

SYNTHESES AND EVALUATION OF BENZODIAZABORINE COMPOUNDS AGAINST *M. TUBERCULOSIS* H₃₇R_v IN VITRO

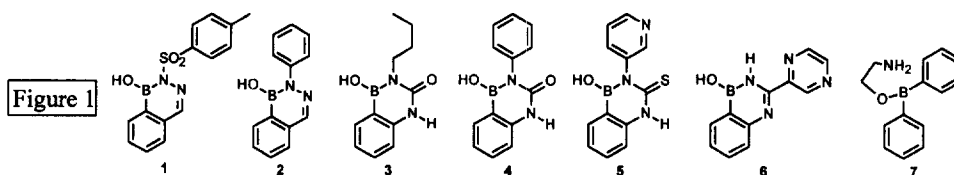
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Abstract: The synthesis of six benzo[e]diazaborine compounds and their in vitro evaluation against *M. tuberculosis* H₃₇R_v is described. The compounds 1,2-dihydro-1-hydroxy-2-phenyl-2,4,1-benzo[e]diazaborin-3(4*H*)-one, **4**, and 1,2-dihydro-1-hydroxy-2-(3-pyridyl)-2,4,1-benzo[e]diazaborin-3(4*H*)-thione, (**5**), showed the greatest inhibitory activity. © 1998 Elsevier Science Ltd. All rights reserved.

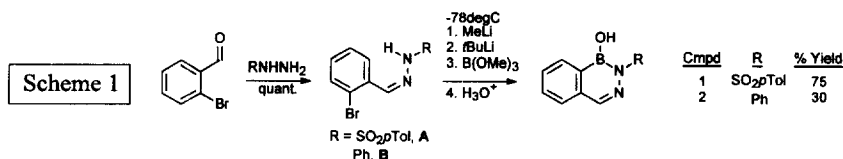
The disease tuberculosis (TB) results from respiratory infection by *M. tuberculosis*¹ and due to the rising incidence of TB worldwide, new drugs are necessary for its treatment. The antibacterial activities of 1,2-dihydro-1-hydroxy-2-(organosulfonyl)-2,3,1-benzo- and thieno[e]diazaborines are well documented in the literature.^{2–5} Recently, it has been proposed that the mechanism of action of diazaborines in *E. coli* is by complexation of NAD⁺ through the boron atom and inhibition of a fatty enoyl-acylcarrierprotein reductase, EnvM.^{6,7} Mycobacteria have a similar enzyme, InhA, required for mycolic acid biosynthesis.⁸ Therefore, we anticipated diazaborine compounds, Figure 1, would exhibit growth inhibitory activity against this organism.



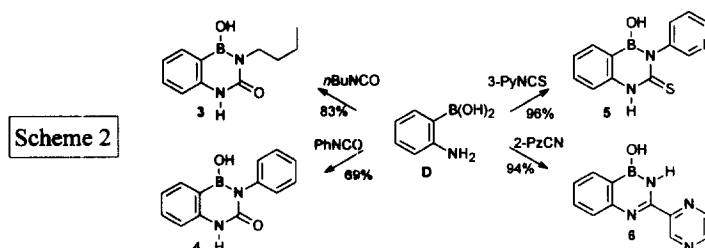
Chemistry

In the 2,3,1-benzo[e]diazaborine series the known structural variation is almost exclusively restricted to the organosulfonyl side chain.⁵ We synthesized 2 previously reported 2,3,1-benzo[e]diazaborines by a dianion method of Sharp and Skinner,⁹ each substituted at N-2 with *p*-tosyl, **1**,⁵ or phenyl, **2**.¹⁰ Also, the isosteric 2,4,1-benzo[e]diazaborines, **3–6**, were synthesized by the procedures of Groziak et al.¹¹ and Hughes et al.¹² and have not been previously reported.

Compounds **1** and **2** were synthesized from the corresponding bromo-hydrazones in a one-pot procedure, Scheme 1.



The 2,4,1-benzo[e]diazaborines **3–6** were prepared by condensation reaction of 2-aminophenylboronic acid,¹¹ **D**, with isocyanates, isothiocyanate and carbonitrile, Scheme 2.



