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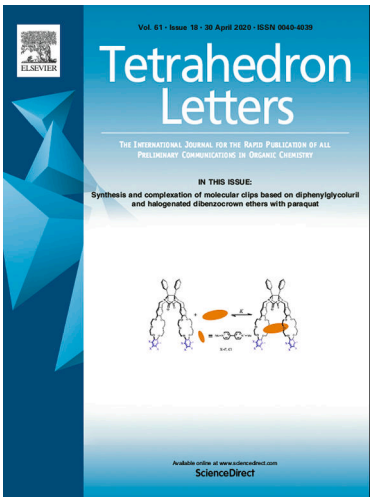

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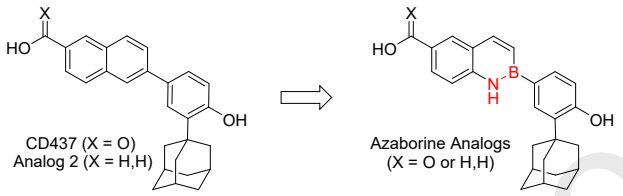

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Synthesis and biological evaluation of an antibacterial azaborine retinoid isostere[#]

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[#]*Dedicated to Prof. Dale Boger for his scholarship in the field of medicinal chemistry and continued support for the next generation of scientists*

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ABSTRACT

Our continued synthetic interest in this class of retinoids, CD437 and its analogs, against methicillin-resistant *Staphylococcus aureus* (MRSA) has brought us to explore further isosteric substitutions within the scaffold. Although our previous findings have shown promising activity against gram-positive pathogens, their therapeutic viability remained an issue. Specifically, through preliminary analysis, our best performing compound, analog 2, displayed low solubility within serum as well as high affinity for retinoid binding proteins with a concentration dependent relationship. To circumvent this issue, we proposed a class of analogs containing an azaborine substitution in place of the naphthalene moiety. Azaborines have a nitrogen-boron bond substituting a carbon-carbon double bond that alters the electronics of the parent scaffold. This motif has been explored successfully in cancer research but to the best of our knowledge has yet to be applied to antibiotics. Herein, we describe the synthesis of the desired analogs, antimicrobial activity, and surprising physiochemical properties.

Introduction

According to the Centers for Disease Control and Prevention, more than 2.8 million antibiotic resistant infections occur annually in the United States alone.[1] One of the most prevalent antibiotic resistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA), which is also known to develop persistent populations. Persister cells are dormant bacterial cells that are genetically identical, but phenotypically different than the wild type.[2] These cells have slowed growth rates, diminished metabolism, and are found in many *S. aureus* populations.[3] As most antibiotics target metabolic and growth processes, persister cells are often difficult to eradicate.[4]

Previously, our laboratory, in collaboration with the Mylonakis laboratory at Brown University, disclosed the potent activity of the synthetic retinoid CD437 (**1**) at eradicating both wild type and persistent MRSA infections *via* membrane perturbation with a minimum inhibitory concentration (MIC) of 1 μg/mL (Fig. 1).[5] Since this finding in 2018, we have disclosed three generations of analogs to further explore the structure activity relationship of this class of retinoids.[6-7] In our investigations, our lead compound was found to be the primary alcohol derivative we deemed analog 2 (**2**). This compound exhibited an MIC of 2 μg/mL while also reducing the toxicity of the parent compound.

