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Pharmacologically active boranes*

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Abstract: Novel methods are described for the preparation of alkyldimethylamine cyanoboranes and β-hydroxylalkyldimethylamine cyanoboranes by C-lithiation of trimethylamine cyanoboranes followed by reaction with alkyl halides, aldehydes, and ketones. Lithiation of the monobromo derivatives of amine cyanoboranes led to the synthesis of the first examples of diborane derivatives of amine cyanoboranes. Bromo derivatives of amine cyanoboranes and amine carboxyboranes have been synthesized by new simple and efficient methods. Amine fluorocyanoboranes and amine fluorocarboxyboranes, new classes of compounds, have been prepared from the bromo precursors by fluorine/bromine exchange using fluorinating reagents such as AgF and $Et_3N{\cdot}3HF$. Eight different derivatives of oxazaborolidines were synthesized and evaluated for their affect on *Streptococcus mutans* viability, adhesion, and biofilm formation using ^{3}[H]-thymidine labeled bacteria, and fluorescent stained bacteria. This is the first reported antibacterial activity of this class of compounds. The minimal inhibitory concentration (MIC) values ranged from 0.26 to 10 mM. Structure–activity relationship was observed. The B-butyl moiety of the oxazaborolidines contributed an anti-adhesion effect for all derivatives, while its effect diminished when the boron atom was incorporated in a fused heterocyclic ring. The B-phenyl group induced bacterial adhesion in all tested compounds. In a separate study for boronated saccahrides and enzymatic inhibition, the complex formation between *N*-butylboronic acid and a series of monosaccharides was investigated by ^{1}H, ^{13}C, and ^{11}B NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS). Then, a series of boronic acid compounds with protease inhibition properties were prepared. The effect of added mono-, di-, and polysaccharides on the inhibitory activity of these compounds was studied. Potassium organotrifluoroborates were found to be reversible competitive inhibitors of α-chymotrypsin and trypsin. Based on ^{19}F NMR, it was speculated that they inactivate the enzymes as a result of the formation of hydrogen bonds between fluorine atoms of the inhibitors and the serine protease.

Keywords: cyanoboranes; C-lithiation; amine cyanoboranes; amine carboxyboranes; oxazaborolidines; antibacterial; bacterial adhesion.

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AMINE CYANOBORANES AND DERIVATIVES

Introduction

Amine-borane compounds such as α-aminoboronic acids, amine carboxyboranes, amine cyanoboranes, and related compounds are considered as isoelectronic and isostructural analogs of many biologically active compounds such as amino acids, neurotransmitters, nucleosides, and nucleic acids. Hence, they can mimic the biological activity of such compounds in the body [1]. Amine carboxyboranes can be regarded as isoelectronic analogs of protonated α-amino acids [2]. While the compounds are similar in size and geometry, they have very different electronic and hydrogen-bonding properties and, therefore, different biological responses [3]. Amine carboxyboranes and amine cyanoboranes have been shown to be anti-hyperlipidemic in laboratory animals and showed considerable reduction in serum cholesterol levels [4]. Additional physiological activities include anti-inflammatory [5], anti-osteoporotic, and anti-obesity [6]. A number of derivatives of amine carboxyboranes were prepared recently, such as amine carboxyborane alkyl esters [7–10], amine dicarboxyboranes and their dialkylesters [11–14], amine carbomylboranes [15], amine bromocarboxyboranes and bromo(methoxycarbonyl) boranes [16–18], and amine alkyl [(ethylimino)alkoxymethyl] boranes [19]. C-Hydroxyamine cyanoboranes can be regarded as protected hemi-aminals. Simple hemi-aminals are unstable due to the nitrogen loan pair. However, C-hydroxyamine cyanoboranes were found to be very stable. When the loan pair on nitrogen is occupied as in amides [20], the corresponding amido hemi-aminals are stable. They also possess interesting pharmacological activity such as antifungal [21], peptidomimetic [22], and protease inhibition [23]. It is clear that the progress of synthetic chemistry has allowed a large number of boronated biomolecular analogs to be prepared, which can play a major role in therapy, and boron-based imaging.

C-Lithiation/alkylation of trimethylamine cyanoborane

Amine cyanoboranes can be prepared by the reaction of cyanide and amine iodoboranes [24], or reaction of $NaBH_3CN$ with X_2 and conversion of the oligomer with amines [25]. They can also be obtained by the reaction of amine-boranes with hydrochlorides [26]. The amino group of the amine cyanoboranes can be replaced by stronger Lewis base (amino or phosphino groups) to afford new cyanoborane derivatives by amine exchange [27]. Amine alkyl cyanoboranes can be prepared from alkyl trihydroborates and mercuric cyanide [28].

In the present work, we have succeeded in preparing more complex derivatives of amine cyanoboranes via a novel method that is based on C-lithiation/alkylation of trimethylamine cyanoborane, **1**. Amine boranes and other Lewis acid complexed boranes can be lithiated [29–34]. Lithiation of amine cyanoboranes should in fact be more facile than the corresponding lithiation of amine.BH_3 complexes due to the cyano group. This in fact has proven to be the case as we recently reported our initial investigations into alkyl lithiation of trimethylamine cyanoborane and its reaction with various electrophiles [35]. The method is applicable to alkylhalides, such as propyl iodide, butyl iodide, allyl bromide, and bromotrimethyl silane, aldehydes, and ketones (Fig. 1).

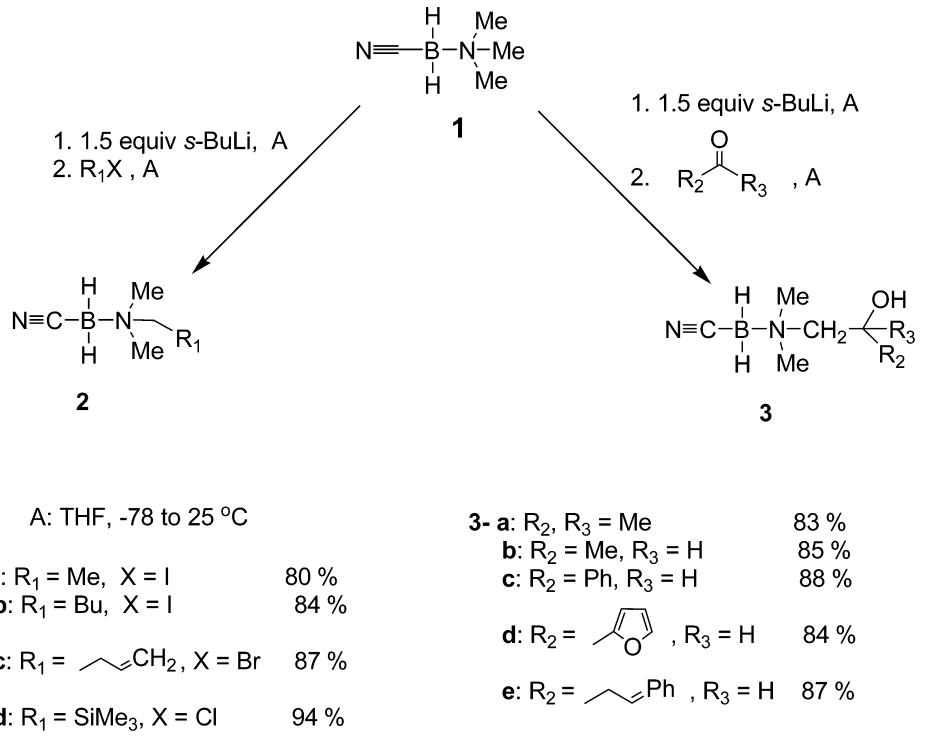

Fig. 1 Synthesis of alkyldimethylamine cyanoborane derivatives following the C-lithition/alkylation method.

Crystals suitable for X-ray molecular structure determination were successfully obtained for compound **3-c**. The molecular structure determined is shown in Fig. 2. The structure reveals that the geometry around the boron atom is tetrahedral, the B–C≡N moiety has bent geometry as indicated by the bond angle at C being 176.97(16)°. The B–N [1.611(2) Å], B–C [1.587(2) Å], and C–N [1.148(2) Å] distances in the BH_2CN group of the title compound **3-c** are in accord with those found in other cyanoboranes [36–38].

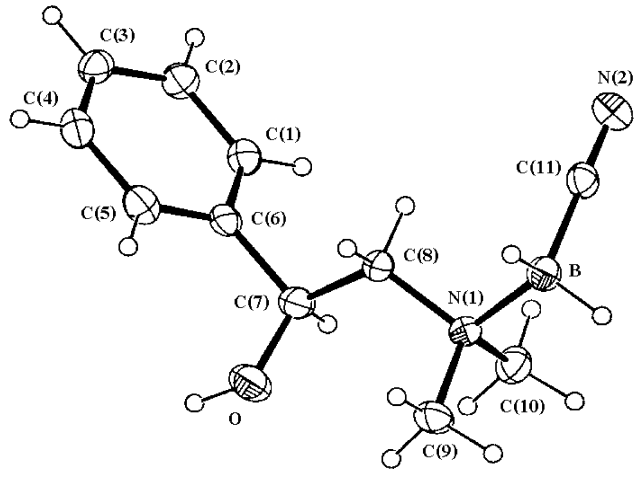

Fig. 2 Molecular structure of compounds **3-c**.

Amine halocyanoboranes and halocarboxyboranes

Although halogenated aromatic amino acids (bromides, chlorides, and iodides on the aromatic ring of histidine, tryptophan, and tyrosine) are well known [39–41], the synthesis and biological studies of the halogenated derivatives of amine cyanoboranes and carboxyboranes have gained less attention. Halogenated aromatic amino acids are especially abundant in marine environments and are used by the organisms for different purposes usually for chemical defense from predators [42]. Halogenated L-phenylalanine has shown significant neuoroprotective action [43]. Conversely, naturally occurring organofluorine compounds are quite rare. Despite this scarcity, they are metabolically active substances and are very interesting, particularly in medicinal chemistry [44]. This is due to the fact that fluorinated amino acids were recognized as inhibitors of specific enzymes, substrates for incorporation into proteins and peptides, or precursors of other critical biomolecules like aminergic neurotransmitters or lethal substances such as fluoroacetate or fluorocitrate [45]. However, α-halo-α-amino acids are intrinsically unstable due to the available loan pair on nitrogen. This is different from the amine halocarboxyboranes where the loan pair is occupied in a dative B–N bond, rendering these compounds very stable.

