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Synthesis and anti-tubercular activity of 3-substituted benzothiophene-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 TC50 = 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 (TC50 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.

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

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Introduction

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

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

Materials and Methods

Determination of minimum inhibitory concentration (MIC)

We used *M. tuberculosis* H37Rv (London Pride), a laboratory-passaged derivative of H37Rv (ATCC 25618), which has been sequenced, as described in [5]. MICs were run as described [4]; briefly MICs were determined against *M. tuberculosis* grown in Middlebrook 7H9 medium containing 10% OADC (oleic acid, albumin, dextrose, catalase) supplement (Becton Dickinson) and 0.05% w/v Tween 80 (7H9-Tw-OADC) under aerobic conditions. Compounds were prepared as 10-point two-fold serial dilutions in DMSO with a starting concentration of 20 µM (lowest compound concentration 40 nM). The final concentration of DMSO in the assay was 2%. Bacterial growth was measured by OD_{590} after 5 days of incubation at 37°C and % growth measured. Growth inhibition curves were plotted and fitted using the Gompertz model. The MIC was defined as the minimum concentration required for >99% growth inhibition.

Vero cytotoxicity assay

CellTiter-Glo® Luminescent Cell Viability Assay (Promega) was used to measure ATP as a indicator of cell viability. The Vero cell line (ATCC CCL81) was grown in Dulbecco's Modified Eagle Medium (DMEM), High Glucose, GlutaMAX™ (Invitrogen), 10% FBS (Fetal Bovine Serum), and 1x of Penicillin-Streptomycin Solution (100 units/mL of penicillin, 100 µg/mL of streptomycin). Compounds were solubilized in DMSO (dimethyl sulfoxide) and assayed using a 10-point three-fold serial dilution starting at the highest concentration of 50 µM. CellTiter-Glo® Reagent (Promega) was added to 96-well plates after 2 days of incubation at 37°C, 5% CO_2. Relative luminescent units (RLU) were measured using Perkin Elmer Wallac 1420 Victor2 plate reader. Inhibition curves were fitted using the Levenberg–Marquardt algorithm. Toxic concentration (TC_{50})was defined as the concentration of compound that gave 50% inhibition of growth. Selectivity index was calculated as MIC/TC_{50}. For published data [3], SI was calculated as IC_{90}/TC_{50}.

Analysis of compounds

^{1}H and NMR spectral data were recorded in $CDCl_3$ or Acetone-d6 on a 300 MHz Bruker NMR spectrometer. Column chromatography was conducted on a Revelaris flash chromatography system. Reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. HPLC analysis was conducted on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm × 150 mm, 5 µm, 1.0 mL/min, UV 254nm, room temperature) with MeCN/H_2O (0.05% TFA or HCOOH buffer) gradient elution. HPLC-MS was performed on a Gilson 321 HPLC with detection performed by a Gilson 170 DAD and a Finnigan AQA mass spectrometer operating in electrospray ionisation mode using a Phenomenex Gemini C18 150x4.6mm column. Compounds **3a, b, c, s, t** and **u** were purchased from ChemBridge Corporation.

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

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

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

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

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

Yield **3d**: (95 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$): 3.9 (3H, OCH_3. s); 7.0 – 8.0 (m, 9H). LCMS – ESI $(M+H)^+$: 373.0.

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

Yield **3e**: (122 mg, 39%). ^{1}H NMR (300 MHz, Methanol-d_4): 7.5 – 8.1 (m, 8H). LCMS – ESI $(M+H)^+$: 377.0.

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

Yield **3f**: (87 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$): 6.6 (1H, s); 7.6 – 8.1 (m, 6H). LCMS – ESI $(M+H)^+$: 282.0.

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

Yield **3g**: (25 mg, 9%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.7 – 8.0 (m, 10H). LCMS – ESI $(M+H)^+$: 358.0.

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

Yield **3h**: (65 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.3 – 8.1 (m, 9H). LCMS – ESI $(M+H)^+$: 332.0.

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

Yield **3i**: (25 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.4 (1H, s); 7.5 – 8.1 (m, 7H). LCMS – ESI $(2M+H_2O)^+$: 754.9.

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

Yield **3j**: (105 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.5 (3H, d); 4.1 (2H, t); 7.1 – 7.9 (m, 8H). LCMS – ESI $(M+H)^+$: 376.0.

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

Yield **3k**: (26 mg, 11%). ^{1}H NMR (300 MHz, $CDCl_3$): 2.6 (3H, CH_3, s); 7.7 – 8.0 (m, 9H). LCMS – ESI $(M+H)^+$: 297.0.

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

Yield **3l**: (16 mg, 7%). ^{1}H NMR (300 MHz, $CDCl_3$): 6.8 (1H, s); 7.3 (2H, NH_2, s); 7.6 – 7.8 (m, 4H). Yield **20**: (5 mg, 2%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.1 (2H, s); 7.6 – 7.8 (m, 4H); 8.8 (1H, SH, s). LCMS – ESI $(M+H)^+$: 298.0.

