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Synthesis, reactivity and antimicrobial properties of boron-containing 4-ethyl-3-thiosemicarbazide derivatives

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Abstract: The addition of 4-ethyl-3-thiosemicarbazide to benzaldehyde and boronic acid-containing derivatives afforded the corresponding thiosemicarbazones (**1**-**3**) or benzodiazaborines (**4**-**6**) depending on the position of the boronic acid within the ring. All compounds have been characterized fully including an X-ray diffraction study of the methoxy-containing benzodiazaborine **6**. Attempts to coordinate thiosemicarbazones **2** and **3** to palladium(II) acetate were unsuccessful, however, addition of the non-boron-containing derivative **1** to palladium afforded complex **7** whose molecular structure was determined by an X-ray diffraction study. The initial bioactivities of compounds **1**-**7** were examined against two fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, and two bacteria, *Bacillus cereus* and *Pseudomonas aeruginosa*.

Key words: antimicrobial, benzodiazaborines, boron, palladium, thiosemicarbazones.

Introduction

There has been recent considerable interest in boron-containing molecules for their use in pharmaceutical chemistry.[1] Among the many types of boron compounds being investigated for their therapeutic potential, diazaborines are a privileged class of heterocyclic compounds that have been studied extensively for their antimicrobial properties (Figure 1a).[2] The mechanism of action of diazaborines is believed to involve the inhibition of fatty acid biosynthesis in *Escherichia coli*, where the boron compound inhibits maturation of rRNAs for the large ribosomal subunit.[2f,j] Interestingly, while the bioactivities of related acyclic thiosemicarbazides (Figure 1b) and thiosemicarbazones (Figure 1c) are well known,[3] relatively little is known about boron-containing derivatives of these species.[4] Indeed, Groziak and co-workers[2o] along with our group[2n,s] have shown that thiosemicarbazones containing boronic acid [-$B(OH)_2$] groups display significant antimicrobial activities. In order to expand our understanding of these promising compounds, we have undertaken a study to make boron-containing diazaborines and thiosemicarbazones derived from readily available 4-ethyl-3-thiosemicarbazide, the results of which are presented herein.

[insert Figure 1]

Experimental

Materials and methods

Reagents and solvents used were obtained from Sigma-Aldrich. Compound **1**[5] was synthesized as previously reported. Nuclear magnetic resonance (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 deuterated 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), triplet (t), quartet (q), multiplet (m), broad (br), or overlapping (ov) with coupling constants (*J*) reported in Hertz. Fourier transform infrared (FTIR) spectra were obtained with a Thermo Fisher Scientific Nicolet iS5 FT-IR spectrometer in attenuated total reflections (ATR) mode and are described as strong (s), medium (m), weak (w) or broad (br) and are reported in cm^{-1}. Melting points were measured uncorrected with a Stuart SMP30 apparatus. Elemental analyses for C, H, and N were carried out at Laboratoire d'Analyse Élémentaire de l'Université de Montréal (Montréal, QC).

Synthesis of (*E*)-(3-((2-(ethylcarbamothioyl)hydrazono)methyl)phenyl)-boronic acid (2)

To a stirred suspension of 4-ethyl-3-thiosemicarbazide (500 mg, 4.19 mmol) in H_2O (25 mL) was added 3-formylphenylboronic acid (629 mg, 4.19 mmol) and 1 drop of formic acid. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to RT. The resulting white precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford **2** as a white solid. Yield: 1.00 g (95%); mp: 200-202 °C. ^{1}H NMR (400 MHz,

DMSO-d_6) δ: 11.39 (s, 1H, N*H*), 8.44 (t, *J* = 6.1 Hz, 1H, N*H*CH_2), 8.14 (s, 2H, B(O*H*)$_2$), 8.03 (s, 1H, C(*H*)=N), 7.95-7.93 (ov m, 2H, Ar), 7.76 (d, *J* = 7.6 Hz, 1H, Ar), 7.35 (app t, *J* = 7.6 Hz, 1H, Ar), 3.56 (m, 2H, CH_2CH_3), 1.11 (t, *J* = 6.9 Hz, 3H, CH_2CH_3). ^{11}B NMR (128 MHz, THF) δ: 27 (br). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ: 177.1, 142.9, 136.1, 135 (br, *C*B), 134.5, 133.7, 128.4, 128.3, 38.8, 15.2. Anal. calc. for $C_{10}H_{14}N_3BO_2S$ (251.11 g/mol) (%): C 47.83, H 5.62, N 16.73. Found: C 48.04, H 5.40, N 16.80. IR: 3323 (br m), 3194 (br m), 1616 (w, $\nu_{C=N}$), 1549 (s), 1496 (s), 1339 (m), 1219 (m), 1143 (m), 1080 (m), 945 (m), 807 (m).