Synthesis of amine bromocyanoboranes

Reaction of the corresponding alkyl amine cyanoborane ($R_3NBH_2C{\equiv}N$), with 1.2 equiv of Br_2 in double distilled water (DDW) at 0 °C for a period of 4 h, produces the monobromo derivatives ($R_3NBHBrC{\equiv}N$). Performing the same reaction with 2.2 equiv of Br_2 and allowing the reaction to warm to room temperature with stirring for 16 h, produced total conversion of the corresponding amine cyanoboranes to the dibrominated derivatives ($R_3NBBr_2C{\equiv}N$) (Fig. 3).

$$R_3NBH_2CN + Br_2 \longrightarrow R_3NBHBrCN + HBr \quad (1)$$

$$R_3NBHBrCN + Br_2 \longrightarrow R_3NBBr_2CN + HBr \quad (2)$$

Fig. 3 Preparation of amine monobromo and dibromocyanoborane derivatives from amine cyanoboranes.

The molecular structures of the prepared trimethylamine dibromocyaborane **4** and triethylamine dibromocyanoborane **5** were determined by X-ray crystallography (Fig. 4). The covalent parameters exhibit standard values characteristic to boron in tetrahedral sp^3 hybridization. Those of the $N–BBr_2–C{\equiv}N$ are of a particular significance in the present context, and are summarized in Table 1. The observed data are comparable to those found in related compounds that contain the $N–BBr_2–C$ fragment [46–51]. The conformation around the boron atom is nearly ideally tetrahedral, with Br–B–Br and N–B–C bond angles of 109º, and the conformation around the carbon atom in B–C≡N moiety is nearly linear with B–C≡N bond angles of 178.5º and 176.7º.

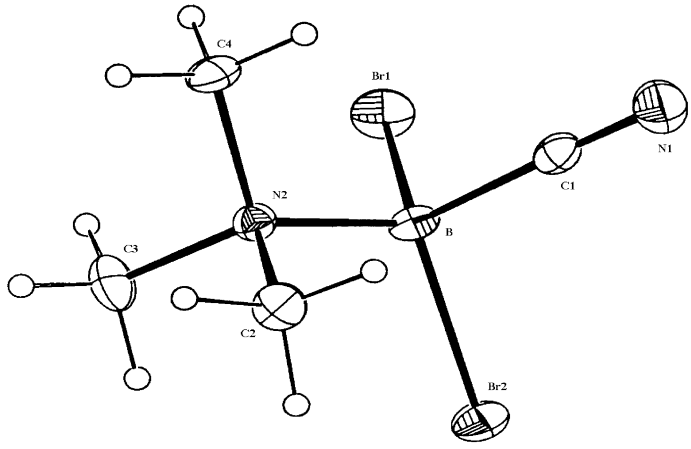

Fig. 4 Molecular structure of compound **4**.

Table 1 Covalent parameters of the N–BBr_2–C≡N in compounds **4** and **5**.

	4	**5**
B–N(sp^3)/Å	1.592	1.613
B–C(sp^2)/Å	1.594	1.596
B–Br/Å	2.013, 2.019	2.016, 2.027
C≡N/Å	1.118	1.134
Br–B–Br/°	109.21	109.2
N–B–C/°	109.2	111.1
N–B–Br/°	111.1, 110.92	113.8, 110.6
C–B–Br/°	108.6, 107.8	106.5, 105.3
B–C≡N/°	178.5	176.7

Synthesis of amine bromocarboxyborane esters

We have developed a new method for the preparation of esters of trimethylamine dibromocarboxyborane from trimethylamine carboxyborane, in a one-pot reaction. The reaction involves the treatment of trimethylamine carboxyborane with 2.5 equiv of Br_2 in the appropriate alcohol for 4 h at 0 °C, for the monobromoderivatives, and 5 equiv of Br_2 for 8 h under the same conditions for the dibromoderivatives. Molecular structures for the compounds **6–9** were determined by X-ray crystallography, and the structure of **6** is shown in Fig. 5.

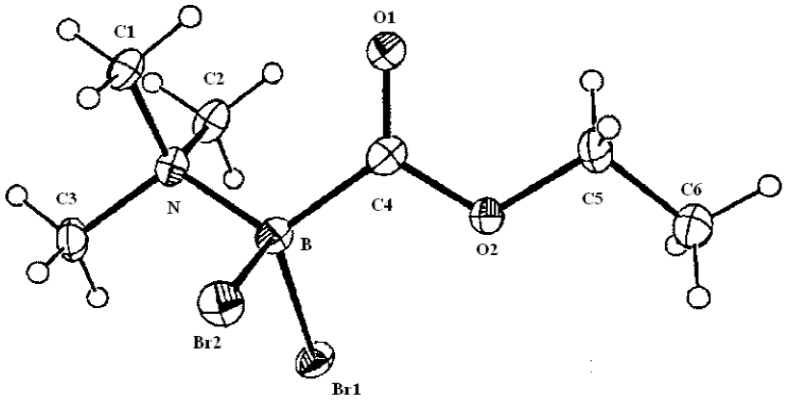

Fig. 5 Molecular structure of compound **6**.

The covalent parameters exhibit standard values characteristic of boron in tetrahedral sp^3 hybridization. Those of the N–BBr_2–C=O are of a particular significance in the present context, and are summarized in Table 2. The conformation around the boron atom is nearly ideally tetrahedral, with Br–B–Br bond angles of 109º and slightly larger N–B–C bond angles of 112º. The observed data are comparable to those found in related compounds that contain a N–BBr_2–C fragment [47,51].

Table 2 Covalent parameters of the N–BBr_2–C=O in compounds **6**–**9**.

	6	**7**	**8**	**9**
B–N(sp^3)/Å	1.605	1.610	1.603	1.603
B–C(sp^2)/Å	1.626	1.633	1.624	1.695
B–Br/Å	2.021, 2034	2.023, 2.024	2.024, 2.031	2.026, 2.030
Br–B–Br/º	109.2	109.3	108.8	109.14
N–B–C/º	112.0	112.2	112.2	113.6
N–B–Br/º	109.1, 109.5	109.0, 109.6	109.3, 109.4	109.4, 108.5
C–B–Br/º	105.5, 111.1	106.7, 110.0	108.4, 108.8	108.9, 107.2

The corresponding crystal structures reveal efficient intermolecular packing, which prevents any possible conformational disorder. These are stabilized by dispersion forces, including intermolecular van der Waals contacts between electron-rich and -deficient surfaces of neighboring species, as represented by the Br···H (3.0 Å) and O···H (2.6 Å) interactions. The crystal packing of **6**, which shows further stabilization of the structure by roughly parallel alignment of the alkyl residues (Fig. 6) is noteworthy.

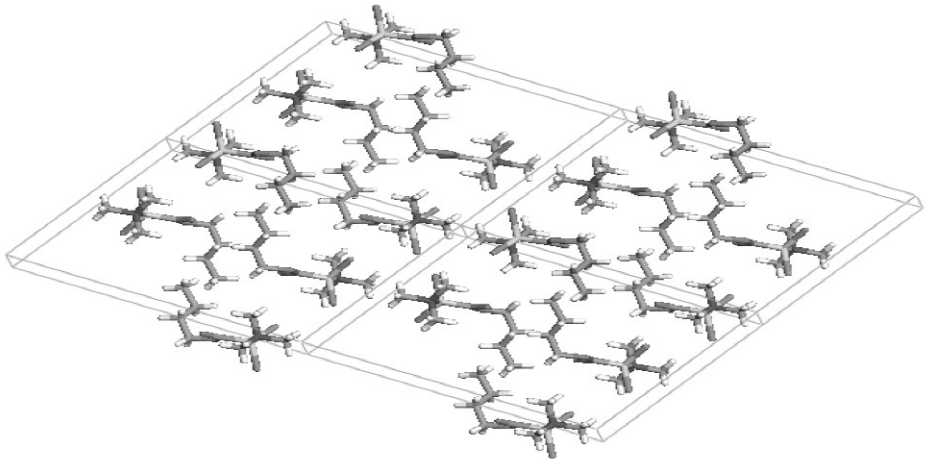

Fig. 6 Crystal structure of compound **6**, viewed the short *b*-axis.

Synthesis of amine fluorocarboxyboranes and amine bromofluorocarboxy-boranes

As stated above, fluoro-containing amino acids are potentially very interesting pharmacological compounds. Our novel approach to amine fluorocarboxyboranes, a new class of compounds, is based on the exchange of bromide by fluoride using AgF [52]. We have found that sonication of the amine bromocarboxyboranes with excess AgF results in the rapid incorporation of fluorine into the compounds (Fig. 7). This was determined by various NMR techniques such as ^{19}F, ^{11}B NMR, etc. that unequivocally indicated the formation of amine difluorcarboxyboranes (a quartet was observed in the ^{19}F while a triplet was seen in the ^{11}B NMR indicative of the spins of the respective nuclei) (Fig. 8). In addition, the amine bromofluorocarboxyboranes were prepared by bromination of the amine monofluorocarboxyboranes as described above.

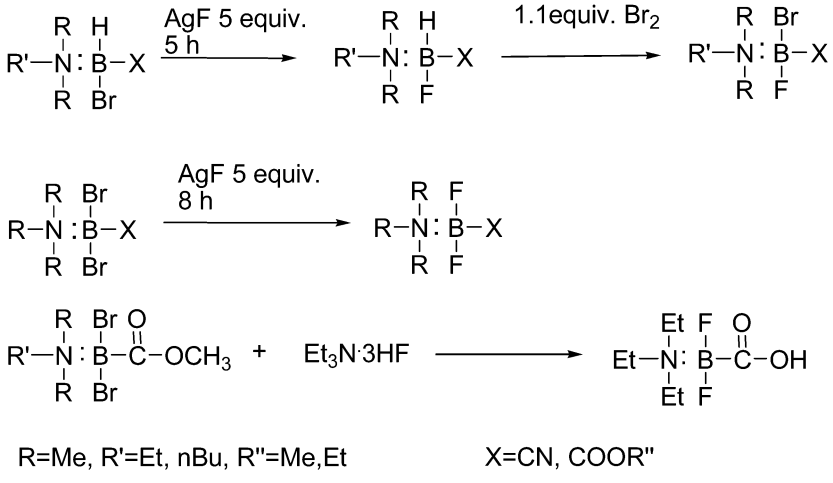

Fig. 7 Preparation of amine fluorocarboxyboranes and amine bromofluorocarboxyboranes from the corresponding amine bromocyanoboranes.

