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Article

Synthesis, Characterization and Antimicrobial Activities of Pentacyclic Oxadiazadiborinanes

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In this study we have expanded upon a family of substituted pentacyclic oxadiazadiborinane derivatives from the condensation of carbohydrazide with *ortho*-formylphenyl boronic acid derivatives containing a variety of chemical and physical properties. All new complexes have been characterized fully including two single crystal X-ray diffraction studies which confirm the solid-state structure of these species along with an unusual methanol activation product, which appears to be a minor solid-state side product. The lack of inherent solubility of these compounds in common physiological media precluded us from doing traditional antimicrobial activities. To circumvent this problem, we incorporated these boron compounds into chitosan films. Only weak or moderate activities against Gram-negative and Gram-positive bacteria were observed in this study.

Keywords: antimicrobial, boron, chitosan, heterocyclic, X-ray diffraction

Introduction

Although α-aminoboronic acids have been recognized as potent inhibitors of serine proteases for several decades,[1-3] boron-containing compounds have traditionally been ignored by the pharmaceutical industry until the relatively recent discovery that the boropeptide Velcade® (aka bortezomib, Figure 1a) displays considerable efficacy for the treatment of multiple myeloma and mantle cell lymphoma.[2]

Since this remarkable discovery, significant effort has focused on designing and developing new small molecule boron-containing compounds for their potential use in medicinal chemistry.[3] The next-generation derivative Ninlaro® (aka ixazomib citrate, Figure 1b) has subsequently displayed promise in the treatment of relapsed multiple myeloma. While Velcade® must be administered by injection, Ninlaro® can be taken orally, which marks a substantial improvement and advancement in the field of boron pharmaceutical chemistry.[4] Remarkably, both complexes are quite similar in structure varying mainly by the aromatic group at the head of the molecule and the citrate protecting group replacing the boronic acid groups, which are known to impart reduced solubility.[4]

Over the years, other small molecule boron compounds have also shown promise for their potential use in the

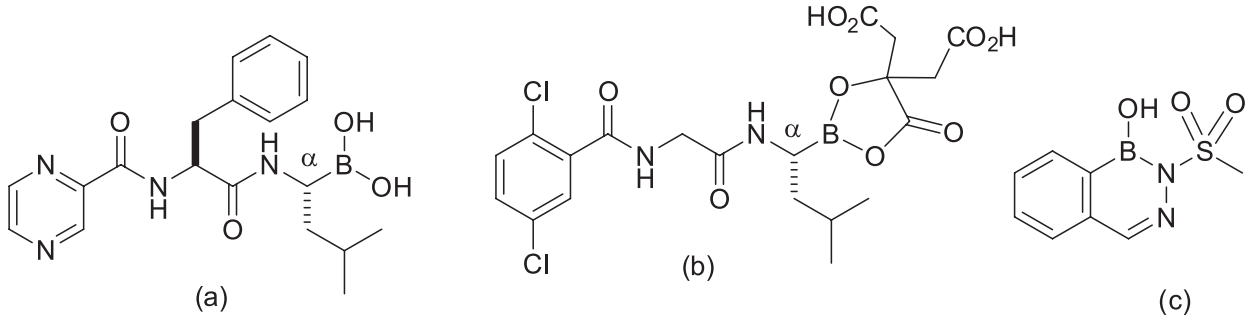

Figure 1. Bioactive small molecule boron compounds.

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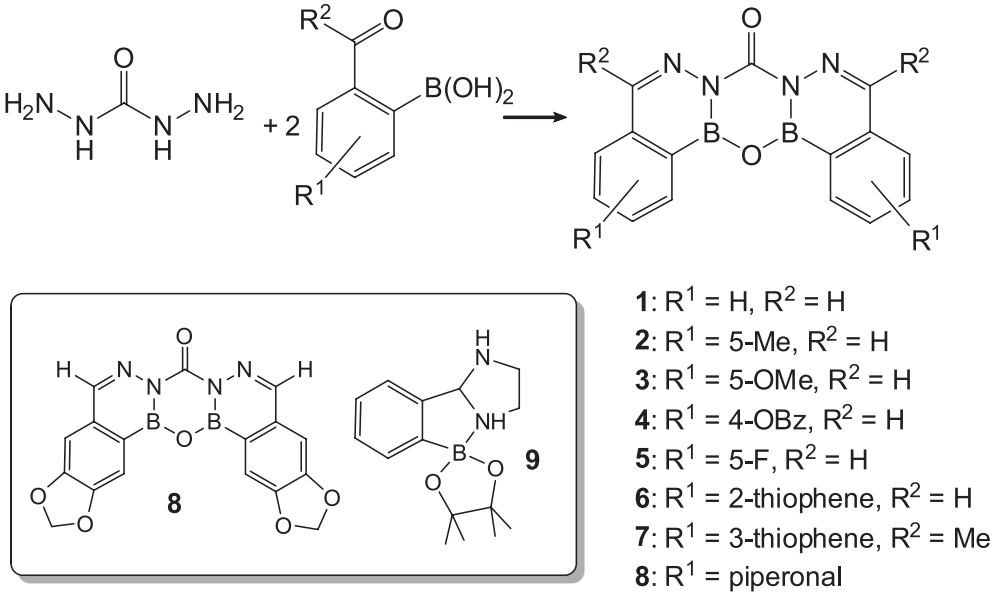
pharmaceutical industry. For additional information on the current state of bioactive boron compounds it is suggested to read the elegant review by dos Santos and co-workers.[5] Within this review are discussed diazaborines, which are a unique class of heterocyclic compounds that have potent antimicrobial properties (a representative example is shown in Figure 1c).[6-17] Although no diazaborines have yet to reach clinical trials, the mechanism of action of these compounds is believed to involve the inhibition of fatty acid biosynthesis in *Escherichia coli*, in which the boron compound inhibits maturation of rRNAs (ribosomal ribonucleic acids) for the large ribosomal subunit. Numerous groups, including Groziak and co-workers,[18-20] have reported derivatives of this important structural scaffold and shown they display significant antimicrobial properties. As part of our ongoing program for generating bioactive boron compounds,[21,22] we have also been designing derivatives of diazaborines and our latest findings are presented within which expands on some of the earlier work by Groziak and co-workers.[18-20]