Synthesis of (*E*)-(4-((2-(ethylcarbamothioyl)hydrazono)methyl)phenyl)-boronic acid (3)

To a stirred suspension of 4-ethyl-3-thiosemicarbazide (500 mg, 4.19 mmol) in H_2O (25 mL) was added 4-formylphenylboronic acid (629 mg, 4.19 mmol) and 1 drop of formic acid. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to RT. The resulting white precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford **3** as a white solid. Yield: 1.01 g (96%); mp: 218-220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.42 (s, 1H, N*H*), 8.55 (t, *J* = 5.5 Hz, 1H, N*H*CH_2), 8.12 (s, 2H, B(O*H*)$_2$), 8.01 (s, 1H, C(*H*)=N), 7.79 (d, *J* = 8.2 Hz, 2H, Ar), 7.71 (d, *J* = 8.2 Hz, 2H, Ar), 3.55 (m, 2H, CH_2CH_3), 1.11 (t, *J* = 7.4 Hz, 3H, CH_2CH_3). ^{11}B NMR (128 MHz, THF) δ: 28 (br). $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d_6) δ: 177.1, 142.3, 136.2, 134.8, 134 (br, *C*B), 126.7, 38.8, 15.2. Anal. calc. for $C_{10}H_{14}N_3BO_2S$

(251.11 g/mol) (%): C 47.83, H 5.62 N 16.73. Found: C 47.82, H 5.76, N 16.58. IR: 3327 (br m), 3154 (br m), 2972 (w), 1607 (w, $\nu_{C=N}$), 1523 (s), 1403 (s), 1324 (s), 1235 (s), 1077 (m), 928 (m), 794 (m).

Synthesis of *N*-ethyl-1-hydroxybenzo[d][1,2,3]diazaborinine-2(1*H*)-carbothioamide (4)

To a stirred suspension of 4-ethyl-3-thiosemicarbazide (500 mg, 4.19 mmol) in H_2O (25 mL) was added 2-formylphenylboronic acid (629 mg, 4.19 mmol) and 1 drop of formic acid. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to RT. The resulting white precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford **4** as a white solid. Yield: 747 mg (76%); mp: 103-105 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.61 (s, 1H, B(O*H*)), 10.36 (br t, *J* = 5.5 Hz, 1H, N*H*CH_2), 8.25 (s, 1H, C(*H*)=N), 8.08 (dd, *J* = 7.3, 0.9 Hz, 1H, Ar), 7.85 (dd, J = 7.3, 0.9 Hz, 1H, Ar), 7.79 (ov ddd, *J* = 7.3, 7.3, 0.9 Hz, 1H, Ar), 7.68 (ov ddd, *J* = 7.3, 7.3, 0.9 Hz, 1H, Ar), 3.64 (dq, *J* = 6.9, 5.5 Hz, 2H, C*H*$_2$$CH_3$), 1.17 (t, *J* = 6.9 Hz, 3H, CH_2C*H*$_3$). ^{11}B NMR (128 MHz, THF) δ: 29 (br). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ: 182.5, 141.5, 133.9, 133.2, 132.3, 131.4, 131 (br, *C*B), 128.6, 39.2, 13.7. Anal. calc. for $C_{10}H_{12}N_3BOS$ (233.10 g/mol) (%): C 51.53, H 5.19, N 18.03. Found: C 51.89, H 5.35, N 17.74. IR: 3311 (m), 2965 (br m), 1618 (w, $\nu_{C=N}$), 1492 (m), 1446 (m), 1398 (m), 1291 (m), 1253 (m), 1169 (m), 1077 (m), 959 (m), 740 (s), 707 (m).

Synthesis of *N*-ethyl-5-fluoro-1-hydroxybenzo[d][1,2,3]diazaborinine-2(1*H*)-carbothioamide (5)

To a stirred suspension of 4-ethyl-3-thiosemicarbazide (500 mg, 4.19 mmol) in H_2O (25 mL) was added 3-fluoro-2-formylphenylboronic acid (708 mg, 4.19 mmol) and 1 drop of formic acid. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to RT. The resulting white precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford **5** as a white solid. Yield: 957 mg (91%); mp: 153-155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.71 (s, 1H, B(O*H*)), 9.48 (br s, 1H, N*H*), 8.24 (s, 1H, C(*H*)=N), 8.02 (dd, J_{HH} = 7.8, 0.9 Hz, 1H, Ar), 7.61 (ov ddd, J_{HH} = 7.8, 7.8, J_{HF} = 5.0 Hz, 1H, Ar), 7.35 (ov ddd, J_{HF} = 10.1 Hz, J_{HH} = 7.8, 0.9 Hz, 1H, Ar), 3.74 (dq, J = 7.4, 5.5 Hz, 2H, C*H*$_2$CH$_3$), 1.36 (t, J = 7.4 Hz, 3H, CH$_2$C*H*$_3$). ^{11}B NMR (128 MHz, $CDCl_3$) δ: 29 (br). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 183.2, 159.6 (d, J_{CF} = 257 Hz), 134 (br, *C*B), 133.5 (d, J_{CF} = 5.7 Hz), 132.5 (d, J_{CF} = 7.6 Hz), 128.4 (d, J_{CF} = 3.8 Hz), 121.8 (d, J_{CF} = 10.5 Hz), 118.2 (d, J_{CF} = 20.1 Hz), 39.3, 13.6. ^{19}F{^{1}H} NMR (188 MHz, $CDCl_3$) δ: -123.6. Anal. calc. for $C_{10}H_{11}N_3BFOS$ (251.09 g/mol) (%): C 47.83, H 4.42, N 16.74. Found: C 47.64, H 4.43, N 16.80. IR: 3309 (m), 2901 (m), 1603 (m, $\nu_{C=N}$), 1529 (s), 1421 (m), 1293 (m), 1168 (m), 1076 (m), 801 (m), 753 (m).