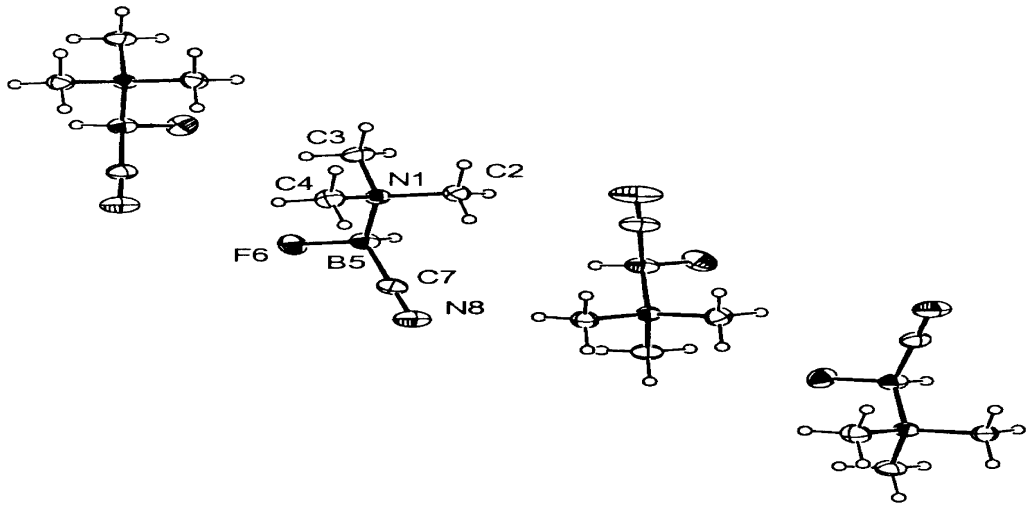

Fig. 8 Molecular structure of trimethylaminefluorocyanoborane.

The pK_a value for the triethylamine difluorocarboxyborane was determined and found to be 5.15 compared to 8.38 for $Me_3N{:}BH_2COOH$, in line with expectations. Molecular structure for trimethylamine fluorocyanoborane was determined by X-ray crystallography. The asymmetric unit of this structure contains four crystallographically independent molecules of $(CH_3)_3NB(H)(F)CN$. This should be attributed to the conformational disorder around the sp^3 boron atoms, which is characterized by alternating orientations of the B–H and B–F bonds. These bonds occupy about the same volume in space, and thus can occupy either of the two possible directions around the tetrahedral boron. In the observed structure, the refined structural model of best fit to the diffraction data is represented by four molecular species with differently populated twofold conformational disorder (with 64–70 to 30–36 % ratio) of this type. Figure 8 shows the four molecules of this compound in their major conformation.

The covalent parameters exhibit standard values characteristic to boron in tetrahedral sp^3 hybridization, with B–N, B–C, and B–F (major conformation) within 1.603–1.609, 1.614–1.617, and 1.333–1.364 Å, respectively. The conformation around the boron atom is nearly ideally tetrahedral with N–B–C bond angles within 107.9–108.6º, F–B–C angles within 110.4–110.9º, and N–B–F angles within 109.8–110.3º.

The molecules are rather loosely packed in the crystal structure (as reflected by the calculated density of only 1.15 g·cm^{-3}), which explains the difficulties encountered in growing diffraction-quality crystals of this compound. Correspondingly, all interatomic distances between neighboring molecules in the crystal are equal to, or longer than, the sums of the corresponding van der Waals radii. Indeed, not even weak intermolecular hydrogen bonding interactions of the C≡N…H–C or C–F…H–H could be detected in this structure, due to the lack of adequately acidic protons and the "hard" nature of the potential N and F acceptors. The loose crystal packing facilitates the occurrence of the conformational disorder described above.

For Me_3NBH_2CN, the ^{11}B NMR spectra showed a triplet at –13.72 ppm, while for $Me_3NBHBrCN$, a doublet was obtained at –11.12 ppm; and for $Me_3NBHFCN$ 1, a double of doublets was obtained at –2.3 ppm. In the monofluoro derivatives, the ^{11}B NMR peaks were obtained as a doublet of doublets due to splitting by both hydrogen and fluorine atoms attached to boron (Fig. 9, a), while a triplet was observed in the difluoro derivatives. In the ^{19}F NMR, a quartet was observed for the amine difluorocyanoborane derivatives, and the amine diflourocarboxyborane esters. This was expected due to coupling to the ^{11}B atom which has spin of 3/2. A quartet of doublets were observed for all the amine monofluorocyanoboranes and the amine monoflourocarboxyborane esters, because of the splitting of

both boron and hydrogen neighbor atoms (Fig. 9b). We found that the differences in the chemical shifts ranges in the mono- and difluoro derivatives in all cases are large ~145 ppm (–25 to –27 ppm for the monofluoro derivatives and –165 to –167 ppm for the difluoro derivatives). This is reasonable because in ^{19}F NMR, it is known that the fluorine atom is much more sensitive to the local environment than hydrogen. The reason for this is that a fluorine nucleus is on average surrounded by 9 electrons, rather than a single electron as in the case of hydrogen.

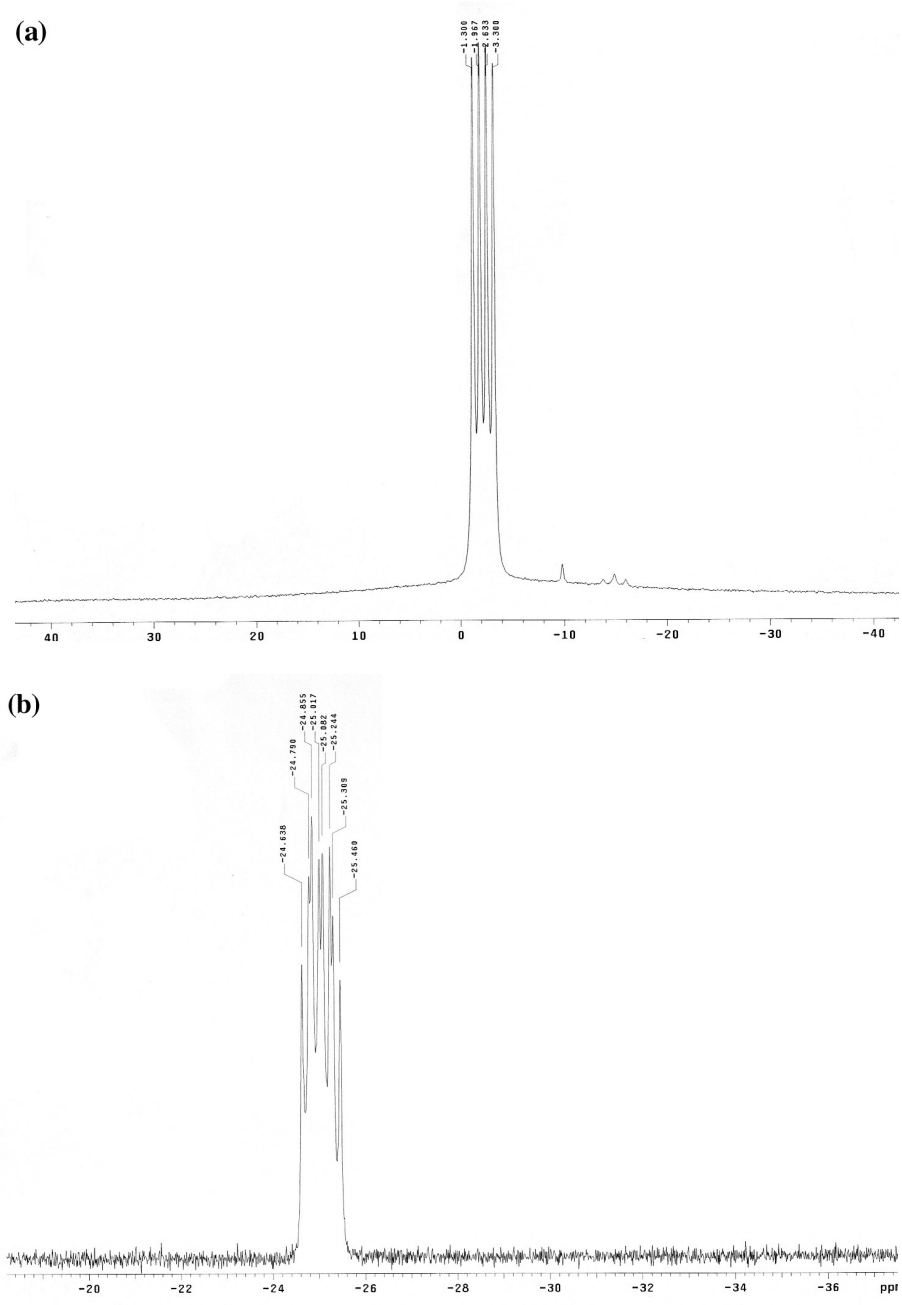

Fig. 9 (a) ^{11}B NMR spectra for trimethylamine monofluorocyanoborane; (b) ^{19}F NMR spectra for trimethylamine monofluorocyanoborane.

Diboron derivatives of amine cyanoboranes

Diboron compounds with a boron–boron single bond are important intermediates, and their structural complexity lies between simple monoboron derivatives and polyhedral electron-deficient compounds. Since the initial discovery of B_2Cl_2 by A. Stock et al. in 1925 [53], a continual effort has been focused on the synthesis of diboron compounds, particularly the single derivatives of B_2X_2 type. These boron-containing compounds provide the simplest examples of catenation in boron chemistry and offer suitable systems to study properties of the covalent B–B bond and the characteristic chemistry of compounds containing this linkage. Diboron derivatives have been utilized as synthetic intermediates, functional molecules, functional polymers, and biologically active compounds [54]. Diboration, the addition of diboron tetrahalides B_2X_4 (X=F, Cl, Br), to unsaturated hydrocarbons is a straightforward method to introduce boryl groups into organic molecules, but their synthetic use has been severely limited because of the instability and limited availability of the reagents [54]. Diboron compounds can also be used in boron neutron capture therapy (BNCT), first proposed in 1936 [55]. Critical to the development of BNCT is the synthesis of boron-containing compounds that selectively target tumor cells [56]. Numerous boron-containing compounds have been tested [57–60]. These agents may be categorized into those containing a single boron atom and those containing multiple boron atoms as boron clusters having B–B bonds. The advantage of the latter is that higher boron concentration in a tumor can be achieved [61].

In this work we have reported a novel method for the preparation of diborane (**4**) derivatives of amine cyanoboranes, the first example of B–B diboron compounds derived from such compounds. The diborane (**4**) derivatives of the corresponding amine cyanoborane were synthesized as their 2LiBr complexes from the monobromo derivative of the corresponding amine cyanoborane, followed by B–B coupling using elemental sodium or *n*-BuLi.

