

Synthesis and anti-tubercular activity of 3-substituted benzo[thiophene-1,1-dioxides

We demonstrated that the 3-substituted benzo[thiophene-1,1-dioxide class of compounds are effective inhibitors of *Mycobacterium tuberculosis* growth under aerobic conditions. We examined substitution at the C-3 position of the benzo[thiophene-1,1-dioxide series systematically to delineate structure-activity relationships influencing potency and cytotoxicity. Compounds were tested for inhibitory activity against virulent *M. tuberculosis* and eukaryotic cells. The tetrazole substituent was most potent, with a minimum inhibitory concentration (MIC) of 2.6 μM . However, cytotoxicity was noted with even more potency (Vero cell TC_{50} = 0.1 μM). Oxadiazoles had good anti-tubercular activity (MICs of 3–8 μM), but imidazoles, thiadiazoles and thiazoles had little activity. Cytotoxicity did not track with anti-tubercular activity, suggesting different targets or mode of action between bacterial and eukaryotic cells. However, we were unable to derive analogs without cytotoxicity; all compounds synthesized were cytotoxic (TC_{50} of 0.1–5 μM). We conclude that cytotoxicity is a liability in this series precluding it from further development. However, the series has potent anti-tubercular activity and future efforts towards identifying the mode of action could result in the identification of novel drug targets.

2 **Synthesis and anti-tubercular activity of 3-substituted benzo[b]thiophene-1,1-**
3 **dioxides**

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15 Introduction

16 Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* is the second leading cause
17 of death from an infectious disease and is a major global health problem. In 2010, according to
18 the World Health Organization (WHO) 8.8 million new cases and 1.4 million deaths from the
19 disease were reported [1]. In addition, one third of the world population has latent TB, 10% of
20 whom are expected to develop active TB at some point in their lives. Currently the recommended
21 first-line TB treatment regimens require a minimum of 6 months of multidrug therapy, resulting
22 in challenges with patient adherence. The result of inadequate therapy and poor compliance has
23 contributed to a rise in the emergence of multidrug resistant (MDR), resistant to isoniazid and
24 rifampicin, and extensively drug-resistant (XDR) strains, resistant to a fluoroquinolone and at
25 least one injectable drug, of *M. tuberculosis* [1]. Consequently, there is an urgent need for the
26 development of novel anti-TB drugs that are effective against both drug sensitive and resistant *M.*
27 *tuberculosis* [2].

28 The benzo[b]thiophene-1,1-dioxide (BTD) series was reported to have activity against *M.*
29 *tuberculosis* in a phenotypic assay [3]. Fourteen compounds were tested from this series; five of
30 these, all of which had heteroarylthio groups, had some inhibitory activity against *M.*
31 *tuberculosis*. As a part of our ongoing TB drug discovery program, we were interested in
32 exploring the potential of the BTD series to be developed as a lead series for TB treatment. We
33 conducted an exploratory chemistry study and evaluated the series for their activity against *M.*
34 *tuberculosis* as well as cytotoxicity for eukaryotic cells.

35 Materials and Methods

36 Determination of minimum inhibitory concentration (MIC)

37 We used *M. tuberculosis* H37Rv (London Pride), a laboratory-passaged derivative of H37Rv
38 (ATCC 25618), which has been sequenced, as described in [5]. MICs were run as described [4];
39 briefly MICs were determined against *M. tuberculosis* grown in Middlebrook 7H9 medium
40 containing 10% OADC (oleic acid, albumin, dextrose, catalase) supplement (Becton Dickinson)
41 and 0.05% w/v Tween 80 (7H9-Tw-OADC) under aerobic conditions. Compounds were prepared
42 as 10-point two-fold serial dilutions in DMSO with a starting concentration of 20 μM (lowest
43 compound concentration 40 nM). The final concentration of DMSO in the assay was 2%.
44 Bacterial growth was measured by OD₅₉₀ after 5 days of incubation at 37°C and % growth
45 measured. Growth inhibition curves were plotted and fitted using the Gompertz model. The MIC
46 was defined as the minimum concentration required for >99% growth inhibition.