Results and Discussion

The *in vitro* growth inhibition activity of **1–7** and isoniazid (INH) and pyrazinamide (PZA), two ‘first-line’ TB drugs, are shown in Table I. Two different media were used, BACTEC 12B (pH 7)¹³ or 6A (pH 6)¹³, reflecting the different populations of mycobacteria found in infections.¹⁴ None of the compounds has activity such as INH and **1**, **2**, **4**, **5**, and **7** all have greater activity than PZA. For comparison, in *E. coli* **1** has an MIC of 83 μ M⁵ while in *M. tuberculosis* **1** has an MIC of 320 μ M and 40 μ M at pH 7 and 6, respectively. The structure–activity relationship between **3** and **4** is interesting, the exchange of *n*-butyl for phenyl extinguishes the activity of the ligand. However, the combined replacement of sulfur for oxygen and pyridine for phenyl did not change activity, compare **4** and **5**. The lack of activity of **6** may indicate the necessity for carbonyl or thiocarbonyl at C-3. The borinic acid **7** has modest antimycobacterial activity. The preparation and antimycobacterial evaluation of additional members of the 1,4,2-diazaborine series is planned.

Table I. Minimum inhibitory concentration (MIC)[‡] of the target compounds.

| Bactec 6A Media MIC | | COMPD | Bactec 12B Media MIC | |
|---|---------|----------|-------------------------|---------|
| μ g/mL | μ M | | μ g/mL | μ M |
| <0.02 | <0.02 | INH | <0.02 | <0.16 |
| 197 | 1600 | PZA | >197 | >1600 |
| 12 | 40 | 1 | 96 | 320 |
| | | 2 | | |
| >128 | >587 | 3 | >128 | >587 |
| 8 | 33.6 | 4 | 16 | 67.2 |
| 8 | 31.4 | 5 | 16 | 63 |
| >128 | >572 | 6 | 128 | 572 |
| 16 | 71 | 7 | 64 | 285 |
| Compd 1 in <i>E. coli</i> MIC = 83 μ M, 25 μ g/mL ⁵ | | | | |

[‡]MIC is defined as the lowest concentration of test compound that results in 99% growth inhibition.

Experimental Procedures

Susceptibility testing: The radiometric method¹⁵ was used for determining the MIC of test samples against *M. tuberculosis* H₃₇R_v (ATCC 27294) using the BACTEC 460 system¹³ with BACTEC 6A or 12B media¹³. INH,¹⁶ PZA,¹⁶ and 7¹⁷ were purchased and used as received.

Synthetic Procedures

General methods: Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained for all compounds using a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are reported in ppm downfield (δ) from internal tetramethylsilane-*d*₁₂. Elemental analyses were performed by Desert Analytics, Tucson. Purified products were shown to be homogeneous by thin-layer chromatography (TLC) on silica gel plates (1.25 × 3 cm). Column chromatography was performed using low pressure or flash liquid chromatography with glass columns packed with silicic acid,¹⁷ 60 Å (70–230 mesh). All the reactions requiring inert atmosphere were carried out under anhydrous N₂ in oven-dried borosilicate glassware using septum techniques. Solvents and gases were dried accordingly: tetrahydrofuran (THF) was distilled from Na-K alloy; diethyl ether (Et₂O) and dioxane were each distilled from LiAlH₄; N₂ was passed through a column of Drierite. 2-Aminophenylboronic acid (**D**)^{11,12} and 3-isocyanatopyridine were prepared in our laboratory. Alkyl lithium solutions, triisopropoxyboron, phenylisocyanate, pyrazinecarbonitrile, *n*-butylisocyanate and 2-bromobenzaldehyde were purchased and used as received.¹⁷

1,2-Dihydro-1-hydroxy-2-butyl-2,4,1-benzo[*e*]diazaborin-3(4*H*)-one (3).¹⁸ To 76 mg (0.56 mmol) of **D** dissolved in 1 mL of dioxane was added 69 μ L of *n*-butylisocyanate (0.61 mmol). The solution was heated to 70 °C for 5 min then allowed to cool to room temperature. After 2 h a precipitate was filtered and rinsed with CH₃CN. The compound was dried in vacuo to yield 100 mg (83%) as a pale-grey solid.

1,2-Dihydro-1-hydroxy-2-phenyl-2,4,1-benzo[*e*]diazaborin-3(4*H*)-one (4).¹⁸ To 100 mg (0.7 mmol) of **D** dissolved in 1.5 mL of dioxane was added 87 mg (0.08 mL, 0.7 mmol) of phenylisocyanate and the mixture was refluxed for 2 h. After cooling to room temperature, the title compound precipitated as fine crystals, filtered and rinsed with dioxane. Drying in an 80 °C oven provided 0.12 g (69%) of product.