Results and Discussion

Chemistry

Pentacyclic compounds derived from carbohydrazides and 2-formylphenyl boronic acid have been elegantly prepared by Groziak and co-workers[20] previously and were demonstrated to display moderate antimicrobial activities. In this previous study only a few derivatives were examined so in an effort to expand on these interesting compounds we decided to generate a larger family of these species containing substituents with various physicochemical properties. The unsubstituted derivative **1** along with the thiophene derivative **6** have been prepared previously,[20] but are used as biological controls in this study (Scheme 1).

In this study we have investigated variations within the aryl group initially bound to the 2-formylphenyl boronic acid group, altering the substitution pattern and the electron withdrawing and donating properties. Compound **7** is a thiophene-containing compound derived from the corresponding ketone. Interestingly, compound **8** is derived from piperonal, commonly known as heliotropin, which is found in fragrances and flavors. Reactions of carbohydrazide with two equivalents of the substituted 2-formylphenyl boronic acid derivatives in aqueous media all proceeded to give the corresponding pentacyclic oxadiazadiborinanes in high isolated yields (78-98%). All new compounds were characterized fully using a number of physical techniques including elemental analysis and multinuclear nuclear magnetic resonance (NMR) (^{1}H, ^{11}B, ^{13}C, and ^{19}F (for compound **5**)) spectroscopy except in the case of **8** which proved to be insoluble in common organic and aqueous deuterated solvents. For instance, the ^{11}B NMR spectra show a broad peak around 28 ppm, which is indicative of a trigonal BCNO environment typical of these compounds.[18-22] Likewise, a sharp singlet at ca. 8.6-8.4 ($CDCl_3$) or ca. 8.3-8.1 ppm (dimethyl sulfoxide (DMSO-d_6)) for the iminic C(*H*)=N peak is significantly shifted upfield from the starting aldehyde at ca. 10 ppm, which is no longer present in these reactions showing complete conversion of the starting materials. The appearance of a single resonance between 146 and 143 ppm in the ^{13}C{^{1}H} spectra is also indicative of the formation



Scheme 1. Synthesis of polycyclic boron compounds to be examined for antimicrobial properties.

of a new C=N bond. The diagnostic C=N stretching bands (1615-1610 cm^{-1}) in the Fourier transform infrared (FTIR) spectra also suggest formation of the oxadiazadiborinanes.

To confirm the solid-state structures of these compounds we carried out a single crystal X-ray diffraction study on **3**, which was recrystallized from methanol. To our surprise the structure did not correspond to the solution data but rather showed an activated methanol group, where the methoxy group (MeO–) was bound to one boron atom while one imine nitrogen had also been protonated (Figure 2).[23]

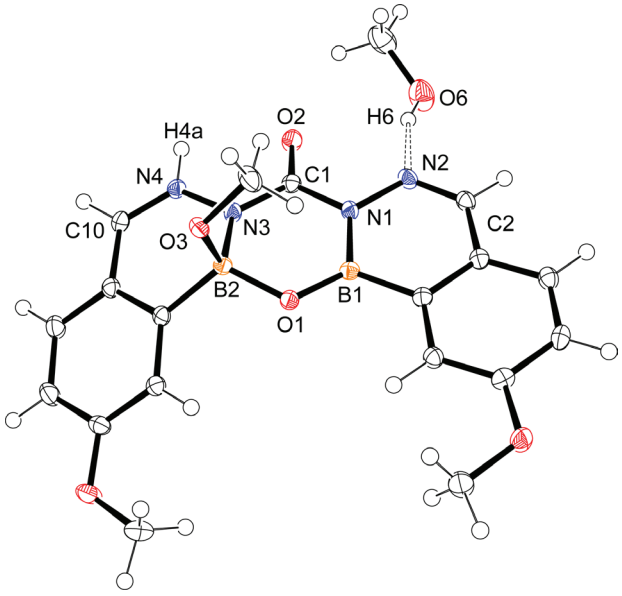


Figure 2. The molecular structure of **3**·2MeOH drawn at the 50% probability level. Select bond distances and angles: B(1)–N(1) 1.4558(17), B(1)–O(1) 1.3424(17), B(1)–C(8) 1.5409(19), C(1)–N(1) 1.4016(16), C(1)–O(2) 1.2158(15), C(1)–N(3) 1.3765(16), N(1)–N(2) 1.3913(14), O(1)–B(2) 1.4518(16), B(2)–N(3) 1.5883(17), B(2)–O(3) 1.4692(17), B(2)–C(16) 1.6014(19); N(1)–B(1)–C(8) 115.22(11), O(1)–B(1)–N(1) 121.07(12), O(1)–B(1)–C(8) 123.70(12), O(2)–C(1)–N(1) 124.38(12), O(2)–C(1)–N(3) 123.63(12), N(3)–C(1)–N(1) 111.98(10), C(1)–N(1)–B(1) 123.44(11), N(2)–N(1)–B(1) 124.38(10), N(2)–N(1)–C(1) 111.99(10), B(1)–O(1)–B(2) 122.93(10), O(1)–B(2)–N(3) 106.67(10), O(1)–B(2)–O(3) 111.66(10), O(1)–B(2)–C(16) 114.45(10), N(3)–B(2)–C(16) 106.19(10), O(3)–B(2)–N(3) 109.79(10), O(3)–B(2)–C(16) 107.90(10).