Synthesis of *N*-ethyl-1-hydroxy-6-methoxybenzo[d][1,2,3]diazaborinine-2(1*H*)-carbothioamide (6)

To a stirred suspension of 4-ethyl-3-thiosemicarbazide (500 mg, 4.19 mmol) in H_2O (25 mL) was added 2-formyl-4-methoxyphenylboronic acid (754 mg, 4.19 mmol) and 1 drop of formic acid. The reaction mixture was heated at reflux for 2 h, at which point the reaction was allowed to cool to RT. The resulting white precipitate was collected by suction filtration and washed with Et_2O (2 × 10 mL) to afford **6** as a white solid. Yield: 1.07 g (97%); mp: 141-142 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.50 (s, 1H, B(O*H*)), 9.45 (br s, 1H, N*H*), 8.16 (d, *J* = 8.7 Hz, 1H, Ar), 7.87 (s, 1H, C(*H*)=N), 7.20 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar), 7.02 (d, *J* = 2.3 Hz, 1H, Ar), 3.91 (s, 3H, OCH_3), 3.73 (dq, *J* = 7.3, 5.5 Hz, 2H, CH_2CH$_3$), 1.35 (t, *J* = 7.3 Hz, 3H, CH$_2$CH_3). ^{11}B NMR (128 MHz, $CDCl_3$) δ: 29 (br). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ: 183.3, 162.9, 140.6, 135.5, 134.6, 124 (br, *C*B), 118.9, 110.2, 55.5, 39.2, 13.6. Anal. calc. for $C_{11}H_{14}N_3BO_2S$ (263.12 g/mol) (%): C 50.21, H 5.37, N 15.97. Found: C 50.03, H 5.42, N 16.10. IR: 3295 (m), 2984 (m), 1596 (m, $\nu_{C=N}$), 1534 (s), 1408 (m), 1276 (m), 1162 (m), 1070 (m), 867 (m), 833 (m), 740 (m).

Synthesis of compound 7

To a stirred EtOH (10 mL) solution of $Pd(OAc)_2$ (100 mg, 0.45 mmol) was added compound **1** (187 mg, 0.90 mmol) as a solid. The reaction was allowed to proceed at RT for 18 h at which point a precipitate was collected by suction filtration and washed with EtOH (2 × 10 mL) to afford **7** as an orange solid. Yield: 182 mg (78%); mp: 173-176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 7.3 Hz, 4H, Ar), 7.42-7.38 (ov m, 4H, C(*H*)=N and Ar), 7.32 (ov dd, *J* = 7.3, 6.8

Hz, 4H, Ar), 4.80 (br t, *J* = 5.2 Hz, 2H, N*H*CH_2), 3.33 (m, 4H, C*H*$_2$$CH_3$), 1.21 (t, *J* = 6.9 Hz, 6H, CH_2C*H*$_3$). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ: 172.2, 156.6, 132.5, 131.1, 130.6, 127.4, 40.5, 14.9. Anal. calc. for $C_{20}H_{26}N_6PdS_2$ (521.01 g/mol): C 46.11, H 5.03, N 16.13. Found: C 46.25, H 5.32, N 16.41. IR: 3366 (m), 1602 (m, $\nu_{C=N}$), 1494 (s), 1472 (s), 1260 (m), 1215 (m), 1150 (m), 1022 (m), 887 (m), 754 (m), 693 (m).

Reactions of $Pd(OAc)_2$ with 2 and 3

To a stirred THF or EtOH (5 mL) solution of $Pd(OAc)_2$ (50 mg, 0.23 mmol) was added a THF or EtOH (2 mL) solution of **2** or **3** (116 mg, 0.46 mmol) respectively. The reaction was allowed to proceed at RT for 2 h at which point the reaction was analyzed by ^{11}B NMR spectroscopy. ^{11}B NMR (128 MHz, THF) δ: 29 (br, minor) and 19.3 (sharp, $B(OH)_3$, major).

Stability testing of compounds

In NMR tubes, compounds **1**-**6** were dissolved in acetone and analyzed by ^{11}B NMR spectroscopy. The solutions were stored at 37 ^oC for 2 d at which point the compounds were reanalyzed by ^{11}B NMR spectroscopy. Compound **7** was observed by ^1H NMR spectroscopy under identical conditions in acetone-d_6. No significant decomposition of the compounds was observed over this time period.

X-ray crystallography

Crystals of **6** were grown from a saturated Et_2O solution stored at RT. Crystals of **7** were grown from a saturated EtOH solution stored at RT. Crystals were attached to the tip of a 400 µm MicroLoop with Paratone-N oil. Measurements were made on a Bruker APEXII CCD equipped diffractometer (30 mA, 50 mV) using monochromated Mo Kα radiation (λ = 0.71073 Å) at 125 K. The initial orientation and unit cell were indexed using a least-squares analysis of a random set of reflections collected from three series of 0.5° wide scans, 10 seconds 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, 10 second (**6**) and 30 second (**7**) 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 hemisphere of data was collected. Cell refinement and data reduction were performed with the Bruker SAINT software, which corrects for beam inhomogeneity, possible crystal decay, Lorentz and polarization effects. Data processing and a multi-scan absorption correction was applied using the APEX2 software package.[6] The structure of **6** was solved using direct methods[7] and all non-hydrogen atoms were refined anisotropically using the shelXle[8] graphical user interface and the SHELXL.[7] Hydrogen atoms were included at geometrically idealized positions and were fixed (O-H, N-H, Ar-H, CH_2) or in the case of methyl roups, the dihedral angle of the idealized tetrahedral CH_3 fragment was allowed to refine. The structure of **7** was solved using SIR92[9] as implemented in WinGX.[10] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included at geometrically

idealized positions with coupled isotropic temperature factors and were fixed (N-H, Ar-H, CH_2) or in the case of methyl groups, the dihedral angle of the idealized tetrahedral CH_3 fragment was allowed to refine.

