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Synthesis and evaluation of functionalized benzoboroxoles as potential *anti*-tuberculosis agents

Mohammad A. Alam[a], **Kriti Arora**[b], **Shirisha Gurrapu**[c], **Sravan K. Jonnalagadda**[c], **Grady L. Nelson**[c], **Paul Kiprof**[d], **Subash C. Jonnalagadda**[a,e,*], and **Venkatram R. Mereddy**[c,d,f,*]

[a]Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08028, USA

[b]Tuberculosis Research Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland 20892, USA

[c]Integrated Biosciences Graduate Program, University of Minnesota, MN 55812, USA

[d]Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN 55812, USA

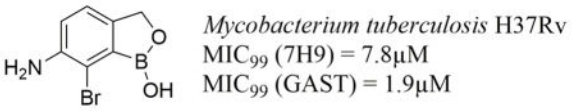
[e]Department of Biomedical and Translational Sciences, Rowan University, Glassboro, NJ 08028, USA

[f]Department of Pharmacy Practice and Pharmaceutical Sciences, University of Minnesota, Duluth, MN 55812, USA

Abstract

Several derivatives of aminobenzoboroxole have been prepared starting from 2-boronobenzaldehyde. All of these derivatives have been evaluated for their *anti*-mycobacterial activity on *Mycobacterium smegmatis* and cytotoxicity on breast cancer cell line MCF7. Based on these studies, all the tested molecules have been found to be generally non-toxic and benzoboroxoles with unsubstituted (primary) amines have been found to exhibit good *anti*-mycobacterial activity. Some of the key compounds have been evaluated for their *anti*-tubercular activity on *Mycobacterium tuberculosis* H37Rv using 7H9 and GAST media. 7-Bromo-6-aminobenzoboroxole **4** has been identified as the lead candidate compound for further development.

Graphical Abstract



*Corresponding author. Tel.: +1-856-256-5452; fax: +1- 856-256-4478; jonnalagadda@rowan.edu.

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Keywords

Benzoxaboroles; nitrobenzoxaborole; aminobenzoxaborole; bromoaminobenzoxaborole; anti-tuberculosis agents

Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is a highly contagious chronic bacterial infection.[1] Nearly one third of world's population has been affected by latent tuberculosis.[1a] This infection spreads through the air and causes millions of deaths every year. The standard treatment of tuberculosis includes a four-drug regimen of isoniazid, rifampicin, ethambutol, and pyrazinamide for six to nine months. The emergence of multi-drug resistant (MDR-TB) and extensively drug resistant strains of this bacterium is a cause of great concern worldwide (eg. WHO estimates indicate that nearly half million people globally have developed MDR-TB).[1a] Hence, new and highly effective therapeutic strategies are urgently required for the better treatment of this epidemic. Owing to the global importance of this disease, there has been an explosive growth of publications in the area of tuberculosis research.[2] Despite this progress, discovery of biologically active small molecules with novel chemical entities is extremely critical to combat this disease in future.

Benzoboroxoles are cyclic boronic acids that are highly stable under strongly acidic and basic conditions.[3] These compounds have been found to have valuable applications in materials[4] and medicinal[3,5] chemistry. While this work was in progress, a patent application has been recently published on the usage of tricyclic benzoboroxoles as *anti*-mycobacterial agents.[6] Our long-standing interest[7] in developing novel functionalized boronic acids and benzoboroxoles as therapeutic agents prompted us to explore the utility of these scaffolds as potential *anti*-tubercular agents.

Results and Discussion

Based on the literature reports as well as our previous experience, it was found that the biological activity of benzoboroxoles greatly diminished upon substitution at the benzylic carbon in the oxaborole ring. Hence, we envisioned the preparation of aromatic ring-substituted benzoboroxoles while leaving the oxaborole methylene group unsubstituted. In this regard, we chose 6-aminobenzoboroxole **3** as the common synthon for the preparation of various functionalized derivatives. The amine **3** was prepared in three steps starting from 2-boronobenzaldehyde (Scheme 1).[7a,8]

The reaction of 2-boronobenzaldehyde with sodium borohydride in THF and water provided benzoboroxoles **1** in 90% yield. Nitration[8a] of **1** with fuming nitric acid followed by reduction of nitro group with ammonium formate in the presence of palladium carbon resulted in the formation of 6-aminobenzoboroxole **3**. Electrophilic aromatic bromination of **3** with bromine in acetic acid provided 7-bromo-6- aminobenzoboroxole **4** in 55% yield along with ~20% of 5,7-dibromo-6-aminobenzoboroxole **5**. Pure **4** could be readily obtained upon simple silica gel column chromatography using hexanes and ethyl acetate as eluents. Addition of excess bromine (2.5 eq) to **3** yielded 53% of **5** (Scheme 1).

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The 6-aminobenzoboroxole **3** was then subjected to reductive amination to prepare monosubstituted derivatives of the amino group.[7a] 6-Aminobenzoboroxole was treated with benzaldehyde, 4-flourobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, and 2-furaldehyde in methanol to form the intermediate imines, followed by reduction of the imines with sodium borohydride to obtain *N*-monoalkylated 6-aminobenzo boroxoles **6–10** respectively (Scheme 2).