Interestingly, a second molecule of methanol is hydrogen bonded to the other imine nitrogen atom. As a result, bond distances within **3**·2MeOH are not equivalent as illustrated by the B(1)–N(1) distance of 1.4558(17) Å compared to the significantly elongated analogous B(2)–N(3) bond distance of 1.5883(17) Å. Likewise, for the BOB linkage, the B(1)–O(1) bond distance of 1.3424(17) Å is noticeably shorter than the B(2)–O(1) bond which is 1.4518(16) Å. The angles around B1 clearly show a trigonal planar environment while those for B2 are closer to a distorted tetrahedral geometry. This is a rare example of a 1,3-zwitterion related to the heterocycle nitrones studied by Kliegel *et al.*[24-26] and James and co-workers.[27,28] Crystallographic data are provided in Table 1. Attempts to generate the **3**·2MeOH adduct by heating compound **3** in MeOH for several hours failed to show any of the zwitterion as ascertained by multinuclear NMR spectroscopy.

It is likely that **3**·2MeOH represents a small sample size in the crystalline solid state and is not indicative of the true nature of **3**. While the mechanism to activate a molecule of methanol using a pentacyclic oxadiazadiborinane derivative at this time remains unclear, it is well documented that Lewis-acidic clusters are known to promote this reaction.[23] We therefore carried out a second single crystal X-ray diffraction on **4** to confirm the structure of these molecules. As expected, compound **4** is nearly planar and symmetrical. The molecular structure of which along with selected bond distances and angles, which are similar to those reported for **1** by Groziak and co-workers[20] previously, are provided in Figure 3.

Figure 4 illustrates the extensive π···π stacking in the unit cell with the distance between planes being 3.48 Å, which suggests a significant intermolecular interaction that may be responsible for the low solubilities observed with these compounds.

Antimicrobial testing

With elementally-pure samples in hand we investigated their antimicrobial properties to determine if these structural changes had any impact on bioactivities.[29-32] Due to the poor solubility of the oxadiazadiborinanes in solvents typically used in the standard Mueller-Hinton disc diffusion method,[33] testing was performed using a less conventional technique employing chitosan as the delivery vehicle. It is well documented that chitosan can be used as a medium for testing biological properties of insoluble metal complexes[34] and various boron-containing molecules.[35-51] Chitosan is produced from deacylation of the polysaccharide chitin, which is the second most abundant biopolymer behind cellulose. Chitin is found naturally in the shells of crustaceans and insects as well as in the cell walls of fungi and some lower plants and animals.[52] Compounds **1**-**9** were incorporated into the chitosan film during the film preparation process at doses of 100, 50, 25 and 12.5 μg with respect to 500 mg of deacetylated chitosan.

Consistent distribution of the boron-containing compounds within the final films was monitored by analyzing three discs cut from each film by FTIR (attenuated total reflection (ATR) mode) for the presence of the distinct C=N bond at ca. 1615 cm^{-1} of the oxadiazadiborinanes; a blank chitosan film was used as

Table 1. Crystallographic data collection parameters for **3** and **4**

Complex	**3**	**4**
Formula	$C_{19}H_{22}B_2N_4O_6$	$C_{31}H_{24}B_2Cl_6N_4O_4$
Molecular weight	424.02	750.86
Crystal system	monoclinic	monoclinic
Space group	P21/c	C 1 2/c 1
a / Å	9.7203(11)	13.1518(4)
b / Å	20.903(2)	12.0377(4)
c / Å	9.8920(11)	20.4104(6)
α / degree	90	90
β / degree	101.7900(10)	90.992(2)
γ / degree	90	90
V / Å^3	1967.5(4)	3230.84(17)
Z	4	4
$\rho_{calc.}$ / (Mg m^{-3})	1.432	1.544
Crystal size / mm^3	0.48 × 0.23 × 0.22	0.24 × 0.15 × 0.14
Temperature / K	125.02	124.99
Radiation	Mo K_α (λ = 0.71073 Å)	Mo K_α (λ = 0.71073 Å)
μ / mm^{-1}	0.105	0.577
Total reflections	24130	24487
Total unique reflections	4727	3890
No. of variables	289	214
θ range / degree	1.949-27.997	1.996-27.995
Largest difference peak/hole / (e Å^{-3})	0.408 and –0.222	0.343 and –0.289
S (goodness-of-fit) on F^2	1.041	1.056
R_1 ($I > 2\sigma(I)$)[a]	0.0390	0.0388
wR_2 (all data)[b]	0.1008	0.0915

[a]$R_1 = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$; [b]$wR_2 = (\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[wF_o^4])^{1/2}$, where $w = 1 / [\sigma^2(F_o^2) + (0.0445P)^2 + (0.7773P)]$ (**3**), $1 / [\sigma^2(F_o^2) + (0.0329P)^2 + (2.1651P)]$ (**4**), where $P = (\max(F_o^2, 0) + 2F_c^2) / 3$. V: volume; Z: number of formula units in the unit cell; ρ_{calc}: calculated density; μ: absorption coefficient; R_1 and R_2: residual factors.

