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SYNTHESES AND EVALUATION OF BENZODIAZABORINE COMPOUNDS AGAINST *M. TUBERCULOSIS* $H_{37}R_V$ IN VITRO

M. C. Davis, S. G. Franzblau† and A. R. Martin*

Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson 85721, U.S.A. †GWL Hansen's Disease Center at Louisiana State University, Baton Rouge 70894, U.S.A.

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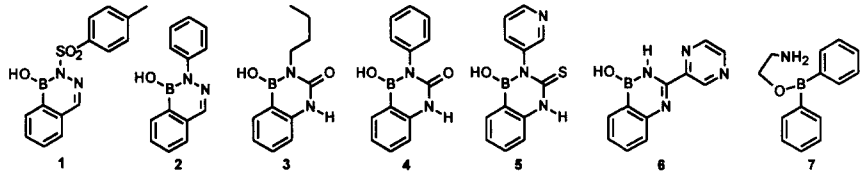
Abstract: The synthesis of six benzo[*e*]diazaborine compounds and thier in vitro evaluation against *M. tuberculosis* $H_{37}R_v$ is described. The compounds 1,2-dihydro-1-hydroxy-2-phenyl-2,4,1-benzo[*e*]diazaborin-3(4*H*)-one, **4**, and 1,2-dihydro-1-hydroxy-2-(3-pyridyl)-2,4,1-benzo[*e*]diazaborin-3(4*H*)-thione, (**5**), showed the greatest inhibitory activity.

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.

Figure 1

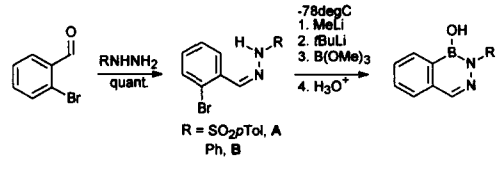


Chemistry

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.

Scheme 1

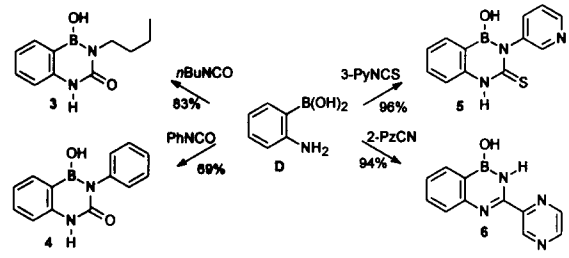


Cmpd	R	% Yield
1	SO_2pTol	75
2	Ph	30

0960-894X/98/$19.00
PII: S0960-894X(98)00126-7

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.

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 μM[5] while in *M. tuberculosis* **1** has an MIC of 320 μM and 40 μM 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			Bactec 12B Media MIC	
μg/mL	μM	COMPD	μg/mL	μM
<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 *E. coli* MIC = 83 μM , 25 μg/mL[5]				

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

Experimental Procedures

Susceptibility testing: The radiometric method[15] was used for determining the MIC of test samples against *M. tuberculosis* $H_{37}R_v$ (ATCC 27294) using the BACTEC 460 system[13] with BACTEC 6A or 12B media[13]. INH,[16] PZA,[16] and **7**[17] 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 N_2 in oven-dried borosilicate glassware using septum techniques. Solvents and gases were dried accordingly: tetrahydrofuran (THF) was distilled from Na-K alloy; diethyl ether (Et_2O) and dioxane were each distilled from $LiAlH_4$; N_2 was passed through a column of Drierite. 2-Aminophenylboronic acid (**D**)[11,12] and 3-isocyanatopyridine were prepared in our laboratory. Alkyllithium solutions, triisopropoxyboron, phenylisocyanate, pyrazinecarbonitrile, *n*-butylisocyanate and 2-bromobenzaldehyde were purchased and used as received.[17]

1,2-Dihydo-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 μL 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 CH_3CN. 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 (CH_2Cl_2/MeOH) and filtered, affording 150 mg of a tan amorphous solid (94%).

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References and Notes

1. Bloom, B. M.; Murray, C. *Science* **1992**, *257*, 1055.
2. Gronowitz, S.; Dahlgren, T.; Namtvedt, J.; Roos, C.; Sjöberg, B.; Forsgren, U. *Acta Pharm. Suec.* **1971**, *8*, 377.
3. Gronowitz, S.; Dahlgren, T.; Namtvedt; J.; Roos, C.; Rosen, G.; Sjöberg, B.; Forsgren, U. *Acta Pharm. Suec.* **1971**, *8*, 623.
4. Bailey, P. J.; Cousins, G.; Snow, G. A.; White, A. J. *Antimicrob. Agents Chemother.* **1980**, *17*, 549.
5. Grassberger, M. A.; Turnowsky, F.; Hildebrandt, J. *J. Med. Chem.* **1984**, *27*, 947.
6. Baldock, C.; Rafferty, J. B.; Sedelnikova, S. E.; Baker, P. J.; Stuitje, A. R.; Slabas, A. R.; Hawkes, T. R.; Rice, D. R. *Science* **1996**, *274*, 2107.
7. Baldock, C., Rafferty, J. B.; Sedelnikova, S. E.; Bithell, S.; Stuitje, A. R.; Slabas, A. R.; Rice, D. W. *Acta Cryst.* **1996**, *D52*, 1181.
8. Quémard, A.; Sacchettini, J. C.; Dessen, A.; Vilcheze, C.; Bittman, R.; Jacobs, W. R.; Blanchard, J. S. *Biochemistry* **1995**, *34*, 8235.
9. Sharp, J. T.; Skinner, C. E. D. *Tetrahedron Lett.* **1986** *27*, 869.
10. Dewar, M.; Dougherty, R. *J. Am. Chem. Soc.* **1964**, *86*, 433.
11. Groziak, M. P.; Ganguly, A. D.; Robinson, P. D. *J. Am. Chem. Soc.* **1994**, *116*, 7597.
12. Hughes, M. P.; Shang, M.; Smith, B. D. *J Org. Chem.* **1996**, *61*, 4510.
13. Becton Dickinson Diagnostic Instrument Systems, Sparks, MD.
14. Young, D. B.; Duncan, K. *Annual Rev. Microbiol.* **1995**, *49*, 641.
15. Heifets, L. B.; Lindholm-Levy, P. J. *Tubercle* **1986**, *68*, 267.
16. Sigma Chemical Co., St. Louis, MO.
17. Aldrich Chemical Co., WI.
18. 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 °C[5]). ^{1}H 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 $BC_{14}H_{13}N_2O_3S$: 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]) ^{1}H 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 $BC_{13}H_{11}N_2O$: C, 70.3; H, 4.96; N, 12.6. Found: C, 70.26; H, 4.92; N, 12.35. **3**: mp 195–200 °C. ^{1}H NMR ($(CD_3)_2SO$): 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). ^{1}H NMR ($(CD_3)_2SO$): 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.). ^{1}H NMR ($(CD_3)_2SO$): 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. ^{1}H NMR ($(CD_3)_2SO$): 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).