Synthesis of diborane (4) derivative of trimethylamine cyanoborane using n-BuLi

Following the previously described procedure for the C-lithiation of trimethylamine cyanoborane [35], lithiation of the trimethylamine monobromocyanoborane derivative ($Me_3NBHBrC{\equiv}N$) with *n*-BuLi produced a B-lithiated intermediate which then coupled with the unlithiated trimethylamine monobromocyanoborane (Fig. 10) to produce the diborane (**4**) derivative as the 2LiBr complex. The nature of the complexes were confirmed by both elemental analysis and by mass spectroscopy. The same product was obtained when 1 equiv of trimethylamine monobromocyanoborane was added 1 h after lithiation.

A series of diboron (**4**) derivatives of amine cyanoboranes was prepared as their 2LiBr complexes using the *n*-BuLi method with different amine monobromocyanoboranes, such as triethylamine, *n*-butyldimethylamine, and dimethylpentylamine, to give the corresponding diborane (**4**) derivatives, as their 2LiBr complexes in high purity and good yields (Fig. 10). Attempted coupling of the amine dibromocyanoboranes under the same reaction conditions failed to give the expected dibromodiborane (**4**) derivative. Only starting reagent was obtained.

Synthesis of diborane (4) derivative of trimethylamine cyanoborane using elemental sodium

Dehalogination of haloborane amines with sodium to give the B–B diboron compounds was previously reported by Abu-Ali et al. [62]. Bis(pyrrolidino)bromoborane was debrominated to give the B–B coupled product, tetra(pyrrolidino)diborane (**4**). We tried this procedure, and obtained the diborane (**4**) derivative of trimethylamine cyanoborane. However, in addition to the coupling product, the monobromo derivative was partially reduced and the trimethylamine cyanoborane was obtained as by-product. This was the major drawback with elemental sodium (Fig. 10).

Brominating the diborane (**4**) derivatives of amine cyanoboranes with excess of Br_2 in DDW, did not give the expected dibromodiborane (**4**) derivative. Instead the B–B bond was cleaved, and the dibrominated monomer (R_3NBBr_2CN) was obtained as a precipitate (Fig. 10). Reducing the number of bromine equivalents into one produced a mixture of the amine dibromocyanoborane and the diborane

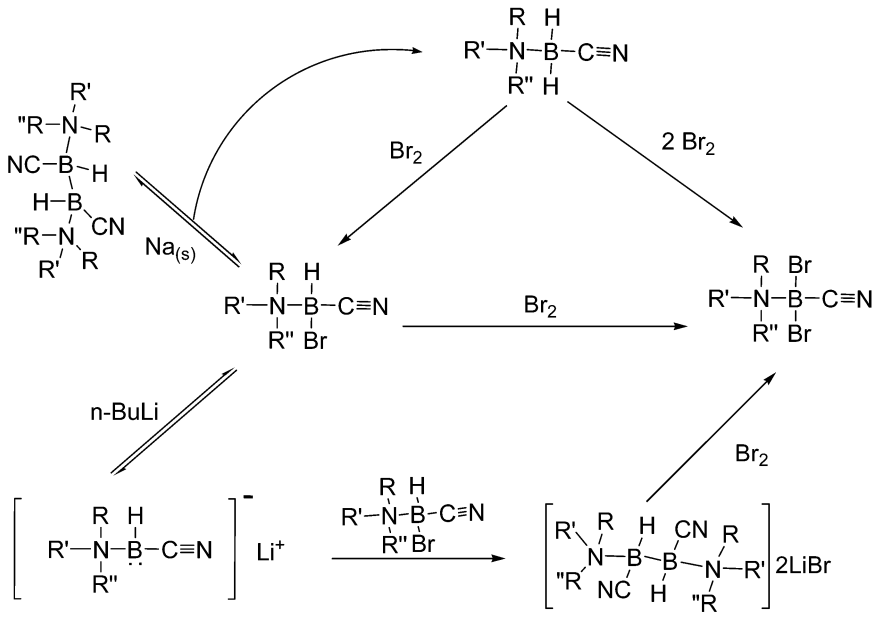

Fig. 10 Preparation of the diborane (**4**) derivatives from the corresponding amine cyanoboranes.

(**4**) compound. This confirms the instability of dibromodiborane (**4**) compounds, and goes with the failure of B–B coupling of the amine dibromocyanoborane derivatives. From these results, it may be concluded that the dibromodiborane (**4**) derivatives are not stable or cannot be obtained by using any of these methods. The instability of the dibromodiborane (**4**) derivatives may be explained in terms of the steric hindrance caused by the large bromine atoms that replace the hydrogen atoms in the diborane (**4**) derivative.

Conclusion

Amine cyanoboranes and amine carboxyboranes are intriguing groups of compounds that have gained pronounced literature attention in the last 35 years. They have been found to possess several promising biological activities. In this work, novel methods to prepare diverse types of new amine cyanoborane and amine carboxyborane derivatives have been approached. These derivatives are new alkyldimethylamine cyanoboranes, β-hydroxylalkyldimethylamine cyanoboranes, amine bromocyanoboranes, amine bromocarboxyborane esters, amine fluorocyanoboranes, amine fluorocarboxyboranes, and diborane (**4**) derivatives of amine cyanoboranes. All prepared compounds were fully characterized, and molecular structures for some examples of each type of derivatives were determined using X-ray crystallography.

EFFECT OF OXAZABOROLIDINES ON *STREPTOCOCCUS MUTANS*

Introduction

Although boron is an essential element for the activity of a variety of enzymatic systems in higher plants and animals [63,64] and the biological role of boron has been the subject of a number of biological studies [65–67], the potential of boron-containing compounds in medicine lags behind other elements. Some success in the use of boron-containing compounds has been achieved in BNCT [68]. Diazaborines have been shown to be active against malaria [69,70]. Naturally occurring borate com-

plexes are used as topical antibiotics [71,72]. Very recently, an α-amido boronic acid, Velcade©, a proteosome inhibitor, has been approved as an antineoplastic agent [73–81]. Nevertheless, taking into account the vast possibilities of structures incorporating boron, the use of boron-containing compounds in medicine has barely been scratched [82]. Compounds containing B–N bonds have been shown to possess biological activity. Thus, carboxyboranes have shown anticancer, hypolipidemic, and antifungal activity [83]. Oxazaborolidines also possess a B–N bond and are readily obtained from an amino alcohol and a boronic acid. Nevertheless, in spite of their ubiquity in organic synthesis [84,85], the effect of oxazaborolidines on bacterial adhesion, biofilm formation, or any other pharmacological activity has never been reported. Based on the biological activity displayed by other boron systems with B–N bonds, oxazaborolidines should be interesting candidates for biological screening. This omission prompted us to undertake an evaluation of this group of compounds. Recently, a borate complex, AI-2 (auto-inducer 2), has been identified as a universal signaling molecule in bacteria [86]. We reasoned that oxazaborolidines somewhat resemble AI-2 and thus may possess some biological activity.

Dental diseases, including tooth decay, gingivitis, and periodontitis, are world-wide oral diseases affecting all ages, ethnic groups, and both sexes. Caries is characterized as a localized pathological disease due to the presence of cariogenic bacteria [87]. These bacteria accumulate on the surface of the tooth (dental plaque biofilm) and initiate demineralization of tooth enamel, resulting in cavitation. Oral biofilms harboring pathogenic bacteria are the major contributing virulence factors associated with these diseases [87]. Adhesion of oral bacteria to the tooth surface is facilitated by physical, chemical, and biological mechanisms [88,89]. Mutans streptococci as *Streptococcus mutans* (*S. mutans*) is one of the bacterial species that play a key role in dental biofilm formation and dental caries [87,88]. Elimination of these bacteria is a fundamental step in preventing and treating dental caries. Several antibacterial drugs are being used for prevention or treatment of tooth decay [90]. Although effective, this approach has many clinical disadvantages, primarily the development of secondary infections, nonspecific activity, and the emergence of resistance bacteria. Possible alternative means to antibacterial therapy for controlling infectious diseases have recently focused on affecting biofilm and bacterial accumulation [91]. Irrespective of the ongoing debate as to the drug of choice for eliminating cariogenic bacteria, new therapeutic approaches are being sought. This review summarizes our initial results on the antimicrobial properties, adhesion effect, and biofilm formation of several derivatives of oxazaborolidines as a new generation of compounds for controlling infectious diseases by affecting bacterial adhesion, biofilm formation, and viability.

Synthesis

The oxazaborolidines were synthesized by reacting either phenyl or butyl boronic acid with an amino alcohol or a diol amine, with the azeotropic removal of water BNO1-6 (Fig. 11). Phenyl and butyl boronic acid were selected as representative structures for nonbonding and π-complex interactions. The amino alcohols selected for the synthesis represent various degrees of steric hindrance and aqueous solubility. BNOO1 and BNOO2 represent highly rigid and charged structures as opposed to the other synthesized compounds which are neutral and not as rigidly structured. These compounds were synthesized in order to study electrostatic interactions.

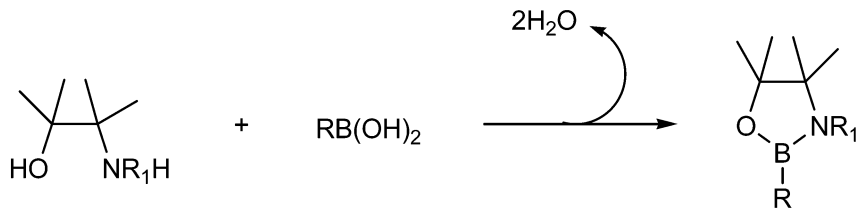

Fig. 11 Oxazaborolidine synthesis.

Results and discussion

All compounds were synthesized and characterized as previously described by Jabbour et al. [92].

Oxazaborolidine antibacterial activity against S. mutans

Minimal inhibitory concentration (MIC) values were used to determine the antibacterial efficacy of oxazaborolidines against *S. mutans*, which is the one of the predominant bacteria in the etiology of dental caries. Since the use of oxazaborolidines described herein is novel, the mechanism of antibacterial action is not known at present. However, BNO1 and BNO5 were most active, which seems to indicate that substitution on nitrogen is desirable, while BNOO1 and BNOO2, which are formally charged, showed the weakest activity. While boronic acids demonstrate no antibacterial activity at their maximal solubility in water (10 mM), all the tested oxazaborolidines demonstrated a significant antibacterial activity at much lower concentrations (Table 3).

Table 3 MICs of oxazaborolidine derivatives against *S. mutans*.