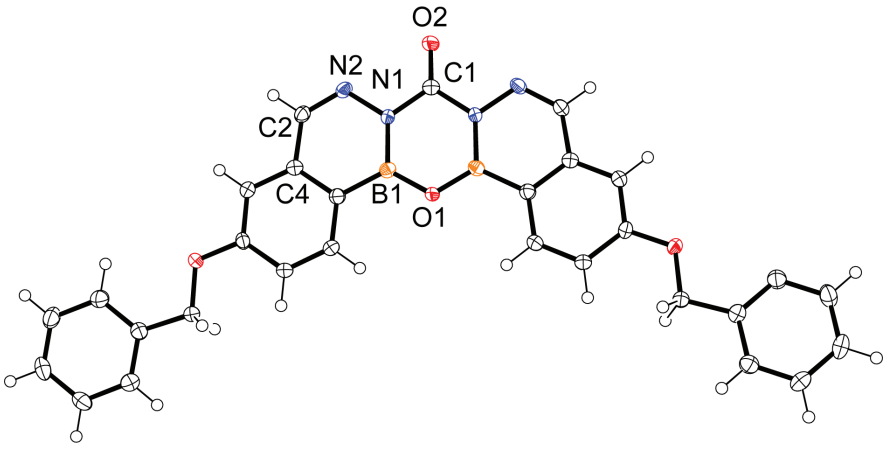


Figure 3. The molecular structure of **4** drawn at the 50% probability level. Select bond distances and angles: B(1)–N(1) 1.443(3), B(1)–O(1) 1.366(2), B(1)–C(4) 1.514(3), C(1)–N(1)#1 1.409(2), C(1)–N(1) 1.409(2), C(1)–O(2) 1.203(3), N(1)–N(2) 1.397(2); N(1)–B(1)–C(4) 117.27(17), O(1)–B(1)–N(1) 119.45(18), O(1)–B(1)–C(4) 123.27(19), N(1)–C(1)–N(1)#1 112.1(2), O(2)–C(1)–N(1)#1 123.93(11), O(2)–C(1)–N(1) 123.93(11), C(1)–N(1)–B(1) 124.47(17), N(2)–N(1)–B(1) 122.73(15), N(2)–N(1)–C(1) 112.80(15), B(1)#1–O(1)–B(1) 120.0(2).

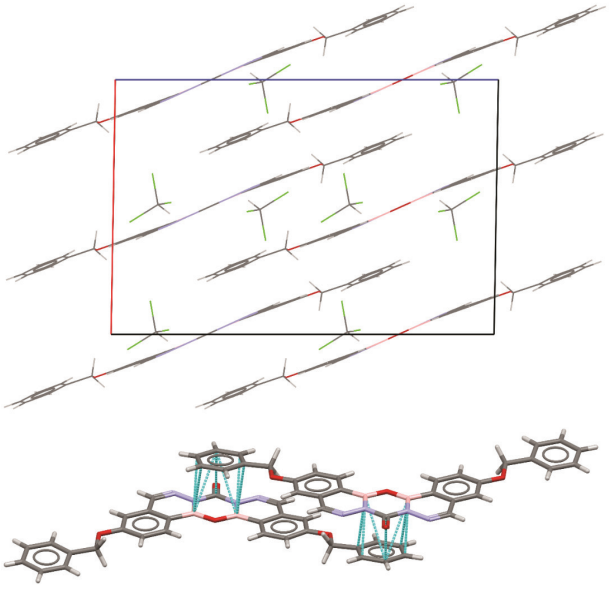


Figure 4. Unit cell for compound **4** showing intermolecular interactions.

the background. As our initial control, biological testing was performed with non-doped chitosan films and in all cases no activity was observed which is consistent with previously reported results from other groups[53] who have used similar techniques. Infusing a chitosan film with a known active compound previously prepared in our lab, 2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]imidazolidine (**9**), and testing its activity demonstrated that, although activity was slightly diminished, these results were consistent with our previous study.[54] No significant activity was observed using the chitosan films. Reduced activity using films has also been observed, for example, as pure oregano essential oil displayed greater activity against *L. monocytogenes* and *E. coli* compared to when it was incorporated into a chitosan film.[55] It was proposed that the decreased activity may be due to a slower diffusion process from a film than from a paper disc. Testing of compounds **1**-**8** revealed no activity against the Gram-negative bacteria *E. coli*, which is consistent with the results reported for the known compounds **1** and **6** previously tested by Groziak and co-workers[20] who observed very minimal antibacterial activity with *E. coli* (Table 2).

Interestingly, significant activity is seen against the other Gram-negative bacteria, *P. aeruginosa*, as compounds **3**-**8** (Figure 5) were active at 100 μg disc^{-1} doses albeit at a much lower level than the control, streptomycin, which was tested at a dose of 10 μg disc^{-1}.

