Synthesis and Evaluation of Oxazaborolidines for Antibacterial Activity against *Streptococcus mutans*

Adel Jabbour,[†] Doron Steinberg,[‡] Valery M. Dembitsky,[†] Arieh Moussaieff,[†] Batia Zaks,[‡] and Morris Srebnik*,[†]

Department of Medicinal Chemistry and Natural Products, School of Pharmacy, and Faculty of Dentistry, Institute of Dental Sciences, Hebrew University, Jerusalem 91120, Israel

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Abstract: Several representative oxazaborolidines have been synthesized and evaluated against *S. mutans* for antibacterial activity. This is the first reported antibacterial activity of this class of compounds. The minimal inhibitory concentration values ranged from 0.53 to 6.75 mM.

Although boron is an essential element for higher plants[1] and the biological role of boron has been the subject of a number of biological studies,[2] 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 boron neutron capture therapy (BNCT).[3] Very recently an α-amido boronic acid, Velcade, a proteosome inhibitor, has been approved as an antineoplastic agent.[4] Nevertheless, taking into account the vast possibilities of structures incorporating boron, the use of boron-containing compounds in medicine has barely been scratched.[5]

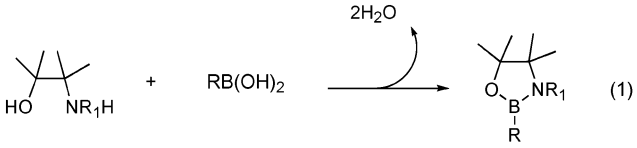
Compounds containing B−N bonds have been shown to possess biological activity. Carboxyboranes have shown anticancer, hypolipidemic, and antifungal activity.[6] Diazaborines have been shown to be active against malaria.[7] Oxazaborolidines also possess a B−N bond and are readily obtained from an amino alcohol and a boronic acid. On the basis of the biological activity displayed by other boron systems with B−N bonds, oxazaborolidines should be interesting candidates for biological screening. Nevertheless, despite their ubiquity in organic synthesis,[8] the biological activity of oxazaborolidines has to date never been reported. This omission prompted us to undertake an evaluation of this group of compounds.

Naturally occurring borate complexes are used as topical antibiotics.[9] Recently, a borate complex, autoinducer 2 (AI-2), has been identified as a universal signaling molecule in bacteria.[10] We reasoned that oxazaborolidines resemble AI-2 in ring size and the ability to form hydrogen bonds and thus may possess some biological activity.

Tooth decay (caries) is a worldwide oral disease affecting all ages, ethnic groups, and both sexes. Caries are characterized as localized pathological disease because of the presence of cariogenic bacteria.[11] These bacteria accumulate on the surface of the tooth (dental plaque biofilm) and initiate demineralization of tooth enamel, resulting in cavitation. Mutans streptococci such as *Streptococcus mutans* (*S. mutans)* are among the cariogenic bacteria highly associated with the prevalence and pathogenicity of tooth decay.[12] 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.[13] Irrespective of the ongoing debate as to which is the drug of choice for eliminating cariogenic bacteria, new drugs are still being sought.

The purpose of this investigation was to synthesize and assess the antimicrobial activity of several derivatives of oxazaborolidines against *S. mutans* as a step in studying potential antibacterial activity of these chemical compounds. Our initial results have been more than satisfying and are the subject of this communication.

The oxazaborolidines were synthesized by the reaction of an amino alcohol, using (−)-ephedrine and (−)-norpseudoephedrine or a diol amine and a boronic acid, with the azeotropic removal of water **1**−**6**:



(1)

After removal of toluene, **1**−**3** and **5** were isolated by distillation in high yields (Figure 1). Compounds **4** and **6** were more difficult to purify. However, **4** could be obtained in high purity by recrystallization. Attempted recrystallization of **6** gave product accompanied by some starting materials (<5% by NMR). Compounds **7** and **8**[14] were isolated by filtration and purified by crystallization in lower yields (Figure 1). For **1** and **4**−**6** the (−)-norpseudoephedrine derivatives were used.