Oxazaborolidines derivatives	MIC range (mM)	MIC range (mM)
BNO1	1.55	0.77–2.33
BNO2	6.00	2–10
BNO3	3.38	2.25–4.5
BNO4	1.33	0.90–1.75
BNO5	0.53	0.26–0.80
BNO6	2.83	1.90–3.75
BNOO1	6.75	4.5–9.0
BNOO2	6.75	4.5–9.0

Effect of oxazaborolidines on S. mutans *adhesion*

The ability of the above oxazaborolidines (Fig. 12) to affect bacterial adhesion to hydroxyapatite was performed in a method similar to Schilling and Bowen [93] by incubating labeled radioactive bacteria with sucrose, hydroxyapatite (HA) beads, and oxazaborolidine derivative at a range of concentration below and above MIC values, for 60 min at 37 ºC. The amount of radioactive bacteria adsorbed onto HA was measured by scintillation counter.

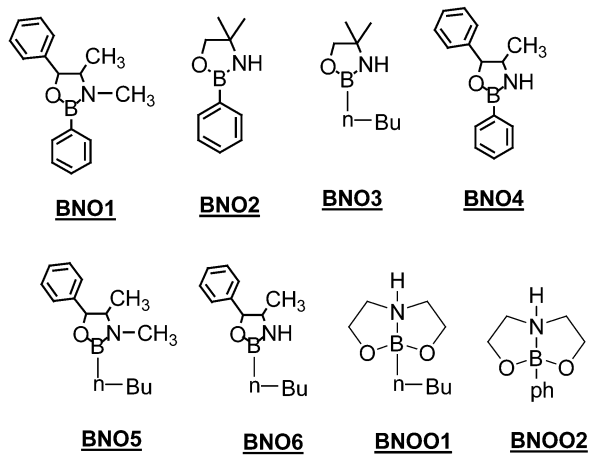

Fig. 12 Structures of oxazaborolidine derivatives.

At low concentrations of up to 1 mM, BNO3, BNO5, and BNOO1 had a minor effect on bacterial adhesion (less than 10 %) (Fig. 13). BNO1, BNO2, and BNO4 lowered bacterial adhesion by about 20 %. BNOO2 promoted adhesion by 22 %, and BNO6 promoted adhesion by 50 % ($p < 0.05$).

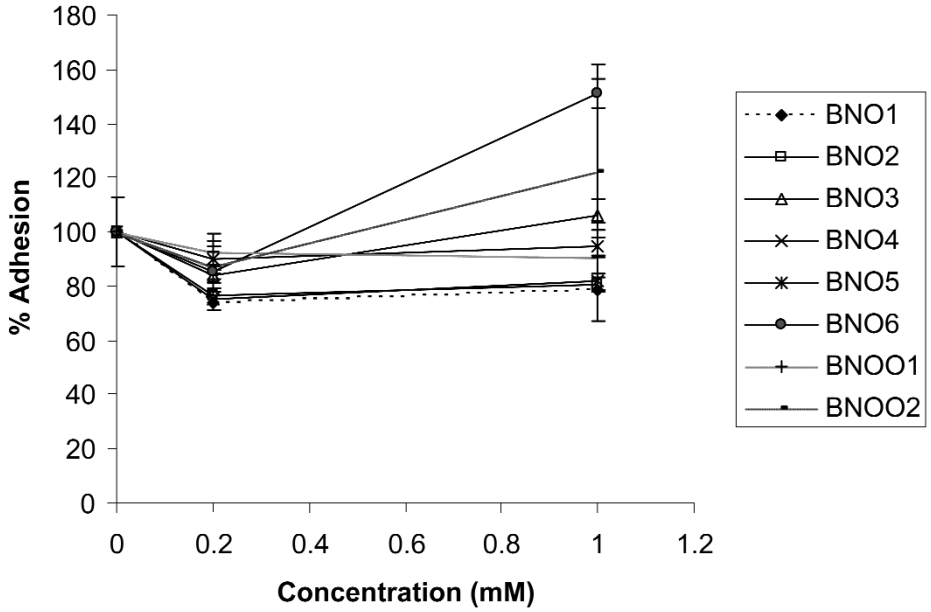

Fig. 13 Effect of oxazaborolidine derivatives on bacterial adhesion at sub-MIC concentrations.

At higher concentrations of oxazaborolidines (between 6–120 mM) a dose–response and structure–activity relationship (SAR), between the different derivatives of the synthesized oxazaborolidines and their effect on bacterial adhesion was observed. In general, compounds that contained B-butyl group (BNO3, BNO5, BNO6, and BNOO1) showed a significant anti-adhesion effect of 21–73 %, at their maximum tested concentration (Fig. 14). Replacing the butyl by phenyl group (BNO1, BNO2, BNO4, BNOO2) created an adverse effect of increased adhesion of 18–62 %.

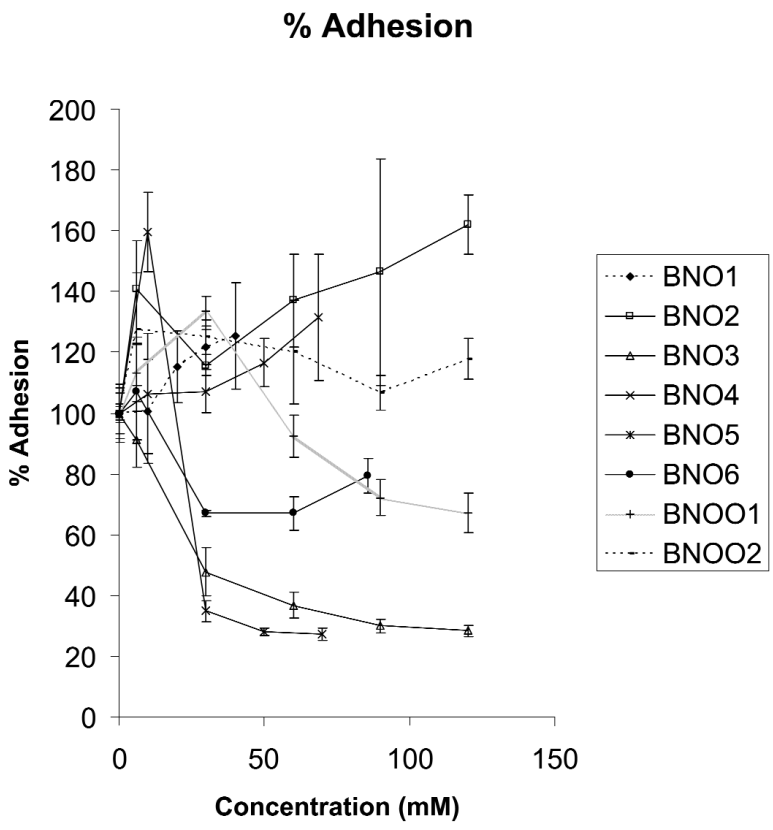

Fig. 14 Effect of oxazaborolidine derivatives on bacterial adhesion above MIC concentrations.

Effect of oxazaborolidines on biofilm formation

We have chosen BNO2 (a phenyl derivative of oxazaborolidine) and BNO3 (butyl derivative) as representatives of the oxazaborolidine series, for further examination of their influence on the biofilm environment. Using a confocal laser scanning microscopy (CLSM) technique, we have demonstrated that biofilm formed in the presence of BNO2 was thicker than that of the biofilm formed in the presence of BNO3 (Fig. 15). Antibacterial effect of BNO2 and BNO3 on *S. mutans* assembled in biofilm indicated that at 0.2 mM, BNO2 maintained the same amount (percentage) of live bacteria similar to the control, while a partial antibacterial effect was observed at 0.9, 9, and 90 mM. BNO3 reduced bacterial survival in biofilm to 40 % at 0.2 mM, but at 90 mM no biofilm growth was detected (Fig. 16).

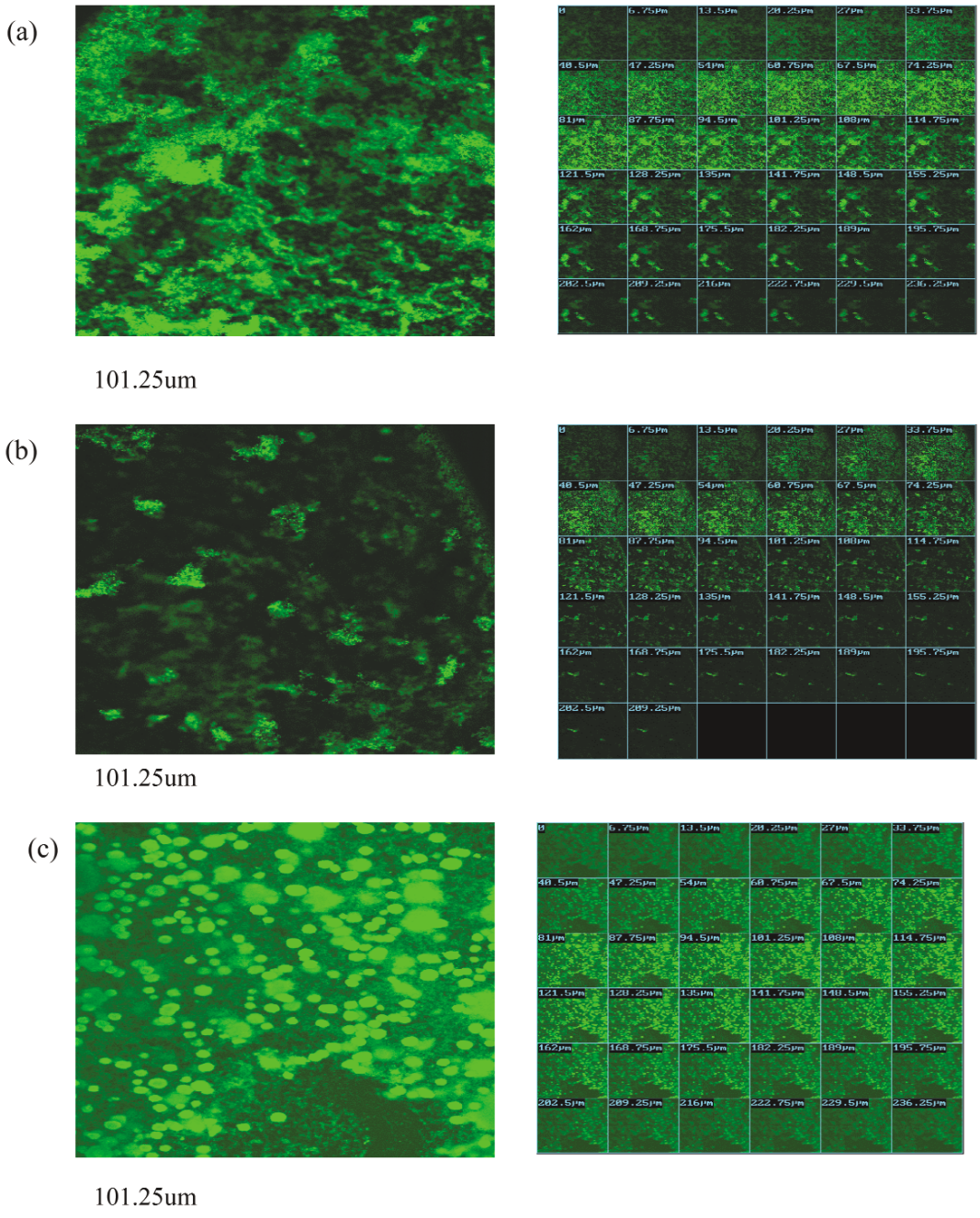

Fig. 15 CLSM image gallery of biofilm which was grown with the presence of: (a) 9 mM BNO2, (b) 9 mM BNO3, and (c) control (no oxazaborolidines were added).