47 **Vero cytotoxicity assay**

48 CellTiter-Glo® Luminescent Cell Viability Assay (Promega) was used to measure ATP as a
49 indicator of cell viability. The Vero cell line (ATCC CCL81) was grown in Dulbecco's Modified
50 Eagle Medium (DMEM), High Glucose, GlutaMAX™ (Invitrogen), 10% FBS (Fetal Bovine
51 Serum), and 1x of Penicillin-Streptomycin Solution (100 units/mL of penicillin, 100 $\mu\text{g}/\text{mL}$ of
52 streptomycin). Compounds were solubilized in DMSO (dimethyl sulfoxide) and assayed using a
53 10-point three-fold serial dilution starting at the highest concentration of 50 μM . CellTiter-Glo®
54 Reagent (Promega) was added to 96-well plates after 2 days of incubation at 37°C, 5% CO₂.
55 Relative luminescent units (RLU) were measured using Perkin Elmer Wallac 1420 Victor2 plate
56 reader. Inhibition curves were fitted using the Levenberg–Marquardt algorithm. Toxic
57 concentration (TC₅₀) was defined as the concentration of compound that gave 50% inhibition of
58 growth. Selectivity index was calculated as MIC/TC₅₀. For published data [3], SI was calculated
59 as IC₉₀/TC₅₀.

60 Analysis of compounds

61 ^1H and NMR spectral data were recorded in CDCl_3 or Acetone- d_6 on a 300 MHz Bruker NMR
62 spectrometer. Column chromatography was conducted on a Revelaris flash chromatography
63 system. Reactions were monitored using thin-layer chromatography (TLC) on silica gel plates.
64 HPLC analysis was conducted on an Agilent 1100 series LC system (Agilent ChemStation
65 Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm \times 150 mm, 5 μm , 1.0 mL/min, UV 254nm, room
66 temperature) with MeCN/ H_2O (0.05% TFA or HCOOH buffer) gradient elution. HPLC-MS was
67 performed on a Gilson 321 HPLC with detection performed by a Gilson 170 DAD and a Finnigan
68 AQA mass spectrometer operating in electrospray ionisation mode using a Phenomenex Gemini
69 C18 150x4.6mm column. Compounds **3a**, **b**, **c**, **s**, **t** and **u** were purchased from ChemBridge
70 Corporation.

71 Synthesis of 3-bromobenzo[b]thiophene 1,1-dioxide (2)

72 To a solution of **1** 1.62 g (7.6 mmol) in 25.0 mL in acetic acid was added 30% aqueous hydrogen
73 peroxide and the mixture was heated for 1 h at 100°C. The mixture was poured into ice cold water
74 and let it stand overnight. The resulting solid was filtered and dried to yield **2** (1.65 g, 89%). ^1H
75 NMR (300 MHz, CDCl_3): δ 6.98 (s, 1H), 7.58 - 7.72 (m, 4H). LCMS – ESI (M+H) $^+$: 214.1.

76 General procedure for the synthesis of 3-substituted benzo[b]thiophene-1,1-dioxides

77 To a solution of 200 mg (0.82 mmol) of **2** in 5 mL of dimethyl formamide was added 2.0 mmol
78 of the thiol reagent followed by 0.5 mL of triethylamine. The reaction was stirred overnight and
79 washed with 20 mL of deionized water and extracted with 50 mL of ethyl acetate. The organic
80 layer was dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The resulting
81 residue was purified by revelaris flash chromatography system to yield the aryl/heteroaryl thio
82 benzo[b]thiophene 1,1-dioxides.

83 3-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3d)

84 Yield **3d**: (95 mg, 31%). ¹H NMR (300 MHz, CDCl₃): 3.9 (3H, OCH₃, s); 7.0 – 8.0 (m, 9H).

85 LCMS – ESI (M+H)⁺: 373.0.

86 3-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3e)

87 Yield **3e**: (122 mg, 39%). ¹H NMR (300 MHz, Methanol-d₄): 7.5 – 8.1 (m, 8H). LCMS – ESI

88 (M+H)⁺: 377.0.

89 3-(thiazol-2-ylthio)benzo[b]thiophene 1,1-dioxide (3f)

90 Yield **3f**: (87 mg, 31%). ¹H NMR (300 MHz, CDCl₃): 6.6 (1H, s); 7.6 – 8.1 (m, 6H). LCMS – ESI

91 (M+H)⁺: 282.0.

92 3-((4-phenylthiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3g)

93 Yield **3g**: (25 mg, 9%). ¹H NMR (300 MHz, CDCl₃): 7.7 – 8.0 (m, 10H). LCMS – ESI (M+H)⁺:

94 358.0.