Positive antibacterial activity at the 100 μg dose against the Gram-positive bacteria *B. cereus* was limited to compound **5**, however, a greater range of activity was observed for the Gram-positive bacteria *S. aureus* as compounds **1** and **5**-**8** all demonstrated some activity, unfortunately, once again with decreased potencies with respect to the control, erythromycin, which was tested at a dose of 15 μg. Of the substrates tested, **5** was the only compound that showed antibacterial properties for the three species in which any activity was observed. Indeed, the inclusion of fluorine has been used in the pharmaceutical industry to improve the bioactivity of small molecules[56-58] due to its size, electrophilicity and electronegativity and could explain the enhanced efficacy of **5** over the other non-fluorine containing substrates. No

Table 2. Antibacterial activity for compounds **1**-**9** incorporated into 5 mm chitosan discs

Compound	*Bacillus cereus*		*Pseudomonas aeruginosa*		*Escherichia coli*		*Staphylococcus aureus*	
	Dose / (μg disc^{-1})	Clear zone[a] ± SD / mm	Dose / (μg disc^{-1})	Clear zone ± SD / mm	Dose / (μg disc^{-1})	Clear zone ± SD / mm	Dose / (μg disc^{-1})	Clear zone ± SD / mm
1	100	inactive	100	inactive	100	inactive	100	5.1 ± 0.6
2	100	inactive	100	inactive	100	inactive	100	inactive
3	100	inactive	100	3.5 ± 0.4	100	inactive	100	inactive
4	100	inactive	100	4.5 ± 0.9	100	inactive	100	inactive
5	100	4.3 ± 0.7	100	4.5 ± 0.8	100	inactive	100	4.0 ± 0.6
6	100	inactive	100	5.4 ± 1.2	100	inactive	100	4.8 ± 0.5
7	100	inactive	100	5.0 ± 0.8	100	inactive	100	4.4 ± 0.7
8	100	inactive	100	5.1 ± 0.3	100	inactive	100	4.5 ± 0.5
9	100	inactive	100	5.9 ± 2.2	100	inactive	100	4.3 ± 0.3
Erythromycin	15	15.1 ± 0.5					15	6.6 ± 0.4
Streptomycin			10	7.5 ± 3.7	10	9.2 ± 0.2		
Chitosan	100	inactive	100	inactive	100	inactive	100	inactive

[a]Clear zone measured from center of disk to end of cell-free region. SD: standard deviation.

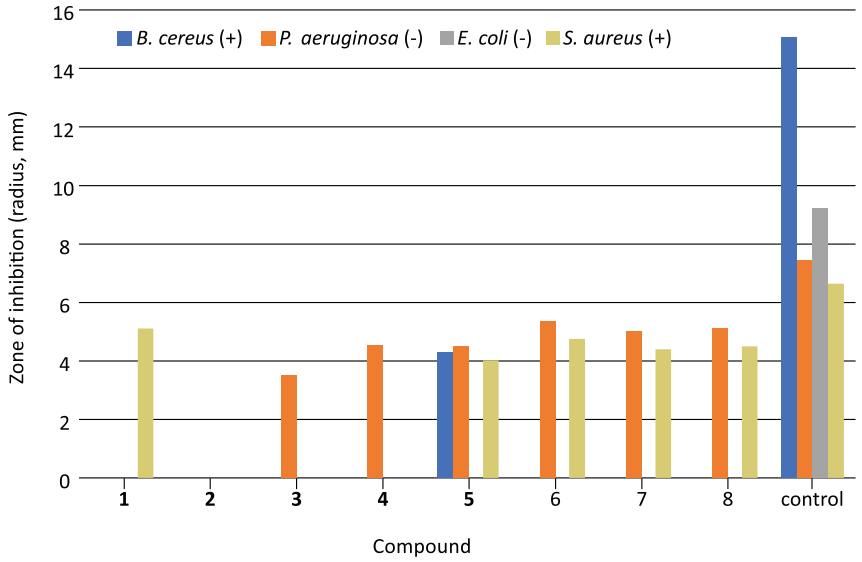


Figure 5. Antibacterial activity of compounds **1**-**8**.

activity was observed with the fungi *Aspergillus niger* or *Saccharomyces cerevisiae* even at the highest dose (100 µg) of oxadiazadiborinanes (data not shown).

Conclusions

In this study we have expanded upon a family of substituted pentacyclic oxadiazadiborinane derivatives from the condensation of carbohydrazide with *ortho*-formylphenyl boronic acid derivatives containing a variety of chemical and physical properties. All new complexes have been characterized fully including two single crystal X-ray diffraction studies which confirm the solid-state structure of compound **4** as well as an unusual methanol activation product observed with compound **3**. The lack of inherent solubility of these compounds in common physiological media precluded us from doing traditional antimicrobial activities. This problem was resolved by incorporating the compounds into chitosan films but, unfortunately, no appreciable activity was observed with these new species and they were only weakly or moderately selective against Gram-negative and Gram-positive bacteria. We will continue to investigate the potential of using small-molecule boron compounds as antimicrobial agents and will report our findings in due course.

Experimental

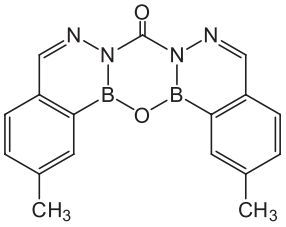
Materials and methods

Reagents and solvents used were obtained from Sigma-Aldrich (Oakville, Ontario, Canada) or Frontier Scientific (Logan, Utah, USA). Compounds **1** and **6** have been reported previously.[20] 2-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]imidazolidine (**9**) was synthesized as previously reported.[54] NMR spectra were recorded on a JEOL JNM-GSX400 FT NMR (^{1}H: 400 MHz; ^{11}B: 128 MHz; ^{13}C: 100 MHz; ^{19}F: 188 MHz) spectrometer. Chemical shifts (δ) are reported in ppm (relative to residual solvent peaks (^{1}H and ^{13}C) and external $BF_3{\cdot}OEt_2$ (^{11}B) or CF_3CO_2H (^{19}F)). Multiplicities are reported as singlet (s), doublet (d), multiplet (m), broad (br), or overlapping (ov) with coupling constants (J) reported in hertz. FTIR spectra were obtained with a Thermo Fisher Scientific Nicolet iS5 FT-IR spectrometer in ATR mode and are described as strong (s), medium (m), weak (w) or broad (br) and are reported in cm^{-1}. Melting and decomposition points were measured uncorrected with a Stuart SMP30 apparatus. Elemental analyses for C, H, and N were carried out at The Centre for Environmental Analysis and Remediation (Saint Mary's University, Halifax, NS).