Cultures

Pure cultures of *Aspergillus niger*, *Saccharomyces cerevisiae*, *Bacillus cereus*, and *Pseudomonas aeruginosa* were revived from strains maintained at -70 °C. *A. niger* was maintained on Sabouraud Dextrose agar, *S. cerevisiae* was maintained on yeast malt agar, and *B. cereus* and *P. aeruginosa* were maintained on tryptic soy agar.

Inoculations

Using aseptic techniques, a small amount (1 cm^2) of agar culture was removed from a plate *via* scalpel and placed into a sterile tissue homogenizer tube. Approximately 3-4 mL of doubly distilled H_2O was then added followed by gentle homogenization. Homogenate (200 μl) was added to an agar plate (Sabouraud Dextrose Agar for fungi; Mueller Hinton II agar for bacteria) and spread evenly to ensure uniform growth.

Compound testing

Disks (5 mm diameter) created from filter paper (Fisherbrand® Filter paper, diameter of 15.0 cm, porosity: coarse, flow-rate: fast (09-795F)) were placed equidistant on an inoculated agar plate (diam. 9 cm) at four points. Set

concentrations of compound (0, 25, 50 and 100 μg for disks 1-4, respectively) in acetone were added to the disks and the cultures were allowed to grow over 48 hours at which point caliper measurements were obtained, measuring from the center of the disc to the nearest presence of fungus or bacteria. Plates were done in triplicate and mean results calculated and reported. Control plates with a known antibiotic were performed with Amphotericin B (Sigma A9528) at a concentration of 100 μg for the fungal species. Control plates for *B. cereus* were performed with erythromycin (BD BBL Sensi-Disc #230793) at 15 μg and for *P. aeruginosa*, streptomycin (BD BBL Sensi-Disc #230942) at 10 μg was used. Negative controls were disks provided with acetone but without compound.

Results and discussion

Chemistry

One of the challenges associated with working with thiosemicarbazones containing boronic acids is that many of these compounds are insoluble in either aqueous or organic solvents, complicating biological studies. In an effort to increase solubilities in organic solvents we chose to investigate reactions with readily available lipophilic 4-ethyl-3-thiosemicarbazide. We have found that the addition of formylphenyl boronic acid derivatives to aqueous suspensions of 4-ethyl-3-thiosemicarbazide resulted in the formation of the corresponding thiosemicarbazones **1**-**3** when the boron group was located in

the *meta*- or *para*- position relative to the aldehyde functionality (Scheme 1, right). However, when the boronic acid moiety was located *ortho* to the aldehyde group the bicyclic diazaborinines **4**-**6** were formed preferentially (Scheme 1, left). Various substituents were examined to investigate the potential bioactivities of these compounds incorporating electron-withdrawing and donating groups using commercially-available 2-phenylboronic acid derivatives. All compounds were analyzed by a number of physical methods including multinuclear NMR and FTIR spectroscopy. The formation of a C=N bond was confirmed by ^{1}H NMR spectroscopy where the aldehyde resonance at *ca.* 10 ppm disappears and a new peak corresponding to the aldimine hydrogen appears between 7.87 and 8.25 ppm for compounds **1**-**6**.[12] Analysis of the ^{13}C{^{1}H} NMR spectra shows the aldehyde carbon at *ca.* 190 ppm disappears and a new C=N resonance is observed between 133 and 143 ppm. The boron atom remains in a three coordinate environment as evidenced by ^{11}B NMR spectra where resonances for compounds **2**-**6** are approximately 29 ppm, indicative of a CBO_2 or CB(O)(N) environment.[11] A single crystal X-ray diffraction study was performed on **6** to confirm the formation of the bicyclic diazaborine, the structure of which is shown in Fig. 2. Crystallographic data are provided in Table 1. The C(4)-N(2) bond distance of 1.2845(19) Å is within the acceptable range for C=N bond lengths. The sum of the three bond angles around boron totals 360^{o} confirming that the boron atom is indeed in a three coordinate environment.

[insert Scheme 1, Figure 2, Table 1]

As part of our ongoing studies into synthesizing metal complexes with boron-containing ligands[12] we decided to examine compounds **2** and **3** as potential ligands for palladium. Unfortunately, reactions with palladium(II) acetate in wet THF or EtOH with the boron-containing thiosemicarbazones resulted in cleavage of the boronic acid group resulting in the formation of boric acid as confirmed by ^{11}B NMR spectroscopy, along with the di-thiosemicarbazonato palladium(II) complex **7**. These results are not surprising as late metals such as platinum or palladium are well known to cleave carbon-boron single bonds, commonly observed in the Suzuki-Miyaura cross-coupling reaction.[13] Indeed, these observations suggest that compounds **2** and **3** could be useful as novel cross-coupling synthons and future efforts in our group will focus on this chemistry. Likewise, future research will also examine the potential of using these compounds as ligands with less reactive copper(II) salts. The control reaction of palladium(II) acetate with the non-boron-containing thiosemicarbazone **1** resulted in the formation of **7** as the only new palladium-containing product (Scheme 2). Coordination of the ligand was confirmed by ^{1}H NMR spectroscopy where the C(H)=N resonance shifted upfield from 7.91 ppm for the free ligand to 7.41 ppm upon complexation to the palladium centre. A single crystal X-ray diffraction study of **7** was undertaken to confirm the exact configuration of the ligand about the palladium centre and is shown in Fig. 3 with crystallographic data presented in Table 1. The

thiosemicarbazone ligands coordinate in a chelating bidentate fashion through the tautomerized thiolate sulfur and the imine nitrogen. The metal centre lies in a roughly distorted square planar environment and the Pd-S and Pd-N bond distances are 2.2895(5) and 2.0355(14), respectively, and are similar to related palladium thiosemicarbazone complexes.[14]

