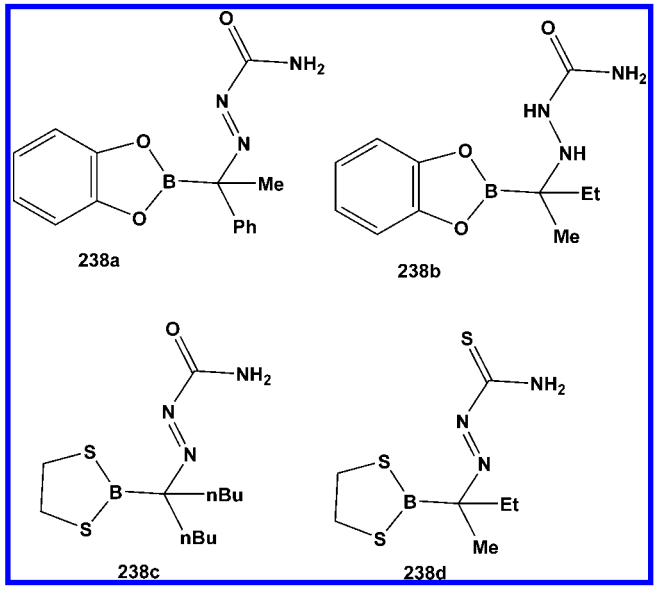


Figure 26. Organoboron semicarbazone **238a**,**b** and thiosemicarbazone complexes **238c**,**d**.

borate esters with the ligand moieties and were active against Gram positive and Gram negative bacteria and against fungi, e.g., *Candida albicans* and *Alternaria solani*. Boron complexes of thiosemicarbazones were more active than the corresponding semicarbazone derivatives due to the presence of NCS group. Also, complexes of ligands derived from aliphatic ketones were more active than those obtained from aldehydes (Figure 26).[154b]

The 3-(dihydroxyboryl)benzoic acid derivatives as inhibitors of the d,d-carboxy-peptidase R39 from *Actinomadura* sp. strain (R39). R39 is a low-molecular-weight PBP that is related to penicillin binding proteins (PBP4) from *Escherichia coli*,[155] PBP4a from *Bacillus subtilis*,[156] and PBP3 from *Neisseria gonorrheae*.[157] The kinetics of peptide-based boronic acid inhibitors of PBP3, PBP4, and PBP5 from *Neisseria gonorrheae* have been studied, and a crystal structure of a peptide boronic acid in complex with PBP5 has been reported.[158] Phenyl- and methylboronic acids **239**−**245** were tested against these same enzymes, and modest activity was observed for phenylboronic acid against PBP3 (Figure 27).[159] With a view to development of potent inhibitors of more clinically important high-molecular-weight PBPs (e.g., PBP1b, PBP2xR6, and the penicillin resistant strain PBP2 × 5204), R39 used as a model system with established kinetic assays for the initial development stages.[160] It is known that 3-(dihydroxyboryl)benzoic acid **246** was a modest inhibitor of R39. The design and synthesis of analogues of **246** were described, from which we developed inhibitors with improved potency for R39. Analogues of an initially identified inhibitor, 3-(dihydroxyboryl)benzoic acid **246**, were prepared via routes involving pinacol boronate esters, which were deprotected via a two-stage procedure involving intermediate trifluoroborate salts that were hydrolyzed to provide the free boronic acids.[160] 3-(Dihydroxyboryl)benzoic acid analogues containing an amide substituent in the meta position were up to 17-fold more potent inhibitors of the R39 penicillin binding proteins (PBP) and displayed some activity against other PBPs. These compounds may be useful for the development of even more potent boronic acid-based PBP inhibitors with a broad spectrum of antibacterial activity (Table 12).