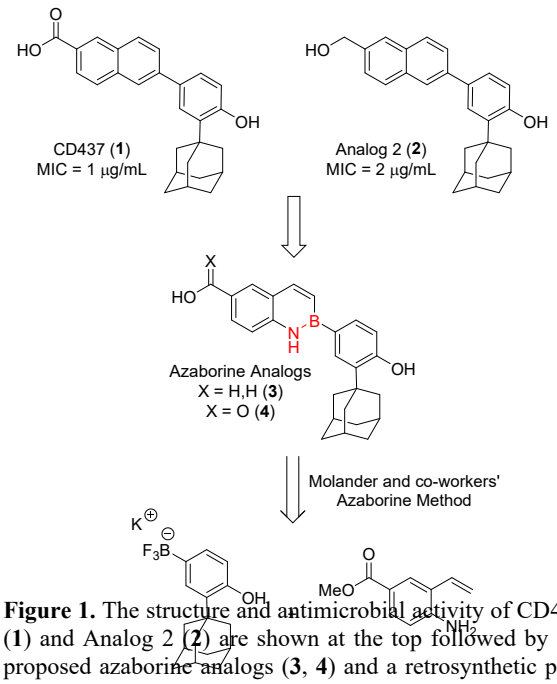
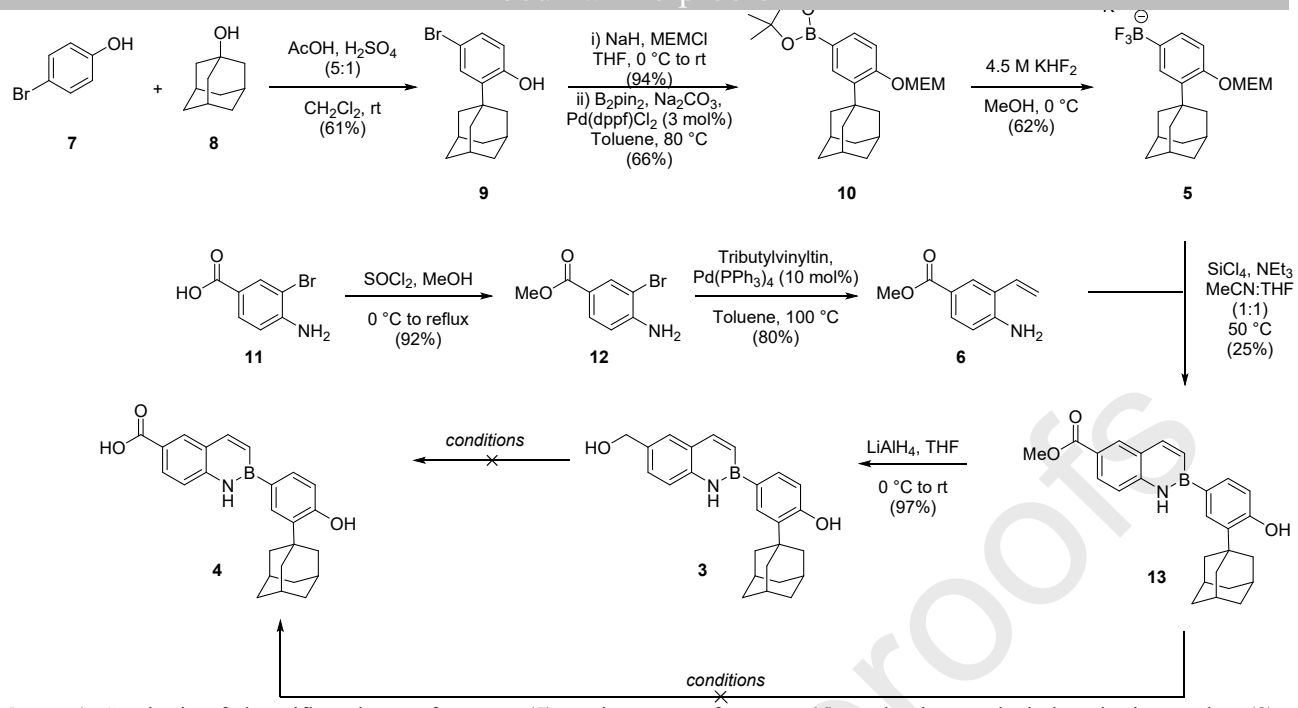


Figure 1. The structure and antimicrobial activity of CD437 (**1**) and Analog 2 (**2**) are shown at the top followed by the proposed azaborine analogs (**3**, **4**) and a retrosynthetic plan invoking the method developed by Molander and co-workers.