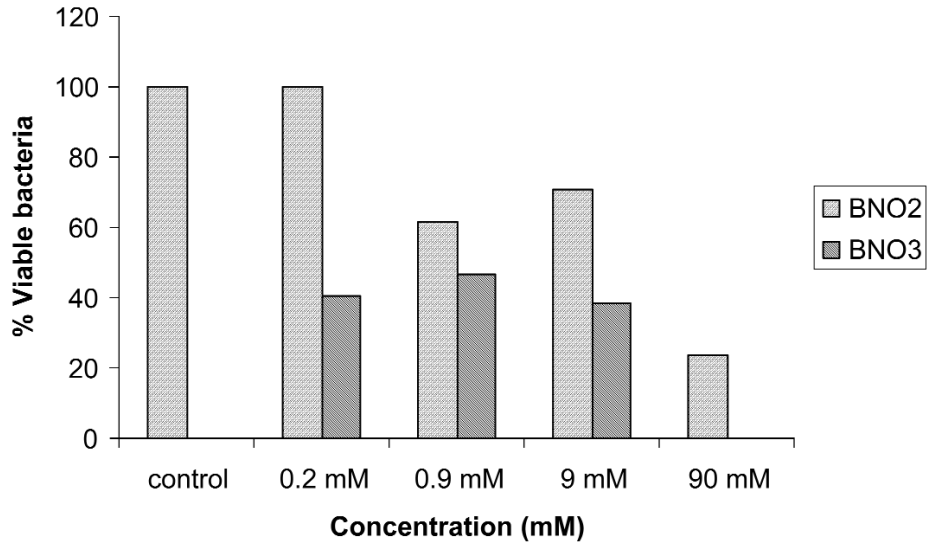

Fig. 16 Ratio of alive/dead bacteria exposed to different concentrations of BNO2 and BNO3.

Conclusions

The eradication of cariogenic bacteria in the oral cavity is a long-lasting goal that has not been successfully accomplished. Thus, applying a novel class of antibiofilm agents opens a potential avenue for combating biofilm-related diseases.

Oxazaborolidines showed antibacterial activity and were capable of affecting biofilm formation. The B-butyl-containing derivatives significantly decreased bacterial adhesion and affected bacterial viability in biofilms. Thus, oxazaborolidines have the ability in vitro to act as novel agents in affecting biofilm formation, and represent additional avenues in preventing biofilm-associated diseases. Since the use of oxazaborolidines as described here is novel, it may serve as a new scientific tool in further elucidation of the mechanism biofilm formation.

BORONIC ACIDS AND TRIFLUOROBORATES: PROTEASE INHIBITION

Introduction

Boronic acids have been shown to form reversibly strong covalent bonds with the diol functionalities of carbohydrates in form of cyclic esters [94–96] with association constants that range from 10 to 10^4 M^{-1} [97]. Most monosaccharides form 1:2 saccharide–boronic acid complexes [98]. Although these sp^2-hybridized species are neutral and favorable to solvent extraction, they are relatively unstable and easily hydrolyzed in H_2O [98]. Several boronic acid derivatives which can selectively bind saccharides have been prepared [99–102]. The interaction of the different boronates with the saccharides is specific depending on the kind of boronic acid and the structure of the saccharide [103–109]. As part of our program to develop drug carrier platforms based on saccharide–boron complexes, the synthesis and the structure of simple derivatives were studied at first.

The two attributes of boronic acids, namely, facile ring formation with suitable functional groups of polar molecules, and relatively small association constants, would seem to make boron–polysaccharide complexes good candidates for the delivery of peptides or peptidomimetics. The colon has been discussed as a potential site of peptidic drug delivery due to apparently lower levels of proteases in this region [110]. While the development of colonic delivery systems for peptides and proteins was our long-term goal, in the short term, we were interested in studying the inhibitory activity of some boronic

acids on various serine proteases found in the GI, such as trypsin, chymotrypsin, elastase, and leucine aminopeptidase, and the effect of added saccharides on the inhibition ability of these boronic acids. Boronic acids are exceptionally potent inhibitors of serine proteases. This characteristic is widely believed to derive from the boronyl group's ability to mimic the transition state of the enzyme-catalyzed reaction [111]. Suenaga et al. [112] proved that the inhibitory effect of phenylboronic acid on chymotrypsin was intensified by added diols but weakened by tripodal additives.

Inhibitors of serine proteases naturally divide into covalent and noncovalent. The former bind covalently to the active site of the enzyme, typically to the nucleophilic serine hydroxyl of the catalytic triad. The resulting tetrahedral intermediate resembles the transition state of a normal substrate hydrolysis. Such inhibitors usually contain electrophiles such as aldehydes, trifluoromethyl ketones, pentafluoroethyl ketones, α-ketoamides, α-ketoesters, α-diketones, α-ketoamides α-ketoaheterocycles, organoboronic acids, and organophosphonate esters. All these inhibitors interact with the protease covalently and reversibly [113–116]. Irreversible covalent inhibitors include a variety of acylating and alkylating agents such as β-lactams and mono-halomethyl ketones [113,115–117]. It is very important that covalent inhibitors such as these are highly selective since nonselectivity may result in toxic side effects [114,117]. For this reason, noncovalent inhibitors are often preferred over covalent inhibitors, and they are commonly studied as potential protease inhibitors [118]. These may be more selective and chemically and metabolically less reactive than covalent inhibitors. The binding of noncovalent inhibitors relies on hydrogen bonds, electrostatic interactions, hydrophobic interactions and/or van der Waals interactions, etc. Thrombin, factor Xa (fXa), and trypsin are the only serine proteases for which small, potent, selective noncovalent inhibitors have been developed, which are ultimately intended as drug development candidates [119–122]. These inhibitors are structural mimics of peptide substrates, maintaining a hydrogen bond array to the enzyme as well as complementing hydrophobic surfaces in the active site.

Several studies have shown that the incorporation of fluorine atoms in the serine protease inhibitors enhances the activity of these inhibitors as a result of the formation of hydrogen bonding between the fluorine atoms and the serine protease [123]. On the other hand, difluoroborane analogs of several 1-acylaminoboronic acids are found to be stable and more easily purified than the acids. Since they hydrolyze readily in aqueous buffer, they are convenient precursor forms for the boronic acid amino acid analog-type transition-state inhibitors [124].

In the third study, we examined the trifluoro(organo)borate salts as inhibitors for trypsin and α-chymotrypsin. Their potential activity could be advantageous since these salts are much more stable than the acids, and are much easier to handle and purify. The molecules that were used were simple compounds and hence their activity was moderate, but in order to observe slow, tight binding characteristics, interactions between inhibitor and enzyme secondary subsites are necessary.

Boronated-saccharide derivatives

Combined ^{1}H, ^{13}C, and ^{11}B NMR and mass spectral assignments of boronate complexes of D-(+)-glucose, D-(+)-mannose, methyl-α-D-glucopyranoside, methyl-β-D-galactopyranoside, and methyl-α-D-mannopyranoside [125]

The complex formation between *N*-butylboronic acid and D-(+)-glucose, D-(+)-mannose, methyl-α-D-glucopyranoside, methyl-β-D-galactopyranoside, and methyl α-D-mannopyranoside under neutral conditions was investigated by ^{1}H, ^{13}C, and ^{11}B NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS). Five derivatives were prepared (Fig. 17). The ^{11}B NMR spectrum of **10** shows two peaks at 30.0 and 36.0 ppm, indicating the formation of six- and five-membered rings, respectively. The *J*(H,H) coupling constants indicate that **10** is in the α-furanose. **10** has a slightly large value for *J*(1,2) (4.1 Hz), which indicates a slightly greater flattening of the furanose ring by the boronate, and this may be due to the trigonal planar nature of the boron that might cause a flatter ring [126]. Furthermore, the