1,2-Dihydro-1-hydroxy-2-(3-pyridyl)-2,4,1-benzo[*e*]diazaborin-3(4*H*)-thione (5).¹⁸ To a solution of 100 mg (0.78 mmol) of **D** in 2 mL of dioxane at room temperature was added dropwise 3-isothiocyanatopyridine (1 equiv, 99.3 mg). With vigorous magnetic stirring the mixture was refluxed for 15 min then cooled to room temperature. The title compound was filtered, rinsed with dioxane, then dried in vacuo to afford 180 mg (96%) of a white powder.

1,2-Dihydro-1-hydroxy-3-(2-pyrazinyl)-2,4,1-benzo[*e*]diazaborine (6).¹⁸ To 25 mL of pyrazinecarbonitrile was suspended 100 mg (0.7 mmol) of **D**. The mixture was refluxed for 2 h. After cooling to room temperature,

a still-head was equipped and the pyrazinecarbonitrile was distilled under reduced pressure. The remaining solid was triturated ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) and filtered, affording 150 mg of a tan amorphous solid (94%).

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- Physical and analytical data: 1,2-Dihydro-1-hydroxy-2-(*p*-tosyl)-2,3,1-benzo[*e*]diazaborine (**1**) mp 150–160 °C (lit. 162–4 °C⁵). ¹H NMR (CDCl_3): δ 2.4–2.46 (s, 3H), 7.3–7.38 (d, 2H), 7.42–7.46 (s, 1H), 7.54–7.78 (m, 3H), 7.9–7.97 (d, 2H), 8.1–8.3 (s, 1H), 8.18–8.25 (dd, 1H). Elemental analysis calcd for $\text{BC}_{14}\text{H}_{13}\text{N}_2\text{O}_3\text{S}$: C, 56; H, 4.3; N, 9.3. Found: C, 55.67; H, 4.42; N, 9.17. 1,2-Dihydro-1-hydroxy-2-phenyl-2,3,1-benzo[*e*]diazaborine (**2**) mp 115–125 °C (lit. 185.5–186.5 °C (anhydride)¹⁰). ¹H NMR (CDCl_3): δ 4.39 (s, 1H), 7.3–7.4 (m, 1H), 7.4–7.6 (d, 3H), 7.6–7.8 (m, 3H), 8.1–8.25 (s, 2H). Elemental analysis calcd for $\text{BC}_{13}\text{H}_{11}\text{N}_2\text{O}$: C, 70.3; H, 4.96; N, 12.6. Found: C, 70.26; H, 4.92; N, 12.35. **3**: mp 195–200 °C. ¹H NMR ($(\text{CD}_3)_2\text{SO}$): δ 10.2 (s, 1H, NH or OH), 9.1 (s, 1H, NH or OH), 7.95 (d, 1H), 7.4 (t, 1H), 7.0 (m, 2H), 3.6 (t, 2H), 1.5 (m, 2H), 1.3 (m, 2H), 0.9 (t, 3H). MS (EI, high-resolution = 10,000) M^+ (218.1222 measured, 218.1229 theoretical). **4**: mp 220 °C (decomp). ¹H NMR ($(\text{CD}_3)_2\text{SO}$): δ 10.4 (s, 1H, NH or OH), 9.05 (s, 1H, NH or OH), 8.0 (d, 1H), 7.34 (dt, 4H), 7.1 (m, 4H). MS (EI, high-resolution = 10,000) M^+ (238.0916 measured; 238.0916 theoretical). **5**: mp 230–235 °C (decomp.). ¹H NMR ($(\text{CD}_3)_2\text{SO}$): δ 12.15 (s, OH or NH), 9.6 (s, OH or NH), 8.5 (d, PyrH), 8.35 (d, PyrH), 8.05 (d, H5), 7.6 (m, 2H), 7.45 (m, 2H), 7.2 (t, PyrH). MS (EI, high resolution = 10,000) M^+ (255.0634 measured, 255.0640 theoretical). **6**: mp 135 °C. ¹H NMR ($(\text{CD}_3)_2\text{SO}$): δ 9.7 (s, NH or OH), 9.5 (s, NH or OH), 8.9 (t, 2H), 8.7 (s, 1H), 8.1 (d, 1H), 7.7 (d, 2H), 7.4 (dd, 1H). MS (EI, high-resolution = 10,000) M^+ (224.0874 measured, 224.0872 theoretical).