Scheme 1. Synthesis of the trifluoroborate fragment (**5**), aminostyrene fragment (**6**), and primary alcohol azaborine analog (**3**). Attempted conditions to achieve the carboxylic acid analog (**4**) are described in the text.

Though this compound showed promise, it displayed high levels of binding to plasma binding proteins, namely retinoid binding proteins (unpublished data), thereby reducing its effectiveness in a therapeutic setting. To combat this issue, our laboratory proposed to investigate an isosteric substitution of the naphthalene scaffold known as an azaborine (**3**, **4**). It is well-known that N-B bonds are isosteric to carbon-carbon double bonds. The N-B bond, although isoelectronic, introduces a local dipole moment with the lone pair of the nitrogen donating into the empty p-orbital of boron.[8-10] This polarity sharply alters the molecular and solid states of the compound, namely widening the HOMO-LUMO gap due to the lowered energy of the HOMO in comparison to parent C=C bonds.

With physical and molecular changes, N-B isosteres have garnered interest within the medicinal community to expand the structural diversity of therapeutic agents. Beginning in the early 1960s, this isostere was mainly explored in diazaborines (B-N-N), which exhibit antibacterial properties against Gram-negative strains.[11-13] Their specific mode of action has been extensively researched and hypothesized to target enoyl reductase; an enzyme involved in fatty acid synthesis.[12] Although diazaborines have been studied in bacterial systems, their sister structure, azaborine, to the best of our knowledge has not. To date, azaborines have been applied in various anti-cancer studies with the focus on T-cell lysozyme targets.[14] Based on this background we proposed to explore this isosteric model within the antibacterial space.

To achieve this target, we employed a process developed by Molander and co-workers at the University of Pennsylvania.[15-16] This method proved to be amenable to our previously disclosed syntheses of the parent compound and allowed us to prepare the azaborine derivative quickly. Herein, we report on the synthesis of the azaborine analog as well as its biological and physiochemical properties in comparison to the parent compounds.

We began by synthesizing the trifluoroborate salt fragment (**5**) utilizing a previously described route for the synthesis of CD437 (Scheme 1).[5-7] Upon achieving the borylated compound, **10** was then subjected to aqueous potassium bifluoride in methanol to afford **5** in 62% yield.[17-18] With the trifluoroborate fragment in hand, we then began synthesis of the aminostyrene fragment (**6**, Scheme 1). Beginning with commercially available 4-amino-3-bromobenzoic acid (**11**), a methyl protection in the presence of thionyl chloride and methyl iodide afforded **12**. This was then subjected to a tin-mediated vinylation to afford **6** in 80% yield.[19]

With both fragments in hand, we then employed the method developed by Molander and co-workers utilizing silicon

Table 1. Antimicrobial activity and toxicity data for analog 2 (**2**) and azaborine analog (**3**).

Compound	Minimum Inhibitory Concentration (μM)						$Lysis_{20}$ (μM)
	MSSA	HA-MRSA	CA-MRSA	*E. faecalis*	*P. aeruginosa*	*E. coli*	
BAC	2	4	4	125	125	32	32
2	2	2	2	>250	>250	>250	125
3	125	64	250	>250	>250	>250	250

All MIC and $Lysis_{20}$ data were acquired through an average of the highest value of three independent trials; all trials were within one dilution.

tetra it proved difficult with our scaffold as the MEM-protecting group was subsequently removed during the reaction progress due to the production of hydrochloric acid *in situ*. We hypothesized that the deprotected free phenol subsequently formed a stable bond with a neighboring boron, eliminating a fluoride anion and thereby limiting the availability of the trifluoroborate in solution. Although triethylamine was added to the reaction to neutralize the hydrochloric acid production, the removal of MEM still occurred regardless of the concentration of triethylamine used. In future use, the addition of a proton sponge would be a potential route to avoid these issues. However, the desired protected azaborine scaffold (**13**) could still be obtained in 25% yield. Although a change in protecting group strategy could potentially circumvent these issues, we sought to pursue a direct path to the desired analog for biological studies.