The 6-aminobenzoboroxole **3** was further derivatized via alkylation of the amine with alkyl and benzyl halides. Amine **3** was treated with allyl bromide, propargyl bromide, 1,4-dibromobutane, benzyl bromide[9] and *p*-xylyl bromide in the presence of potassium carbonate and DMSO to obtain the *N,N*-dialkylated aminobenzoboroxoles **11–15** respectively (Scheme 3).

The 6-aminobenzoboroxole **3** was also functionalized as the corresponding amides **16–20** upon reaction with acetic anhydride[10], butyric anhydride, trifloroacetic anhydride, succinic anhydride[11], and phthalic anhydride respectively (Scheme 4). The amide with phthalic anhydride cyclized intramolecularly to provide the cylic imide **20**. Similarly, a sulfonamide derivative **21** was also prepared by treating the amine **3** with 4-nitrobenzenesulfonyl chloride.[12]

After synthesizing various functionalized benzoboroxoles, these molecules **1–21** were evaluated for their antimycobacterial activity. Before testing their antimycobacterial potential, all the molecules were evaluated for their general cytotoxicity against breast cancer cell line MCF-7. All of the tested compounds did not exhibit any significant cytotoxicity even at 50μM. Encouraged by their lack of cytotoxicity, we screened them for their biological activity against *Mycobacterium smegmatis* (*M. smegmatis*). This strain is especially useful in screening large chemical libraries because of the microbe's speed of growth and its non-pathogenic nature that allows its use in biosafety level II hoods. Several laboratories have reported on the effectiveness of *M. smegmatis* as a surrogate screen for compounds that inhibit the growth of *M. tuberculosis*.[13] We employed Kirby-Bauer disk method for the identification of lead compounds. The compounds that exhibited >3 cm zone of inhibition were further tested for their efficacy against *M. tuberculosis*. Unfortunately, most of the amine substituted derivatives exhibited weak or no inhibitory properties in the Kirby-Bauer disk method. Of all the derivatives, the 6-aminobenzoxaborole **3**, 7-bromo-6-aminobenzoboroxole **4** and 5,7-dibromo-6-aminobenzoboroxole **5** were found to be the most active with zone of inhibition values >5 cm. However, **5** was not selected for further studies due to its high bromine content which may preclude its suitability as a pharmaceutical agent. Consequently, the two aminobenzoboroxoles **3** and **4** were further subjected to detailed biological evaluation against *M. tuberculosis*.

Initially, parent benzoboroxole **1**, amines **3** and **4** were evaluated for their minimum inhibitory concentration (MIC_{99}) under aerobic conditions. Isoniazid was used as the positive control. Compared to **1**, the amine derivatives **3** and **4** exhibited superior inhibitory properties. The amine **3** was found to be ~2–4 times more potent than **1** whereas the bromo derivative **4** was found to be ~8–16 times more potent than **1** in 7H9 medium. The MIC_{99} values of **1**, **3** and **4** were also determined in more sensitive glycerol-alanine-salts-tween

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(GAST) medium.[14] In this study, **3** was found to be ~4 times more potent than **1** and **4** was found to be ~30 times more potent than **1** (Table 1).

We then carried out the minimum bactericidal concentration of **4** under aerobic conditions to evaluate whether these compounds were bacteriostatic or bactericidal. In this case, rifampicin was used as the positive control and DMSO was used as the negative control. We also evaluated the effectiveness of the hydrochloride salt of **4** under these conditions. This compound as well as its hydrochloride salt were found to be bacteriostatic at lower concentrations but at higher concentration of >40μM, they were found to exhibit good bactericidal properties (Table 2).

We then carried out the minimum anaerobicidal concentration study to determine if the candidate compounds have activity under conditions of dormancy. There is very little metabolic activity under these conditions hence most compounds including clinically used isoniazid fail to provide therapeutic benefit. Metronidazole was used as the positive control and DMSO was used as the negative control in this study. Unfortunately, aminobenzoboroxole **4** and its salt did not exhibit any significant activity at lower or higher concentrations (10 and 100μM) under these conditions (Table 3).

Finally, we also carried out the intracellular macrophage-killing assay, which provides information about the ability of the test compounds to kill in macrophages, which are the natural hosts of Mtb. If a compound shows killing of Mtb in macrophages then it has better chances of showing efficacy in an animal model of disease. The candidate benzoboroxole **4** and its salt showed some cidality in the macrophages but not as much as the positive control INH (Table 4).