[insert Scheme 2 and Figure 3]

Biological activity

With elementally pure compounds in hand, we set out to explore the bioactivities of compounds **1**-**7** by studying their effect upon two fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, as well as two bacteria, *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Phenylboronic acid (PBA), 4-ethyl-3-thiosemicarbazide (ETSC), and known controls were also studied and the results are presented in Table 2 and Figure 4. The most promising results were observed in the case of *Saccharomyces cerevisiae* where three of the seven compounds (**1**, **2**, and **4**) showed activity equivalent to that of the control, Amphotericin B. Somewhat puzzling is the observation that altering the boronic acid from the 3- to the 4-position drastically alters the bioactivity as observed in the cases of *Saccharomyces cerevisiae* and the Gram-positive bacterium *Bacillus cereus* where the *meta*-boronic acid derivative (**2**) showed moderate activity in both cases yet, once again, the *para*-derivative (**3**) demonstrated no activity. Unexpectedly, only the

fluorinated benzodiazaborine **5** displayed any significant antifungal activity against *Bacillus cereus*. This result is somewhat surprising as diazaborines are usually more active than their thiosemicarbazone counterparts.

[insert Table 2 and Figure 4]

Conclusions

Two new thiosemicarbazones and three new benzodiazaborines derived from 4-ethyl-3-thiosemicarbazide have been synthesized and characterized fully including an X-ray diffraction study of the methoxy-containing benzodiazaborine **6**. Unfortunately, the boron-containing thiosemicarbazones **2** and **3** did not prove to be viable ligands for palladium as cleavage of the boron group was observed in reactions with palladium(II) acetate in EtOH and THF. Complexation of the non-boron-containing derivative **1** to palladium afforded complex **7** which was analyzed by an X-ray diffraction study. The antifungal and antibacterial activities of all compounds were studied against two fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, as well as two bacteria, *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Significant activity was observed against *Saccharomyces cerevisiae* in the cases of compounds **1**, **2**, and **4**. These results are quite surprising and show the promise of developing thiosemicarbazones as antimicrobial agents. Future studies will be directed at designing more lipophilic thiosemicarbazones

containing pendant boron groups, the results of which will be presented in due course.

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/. Crystallographic information has also been deposited with the Cambridge Crystallographic Data Centre (CCDC 1828794 (**6**) and 1828795 (**7**)). 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 336033or email:deposit@ccdc.cam.ac.uk).

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Table 1. Crystallographic data collection parameters for **6** and **7**.

Complex	**6**	**7**
Formula	$C_{11}H_{14}BN_3O_2S$	$C_{20}H_{24}N_6PdS_2$
Molecular weight	263.12	518.97
Crystal system	Triclinic	Triclinic
Space group	P-1	P-1
a(Å)	7.2345(11)	8.6101(11)
b(Å)	7.2819(11)	10.1516(13)
c(Å)	12.640(2)	12.4510(16)
α(°)	95.072(2)	87.8580(10)
β(°)	100.303(2)	87.369(2)
γ(°)	109.790(2)	79.5170(10)
V(Å^3)	608.44(16)	1068.5(2)
Z	2	2
$\rho_{calc.(}$Mg m^{-3})	1.436	1.613
Crystal size (mm^3)	0.260 x 0.220 x 0.060	0.448 x 0.159 x 0.116
Temperature (K)	125(2)	125(2)
Radiation	Mo-K_α (λ=0.71073 Å)	Mo-K_α (λ=0.71073 Å)
μ (mm^{-1})	0.262	1.083
Total reflections	4060	8855
Total unique reflections	2089	4909
No. of variables	173	267
θ range (°)	3.012-24.997	1.638-28.264
Largest difference peak/hole (e/Å^{-3})	0.251 and -0.196	0.482 and -0.392
S (goodness-of-fit) on F^2	1.086	1.048
R_1 (I>2s(I))[a]	0.0297	0.0219
wR_2 (all data)[b]	0.0829	0.0555

[a] $R_1 = \sum||F_o|-|F_c||/\sum|F_o|$.

[b] $wR_2 = (\sum[w(F_o^2-F_c^2)^2]/\sum[wF_o^4])^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (0.0388P)^2 + (0.1570P)]$ (**6**), $1/[\sigma^2(F_o^2) + (0.0257P)^2 + (0.4734P)]$ (**7**), where $P = (\max(F_o^2, 0) + 2F_c^2)/3$.

Table 2. Antimicrobial activity for compounds **1**-**7**.

Compound	*Aspergillus niger*		*Saccharomyces cerevisiae*		*Bacillus cereus*		*Pseudomonas aeruginosa*	
	Dose (μg disk^{-1})	Clear zone (mm±SD)[a]	Dose (μg disk^{-1})	Clear zone (mm±SD)	Dose (μg disk^{-1})	Clear zone (mm±SD)	Dose (μg disk^{-1})	Clear zone (mm±SD)
PBA[b]	100	Inactive	100	Inactive	100	Inactive	100	Inactive
ETSC[c]	100	Inactive	100	Inactive	100	Inactive	100	Inactive
1	100	Inactive	100	3.9±0.6	100	Inactive	100	Inactive
2	100	Inactive	100	4.0±0.8	100	3.5±0.5	100	Inactive
3	100	Inactive	100	Inactive	100	Inactive	100	Inactive
4	100	Inactive	100	4.3±0.8	100	Inactive	100	Inactive
5	100	Inactive	100	Inactive	100	3.4±0.2	100	Inactive
6	100	Inactive	100	Inactive	100	Inactive	100	Inactive
7	100	Inactive	100	Inactive	100	Inactive	100	Inactive
Amphotericin B	100	4.1±0.5	100	4.1±0.2				
Erythromycin					15	13.5±1.0		
Streptomycin							10	4.6±0.6

[a]Clear zone measured from center of disk to end of cell-free region

[b]PBA = Phenylboronic acid

[c]ETSC = 4-Ethyl-3-thiosemicarbazide

Figure and Scheme Captions

Fig. 1. Structures of benzodiazaborines, thiosemicarbazides, and thiosemicarbazones.