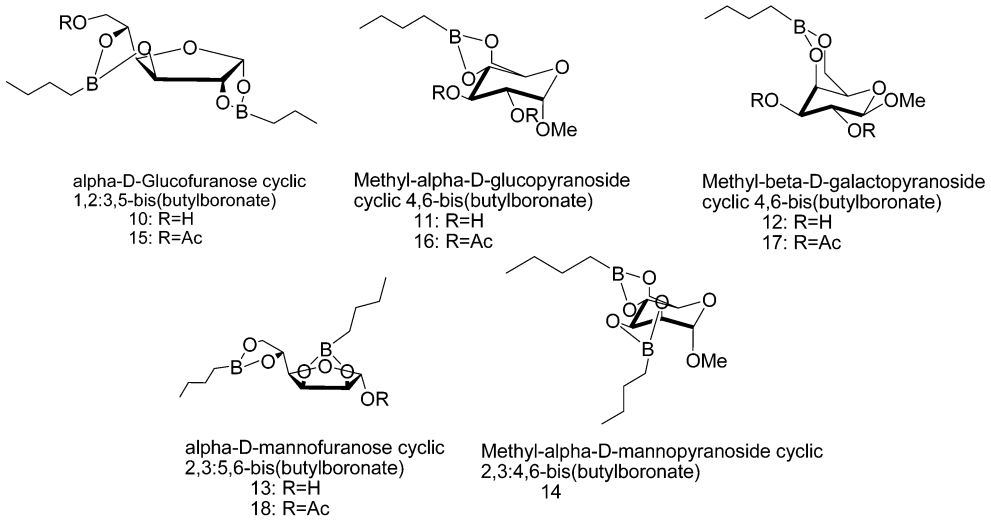

Fig. 17

^1J(C1,H1) coupling constant is unexpectedly high (186 Hz), which gives further evidence of an α-furanose ring complexed in the 1,2-position for glucose as shown also for the 1,2-*O*-isopropylidine derivatives of α-furanoses that give an extremely high ^1J(C1,H1) value of 186 Hz [106]. Therefore, the similarities in the coupling constants suggest an identical conformation for **10** and that it is an envelope ^{3}E [127]. In addition, the acetylated product **15** was investigated by NMR and GC–MS and further confirms that the structure of **10** is α-D-glucofuranose cyclic 1,2:3,5-bis(butylboronate). For the boronic acid complexes of the methyl-α-D-glucopyranoside **11** and the methyl-β-galactopyranoside **12**, ^{1}H, ^{13}C, and ^{11}B NMR data show that one boronic acid is bound in the 4,6-position, forming a six-membered ring product with the sugar rings remaining in the α-pyranose form. Therefore, the monodentate complexes, **11** and **12**, are assigned the structures methyl-α-D-glucopyranoside 4,6-butylboronate and methyl-β-D-galactopyranoside 4,6- butylboronate, respectively. For the D-(+)-mannose complex **13**, ^{11}B NMR gave a single peak at 35.12 ppm, indicating the formation of a five-membered ring only. The data indicate the formation of a 2,3:5,6-ring system with two five-membered rings. The small *J*(H,H) values for *J*(2,3) and *J*(4,5) (4.2–4.4 ppm) indicated the presence of the furanose form of the sugar, thus giving the structure of D-mannofuranose cyclic 2,3:5,6-bis(butylboronate) (**13**). For the methylmannose **14**, the data show that it reacts readily to form the 2,3;4,6-diester with the ring remaining in the pyranose form. This assignment is correlated by the connectivities in the correlation spectroscopy (COSY) and the HSQC assignments. Therefore, the structure of **14** is methyl-α-D-mannopyranoside 2,3:4,6-bis(butylboronate).

Whereas **10**, **13**, and **14** are the only products obtained with 1 equiv of butylboronic acid, **11** and **12** are the only products formed with 2 equiv of butylboronic acid. This must be due to the locked-in *trans*-relationship of the two free hydroxyl groups in **11** and **12**, preventing the formation of an additional ring. On the other hand, all the hydroxyl groups in **10**, **13**, and **14** are *cis*, allowing facile formation of an additional five-membered ring.

Additional observable peaks for excess nonreacted sugars were observed only in the ^{1}H and ^{13}C NMR spectra of mannose and its methyl derivative. In addition, although the NMR parameters are identical for the 1:1 and 1:2 products with the methyl-galactopyranoside **12**, the reaction does not go to completion with a 1:1 ratio, i.e., only 50 % of the sugar reacts. This is in accordance with the results of James et al. [98], indicating that the association constants of boronic acids to monosaccharides decrease in the order D-glucose >> D-galactose > D-mannose.

The methyl derivatives of the sugars were unstable under aqueous conditions, and this was markedly seen for the α-methylglucopyranoside product **11**. The nonbonded interactions between the axial methoxyl group and H-3 and H-5 are held to be responsible for destabilizing [128] the α-complex and also occur in the methyl-α-D-glucopyranoside 4,6-butylboronate **11**, and so it was expected that this ester would show greater susceptibility to hydrolysis than the methyl-β-D-galactopyranoside complex **12** and the methyl-α-D-manno-pyranoside complex **14**.

It is well known that *trans*-six-membered rings are less stable than five-membered rings, but the equilibrium is largely determined by the geometric arrangement of the diols [129,130]. With methyl-β-D-galactopyranose **12**, the 4,6-six-membered ring is a *cis*- rather than a *trans*-arrangement, and it is known from decalin that the *cis*-fused six-membered ring system is conformationally flexible, whereas the *trans*-system is fixed. In addition, the presence of a methyl group at the anomeric positions and the hydroxyls at positions 2 and 3 being *trans* leaves the 4,6-positions for the attachment with boronic acid. Also, with methyl-α-D-mannopyranose **14**, a *trans*-six-membered ring is formed because of the presence of a methyl group at the anomeric position that causes the first butaneboronate to attach itself to the *cis*-2,3 position and the second to form the *trans*-4,6-six-membered ring.

Boronic acids, sugars, and protease inhibition

Inhibition of pancreatic elastase, trypsin, chymotrypsin, and leucine aminopeptidase by boronic acids [131]

Phenylboronic acid, 3-aminophenylboronic acid; 4-fluorophenylboronic acid, CbzAla-(boro)Gly$(OH)_2$, and CbzPhe(boro)Gly$(OH)_2$ (Fig. 18) were all tested for their ability to inhibit trypsin, chymotrypsin, elastase, and leucine aminopeptidase. The progress of the reaction was followed by monitoring the appearance of the absorption band at 410 nm (*p*-nitroaniline).

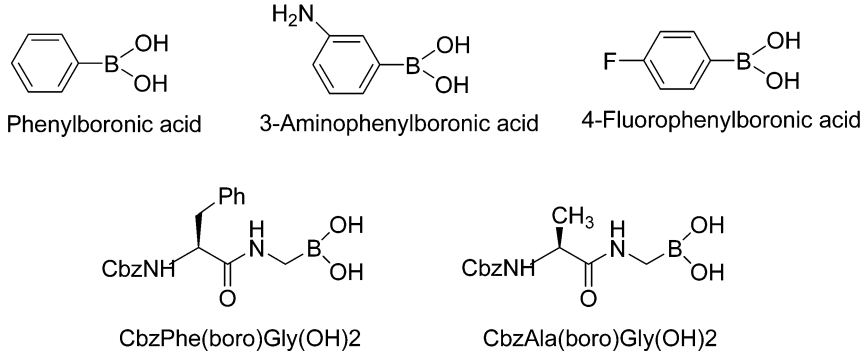

Fig. 18

The values for the dissociation constants, K_i (Table 4), and the Lineweaver–Burk plots for a representative grouping of inhibitors with each enzyme were determined.

Table 4 Inhibition of α-chymotrypsin, trypsin, elastase, and leucine aminopeptidase by boronic acids **1**–**5**.

Boronic acid	Chymotrypsin, K_i (mM)	Trypsin, K_i (mM)	Elastase, K_i (mM)	Aminopeptidase, K_i (mM)
Phenylboronic acid	9.70	>10.0	1.83	>10.0
3-Aminophenylboronic acid	>10.0	>10.0	3.61	>10.0
4-Fluorophenylboronic acid	31.01	>10.0	1.02	>10.0
*Cbz*Ala(boro)Gly$(OH)_2$	1.08	>10.0	0.27	0.35
*Cbz*Phe(boro)Gly$(OH)_2$	1.96	>10.0	0.60	1.02

Researchers [133,134] have demonstrated that the binding mode used by a particular boronic acid inhibitor appears to depend on how well the inhibitor matches the structure of the physiological or natural substrate of the serine protease. In cases in which a boronic acid is a good substrate mimic, formation of a tetrahedral complex with the active site serine is favored. On the other hand, when boronic acids have structures that are not well related to that of the substrate, they tend to coordinate with histidine and serine. Serine proteases are classically categorized by their substrate specificity, notably by the residue at P1 binding being trypsin-like (lys/Arg preferred at P1), chymotrypsin-like (large aromatic hydrophobic residues such as Phe/Tyr/Leu at P1), or elastase-like (small hydrophobic residues such as Ala/Val at P1).

In this context, the boronic acids with aromatic groups were able to inhibit chymotrypsin to different extents depending on the substituents incorporated (Table 4). The peptidyl boronic acids CbzPhe(boro)Gly$(OH)_2$ and CbzAla(boro)Gly$(OH)_2$ were expected [135] to act as cysteine protease inhibitors, but they were proved ineffective up to a concentration of 10 mM. Despite the fact that inhibitors of serine proteases very often inhibit cysteine proteases [136], these two compounds were good inhibitors of chymotrypsin, elastase, and leucine aminopeptidase. They contain amino acids favored by chymotrypsin and elastase (Phe and Ala), thus, inhibited these enzymes in a competitive reversible manner. Alanine is a natural substrate for leucine aminopeptidase [137], therefore, CbzAla(boro)Gly$(OH)_2$ inhibited this enzyme competitively and reversibly. On the other hand, CbzPhe(boro)Gly$(OH)_2$ inhibited the enzyme noncompetitively and irreversibly.

Sugars can increase/decrease the inhibitory effect of boronic acids on the hydrolytic activity of α-chymotrypsin

The hydrolytic activity was affected to different extents by the addition of mono-, di-, and polysaccharides to the boronic-acid-inhibited system. The saccharide-only (no inhibitor) additives had no effect on the activity of the enzyme, therefore, the saccharide effects on the inhibition were due to interactions with boronic acid and not due to direct effect with the enzyme. On the other hand, boronic acids form cyclic esters with saccharides and the reaction occurs reversibly and rapidly at ambient temperature, therefore, the complexation with the boronic acid and binding to the enzyme as the complex must be the reason for increasing or decreasing the apparent inhibition.

In agreement with the data published by Suenaga et al. [112], the addition of D-(+)-glucose, D-(+)-mannose, and D-galactose to phenylboronic acid further suppresses the rate of the hydrolytic reaction with the order: D-(+)-glucose > D-(+)-mannose > D-(+)-galactose. However, methylmono-, di-, and trisaccharides weakened the inhibition ability of phenylboronic acid. It is known that for α-D-glucose mono and bisboronates, the sugar ring has a furanose structure with a conformation between $T^3{}_2$ and E^3. However, methyl-α-D-glucopyranoside reacts with phenylboronic acid to give a crystalline 4,6-cyclic ester which, in turn, forms a 2,3-(diphenylpyroboronate) while phenylboronic acid condenses smoothly with methyl-α-D-mannopyranoside, to give the 2,3:4,6-diester. This difference in the structure of the final product may have the effect of either increasing or decreasing the inhibitory activity.

For CbzPhe(boro)Gly$(OH)_2$ and CbzAla(boro)Gly$(OH)_2$, the monosaccharides weakened the inhibition of chymotrypsin. Lactulose, on the other hand, removed the inhibition of CbzAla(boro)Gly$(OH)_2$ completely. The association constant of the boronate with the lactulose could not be detected by NMR due to instantaneous formation of a complex. This complex in turn was able to bind to the enzyme as a complex, thereby decreasing the apparent inhibition.