Based on the results, complete loss in biological activity was observed upon structural changes on the 6-position primary amino group in benzoboroxole. We observed that the conversion of primary amine to secondary amines (eg. compounds 6–10, Scheme 2), tertiary amines (eg. compounds 11–15, Scheme 3), as well as amides and sulphonamides (eg. compounds 16–21, Scheme 4) resulted in significant loss in biological acvitiy. However, some of the compounds such as 6-aminobenzoboroxole **3**, 7-bromo-6-aminobenzoboroxole **4**, and 5,7-dibromo-6-aminobenzoboroxole **5** exhibit good biological activity, and efforts are on going towards their development as anti-tuberculosis agents.

Conclusion

In conclusion, we have synthesized several derivatives of 6- aminobenzoboroxole starting from 2-boronobenzaldehyde. Some of the protocols employed in the synthesis include electrophilic aromatic bromination, reductive amination, dialkylation, and amide formation. All the synthesized derivatives have been evaluated for their zone of inhibition against *M. smegmatis* and cytotoxic properties against MCF7 cell line. From this study, two lead derivatives **3** and **4** have been identified for further *anti*-mycobacterial activity against *M. tuberculosis* H37Rv using 7H9 and GAST media. **3** and **4** have been found to be several times more potent than their parent benzoboroxole **1** in both these assays. Some of the other biology studies on **3** and **4** include minimum bactericidal concentration under aerobic and

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anaerobic conditions. Based on all these studies, 7-bromo-6- aminobenzoboroxole **4** has been identified as the lead candidate compound for further structure-activity studies to identify a derivative that exhibits much superior properties than the existing clinically used drugs.

Experimental Section

General Methods

All operations were carried out under an inert atmosphere of nitrogen. Glassware for all reactions was oven dried at 125 ºC and cooled under nitrogen prior to use. Liquid reagents and solvents were introduced by oven-dried syringes or cannulas through septa sealed flasks under a nitrogen atmosphere. THF was distilled from sodium benzophenone ketyl. All other solvents and reagents were purchased and used without further purification. The ^{1}H and ^{13}C NMR spectra were plotted on a Bruker-500 spectrometer fitted with a Quad probe. Elemental analysis was performed on Perkin Elmer PE2400 CHN analyzer.

6-amino-7-bromobenzo[c][1,2]oxaborol-1(3H)-ol (4)

To a stirred solution of aminobenzoboroxole **3** (1mmol) in acetic acid (4 mL) was added bromine (1 mmol) at 0°C and stirred for 5h. Upon completion via thin layer chromatography (TLC), reaction mixture was quenched by the addition of saturated $NaHCO_3$ followed by extraction with ethyl acetate (3×15mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated under vacuum and purified via silica gel column chromatography (hexanes: ethyl acetate) to obtain pure compound **4** (55%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 5.12 (s, 2H), 4.81 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 145.2, 143.7, 121.4, 119.5, 109.5, 69.1; CHN Analysis: (Found: C, 36.85%; H, 3.25%; N, 6.25%; $C_7H_7BBrNO_2$ requires: C, 36.90%; H, 3.10%; N, 6.15%).

6-amino-5,7-dibromobenzo[c][1,2]oxaborol-1(3H)-ol (5)

To a stirred solution of 6-aminobenzoboroxole **3** (1mmol) in acetic acid (5 mL) was added bromine (2.5 mmol) and stirred for 5h. Upon completion (TLC), the reaction mixture was quenched by the addition of saturated $NaHCO_3$ and extracted with ethyl acetate (3×15mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under vacuum and purified via silica gel column chromatography (hexanes: ethyl acetate) to obtain pure compound **5** (53%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.11 (s, 1H), 7.51 (s, 1H), 5.24 (s, 2H), 4.85 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 142.3, 142.0, 125.3, 112.3, 109.9, 68.7; CHN Analysis: (Found: C, 27.12%; H, 2.05%; N, 4.34%; $C_7H_6BBr_2NO_2$ requires: C, 27.41%; H, 1.97%; N, 4.57%)

6-(benzylamino)benzo[c][1,2]oxaborol-1(3H)-ol (6)

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.91 (s, 1H), 7.37-7.24 (m, 4H), 7.22 (t, J = 7.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.87 (s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.23 (br s, 1H), 4.82 (s, 2H), 4.28 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.3, 142.0, 140.8, 128.7, 127.6, 127.0, 121.9, 117.0, 112.7, 70.0, 47.1; CHN Analysis: (Found: C, 70.56%; H, 5.78%; N, 5.62%; $C_{14}H_{14}BNO_2$ requires: C, 70.33%; H, 5.90%; N, 5.86%)

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6-((4-fluorobenzyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (7)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.91 (s, 1H), 7.41-7.38 (m, 2H), 7.16-7.07 (m, 3H), 6.86 (s, 1H), 6.76 (d, J = 8.5 Hz, 1H), 6.24 (t, J = 6.0 Hz, 1H), 4.82 (s, 2H), 4.27 (d, J = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.6 (d, J_F = 300.0 Hz), 148.1, 142.1, 136.9, 129.4, 122.0, 117.1, 115.4 (d, J_F = 26.2 Hz), 112.8, 70.0, 46.4; CHN Analysis: (Found: C, 65.24%; H, 4.97%; N, 5.60%; $C_{14}H_{13}BFNO_2$ requires: C, 65.41%; H, 5.10%; N, 5.45%).