Scheme 1. Addition of formylphenyl boronic acid derivatives to 4-ethyl-3-thiosemicarbazide.

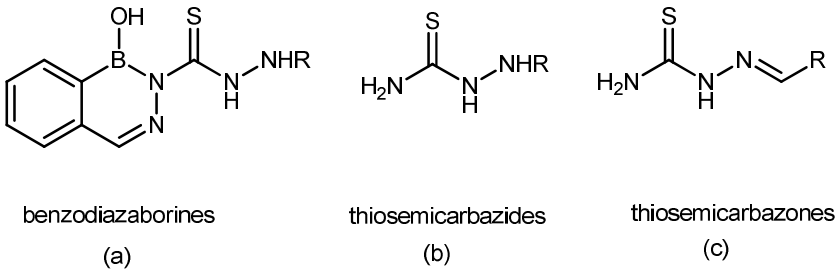
Fig. 2. The molecular structure of **6** with ellipsoids shown at the 50% confidence level. Hydrogen molecules have been omitted for clarity. Selected bond distances (Å) and angles (º): B(1)-O(1) 1.352(2), B(1)-N(1) 1.476(2), C(4)-N(2) 1.2845(19), N(1)-N(2) 1.4030(16), C(1)-S(1) 1.6968(15), C(1)-N(1) 1.4125(19); N(1)-C(1)-N(3) 116.14(13), O(1)-B(1)-N(1) 124.17(14), O(1)-B(1)-C(10) 120.23(14), N(1)-B(1)-C(10) 115.59(13).

Scheme 2. Formation of palladium thiosemicarbazone complex **7**.

Fig. 3. The molecular structure of **7** with ellipsoids shown at the 50% confidence level. Hydrogen molecules have been omitted for clarity. Selected bond distances (Å) and angles (º): Pd(1)-S(1) 2.2895(5), Pd(1)-N(2) 2.0355(14), N(1)-C(1) 1.314(2), N(1)-N(2) 1.385(2), N(2)-C(2) 1.300(2); N(2)a-Pd(1)-N(2) 180.0, S(1)a-Pd(1)-S(1) 180.0, N(2)-Pd(1)-S(1) 83.28(4), N(2)a-Pd(1)-S(1)a 83.24(4), N(2)-Pd(1)-S(1)a 96.72(4), N(2)a-Pd(1)-S(1) 96.72(4).

Fig. 4. Comparison of antimicrobial activity of compounds **1**-**7** (100 μg).

Fig. 1.

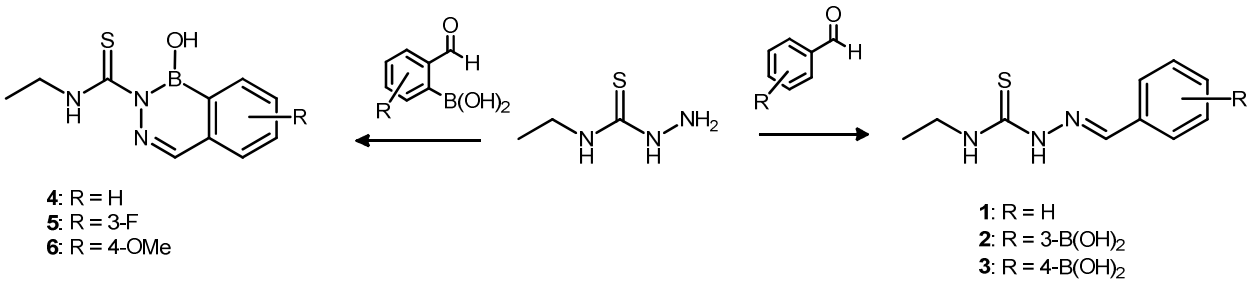


benzodiazaborines (a)

thiosemicarbazides (b)

thiosemicarbazones (c)

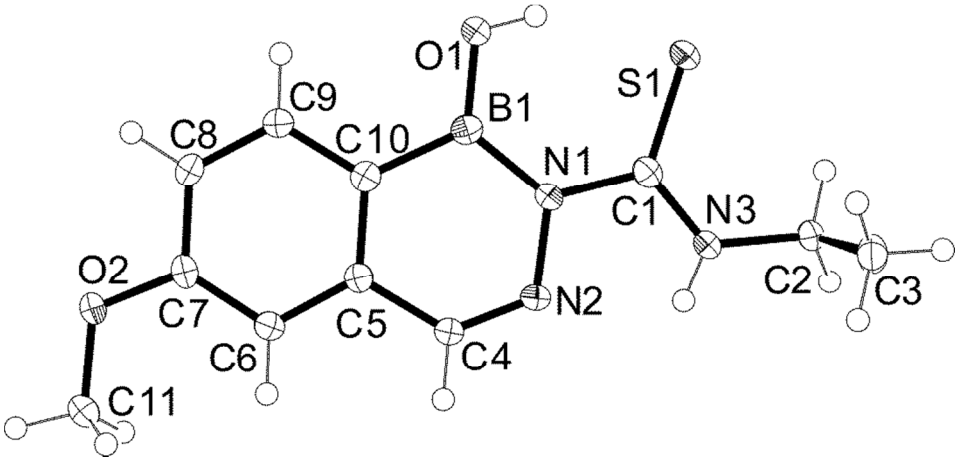
Scheme 1.