Effect of a polysaccharide on the inhibition of α-chymotrypsin, trypsin, elastase, and leucine aminopeptidase by the five boronic acids

Since our interest was with the effect of polysaccharides on the inhibitory activity of boronic acids, the effect of arabinogalactan on the inhibitory properties of all boronic acid with the four enzymes was examined (Fig. 19). Arabinogalactan did not affect the activity of the enzymes directly. However, it decreased the inhibitory activity of both CbzAla(boro)Gly$(OH)_2$ and CbzPhe(boro)Gly$(OH)_2$ with chymotrypsin, elastase, and leucine amino peptidase. The most significant effect was with leucine aminopeptidase, where the inhibition activity was decreased by 50.60 and 40.25 %, respectively. Therefore, it seems that the boronic acids form complexes with arabinogalactan and binds to the enzyme as the complex, and this probably decreased the apparent inhibition.

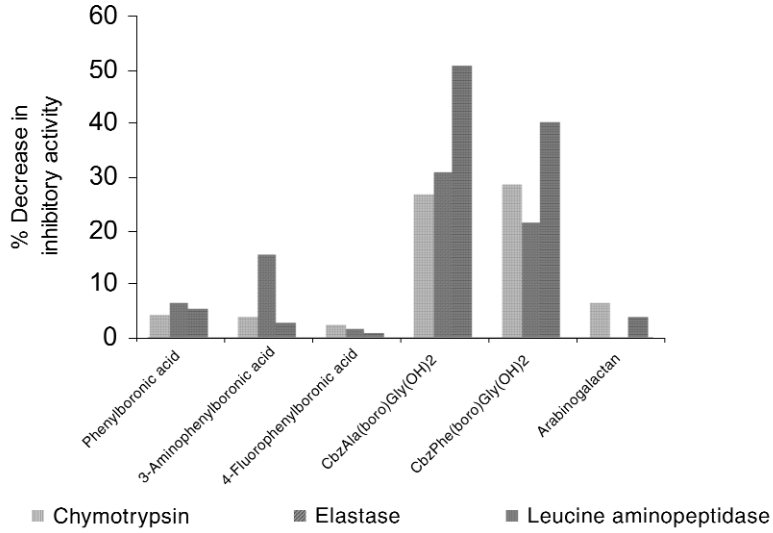

Fig. 19 The decrease in inhibitory activity of boronic acids with chymotrypsin, elastase, and leucine aminopeptidase as a result of the addition of arabinogalactan.

These findings support the view that the enzyme active site recognizes the molecular structure of the boronic acid-saccharide complexes, and that not all saccharides act as co-inhibitors in the boronic acid inhibition system.

The results in this study shows that both CbzAla(boro)Gly$(OH)_2$ and CbzPhe(boro)Gly$(OH)_2$ are good inhibitors for chymotrypsin, elastase, and leucine aminopeptidase. For chymotrypsin, this inhibitory activity can be either unaffected or decreased by the addition of mono-, di-, and a trisaccharide. However, arabinogalactan decreased the inhibitory activity of the two inhibitors CbzAla(boro)Gly$(OH)_2$ and CbzPhA(boro)Gly$(OH)_2$ with all the enzymes. This finding is important since it means that the activity of the inhibitor will be reduced by possible interactions with the polysaccharide.

Trifluoroborates [138]

Synthesis and stability

Organotrifluoroborates are a new class of air-stable boronic acid derivatives. They can be easily prepared [139] (Fig. 20), easy to handle, do not show any sensitivity toward oxygen or moisture, and are thus far more stable than their corresponding organoboronic acid or ester [140]. Unlike trivalent boron substituents, trifluoroborate is an electron-donating substituent [141]. Until now, the only utility of potassium organotrifluoroborates has been in the field of organic synthesis [142,143].

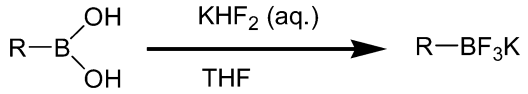

Fig. 20 Formation of potassium organotrifluoroborates.

Since the enzymatic study was done in aqueous environment, it was necessary to determine the stability of the salts (Fig. 21) in this medium. By ^{1}H, ^{11}B, and ^{19}F NMR analysis, the salts in D_2O did not hydrolyze after incubation for 30 min, but after 24 h, partial hydrolysis took place ranging from 10 to 30 %. On dissolving the salts in Tris buffer (in D_2O, pH = 7.00), ^{1}H and ^{11}B NMR indicates the presence of the salts only except for the butyl salt that had about 50 % hydrolysis.

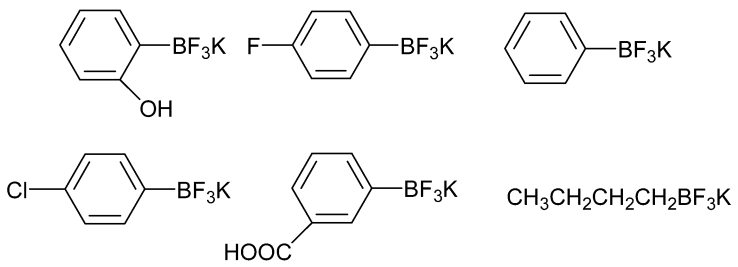

Fig. 21 Potassium organotrifluoroborates.

Enzyme-inhibition studies

For this study, α-chymotrypsin and trypsin were selected as representative serine proteases from bovine pancreas. The inhibitory activity of the trifluoro(organo)borates was evaluated by determining the dissociation constant (K_i) using Dixon plots [132]. These plots show that the inhibition is competitive for all the compounds. The values of the inhibition constants (K_i) are listed in Table 5 for both α-chymotrypsin and trypsin, respectively. As can be seen, all the trifluoroborate salts are much more potent than the corresponding acids. For trypsin, the difference in activity between the trifluoroborate salts and the boronic acids ranges from 12–32-fold increased inhibitory activity, while for chymotrypsin, the increase is 2–47-fold increase. Both potassium fluoride and potassium tetrafluoroborate are used as controls. They show no activity/very weak activity (Table 5).

Table 5 Competitive inhibition of α-chymotrypsin and trypsin by trifluoroborates.

Compound	K_i(mM) (Chymotryspin)		K_i(mM) (Trypsin)	
	X = $(OH)_2$	X = F_3K	X = $(OH)_2$	X = F_3K
PhBX	9.70	0.20	>10	1.11
4-F-PhBX	>10	1.84	>10	0.29
4-Cl-PhBX	0.65	0.57	>10	1.50
2-OH-PhBX	0.61	0.14	>10	1.06
3-HOOC-PhBX	1.42	0.69	2.87	0.26
$CH_3CH_2CH_2CH_2BX$	>10	2.84	>10	0.62
KF (control)	>10		>10	
KBF_4 (control)	>10		>10	

Mode of binding

Until now, it was accepted that only boronic acids inhibit serine proteases. Trifluoroborates were not ever tested biologically for this purpose. Owing to the high electron density, the trifluoroborate group is capable of participating in hydrogen bonding as an electron donor. This property confers additional ways of interaction with enzyme or receptor subsites for trifluoropotassium borates which cannot be found for the fluorine-free compound. The different proteolytic stabilities of the compounds seem to result from a specific interaction between the substrate and the enzyme induced by the high electron density of the BF_3K group.

^{19}F NMR studies on enzyme-inhibitor complex

Fluorine NMR is useful for investigating enzyme-inhibitor interactions because of high sensitivity and lack of background interference from protein [144,145]. A change in the ^{19}F NMR shifts is observed upon changes in ionic character, bond order, and degree of hybridization of the bond involving the fluorine atom. This high sensitivity of the fluorine chemical shift to local environment can be used to probe details of a receptor-ligand complex. Two new broad peaks emerged in the ^{19}F NMR spectra upon addition of α-chymotrypsin/trypsin to the trifluoro-4-fluorophenylboronate. These peaks were downfield-shifted from the original peaks for the $–BF_3K$ and the aromatic fluorine. This was indicative of hydrogen-bond formation between the fluorine atoms and the N–H and O–H groups in the active site of serine protease and other noncovalent enzyme-bound aromatic fluorine with the hydrophobic pocket. The amide N–H groups that constitute the oxyanion hole are believed to stabilize the tetrahedral substrate oxyanion formed during the catalysis (Fig. 22). The increased line widths for the signals of the enzyme-bound species are consistent with other ^{19}F NMR studies on chymotrypsin-bound inhibitors and are attributed to the slower tumbling of the macromolecular complex [146,147].

Saika and Slichter [148] have developed a quantitative theory for the ^{19}F chemical shifts, and these shifts have been related [149] to changes in ionic character, bond order, and degree of hybridization of the bond involving the fluorine atom. In general, an increase in electronegativity of the atom attached to the fluorine decreases the ionic character of the fluorine, which thereby increases the paramagnetic contribution to shielding, i.e., causes a downfield shift.

The downfield shift of the ^{19}F signal of an enzyme-bound species is consistent with other studies. The ^{19}F signals of various *p*-fluorophenylalanine inhibitors are shifted 1–2 ppm downfield upon binding to chymotrypsin [150,151]. Current ^{19}F chemical shift theory attributes these downfield shifts to van der Waals interactions with protein protons and thus to binding of the *p*-fluorophenyl ring into the aromatic ring pocket. Gorenstein and Shah [152] observed ~4 ppm downfield shift for *N*-acetyl-DL-*p*-fluorophenylalinal, and this is one of the largest protein-induced shifts observed for a reversibly bound inhibitor to chymotrypsin.

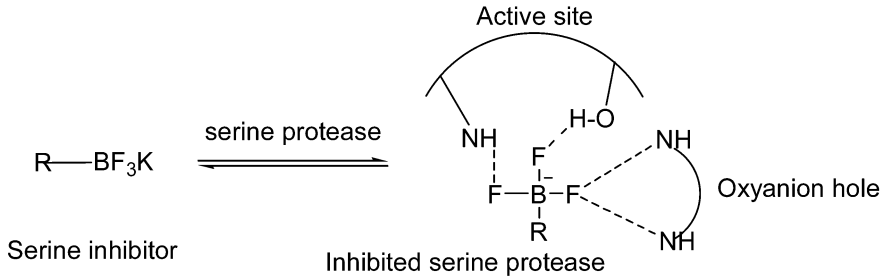

Fig. 22 A trifluoroborate inhibitor forming hydrogen bondings with a serine protease which is subsequently inhibited from further activity.

In this study, the organotrifluoroborates, highly stable toward air and moisture, were found to be simple noncovalent, competitive reversible inhibitors of the serine protease, α-chymotrypsin, and trypsin. The potency and/or selectivity can be improved by taking advantage of binding interactions at the S subsites of the protease.

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