The minimal inhibitory concentrations (MIC)[15] for antibacterial activity of the tested oxazaborolidines was

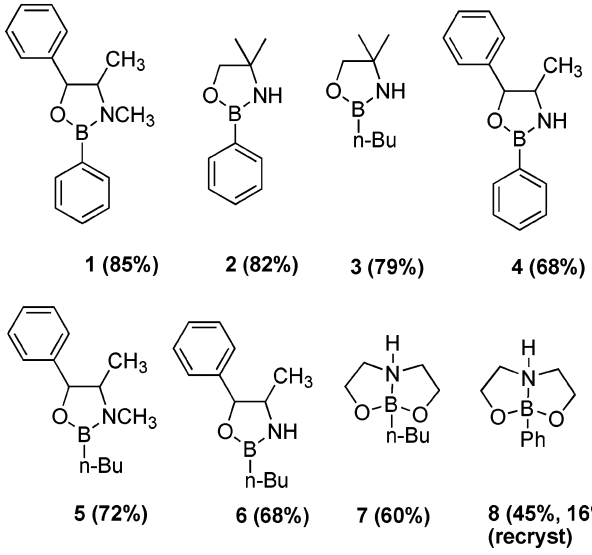


Figure 1. Structures of oxazaborolidines derivatives (isolated yields).

* To whom correspondence should be addressed. Phone: 972 2 675 7301. Fax: 972 2 675 8201. E-mail: msrebni@md.huji.ac.il.
[†] School of Pharmacy.
[‡] Institute of Dental Sciences.

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Table 1. Minimal Inhibitory Concentration (MIC) of Oxazaborolidine Derivatives (**1**−**8**) against *S. mutans*

oxazaborolidine derivatives	MIC (mM)
1	1.55
2	6.00
3	3.38
4	1.33
5	0.53
6	2.83
7	6.75
8	6.75

determined as follows: Sterile microculture dishes containing multiple wells were used to determine the MIC of eight derivatives of oxazaborolidines. Each well contained 120 μL of brain−heart infusion medium, 15 μL of an overnight suspension of *S. mutans* ATCC 27351 adjusted to 1 OD_{540nm}, and 15 μL of the tested compound. Each compound was tested at five different concentrations between 0 and 50 mM. Each experiment was repeated twice. These cultures were incubated at 37 °C in an atmosphere enriched in 5% CO_2 for 24 h. Bacteria growth was determined as the turbidity of the overnight growth and by a computerized ELISA reader ($Thermo_{max}$ microplate reader, Molecular Devices) at 650 nm. Control cultures of bacteria with no oxazaborolidines added and media broth with the oxazaborolidines added but with no bacteria were conducted. MIC was determined as the lowest concentration of the tested agent in which bacteria did not grow.

MIC values were used to determine the antibacterial efficacy of oxazaborolidines **1**−**8** 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. The most active compound in the series is **5,** which contains both an *N*-Me group and *B*-Bu group. Consequently, **1**−**4** and **6**, which do not contain either an *N*-Me or a *B*-Bu group, are less active. Compounds **7** and **8**, which are formally charged, showed the weakest activity. However, the formal charge in itself may only be one of several unknown factors responsible for decreased activity.

While boronic acids demonstrate no classic MIC at their maximal solubility in water (10 mM), all the tested oxzaborolidines demonstrated antibacterial activity at much lower concentrations (Table 1).

The eradication of cariogenic bacteria in the oral cavity is a long-term goal that has not been successfully accomplished. Thus, applying a novel class of antibacterial agents opens another potential avenue for combating this disease that affects most of mankind.

We have described a novel use of readily available and stable oxazaborolidines. These initial results are very encouraging. Structure−activity relationship studies of oxazaborolidines against *S. mutans* are continuing.

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Supporting Information Available: Synthesis and characterization by ^1H NMR, ^{13}C NMR, and MS for **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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