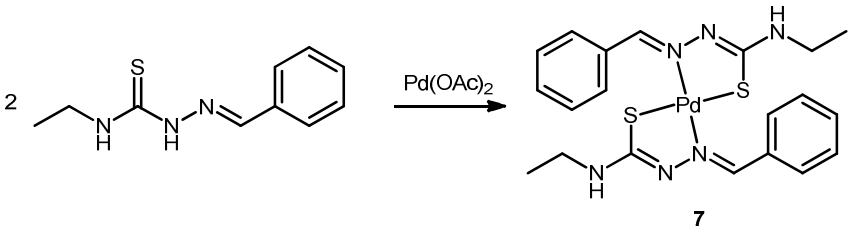
4: R = H
5: R = 3-F
6: R = 4-OMe

1: R = H
2: R = 3-$B(OH)_2$
3: R = 4-$B(OH)_2$

Fig. 2.



Scheme 2.



7

Fig. 3.

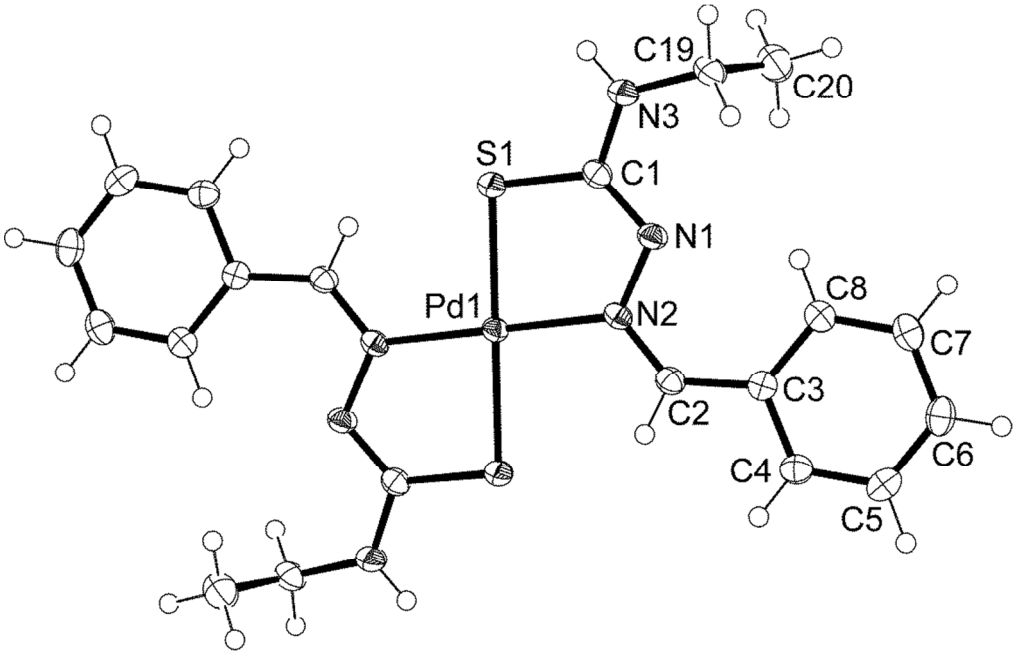
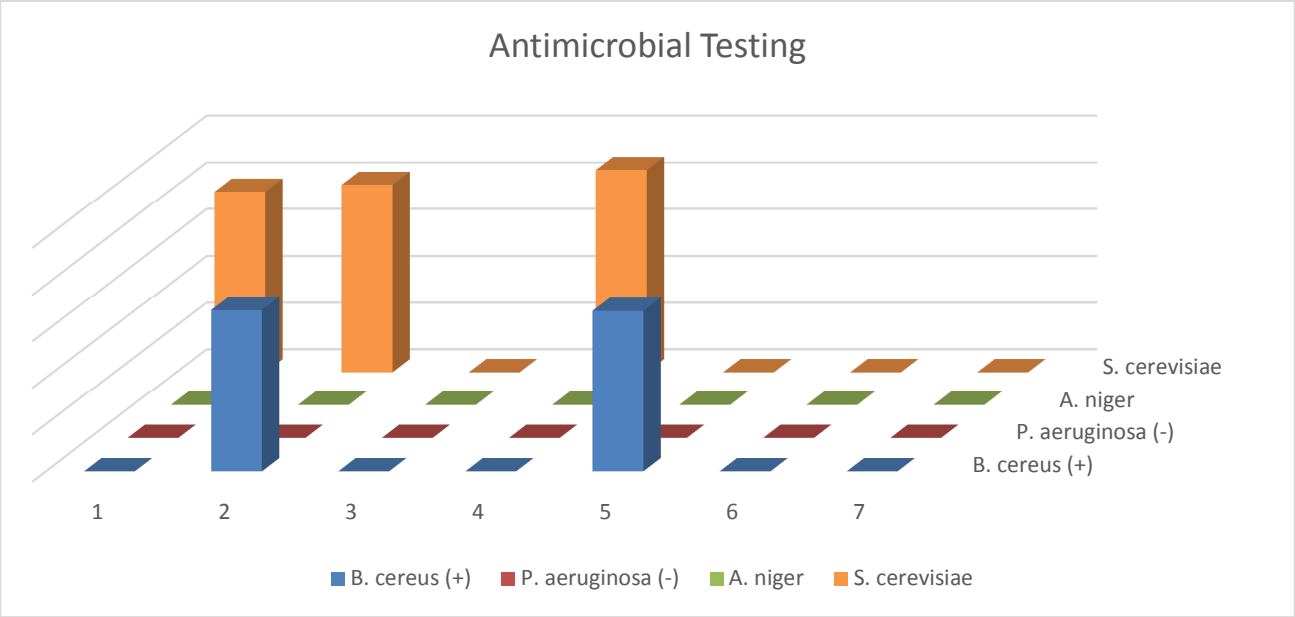