General procedure for the synthesis of pentacyclic oxadiazadiborinanes

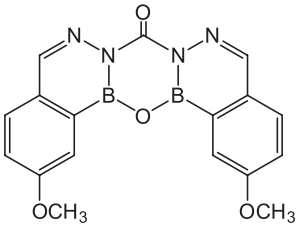
To a stirred H_2O (25 mL) suspension of the appropriate boronic acid-containing aldehyde (2.22 mmol) and 1 drop of formic acid was added an aqueous solution (5 mL) of carbohydrazide (100 mg, 1.11 mmol). Compound **7** was synthesized using MeOH as the solvent. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to room temperature (RT). The resulting precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford the pentacyclic oxadiazadiborinanes as white or off-white solids.

2,14-Dimethyl-8*H*-benzo[4,5][1,2,3]diazaborinino[3,2-*b*]benzo[4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-8-one (**2**)



Yield: 342 mg (94%); mp stable up to 400 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 2H, C(*H*)=N), 8.29 (s, 2H, Ar), 7.72-7.69 (ov m, 4H, Ar), 2.62 (s, 6H, CH_3); ^{11}B NMR (128 MHz, $CDCl_3$) δ 29 (br); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 153.4, 146.3, 141.7, 135.0, 133.5, 132.2, 129 (br, *C*B), 128.2, 22.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 2H, C(*H*)=N), 8.08 (s, 2H, Ar), 7.70 (d, *J* 8.2 Hz, 2H, Ar), 7.58 (d, *J* 8.2 Hz, 2H, Ar), 2.50 (s, 6H, CH_3); anal. calcd. for $C_{17}H_{14}N_4B_2O_2 \cdot 0.25H_2O$ (332.45 g mol^{-1}) (%): C, 61.41; H, 4.41; N, 16.86; found: C, 61.38; H, 4.04; N, 16.88; IR (ATR) ν / cm^{-1} 3028 (w), 1747 (s, $\nu_{C=O}$), 1615 (w, $\nu_{C=N}$), 1493 (m), 1374 (s), 1333 (m), 1279 (m), 1147 (m), 886 (s), 823 (m), 782 (m), 651 (s), 592 (s).

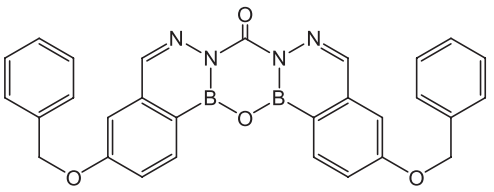
2,14-Dimethoxy-8*H*-benzo[4,5][1,2,3]diazaborinino[3,2-*b*]benzo[4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-8-one (**3**)



Yield: 390 mg (98%); mp 325-328 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 2H, C(*H*)=N), 7.89 (d, *J* 2.3 Hz, 2H, Ar), 7.78 (d, *J* 8.7 Hz, 2H, Ar), 7.43 (dd, *J* 8.7, 2.3 Hz, 2H, Ar), 4.04 (s, 6H, OCH_3); ^{11}B NMR (128 MHz, $CDCl_3$) δ 30 (br); ^{1}H NMR (400 MHz, 50 °C, DMSO-d_6) δ 8.21 (s, 2H, C(*H*)=N), 7.75 (d, *J* 8.7 Hz, 2H, Ar), 7.69 (d, *J* 1.4 Hz, 2H, Ar), 7.29 (dd, *J* 8.7, 1.4 Hz, 2H, Ar), 3.92 (s, 6H, OCH_3); ^{13}C{^{1}H} NMR (100 MHz, 50 °C, DMSO-d_6) δ 161.5, 154.2, 144.1, 136 (br, *C*B), 130.3, 128.2, 119.3, 114.3, 56.1, 56.0; anal. calcd. for $C_{17}H_{14}N_4B_2O_4 \cdot 2H_2O$ (395.98 g mol^{-1}) (%): C, 51.56; H, 4.59; N, 14.15; found: C, 51.33; H, 4.35; N, 14.15; IR (ATR) ν / cm^{-1} 3462 (br m), 3324 (br m), 3059 (w), 1679 (s, $\nu_{C=O}$), 1615 (w, $\nu_{C=N}$), 1592 (m), 1548 (m), 1497 (w), 1429 (s), 1310 (s), 1239 (s), 1107 (m), 1011 (s), 978 (s), 824 (m), 589 (s).