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

Yield **3m**: (5 mg, 2%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.1 (2H, s); 7.6 – 7.8 (m, 4H); 8.8 (1H, SH, s). LCMS – ESI $(M+H)^+$: 298.0.

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

Yield **3n**: (100 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$): 6.7 (1H, s); 7.3 – 7.8 (m, 9H). LCMS – ESI $(M+H)^+$: 315.0.

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

Yield **3o**: (57 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): 3.9 (3H, CH_3. s); 6.4 (1H, s); 7.3 – 7.8 (m, 8H). LCMS – ESI $(M+H)^+$: 329.0.

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

Yield **3p**: (142 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.5 (1H, s); 7.7 – 7.9 (m, 4H); 8.2 (2H, d); 8.5 (1H, s). LCMS – ESI $(M+H)^+$: 360.0.

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

Yield **3q**: (97 mg, 34%). ^{1}H NMR (300 MHz, Methanol-d_4): 3.8 (3H, OCH_3. s); 6.6 (1H, s); 7.0 – 7.7 (m, 7H).

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

Yield **3r**: (115 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): 4.3 (3H, $3CH_3$, s); 6.9 (1H, s); 7.5 – 8.1 (m, 7H). LCMS – ESI $(2M+H)^+$: 561.0.

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

Yield **3v**: (120 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): 6.6 – 8.5 (m, 9H). LCMS – ESI $(M+H)^+$: 276.0.

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

Yield **3w**: (57 mg, 25%). ^{1}H NMR (300 MHz, $CDCl_3$): 6.6 – 8.5 (m, 9H). LCMS – ESI $(M+H)^+$: 276.0.

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

Yield **3x**: (95 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.3 – 8.1 (m, 6H).

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

Yield **3y**: (110 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$): 5.8 (1H, s); 7.6 – 8.1 (m, 11H). LCMS – ESI $(M+2Na)^+$: 671.0.

Results and Discussion

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

We probed the consequences of having oxazoles and oxadiazoles as substituents at the C-3 position. Compounds were tested for efficacy against a virulent strain of *M. tuberculosis* in liquid culture under aerobic growth conditions [4]. All compounds had good activity and the minimum inhibitory concentration (MIC) was very similar (3-8 μM) (**Table 1**). The change in electronics of the phenyl substituents had no effect on potency of the oxadiazole compounds. The addition of the electron donating groups, methyl (**3b**), methoxy (**3d**) or an electron withdrawing Cl-group (**3e**) to the para position of (**3a**) resulted in similar MIC values (**Table 1**). MICs were similar for benzaoxazole **3c** and the phenyl linked oxadiazoles (**3a, b, d, and e**). This confirmed that the series has good anti-tubercular activity. We tested compound activity against eukaryotic cells using the Vero cell line (derived from African green monkey kidney cells). All of the compounds had significant cytotoxicity, with TC_{50} values < 0.3 μM, suggesting that these compounds are even more effective against eukaryotic cells (**Table 1**). Of the compounds we tested, two had

previously been identified as having anti-tubercular activity (**3a** and **3i**)[3]. In this study **3a** appeared to have a selectivity index (SI) of > 33. However, in our assay this compound had a SI of 0.03. The compound **3a** was reported to have anti-tubercular activity with an IC_{90} of 1.3 µM (0.45 µg/mL) and a TC_{50} of 43 µM (calculated from the published data using the equation TC_{50}= SI x IC_{90}). Ananthan *et al.,* calculated IC_{90} in their assay, representing the concentration required to inhibit growth by 90%, but in our experience $IC9_0$ and MIC (which we used) are very similar. In our case it had an MIC of 3.1 µM and a cytotoxicity of 0.1 µM. Therefore, the difference in SI is primarily due to the difference in cytotoxicity data.

Since we had seen good activity with the compounds, but significant cytotoxicity, we determined whether we could separate the two activities to generate potent, non-toxic compounds. We examined the influence of thiazoles and thiadiazoles on the biological activity and cytotoxicity of these BTD compounds. Anti-tubercular activity was diminished by the replacement of an oxazole with either a thiazole or a thiadiazole; these compounds showed MICs ≥20 µM (**Table 2**). The only exception was compound **3k** which showed good activity (9 µM), where the addition of an electron donating ethoxy group to the benzothiazole compound improved its potency to 5 µM (**3i)**. In contrast, addition of an electron-withdrawing group diminished activity in compound **3j** (MIC >20 µM, **Table 2**). Cytotoxicity was also reduced by 10-100-fold, and although the selectivity index (SI) was also improved the compounds were still more active against eukaryotic cells with SI of < 0.2 (**Table 2**). The benzothiazole compound **3i** has previously been reported[3], but in contrast to our results, it had a SI > 150, whereas our data indicate that the SI = 0.5. The compound **3i** was reported to have a TB IC_{90} of < 0.3 µM (< 0.1 µg/mL) and a TC_{50} of 45 µM. In our assays it gave a MIC of 20 µM and a cytotoxicity of 1 µM. In this case the difference in SI is due to both the difference in activity and cytotoxicity data.