95 3-(benzo[d]thiazol-2-ylthio)benzo[b]thiophene 1,1-dioxide (3h)

96 Yield **3h**: (65 mg, 32%). ¹H NMR (300 MHz, CDCl₃): 7.3 – 8.1 (m, 9H). LCMS – ESI (M+H)⁺:

97 332.0.

98 3-((5-chloro-3a,7a-dihydrobenzo[d]thiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3i)

99 Yield **3i**: (25 mg, 30%). ¹H NMR (300 MHz, CDCl₃): 7.4 (1H, s); 7.5 – 8.1 (m, 7H). LCMS – ESI

100 (2M+H₂O)⁺: 754.9.

101 3-((6-ethoxybenzo[d]thiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3j)

102 Yield **3j**: (105 mg, 28%). ¹H NMR (300 MHz, CDCl₃): 1.5 (3H, d); 4.1 (2H, t); 7.1 – 7.9 (m, 8H).

103 LCMS – ESI (M+H)⁺: 376.0.

104 3-((5-methyl-1,3,4-thiadiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3k)

105 Yield **3k**: (26 mg, 11%). ¹H NMR (300 MHz, CDCl₃): 2.6 (3H, CH₃, s); 7.7 – 8.0 (m, 9H). LCMS

106 – ESI (M+H)⁺: 297.0.

107 3-((5-amino-1,3,4-thiadiazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3l)

108 Yield **3l**: (16 mg, 7%). ¹H NMR (300 MHz, CDCl₃): 6.8 (1H, s); 7.3 (2H, NH₂, s); 7.6 – 7.8 (m,

109 4H). Yield **20**: (5 mg, 2%). ¹H NMR (300 MHz, CDCl₃): 7.1 (2H, s); 7.6 – 7.8 (m, 4H); 8.8 (1H,

110 SH, s). LCMS – ESI (M+H)⁺: 298.0.

111 3-((5-mercapto-1,3,4-thiadiazol-2-yl)amino)benzo[b]thiophene 1,1-dioxide (3m)

112 Yield **3m**: (5 mg, 2%). ¹H NMR (300 MHz, CDCl₃): 7.1 (2H, s); 7.6 – 7.8 (m, 4H); 8.8 (1H, SH,

113 s). LCMS – ESI (M+H)⁺: 298.0.

114 3-((1H-benzo[d]imidazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide (3n)

115 Yield **3n**: (100 mg, 39%). ¹H NMR (300 MHz, CDCl₃): 6.7 (1H, s); 7.3 – 7.8 (m, 9H). LCMS –

116 ESI (M+H)⁺: 315.0.

**117 3-((1-methyl-3a,7a-dihydro-1H-benzo[d]imidazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide
118 (3o)**

119 Yield **3o**: (57 mg, 21%). ¹H NMR (300 MHz, CDCl₃): 3.9 (3H, CH₃, s); 6.4 (1H, s); 7.3 – 7.8 (m,

120 8H). LCMS – ESI (M+H)⁺: 329.0.

121 **3-((5-nitro-3a,7a-dihydro-1H-benzo[d]imidazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide**

122 **(3p)**

123 Yield **3p**: (142 mg, 48%). ¹H NMR (300 MHz, CDCl₃): 7.5 (1H, s); 7.7 – 7.9 (m, 4H); 8.2 (2H,

124 d); 8.5 (1H, s). LCMS – ESI (M+H)⁺: 360.0.

125 **3-((5-methoxy-3a,7a-dihydro-1H-benzo[d]imidazol-2-yl)thio)benzo[b]thiophene 1,1-dioxide**

126 **(3q)**

127 Yield **3q**: (97 mg, 34%). ¹H NMR (300 MHz, Methanol-d₄): 3.8 (3H, OCH₃, s); 6.6 (1H, s); 7.0 –

128 7.7 (m, 7H).

129 **3-((1-methyl-1H-tetrazol-5-yl)thio)benzo[b]thiophene 1,1-dioxide (3r)**

130 Yield **3r**: (115 mg, 50%). ¹H NMR (300 MHz, CDCl₃): 4.3 (3H, 3CH₃, s); 6.9 (1H, s); 7.5 – 8.1

131 (m, 7H). LCMS – ESI (2M+H)⁺: 561.0.