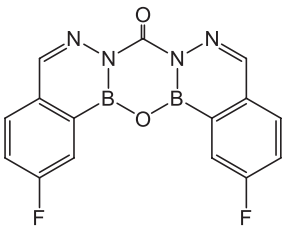
3,13-Bis(benzyloxy)-8*H*-benzo[4,5][1,2,3]diazaborinino[3,2-*b*]benzo[4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-8-one (**4**)

Yield: 474 mg (83%); mp 278-281 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 2H, C(*H*)=N), 8.39 (d, *J* 8.3 Hz, 2H, Ar), 7.48-7.35 (ov m, 12H, Ar), 7.25 (d,



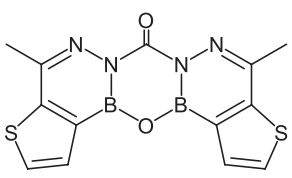
J 2.3 Hz, 2H, Ar), 5.21 (s, 4H, CH_2); ^{11}B NMR (128 MHz, $CDCl_3$) δ 28 (br); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 163.2, 153.4, 146.0, 137.8, 135.9, 134.1, 128.9, 128.5, 127.6, 121 (br, *C*B), 120.3, 111.3, 70.4; anal. calcd. for $C_{29}H_{22}N_4B_2O_4 \cdot 0.5H_2O$ (521.14 g mol^{-1}) (%): C, 66.83; H, 4.46; N, 10.75; found: C, 66.46; H, 4.27; N, 10.72; IR (ATR) ν / cm^{-1} 3436 (br m), 3304 (w), 3030 (br w), 1738 (s, $\nu_{C=O}$), 1687 (m), 1614 (w, $\nu_{C=N}$), 1592 (m), 1514 (m), 1403 (m), 1329 (s), 1234 (s), 1173 (m), 998 (m), 831 (m), 777 (m), 629 (m).

2,14-Difluoro-8*H*-benzo[4,5][1,2,3]diazaborinino[3,2-*b*]benzo[4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-8-one (**5**)



Yield: 292 mg (78%); decomposes > 310 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 2H, C(*H*)=N), 8.17 (dd, J_{HF} 7.3 Hz, J_{HH} 2.3 Hz, 2H, Ar), 7.92 (dd, J_{HH} 8.7 Hz, J_{HF} 5.0 Hz, 2H, Ar), 7.92 (td, J_{HF} 8.7 Hz, J_{HH} 2.3 Hz, 2H, Ar); ^{11}B NMR (128 MHz, $CDCl_3$) δ 28 (br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 2H, C(*H*)=N), 7.82-7.75 (ov m, 4H, Ar), 7.45 (ov dd, J_{HF} 8.2 Hz, 2H, Ar); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 163.6 (d, J_{CF} 249 Hz, *C*F), 154.7, 142.9, 139 (br, *C*B), 130.7 (d, J_{CF} 8 Hz), 130.4, 118.5 (d, J_{CF} 24 Hz), 116.4 (d, J_{CF} 18 Hz); ^{19}F{^{1}H} NMR (376 MHz, DMSO-d_6) δ −109 (br); anal. calcd. for $C_{15}H_8N_4B_2F_2O_2 \cdot 2H_2O$ (372.10 g mol^{-1}) (%): C, 48.44; H, 3.25; N, 15.07; found: C, 48.55; H, 2.94; N, 15.12; IR (ATR) ν / cm^{-1} 3308 (br m), 3079 (br m), 1653 (s, $\nu_{C=O}$), 1613 (w, $\nu_{C=N}$), 1591 (m), 1557 (m), 1495 (m), 1332 (s), 1282 (s), 1262 (s), 999 (m), 887 (m), 822 (m), 790 (w), 753 (w), 745 (w), 706 (m) 689 (m), 593 (m).

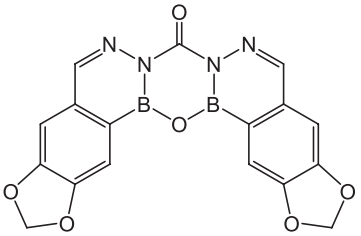
4,10-Dimethyl-7*H*-thieno[3',2':4,5][1,2,3]diazaborinino[3,2-*b*]thieno[3',2':4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-7-one (**7**)



Yield: 340 mg (90%); decomposes > 355 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* 4.6 Hz, 2H, Ar), 7.74 (d, *J* 4.6 Hz, 2H, Ar), 2.78 (s, 6H, CH_3);

^{11}B NMR (128 MHz, $CDCl_3$) δ 27 (br); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 153.6, 149.5, 146.5, 137 (br, *C*B), 130.9, 130.0, 21.9; anal. calcd. for $C_{13}H_{10}N_4B_2O_2S_2 \cdot CH_3OH$ (372.07 g mol^{-1}) (%): C, 45.20; H, 3.79; N, 15.06; found: C, 44.99; H, 3.46; N, 15.19; IR (ATR) ν / cm^{-1} 3075 (w), 3007 (br m), 2952 (br m), 2807 (m), 1742 (s, $\nu_{C=O}$), 1658 (m), 1612 (w, $\nu_{C=N}$), 1507 (s), 1430 (s), 1319 (s), 1256 (m), 1087 (m), 962 (m), 842 (m), 765 (m), 740 (s), 698 (s), 616 (m).

8*H*-[1,3]Dioxolo[4",5":4',5']benzo[1',2':4,5][1,2,3]diazaborinino[3,2-*b*]-[1,3]dioxolo[4",5":4',5']benzo[1',2':4,5][1,2,3]diazaborinino[2,3-*e*][1,3,5,2,6]oxadiazadiborinin-8-one (**8**)



Yield: 418 mg (97%); decomposes > 384 °C; anal. calcd. for $C_{17}H_{10}N_4B_2O_6$ (387.91 g mol^{-1}) (%): C, 52.64; H, 2.60; N, 14.44; found: C, 52.31; H, 2.35; N, 14.35; IR (ATR) ν / cm^{-1} 3026 (w), 2919 (w), 1750 (s, $\nu_{C=O}$), 1610 (w, $\nu_{C=N}$), 1493 (m), 1438 (m), 1400 (m), 1378 (s), 1252 (s), 1166 (m), 1120 (m), 1022 (s), 874 (m), 775 (m), 617 (m).