6-((4-chlorobenzyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (8)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.92 (s, 1H), 7.37-7.22 (m, 4H), 7.07 (d, J = 8.0 Hz, 1H), 6.84 (s, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.28 (t, J = 6.2 Hz, 1H), 4.82 (s, 2H), 4.28 (d, J = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.0, 142.2, 140.0, 131.5, 129.4, 128.7, 122.0, 117.0, 112.8, 70.0, 46.4; CHN Analysis: (Found: C, 61.39%; H, 4.85%; N, 4.95%; $C_{14}H_{13}BClNO_2$ requires: C, 61.48%; H, 4.79%; N, 5.12%).

6-((4-bromobenzyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (9)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.92 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 8.5 Hz, 1H), 6.83 (s, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.28 (br s, 1H), 4.82 (s, 2H), 4.26 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.0, 142.2, 140.4, 131.6, 129.8, 122.0, 119.9, 117.1, 112.8, 70.0, 46.5; CHN Analysis: (Found: C, 52.72%; H, 4.02%; N, 4.49%; $C_{14}H_{13}BBrNO_2$ requires: C, 52.88%; H, 4.12%; N, 4.41%).

6-((furan-2-ylmethyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (10)

^{1}H NMR (500MHz, DMSO-d_6) δ 8.94 (s, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.10 (d, J = 8.5 Hz, 1H), 6.95 (s, 1H), 6.81-6.84 (m, 1H), 6.39-6.28 (m, 2H), 6.06 (t, J = 6.2 Hz, 1H), 4.84 (s, 2H), 4.25 (d, J = 6.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.9, 147.9, 142.3, 121.9, 117.1, 112.9, 110.8, 107.3, 70.0, 31.2; CHN Analysis: (Found: C, 62.79%; H, 5.37%; N, 6.20%; $C_{12}H_{12}BNO_3$ requires: C, 62.93%; H, 5.28%; N, 6.12%).

6-(diallylamino)benzo[c][1,2]oxaborol-1(3H)-ol (11)

To a stirred solution of aminobenzoboroxole **3** (1mmol) in DMSO (5 mL) was added allyl bromide (6 mmol) and K_2CO_3 (3.0 mmol). The reaction mixture was stirred for 12h. Upon completion (TLC), reaction mixture was extracted with ethyl acetate and water. The combined organic layers were dried over anhydrous $MgSO_4$, concentrated under vacuum and purified via silica gel column chromatography (hexanes: ethyl acetate) to obtain pure compound **10** (76%). ^{1}H NMR (500MHz, DMSO-d_6): δ 8.97 (s, 1H), 7.17 (d, J = 8.0 Hz, 1H), 7.03 (s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 5.90-5.84 (m, 2H), 5.18-5.12 (m, 4H), 4.87 (s, 2H), 3.94-3.91 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 147.9, 141.9, 134.9, 128.6, 122.0, 116.3, 113.5, 70.0, 53.1; CHN Analysis: (Found: C, 68.25%; H, 6.92%; N, 6.02%; $C_{13}H_{16}BNO_2$ requires: C, 68.16%; H, 7.04%; N, 6.11%).

6-(di(prop-2-yn-1-yl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (12)

^{1}H NMR (500MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.30-7.27 (m, 2H), 7.11 (d, J = 8.5 Hz, 1H), 4.92 (s, 2H), 4.14 (s, 4H), 2.51 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 147.1, 145.1,

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122.1, 119.7, 116.9, 80.3, 75.6, 70.0, 40.8; CHN Analysis: (Found: C, 69.23%; H, 5.29%; N, 6.29%; $C_{13}H_{12}BNO_2$ requires: C, 69.38%; H, 5.37%; N, 6.22%).

6-(pyrrolidin-1-yl)benzo[c][1,2]oxaborol-1(3H)-ol (13)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.94 (s, 1H), 7.18 (d, J = 8.5 Hz, 1H), 6.86 (s, 1H), 6.69 (d, J = 8.5 Hz, 1H), 4.87 (s, 2H), 3.22 (t, J = 6.0 Hz, 4H), 1.97-1.95 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 147.6, 141.1, 122.0, 115.7, 112.7, 70.0, 48.0, 25.4; CHN Analysis: (Found: C, 64.96%; H, 7.02%; N, 6.79%; $C_{11}H_{14}BNO_2$ requires: C, 65.07%; H, 6.95%; N, 6.90%).

6-(dibenzylamino)benzo[c][1,2]oxaborol-1(3H)-ol (14)

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.95 (s, 1H), 7.35-7.23 (m, 10H), 7.12 (d, J = 8.5 Hz, 1H), 7.07 (s, 1H), 6.84 (d, J = 8.0 Hz, 1H), 4.84 (s, 2H), 4.71 (s, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.0, 142.2, 139.4, 128.9, 127.2, 127.1, 122.1, 116.4, 113.6, 70.0, 54.8; CHN Analysis: (Found: C, 76.35%; H, 6.30%; N, 4.16%; $C_{21}H_{20}BNO_2$ requires: C, 76.62%; H, 6.12%; N, 4.25%).