132 **3-(pyridin-2-ylthio)benzo[b]thiophene 1,1-dioxide (3v)**

133 Yield **3v**: (120 mg, 53%). ¹H NMR (300 MHz, CDCl₃): 6.6 – 8.5 (m, 9H). LCMS – ESI (M+H)⁺:

134 276.0.

135 **3-(pyridin-4-ylthio)benzo[b]thiophene 1,1-dioxide (3w)**

136 Yield **3w**: (57 mg, 25%). ¹H NMR (300 MHz, CDCl₃): 6.6 – 8.5 (m, 9H). LCMS – ESI (M+H)⁺:

137 276.0.

138 **3-(isoquinolin-3-ylthio)benzo[b]thiophene 1,1-dioxide (3x)**

139 Yield **3x**: (95 mg, 36%). ¹H NMR (300 MHz, CDCl₃): 7.3 – 8.1 (m, 6H).

140 **3-(naphthalen-2-ylthio)benzo[b]thiophene 1,1-dioxide (3y)**

141 Yield **3y**: (110 mg, 42%). ¹H NMR (300 MHz, CDCl₃): 5.8 (1H, s); 7.6 – 8.1 (m, 11H). LCMS –
142 ESI (M+2Na)⁺: 671.0.

143 **Results and Discussion**

144 BTD analogs were synthesized as outlined in **Scheme 1**. The oxidation of commercially available
145 3-bromothianaphthalene (**1**) with hydrogen peroxide afforded 3-bromobenzothiophene-1,1-
146 dioxide (**2**). This in turn was reacted with the corresponding thiols to afford the 3-substituted
147 BTDs. To investigate the biological activity, we conducted a systematic exploration of the
148 aryl/heteroaryl substituents linked via a thioether to the C-3 position of the benzo[b]thiophene-
149 1,1-dioxide compound.

150 We probed the consequences of having oxazoles and oxadiazoles as substituents at the C-3
151 position. Compounds were tested for efficacy against a virulent strain of *M. tuberculosis* in liquid
152 culture under aerobic growth conditions [4]. All compounds had good activity and the minimum
153 inhibitory concentration (MIC) was very similar (3-8 μM) (**Table 1**). The change in electronics
154 of the phenyl substituents had no effect on potency of the oxadiazole compounds. The addition of
155 the electron donating groups, methyl (**3b**), methoxy (**3d**) or an electron withdrawing Cl-group
156 (**3e**) to the para position of (**3a**) resulted in similar MIC values (**Table 1**). MICs were similar for
157 benzaoxazole **3c** and the phenyl linked oxadiazoles (**3a, b, d, and e**). This confirmed that the
158 series has good anti-tubercular activity. We tested compound activity against eukaryotic cells
159 using the Vero cell line (derived from African green monkey kidney cells). All of the compounds
160 had significant cytotoxicity, with TC₅₀ values < 0.3 μM, suggesting that these compounds are
161 even more effective against eukaryotic cells (**Table 1**). Of the compounds we tested, two had

162 previously been identified as having anti-tubercular activity (**3a** and **3i**)³. In this study **3a**
163 appeared to have a selectivity index (SI) of > 33. However, in our assay this compound had a SI
164 of 0.03. The compound **3a** was reported to have anti-tubercular activity with an IC₉₀ of 1.3 μM
165 (0.45 μg/mL) and a TC₅₀ of 43 μM (calculated from the published data using the equation TC₅₀=
166 SI x IC₉₀). Ananthan *et al.*, calculated IC₉₀ in their assay, representing the concentration required
167 to inhibit growth by 90%, but in our experience IC₉₀ and MIC (which we used) are very similar.
168 In our case it had an MIC of 3.1 μM and a cytotoxicity of 0.1 μM. Therefore, the difference in SI
169 is primarily due to the difference in cytotoxicity data.