X-ray crystallography

Crystals of **3** were grown from a saturated MeOH solution stored at RT. Crystals of **4** were grown from a saturated $CHCl_3$ solution stored at RT. Crystals were attached to the tip of a 200 μm MicroLoop with paratone-N oil. Measurements were made on a Bruker APEXII charge-coupled device (CCD) equipped diffractometer (30 mA, 50 mV) using monochromated Mo Kα radiation (λ = 0.71073 Å) at 125 K. APEX2 software[59] was used for the initial orientation and unit cells were indexed using a least-squares analysis of a random set of reflections collected from three series of 0.5° wide scans, 5 s *per* frame and 12 frames *per* series that were well distributed in reciprocal space. For data collection, four ω-scan frame series were collected with 0.5° wide scans, 30 s *per* frames and 416 frames *per* series at varying φ angles (φ = 0°, 90°, 180°, 270°). The crystal to detector distance was set to 6 cm and a complete sphere of data was collected. Cell refinement and data reduction were performed with the Bruker APEX3 software,[60] which corrects for beam inhomogeneity, possible crystal decay, Lorentz and polarization effects. Data processing and a multi-scan absorption correction was applied using the APEX3 software package.[60] The structures of **3** and **4** were solved using Intrinsic phasing[61] and all non-hydrogen atoms were refined anisotropically using SHELXL[61] with the ShelXle graphical user interface.[62] Hydrogen atoms were placed in calculated positions using an appropriate riding model and coupled isotropic temperature factors, with the exception of H4a in compound **3**. H4a was allowed to freely refine with the exception of isotropic thermal factor which was 1.2 times the value of the atom H4a is attached to (N4). Figures were made using ORTEP-3 for Windows.[63] For the crystallographic information, see Supplementary Information section.

Biological testing

Chitosan solubilization

To a film forming solution composed of a stirring suspension of medium molecular mass and degree of deacetylated chitosan (500 mg) in Milli-Q water (25 mL), the appropriate amount of compound was added. Solubilization was initiated with the addition of acetic acid (250 μL) and the reaction mixture was allowed to stir for 6 h before being poured into a sterile 100 × 15 mm polystyrene Petri dish (VWR, Ville Mont-Royal, Québec, Canada). The films were dried under vacuum for 72 h before being sealed with parafilm and stored at 4 °C until further analysis.

Stability testing of boron-containing complexes in the presence of acetic acid

In NMR tubes, compounds **1**-**7** and **9** (5 mg) were dissolved or suspended in $CDCl_3$ (1 mL) to which 1 drop of acetic acid was added. The mixtures were allowed to sit at RT for 24 h at which point the samples were analyzed by ^{1}H and ^{11}B NMR spectroscopies and compared with original NMR data. All compounds proved stable to these replicated chitosan oligomerization conditions.

Cultures

Four species of pathogenic bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*) and two species of fungi (*Aspergillus niger* and *Saccharomyces cerevisiae*) were revived from strains stored at −80 °C. Bacterial stock cultures were maintained on tryptic soy agar while fungal cultures were maintained on Sabouraud dextrose agar.

Inoculations

Using aseptic techniques, a small amount (1 cm^2) of agar culture was removed from the stock plate via scalpel and placed into a sterile tissue homogenization tube. Doubly

distilled water (ca. 3-4 mL) was then added followed by gentle homogenization. The homogenate (100 µL) was then added to an agar plate and spread evenly to ensure uniform growth.

Compound testing

Discs (5 mm) were created from chitosan films and placed equidistant on an inoculated agar plate (diameter 100 mm) at three points. Set doses of compound (100, 50, 25, 12.5, and 0 µg disc^{-1}) were incorporated into the discs during oligomerization and the culture was allowed to grow over 48 h. During this time *B. cereus* was maintained at 30 °C while all other species were held at 37 °C. After 48 h, caliper measurements were obtained, measuring the radius of the clear zone from the center of the disc to the nearest microbial growth detected. Plates were prepared in duplicate and the mean results were calculated. Control plates for the Gram-positive bacteria *B. cereus* and *S. aureus* were performed with erythromycin (BD BBL Sensi-Disc No. 230793) at 15 µg and the Gram-negative strains *P. aeruginosa* and *E. coli* were performed with streptomycin (BD BBL Sensi-Disc No. 230942) at a 10 µg *per* dose. Control plates with a known antifungal were performed with amphotericin B (Sigma A9528, Oakville, Canada) at a concentration of 100 µg disc^{-1} on discs from filter paper (Fisherbrand® Filter paper, diameter of 15.0 cm, porosity: coarse, flow-rate: fast (09-795F)) for *A. niger* and *S. cerevisiae*. Negative controls were chitosan discs without the addition of compound.

Supplementary Information

Crystallographic information has also been deposited with the Cambridge Crystallographic Data Centre (CCDC 1961256 and 1961257). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK fax: + 44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Supplementary data associated with this article (NMR spectra of compounds) can be found available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Patrick T. Gormley, Stephen J. Geier, Christopher M. Vogels and B. Ninh Khuong contributions in this study include data curation and methodology; Jason D. Masuda was responsible for data curation and formal analysis and Tyson J. MacCormack was responsible for supervision. Stephen A. Westcott was responsible for funding acquisition, project administration, supervision, writing original draft, reviewing and editing.

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