In achieving the formation of the desired scaffold, we proceeded with reduction and deprotection pathways to afford our planned primary alcohol and carboxylic acid analogs, respectively. Firstly, protected **13** was subjected to lithium aluminum hydride to afford the desired primary alcohol azaborine (**3**) in 97% yield. Upon obtaining **3**, we then turned to deprotection of the methyl ester **13**. Several hydrolysis methods were attempted to achieve the desired carboxylic acid analog (**4**) including base mediated hydrolysis (NaOH, KOH), acid-mediated hydrolysis (HCl, H_2SO_4), as well as milder approaches with pig liver esterase (PLE). However, each method proved unsuccessful. The base mediated hydrolysis showed degradation in which the proposed degradative mechanism involved the hydroxide anion attacking the empty p-orbital of the boron, forming a stable O-B bond and eliminating the nitrogen, thereby breaking apart the scaffold. This degraded product was confirmed by NMR spectroscopy (data not shown). Additionally, the acid mediated hydrolysis and the PLE-mediated hydrolysis reactions yielded only recovered starting material.

Table 2. Experimentally determined P and logP for analog 2 (**2**) and azarborine analog (**3**) as well as quantum mechanical calculated dipole expressed as debye (D).

	2	**3**
P	9.16	1.39
logP	0.93 ± 0.20	0.12 ± 0.19
cP[a]	7.687	6.792
clogP[a]	0.89	0.83
QM Dipole (D)	2.42	1.34

Partition coefficients shown as an average over 3 independent trials. logP data shown with + / – standard deviation. [a] "c" indicates calculated data for the partition coefficient utilizing the ChemDraw3D program.

To avoid degradation of the scaffold, we attempted utilizing oxidation pathways from the primary alcohol **3**. First, we implemented a Swern-Pinnick oxidation sequence that yielded starting material as well as minor degradative products. Additionally, we attempted a TEMPO oxidation that yielded only recovered starting material. After these failed attempts to access did mimic our best in class analog, analog 2 (**2**).

The antimicrobial activity of **3** was analyzed along with **2** against a panel of bacterial strains including three different strains of *Staphylococcus aureus* (SH1000, ATCC 33591, USA 300-0114), *Pseudomonas aeruginosa* (PAO1), *Enterococcus faecalis* (OG1RF), and *Escherichia coli* (MC4100).[20] We chose to use benzalkonium chloride (BAC) as a positive control since it is a common, commercially available quaternary ammonium compound that also induces bacterial killing *via* membrane perturbation. In addition to antimicrobial activity, the toxicity was analyzed using a red blood cell (RBC) lysis assay utilizing mechanically defibrillated sheep's blood. These values are indicated as $Lysis_{20}$ and are measured as the concentration that lyse 20% or less of RBC. Surprisingly, the azaborine analog (**3**) possessed a higher MIC than its isosteric partner (**2**, Table 1) across all strains. However, the toxicity was comparable to the parent compound (Table 1).

To further understand this decrease in biological activity, partition coefficients (P) for both the analog 2 (**2**) and azaborine analog (**3**) were theoretically calculated *via* ChemDraw-3D and experimentally determined through extraction protocols with *n*-octanol and deionized water.[21-22] This experiment was performed in triplicate, and the average P was determined. From this data, log(base 10) of P was calculated. Somewhat surprisingly, we found that **2** had a logP of 0.93 and **3** had a logP of 0.12 (Table 2). These results indicate that the isosteric substitution in **3** switches the compound to become more hydrophilic in comparison to **2**.