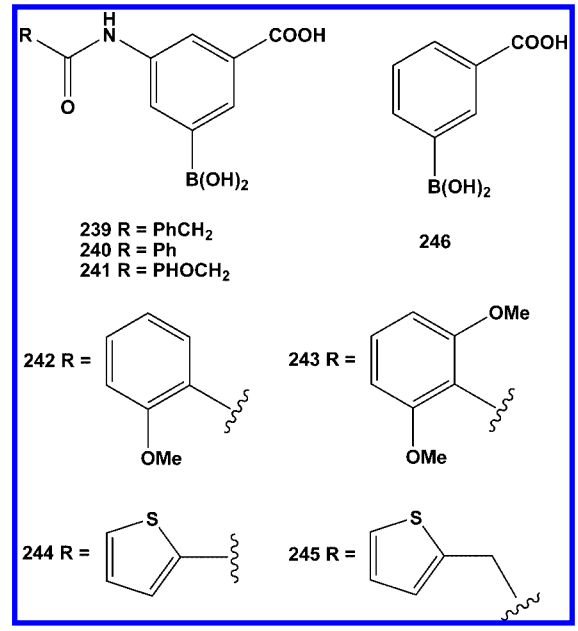


Figure 27. Boronic acids **239**−**246**.

Chelated borate esters **247**−**249** were prepared and showed remarkable inhibitory activity against pathogenic fungi *Fusarium oxysporum*, *Alternaria alternata, Rhizoctonia bataticola*, and bacteria *Staphylococcus aureus* and *Xanthomonas compestris* (Figure 28).[161]

Antibacterial boron-containing compounds **250**−**255** were prepared and tested against two-strain pathogenic bacteria.[162] Compounds of the dithiazolyl borinic acid series were more active than compounds of the diazaborine series in growth inhibition of *Escherichia coli* 198 (ATCC 11229) and *Proteus mirabilis* P1. The most active compound described here was **252**, which inhibited the growth of *E. coli* 198 by 50% at 0.28 μM (Figure 29).

Table 12. Inhibitory Activity of Aryl-Boronic Acids against d,d-Carboxypeptidase R39 from *Actinomadura* sp. Strain (R39)

inhibitor	residual activity (1 mM) (%)[a]	IC_{50} (μM)
239	3 ± 0	88 ± 2.6
240	9 ± 31	34 ± 3
241	2 ± 10	28 ± 0.6
242	10 ± 19	23 ± 1
243	84 ± 2	ND
244	18 ± 8	32 ± 2.2
245	5 ± 2	78 ± 5.5
246	20 ± 5	400 ± 19

[a] Quoted values are mean ± standard deviation over three replicate experiments.

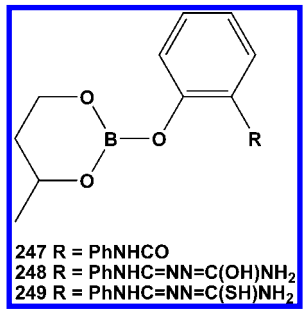


Figure 28. Bononic esters **247**−**249**.

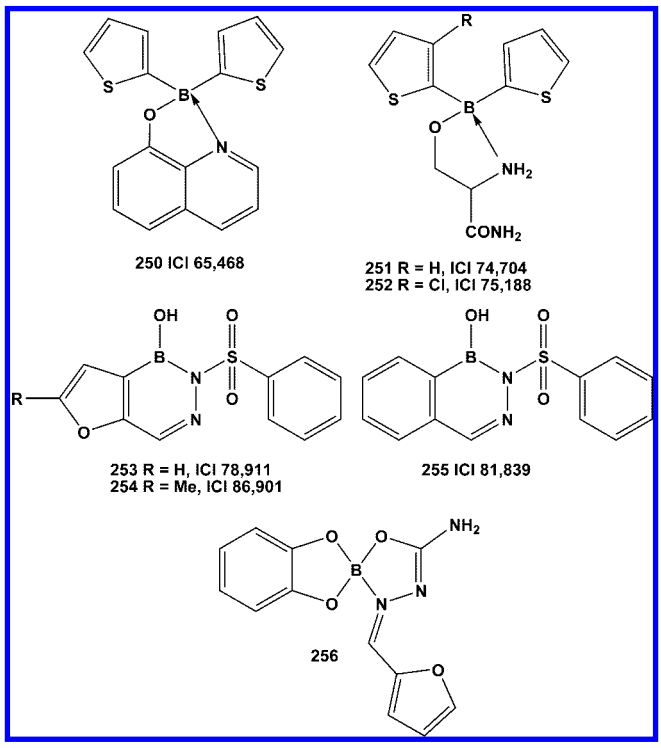


Figure 29. Boronic complexes **250**−**256**.