Fig. 4.



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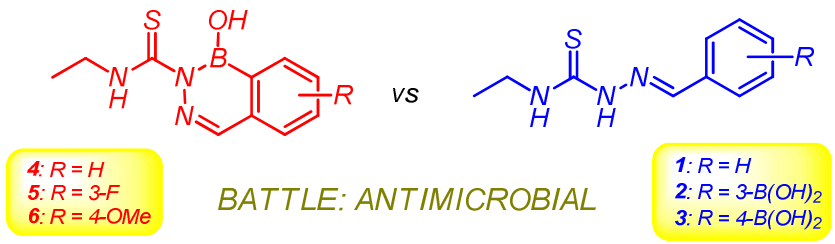
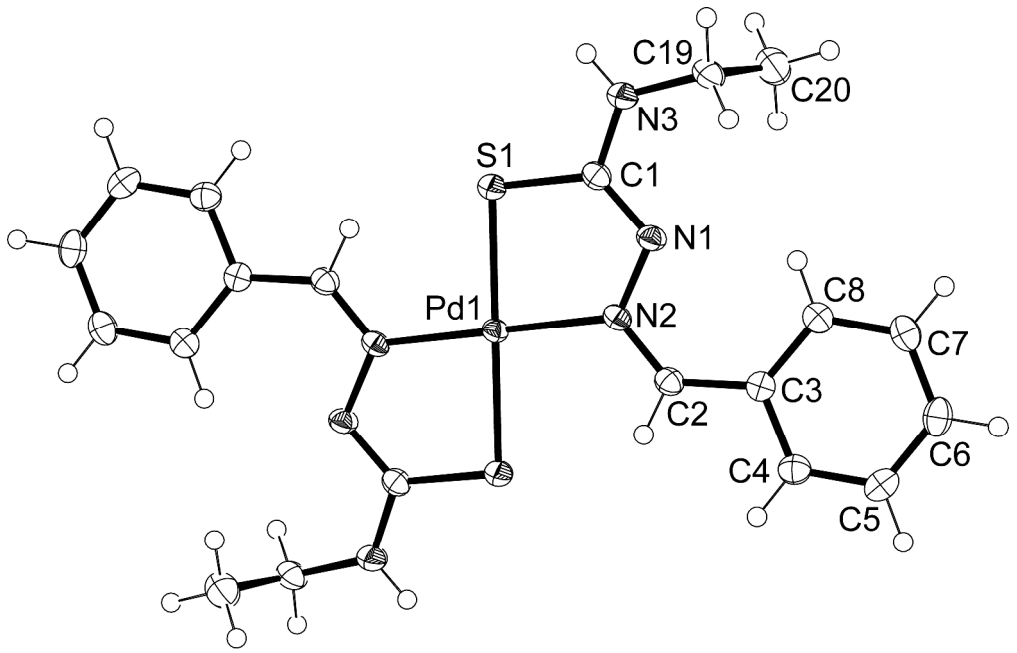
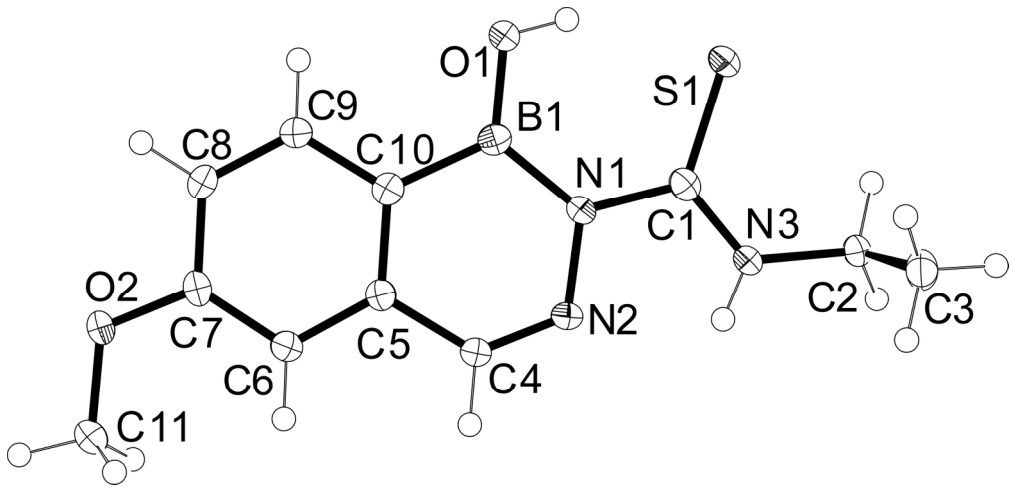
Graphical abstract







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