170 Since we had seen good activity with the compounds, but significant cytotoxicity, we determined
171 whether we could separate the two activities to generate potent, non-toxic compounds. We
172 examined the influence of thiazoles and thiadiazoles on the biological activity and cytotoxicity of
173 these BTB compounds. Anti-tubercular activity was diminished by the replacement of an
174 oxazole with either a thiazole or a thiadiazole; these compounds showed MICs ≥20 μM (**Table**
175 **2**). The only exception was compound **3k** which showed good activity (9 μM), where the
176 addition of an electron donating ethoxy group to the benzothiazole compound improved its
177 potency to 5 μM (**3i**). In contrast, addition of an electron-withdrawing group diminished activity
178 in compound **3j** (MIC >20 μM, **Table 2**). Cytotoxicity was also reduced by 10-100-fold, and
179 although the selectivity index (SI) was also improved the compounds were still more active
180 against eukaryotic cells with SI of < 0.2 (**Table 2**). The benzothiazole compound **3i** has
181 previously been reported³, but in contrast to our results, it had a SI > 150, whereas our data
182 indicate that the SI = 0.5. The compound **3i** was reported to have a TB IC₉₀ of < 0.3 μM (< 0.1
183 μg/mL) and a TC₅₀ of 45 μM. In our assays it gave a MIC of 20 μM and a cytotoxicity of 1 μM.
184 In this case the difference in SI is due to both the difference in activity and cytotoxicity data.

185 We then investigated the effect of C-3 imidazoles to see if we could improve the SI. Similar to
186 the thiazoles and thiadiazoles, this resulted in diminished activity (MIC > 20 μ M) (**3n – 3q**)
187 (**Table 3**). Cytotoxicity was similar to those seen with the thiazole and thiadiazole groups.
188 Methylation of the N-1 of the imidazole (**3o**) had no effect on activity (**3n, 3p and 3q**). The
189 tetrazole compound (**3r**) showed the best activity of all the compounds synthesized (MIC = 2.6
190 μ M), but also had significant cytotoxicity (**Table 3**).

191 Finally, we explored the influence of having six membered heterocycles in the C-3 position. We
192 synthesized compounds with pyrimidyl (**3t**), pyridyl (**3v, 3w**), quinolinyl (**3u**), or isoquinolinyl
193 (**3x**) groups and a non-heterocyclic compound with a naphthyl group (**3y**). All these analogs were
194 inactive suggesting that the BTB series requires a five membered heterocyclic substituent at the
195 C-3 position linked via a thioether for its activity against *M. tuberculosis* (**Table 4**).

196 **Conclusions**

197 We conducted a systematic exploration of the aryl/heteroaryl thioether substituents at the C-3
198 position of the benzo[b]thiophene-1,1-dioxide compound series for its inhibitory activity against
199 *M. tuberculosis*. The series exhibited encouraging activity with some MIC values <10 μ M. The
200 tetrazole, oxazole and the oxadiazoles were the most potent compounds tested, whereas
201 compounds bearing six-membered aromatic substituents at the C-3 position were inactive.
202 However, the BTB series was also active against eukaryotic cells showing significant toxicity
203 against the Vero cell line; in fact cytotoxicity was more pronounced than the anti-mycobacterial
204 activity. Our data are in contrast to that previously reported in which cytotoxicity was not
205 observed in selected members of the series [3]. Differences in cytotoxicity could be due to the
206 exact assay method and the cell line used; in this case we used the same Vero cell line.
207 However, the assays conditions were different; we used passaged cells which were actively

208 replicating as opposed to cells recovered directly from frozen. Since the majority of
209 cytotoxicity is manifested during cell division, this may account for our increased sensitivity. In
210 any case, we found that the series as a whole was cytotoxic. We were unable to reduce
211 cytotoxicity in this series, even after significant modifications of the third position substituent. On
212 this basis we concluded that the series lacks further potential for drug development. However, the
213 target of the series may still be of interest, since one might find alternative scaffolds with
214 specificity. Thus, in future, we are interested in finding the target of these compounds.

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Table 1 (on next page)

Activity of oxazole and oxadiazole analogs of the BTD series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments \pm standard deviation. b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs \pm standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀. For comparison, MIC of rifampicin is 0.003 μ M and isoniazid is 0.2 μ M [4] .

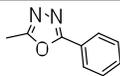
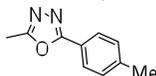
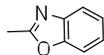
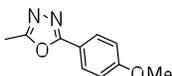
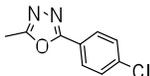
| Compound | R-group | MIC (μM) ^a | TC ₅₀ (μM) ^b | SI ^c |
|-----------|---|------------------------------------|---|-----------------|
| 3a |  | 3.1 ± 0.07 | 0.1 ± 0 | 0.03 |
| 3b |  | 8.2 ± 0.6 | 0.2 ± 0 | 0.02 |
| 3c |  | 5.7 ± 2.9 | 0.2 ± 0.07 | 0.04 |
| 3d |  | 7.2 ± 0.3 | 0.3 ± 0.3 | 0.04 |
| 3e |  | 3.9 ± 1.7 | 0.3 ± 0.2 | 0.08 |

Table 1. Activity of oxazole and oxadiazole analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments ± standard deviation.

b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs ± standard deviation.