Heterocyclic aldimines of 2-furaldehyde, 2-thiophenecarbaldehyde, 2-pyridine-carbaldehyde, and 3-indolecarbaldehyde with semicarbazide hydrochloride and thiosemicarbazide react with 2-isopropoxybenzo-1,3-dioxa-2-borole [OC6H4OB(OCHMe2)], giving complexes OC6H4OB(X-N)] (where X = O or S).[163] Boron complex **256** showed inhibitory activity against a number of pathogenic bacteria and fungi.

Nineteen boron chelates were synthesized from 2-aminopyridine derivatives by various methods and were tested for antiviral activity in vitro, in ovo, and in vivo. The boron complexes **257**−**259** showed broad-spectrum antiviral activity against both DNA and RNA viruses (Figure 30).[164]

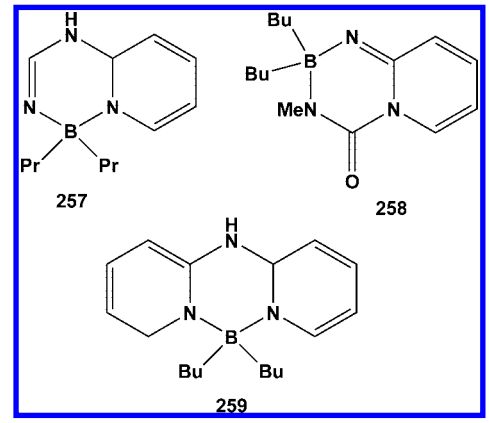


Figure 30. Boronic complexes **257**−**259**.

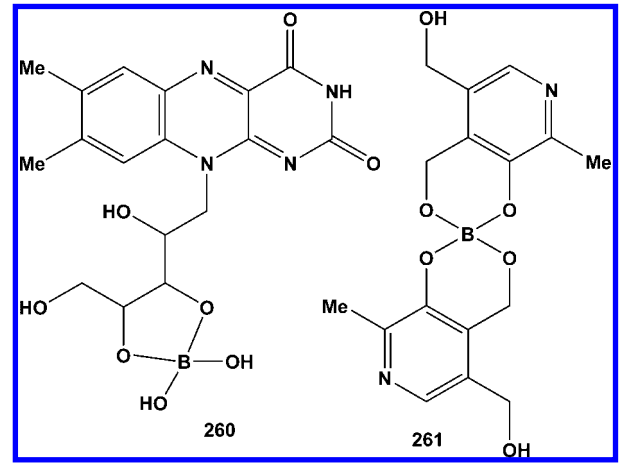


Figure 31. Riboflavin−boron complexes **260**, **261**.

The water-soluble riboflavin−boron complexes **260**, **261** of good stability were prepared by heating an aquatic solution of riboflavin and up to 5% HBO_3 at pH 6.5 for 3 h at 95°. Isotonic prepared from the riboflavin−boron complex was self-sterilizing toward molds and bacteria.[165] Many microorganimsms produced pyridoxine and/or their glucosides.[166] Pyridoxine is known as a versatile complexing agent. The predominant coordination modes in its metal complexes are neutral, monoanionic, or dianionic chelates involving the deprotonated phenolic and the deprotonated neighboring hydroxymethyl groups. Pyridoxine−boron complex was prepared (Figure 31).[167]

9. Concluding Remarks

This review described the effect of various classes of natural and synthetic boron-containing small molecules as potential inhibitors of bacterial and fungal quorum sensing. The chemical synthesis of these boron containing compounds and/or the extraction from natural resourses has been described in detail. Major emphasis has been placed upon Matteson's method for the synthesis of α-aminoboronic acids (stabilized as amides) and their potent role as bacterial enzyme inhibitors including serine proteases, β-lactamase, and others. In addition, oxazaborolidine compounds prepared in our lab were found to be effective against bacterial adhesion and biofilm formation. These compounds contain a five-membered ring boron heterocycle which might be analogous to autoinducers-2. We hope that this review will provide useful knowledge to the medicinal chemist community with the wide scope of the biological role of boron compounds.

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