6-(bis(4-methylbenzyl)amino)benzo[c][1,2]oxaborol-1(3H)-ol (15)

^{1}H NMR (500MHz, DMSO-d_6): δ 8.97 (s, 1H), 7.21-7.07 (m, 10H), 6.83-6.85 (m, 1H), 4.85 (s, 2H), 4.62 (s, 4H), 2.28 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.2, 142.2, 136.3, 129.5, 129.1, 127.1, 122.1, 116.6, 113.8, 70.0, 54.6, 21.2; CHN Analysis: (Found: C, 77.20%; H, 6.62%; N, 3.83%; $C_{23}H_{24}BNO_2$ requires: C, 77.33%; H, 6.77%; N, 3.92%).

N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)acetamide (16)

To a stirred solution of aminobenzoboroxole **3** (1mmol) in dioxane (5 mL) was added acetic anhydride (1.2 mmol) and stirred for 1h. Upon completion (TLC), reaction mixture was quenched by the addition of dilute HCl followed by extraction with ethyl acetate. The organic layers were dried under anhydrous $MgSO_4$, concentrated under vacuum and purified via silica gel column chromatography (hexanes: ethyl acetate) to obtain amidobenzoboroxole **16** (67%).

N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6yl)butyramide (17)

^{1}H NMR (500MHz, DMSO-d_6): δ 9.89 (s, 1H), 9.20 (s, 1H), 8.02 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 4.94 (s, 2H), 2.31 (t, J = 7.2 Hz, 2H), 1.66-1.61 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.5, 148.9, 138.6, 122.8, 121.9, 121.5, 70.2, 38.8, 19.1, 14.1; CHN Analysis: (Found: C, 60.13%; H, 6.38%; N, 6.45%; $C_{11}H_{14}BNO_3$ requires: C, 60.32%; H, 6.44%; N, 6.39%).

2,2,2-trifluoro-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)acetamide (18)

^{1}H NMR (500MHz, DMSO-d_6): δ 11.30 (s, 1H), 9.32 (s, 1H), 8.07 (s, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 4.99 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.1 (q, J_F = 36.0 Hz), 151.6, 135.5, 124.6, 123.6, 122.4, 118.5 (q, J_F = 285.0 Hz), 70.2; CHN Analysis: (Found: C, 44.01%; H, 2.99%; N, 5.60%; $C_9H_7BF_3NO_3$ requires: C, 44.13%; H, 2.88%; N, 5.72%).

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4-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)amino)-4-oxobutanoic acid (19)

^{1}H NMR (500MHz, DMSO-d_6): δ 12.13 (br s, 1H), 9.99 (s, 1H), 9.20 (s, 1H), 8.01 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 4.94 (s, 2H), 2.55 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.4, 170.5, 148.9, 138.6, 122.6, 121.93, 121.3, 70.2, 31.5, 29.3; CHN Analysis: (Found: C, 53.18%; H, 4.74%; N, 5.49%; $C_{11}H_{12}BNO_5$ requires: C, 53.05%; H, 4.86%; N, 5.62%).

2-((1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)carbamoyl)benzoic acid (20)

^{1}H NMR (500MHz, DMSO-d_6): δ 9.36 (s, 1H), 8.00-7.93 (m, 4H), 7.78 (s, 1H), 7.59-7.55 (m, 2H), 5.08 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.7, 154.1, 135.2, 132.1, 131.2, 130.3, 129.8, 123.9, 122.5, 70.4; CHN Analysis: (Found: C, 64.38%; H, 3.44%; N, 5.18%; $C_{15}H_{10}BNO_4$ requires: C, 64.56%; H, 3.61%; N, 5.02%).

N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-4-nitrobenzene sulfonamide (21)

To a stirred solution of aminobenzoboroxole **3** (1mmol) in dioxane (5 mL) was added 4-nitrobenzenesulfonyl chloride (1.1 mmol) and stirred for 1h. Upon completion (TLC), reaction mixture was quenched by the addition of dilute HCl followed by extraction with ethyl acetate. The combined organic layers were dried over anhydrous $MgSO_4$, concentrated under vacuum and purified by silica gel column chromatography (hexanes: ethyl acetate) to obtain pure compound **21** (40%). ^{1}H NMR (500MHz, DMSO-d_6): δ 9.34 (s, 1H), 7.99-7.90 (m, 4H), 7.76 (s, 1H), 7.57-7.52 (m, 2H), 5.06 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.7, 154.1, 135.2, 132.1, 131.1, 130.3, 129.8, 123.9, 122.4, 70.4; CHN Analysis: (Found: C, 46.64%; H, 3.19%; N, 8.25%; $C_{13}H_{11}BN_2O_6S$ requires: C, 46.73%; H, 3.32%; N, 8.38%).