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀.

For comparison, MIC of rifampicin is 0.003 μM and isoniazid is 0.2 μM [4].

Table 2(on next page)

Activity of thiazole and thiadiazole analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments \pm standard deviation. b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs \pm standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀. For comparison, MIC of rifampicin is 0.003 μ M and isoniazid is 0.2 μ M [4] .

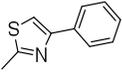
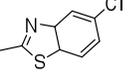
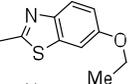
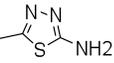
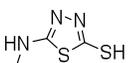
| Compound | R-group | MIC (μM) ^a | TC ₅₀ (μM) ^b | SI ^c |
|-----------|---|------------------------------------|---|-----------------|
| 3g |  | 20 | 1 | 0.05 |
| 3h |  | 20 | 3 ± 1.2 | 0.2 |
| 3i |  | 20 | 1 | 0.05 |
| 3j |  | >20 | 1 ± 0.4 | NC |
| 3k |  | 9.0 ± 4.7 | 1 ± 0.1 | 0.1 |
| 3l |  | >20 | 1 ± 0.4 | NC |
| 3m |  | >20 | 3 ± 1 | NC |
| 3n |  | 20 | 3 ± 0.7 | 0.2 |

Table 2. Activity of thiazole and thiadiazole analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments ± standard deviation.

b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs ± standard deviation.

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀.

NC = not calculated.

Table 3(on next page)

Activity of imidazole and tetrazole analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments \pm standard deviation. b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs \pm standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀. NC = not calculated. ND = not determined.

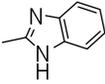
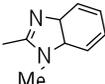
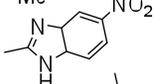
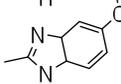
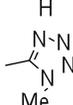
| Compound | R-group | MIC (μM) ^a | TC ₅₀ (μM) ^b | SI ^c |
|----------|---|------------------------------------|---|-----------------|
| 3n |  | >20 | 1 | NC |
| 3o |  | >20 | 5 | NC |
| 3p |  | >20 | ND | NC |
| 3q |  | >20 | 0.3 \pm 0.07 | NC |
| 3r |  | 2.6 | 0.1 \pm 0 | 0.004 |

Table 3. Activity of imidazole and tetrazole analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments \pm standard deviation.

b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs \pm standard deviation.

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀.

NC = not calculated.

ND = not determined.

Table 4(on next page)

Activity of six membered heterocyclic analogs of the BTD series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments \pm standard deviation. b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs \pm standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀. NC = not calculated. ND = not determined.

| Compound | R-group | MIC (μM) ^a | TC ₅₀ (μM) ^b | SI ^c |
|----------|---|------------------------------------|---|-----------------|
| 3s |  | >20 | 10 ± 1 | NC |
| 3t |  | >20 | ND | NC |
| 3u |  | >20 | ND | NC |
| 3v |  | >20 | ND | NC |
| 3w |  | >20 | ND | NC |
| 3x |  | >20 | ND | NC |
| 3y |  | >20 | ND | NC |

Table 4. Activity of six membered heterocyclic analogs of the BTB series against *M. tuberculosis* and Vero cell line.

a = MIC is the minimum concentration required to inhibit growth completely of *M. tuberculosis* in liquid culture⁴. MICs of active compounds are the average of two independent experiments ± standard deviation.

b = TC₅₀ is concentration required to inhibit growth of Vero cells by 50%. TC₅₀ is the average of two runs ± standard deviation.

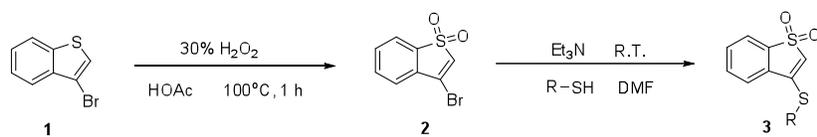
c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC₅₀.

NC = not calculated.

ND = not determined.

Table 5(on next page)

Synthesis of 3-substituted benzo[b]thiophene-1,1-dioxides.



Scheme 1. Synthesis of 3-substituted benzo[b]thiophene-1,1-dioxides.