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

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

Conclusions

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

replicating as opposed to cells recovered directly from frozen. Since the majority of cytotoxicity is manifested during cell division, this may account for our increased sensitivity. In any case, we found that the series as a whole was cytotoxic. We were unable to reduce cytotoxicity in this series, even after significant modifications of the third position substituent. On this basis we concluded that the series lacks further potential for drug development. However, the target of the series may still be of interest, since one might find alternative scaffolds with 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation. b = TC_{50} is concentration required to inhibit growth of Vero cells by 50%. TC_{50} is the average of two runs ± standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ $TC_{50.}$ For comparison, MIC of rifampicin is 0.003 µM and isoniazid is 0.2 µM [4] .

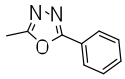
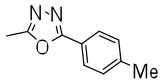
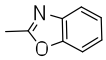
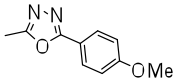
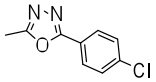
Compound	R-group	MIC (μM) [a]	TC_{50} (μM) [b]	SI [c]
3a		3.1 ± 0.07	0.1 ± 0	0.03
3b		8.2 ± 0.6	0.2 ± 0	0.02
3c		5.7 ± 2.9	0.2 ± 0.07	0.04
3d		7.2 ± 0.3	0.3 ± 0.3	0.04
3e		3.9 ± 1.7	0.3 ± 0.2	0.08

Table 1. Activity of oxazole and oxadiazole analogs of the 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation.

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

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC_{50}.

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 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation. b = TC_{50} is concentration required to inhibit growth of Vero cells by 50%. TC_{50} is the average of two runs ± standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ $TC_{50.}$ For comparison, MIC of rifampicin is 0.003 µM and isoniazid is 0.2 µM [4] .

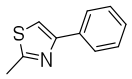
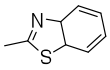
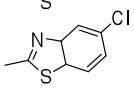
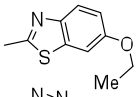
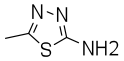
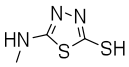
Compound	R-group	MIC (μM) [a]	TC_{50} (μM) [b]	SI [c]
3g		20	1	0.05
3h		20	3 ± 1.2	0.2
3i		20	1	0.05
3j		>20	1 ± 0.4	NC
3k		9.0 ± 4.7	1 ± 0.1	0.1
3l		>20	1 ± 0.4	NC
3m		>20	3 ± 1	NC
3n		20	3 ± 0.7	0.2

Table 2. Activity of thiazole and thiadiazole analogs of the 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation.

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

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC_{50}.

NC = not calculated.

Table 3(on next page)

Activity of imidazole and tetrazole 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation. b = TC_{50} is concentration required to inhibit growth of Vero cells by 50%. TC_{50} is the average of two runs ± standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ $TC_{50.}$ NC = not calculated. ND = not determined.

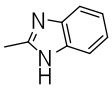
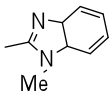
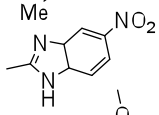
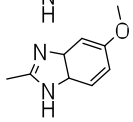
Compound	R-group	MIC (μM) [a]	TC_{50} (μM) [b]	SI [c]
3n		>20	1	NC
3o		>20	5	NC
3p		>20	ND	NC
3q		>20	0.3 ± 0.07	NC
3r		2.6	0.1 ± 0	0.004

Table 3. Activity of imidazole and tetrazole 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation.

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

c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC_{50}.

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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation. b = TC_{50} is concentration required to inhibit growth of Vero cells by 50%. TC_{50} is the average of two runs ± standard deviation. c = SI is the selectivity index. Selectivity index is calculated as MIC/ $TC_{50.}$ NC = not calculated. ND = not determined.

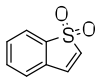
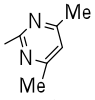
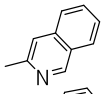
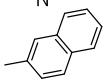
Compound	R-group	MIC (μM) [a]	TC_{50} (μM) [b]	SI [c]
3s		>20	10 ± 1	NC
3t		>20	ND	NC
3u		>20	ND	NC
3v		>20	ND	NC
3w		>20	ND	NC
3x		>20	ND	NC
3y		>20	ND	NC

Table 4. Activity of six membered heterocyclic analogs of the 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 culture[4]. MICs of active compounds are the average of two independent experiments ± standard deviation.

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

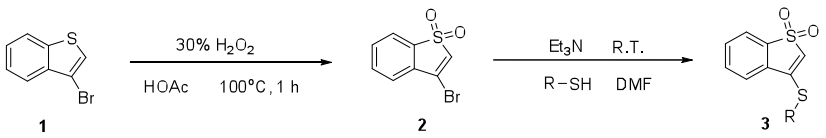
c = SI is the selectivity index. Selectivity index is calculated as MIC/ TC_{50}.

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.