Sulforhodamine-B assay for cytotoxicity

MCF-7 cells were cultured in Iscove's Modified Eagle Medium containing 10% Hyclone-III and 1% antibiotic (500,000 units penicillin-streptomycin). The cells were seeded at a concentration of ~2×10^4 cells per well in 48 well plates and incubated for 18–24 hours at 37ºC and 5% CO_2 atmosphere. The test compounds were initially diluted in DMSO and further diluted 1000 times in growth media so that the final DMSO concentration was <0.1%. Growth media was removed from 48 well plates and test compound in 400μL of growth media were added. Taxol was used as the positive control whereas DMSO and growth media were used as negative controls. All the compounds were tested in triplicates at 50 and 10μM. The plates were incubated for 72 hours and growth media was removed. The wells were washed with 1mL of 1% DPBS and the plates were dried. 100μL of 0.5% SRB (in 1% acetic acid) was added in each well and incubated at 37ºC for 45 minutes. The wells were washed 5 times with 1% acetic acid solution to remove residual SRB and the plates were dried. The cellular protein was dissolved in 400μL of 10mM tris base (pH 10.2) and absorbance was recorded at 540nm. %Survival was calculated using the formula (Absorbance of test compound/Absorbance of DMSO control) × 100%.

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Bacterial strains, media and growth conditions

M. tuberculosis H37Rv was grown in Middlebrook 7H9 broth medium (Difco) supplemented with 0.2% (v/v) glycerol, 0.05% (v/v) tween 80 and albumin dextrose catalase (ADC): 5g/L bovine serum albumin (BSA); 2g/L dextrose; and 0.81g/L NaCl. *M. tuberculosis* was also cultured in GAST medium consisting of 0.3g/L bacto-casitone, 4g/L dibasic potassium phosphate, 2g/L citric acid, 1g/L L-alanine, 1.2g/L magnesium chloride hexahydrate, 0.6g/L potassium sulfate, 2g/L ammonium chloride, 1% glycerol (v/v) and 0.05% tween 80 (v/v) at pH 6.6. For generating non-replicating persistent cells *M. tuberculosis* was cultured in Middlebrook Dubos medium (Difco) supplemented with 5g/L BSA, 7.5g/L glucose and 0.81g/L NaCl. For determination of viable cell counts, *M. tuberculosis* cells were plated on Middlebrook 7H11 plates supplemented with 0.5% glycerol (v/v) and oleic albumin dextrose catalase (OADC): 0.06% oleic acid; 4mg/L NaOH; 50g/L BSA; 2g/L dextrose; and 0.81g/L NaCl. All cultures were incubated at 37ºC.

Minimum inhibitory concentration (MIC_{99})

MIC_{99} was determined in 7H9 as well as GAST medium by micro-dilution broth method. Briefly, *M. tuberculosis* was grown to an optical density at 650nm (OD_{650}) of 0.2–0.3 in 7H9 or GAST medium. Cells were diluted to final OD_{650} of 0.0002 (1:1000 of parent culture) in desired medium. Ten-fold serial dilution of the test compounds was made in triplicate rows of a 96-well plate. The above dilution of cells was then added to all the wells of the 96-well plate. The assay was performed in duplicate and each compound dilution was set-up in triplicate rows per plate. The plates were incubated at 37ºC and observations were noted on day-7, and day-14. MIC_{99} was noted as the lowest concentration of test compound that inhibited visible growth after 14 days of incubation.

Minimum bactericidal concentration

M. tuberculosis was grown to OD_{650} 0.2–0.3 in 7H9 medium and cells were then diluted to OD_{650} 0.002 (1:100 of parent culture). For enumeration of viable numbers on day 0, serial dilutions of the diluted cultures were plated in duplicate on 7H11 plates (supplemented with OADC). 1mL aliquots of the diluted culture were then dispensed in duplicate wells of 24-well plates. Treatments with chosen test compounds were carried out at 4μM and 40μM for 7 days. Serial dilutions were then plated on 7H11 medium (supplemented with OADC) and colonies were counted after 3-week incubation at 37ºC and CFU/ml were determined.

Minimum anaerobicidal concentration

Non-replicating persistent (NRP) cells of *M. tuberculosis* were obtained by culturing the cells in Wayne model system.[15] Briefly, *M. tuberculosis* was cultured in Dubos medium to an OD_{650} of 0.2–0.3. Cells were then diluted to OD_{650} 0.005 and dispensed in glass bottles leaving a head-space ratio of 0.5. The bottles were sealed with wax and placed on a magnetic stir plate at 37ºC. The cells were allowed to stir for 21 days at which point the culture reached NRP2 state. The bottles were removed from the stir plate and opened in an anaerobic chamber. For determination of day 0 cell numbers, serial dilutions of the culture were plated on 7H11 plates (supplemented with OADC). 1mL cells were dispensed in duplicate wells of 24-well plates and treated with the desired concentrations of the candidate

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compounds for 7 days in the anaerobic chamber at 37ºC. After 7 days, the plates were removed from the chamber and serial dilutions were then made and plated on 7H11 plates (supplemented with OADC). Viable numbers were counted after 4 weeks of incubation at 37ºC and CFU/ml was determined.