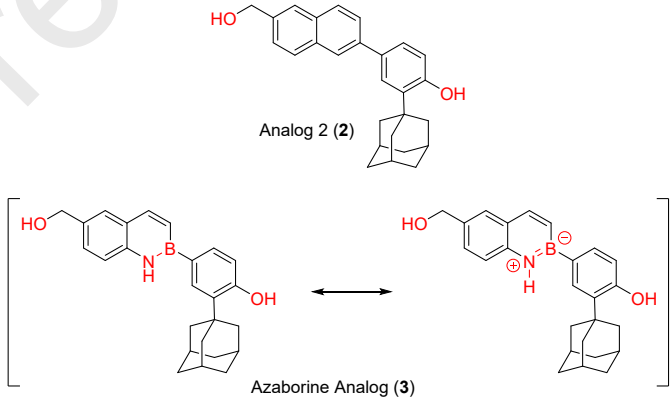


Figure 2. The structures of Analog 2 and the azaborine analog, displaying contributing resonance structures. Highlighted in red are hydrogen-bond donors and acceptors.

To further explore this surprising finding, we performed a quantum mechanical analysis with Schrodinger 2020-3 suite of modelling software to analyze the dipole moment of both compounds. Through these calculations, we found that analog 2 had a higher dipole moment of 2.42 D vs 1.34 D for azaborine (Table 2). As was previously disclosed by our group, the proposed mechanism of action of this class of molecules is membrane perturbation *via* amphiphilic attachment to the phospholipids. We propose that the presence of the N-B bond greatly reduces the molecular dipole moment of the primary alcohol, thereby limiting its amphiphilicity. Additionally, within the dipole analysis it was shown that the phenol dipole moment was greatly reduced in comparison to the parent molecule. This is most likely due to the resonance induction into the boron atom (not shown). Also, as highlighted in Figure 2, the azaborine analog has more hydrophilic regions, explaining the observed differences between clogP and logP (Fig. 2). Therefore, with a

bonding with water, **3** is likely unable to associate strongly with the lipid bilayer thereby reducing its biological activity.

To confirm that the dipole moment, and not chemical stability, was the reason for differential activity we characterized the stability of **3** in water. Toward this end, we suspended the compound in deionized water and heated it to 37 ºC for 23 h to mimic the *in vitro* conditions employed in our biological assays. The resulting mixture was then evacuated and analyzed by NMR spectroscopy, which proved the compound to be stable in neutral water. We recognize that there are limitations in this evaluation as we chose deionized water over the growth media used. However, we wanted a simple system to quantify degradation with minimal background noise in the NMR spectroscopy analysis and did not anticipate the additional nutrients found in growth media to cause further degradation. Overall, we found that the azaborine isosteric substitution is not a viable option for improving the biological activity of our retinoid class of antibacterial agents.

In summary, we successfully completed the synthesis of the first azaborine isostere of a retinoid derivative. Although, the biological activity was inferior to our lead compound we did uncover some surprising physiochemical properties. Namely that the naphthalene to azaborine isosteric substitution increased water solubility, which could find promising applications to other drug candidates. This isosteric substitution warrants further analysis in other antimicrobial natural products to fully understand its availability for drug discovery.

Acknowledgments

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Highlights: Synthesis and biological evaluation of an antibacterial azaborine retinoid isostere

- First disclosure of an azaborine isosteric substitution for a retinoid antibiotic.
- Discovery of differences in the chemical properties of an azaborine.
- The surprising difference in antibacterial activity between these two scaffolds.

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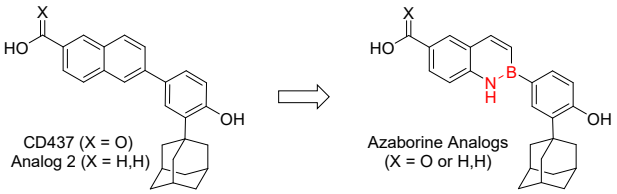

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