Intracellular macrophage killing

Bone marrow derived macrophages were obtained from C57/BL6 mice. Briefly femurs obtained from the mice were flushed with RPMI supplemented with 10% heat-inactivated fetal bovine serum (RPMI+10% FBS). The collected material was then strained through a 70μm nylon strainer. The cells were counted in cellometer and plated at a density of 4×10^5 cells/ml in tissue culture petri-dishes in RPMI medium containing 20% FBS, 1% penicillin-streptomycin, 40% L-cell supernatant and allowed to adhere for 7 days at 37ºC with medium replenishment on day 3. After the monocytes had differentiated in to macrophages the adherent cells were removed by discarding the medium and rinsing the monolayer with ice-cold 1XPBS+3mM EDTA. The removed cells were collected by centrifugation at 400Xg for 10mins at 4ºC. The cells were resuspended in fresh RPMI+10%FBS and counted. The cells were then replated in wells of 24 well plates at a density of 5×10^5 cells/well and left overnight at 37ºC to adhere. Cells were then infected with a culture of Mtb grown in 7H9 to OD_{650}=0.2–0.3. The bacterial culture was diluted to 5×10^5 CFU/ml (OD_{650}=0.2 is approximately 2×10^8 CFU/ml) and added to the wells of the 24-well plates (m.o.i=1). Infection was allowed to establish for 24hrs at 37ºC and then the wells were rinsed with fresh medium to remove uninternalized bacteria. Treatments with the chosen compound **4** and its hydrochloride salt were initiated at the desired concentrations (4 and 40μM). For enumeration of viable bacterial numbers on day 0, macrophages in duplicate infected wells were lysed with 0.1% SDS and serial dilutions were plated on 7H11 plates (supplemented with OADC). After 6 days of treatment, all macrophages were lysed (by adding 0.1% SDS) and serial dilutions were plated on 7H11 plates (supplemented with OADC). Isoniazid (35μM) was used as a positive control and DMSO was used as a negative control.

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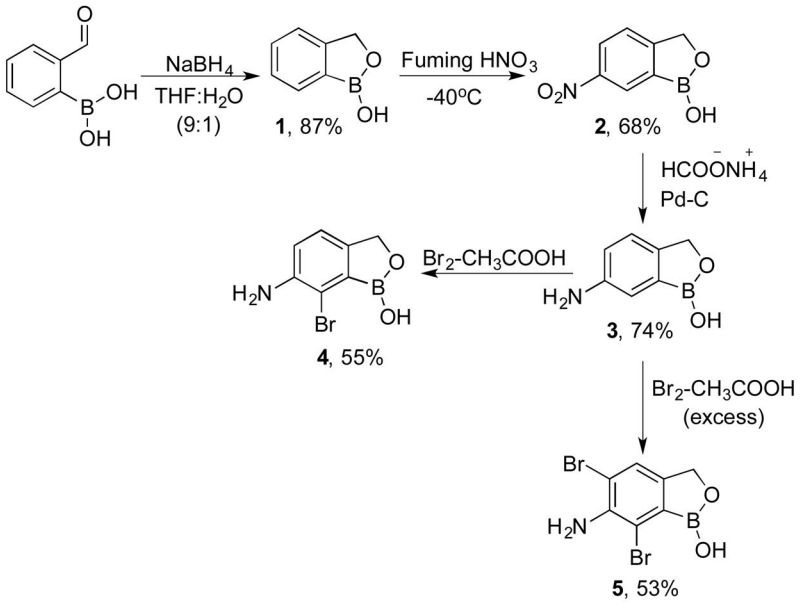

Scheme 1.
Preparation of brominated aminobenzoboroxoles

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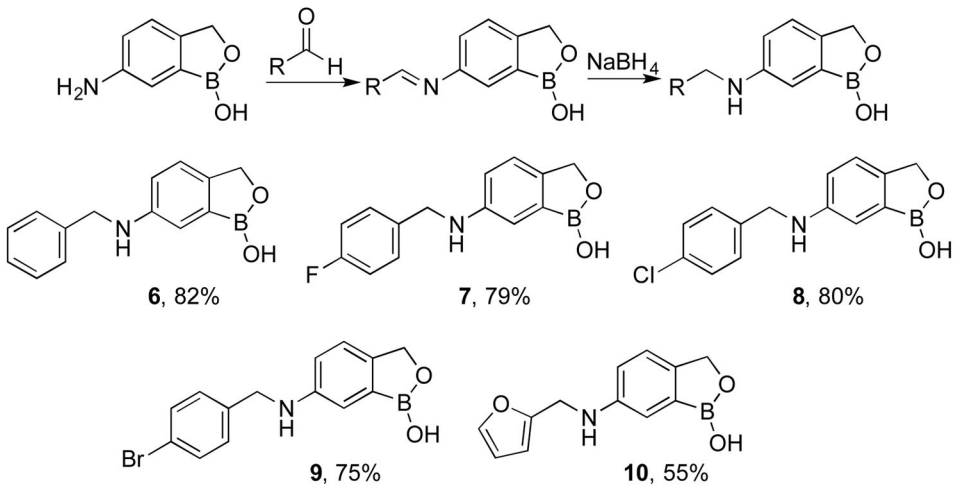

Scheme 2.
Reductive amination of 6-aminobenzoboroxoles

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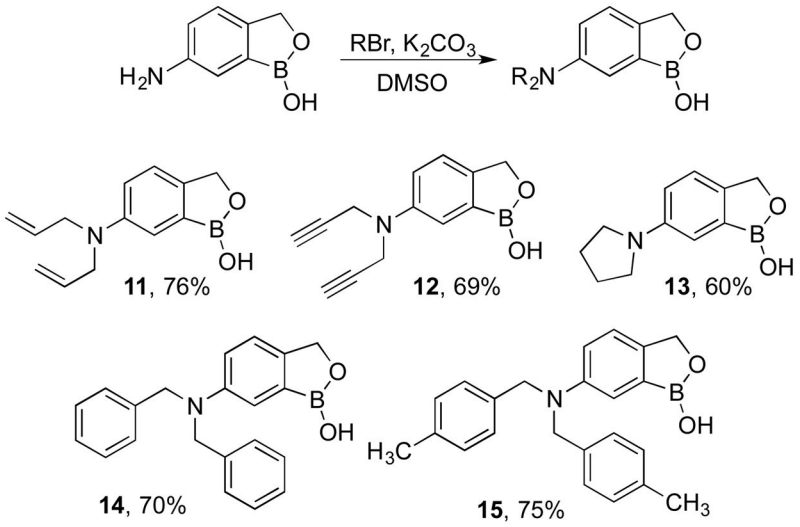

Scheme 3.
Dialkylation of 6-aminobenzoboroxoles

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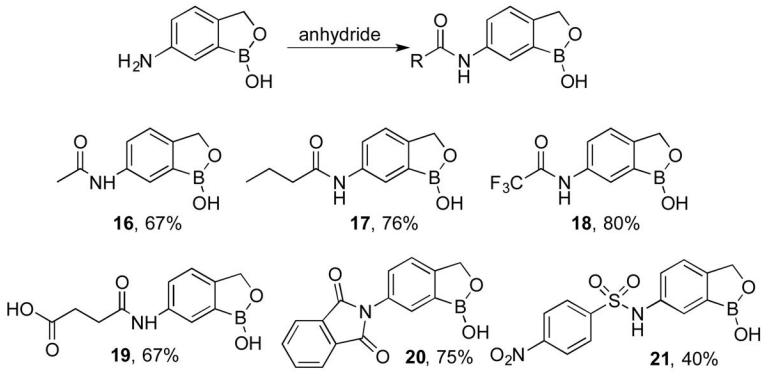

Scheme 4.
Synthesis of 6-amidobenzoboroxoles

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Table 1

Minimum Inhibitory Concentration

Compound	MIC_{99} * (7H9)		MIC_{99}* (GAST)
	Week 1	Week 2	Week 2
1	62.5	62.5–125	62.5
3	15.6	31.25	15.6
4	3.9	7.8	1.9
Isoniazid	0.7	0.7	0.15

* MIC99 expressed in μM

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Table 2

Minimum Bactericidal Concentration

Compound	Conc.	CFU*/mL	Conc.	CFU*/mL
DMSO	-	3.8×10^8	-	1.06×10^8
Rifampicin	0.1 μM	290	1.0 μM	165
4	4 μM	4.59×10^5	40 μM	750
4**	4 μM	5.51×10^4	40 μM	1475

* CFU = colony forming units

** Hydrochloride salt of bromo-aminobenzoboroxole **4** was used.

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Table 3

Minimum Anaerobicidal Concentration

Compound	Conc. (μM)	CFU*/mL	Conc. (μM)	CFU*/mL
DMSO		1.32×10^7	-	-
Metronidazole	100	1.73×10^5	-	-
Isoniazid	10	1.36×10^7	-	-
4	10	7.13×10^6	100	4.81×10^6
4**	10	6.55×10^6	100	4.30×10^6

*CFU = colony forming units

Hydrochloride salt of 7-bromo-6-aminobenzoboroxole **4 was used.

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Table 4

Intracellular Macrophage Killing Assay

Compound	Conc. (μM)	Day 1 CFU*/mL	Conc. (μM)	Day 6 CFU*/mL
Untreated	-	2.16×10^5	-	1.80×10^5
Isoniazid	4	1.34×10^3	-	-
4	4	4.31×10^4	40	2.28×10^4
4**	4	6.09×10^4	40	2.44×10^4

* CFU = colony forming units,

** Hydrochloride salt of 7-bromo-6-aminobenzoboroxole **4** was used.