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Synthesis and Antitubercular Activity of New Benzo[*b*]thiophenes

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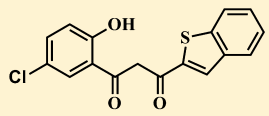
Supporting Information

ABSTRACT: *In vitro* and *ex vivo* efficacies of four series of benzo[*b*]thiophene-2-carboxylic acid derivatives were studied against *Mycobacterium tuberculosis* H37Ra (MTB). Benzo[*b*]thiophenes were also tested *in vitro* against multidrug resistant *Mycobacterium tuberculosis* H37Ra (MDR-MTB), and **7b** was found to be highly active against A- and D-MDR-MTB/MTB (MIC ranges 2.73−22.86 μg/mL). The activity of all benzo[*b*]-thiophenes against *M. bovis* BCG (BCG) was also assessed grown under aerobic and under conditions of oxygen depletion. Compounds **8c** and **8g** showed significant activity with MICs of 0.60 and 0.61 μg/mL against dormant BCG. The low cytotoxicity and high selectivity index data against human cancer cell lines, HeLa, Panc-1, and THP-1 indicate the potential importance of the development of benzo[*b*]thiophene-based 1,3-diketones and flavones as lead candidates to treat mycobacterial infections. Molecular docking studies into the active site of DprE1 (Decaprenylphosphoryl-β-D-ribose-2′-epimerase) enzyme revealed a similar binding mode to native ligand in the crystal structure thereby helping to understand the ligand−protein interactions and establish a structural basis for inhibition of *MTB*. In summary, its good activity in *in vitro* and *ex vivo* model, as well as its activity against multidrug-resistant *M. tuberculosis* H37Ra in a potentially latent state, makes **7b** an attractive drug candidate for the therapy of tuberculosis.



Ex vivo H37Ra (MIC)	MDR H37Ra (MIC)
Active: 2.83	Active: 5.3
Dormant: 0.91	Dormant: 2.73

KEYWORDS: *Benzo[b]thiophene, MDR-MTB, M. Bovis BCG, cytotoxicity, molecular docking*

Among infectious widespread diseases, tuberculosis (TB) remains an active and major health problem worldwide. TB is the deadliest communicable disease, caused by the global emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of TB along with acquired, primary, and cross resistance *mycobacterium* TB (*MTB*) strains. Despite the availability of first line and second line drugs regimen to treat the disease, still unacceptably TB continues to have high mortality and a global health threat. Resistance to first line anti-TB drugs has been linked to spontaneous mutations in many genes.[1] The probability of resistance arising when rifampicin and isoniazid are used in combination is only one in 10^{14}, sufficiently low to prevent resistance for either drug.[2] As per WHO, the direct observed therapy strategy (DOTS) is the most effective means to prevent the emergence of drug resistance.[3] As for those with HIV, several other inter-related factors like poverty, mobility of people from the countries where TB is prevalent, the long and complex TB regimen, and poor management of TB control programs[4] pose the resurgence of TB.

Mycobacterium bovis Bacillus Calmette Guerin (BCG), an attenuated strain closely related to *MTB*,[5] infections in humans have been reported from many centuries ago. *M. bovis* infections have re-emerged and are causing TB in humans, due to mutations during the long *in vitro* propagation of this strain and, in particular, those who are HIV-positive. Owing to the appearance of drug resistance surveillance, the significant side effects and drug interactions of present agents, there is still an urgent need for the development of new ideal anti-TB agents with low toxicity and those that are active to treat against MDR and XDR bacteria and latent diseases.

Based on these facts, efforts have been continued to discover new and effective chemotherapeutic agents for the tuberculosis treatment. We recently reported the antitubercular activity of several new molecules with good minimum inhibitory concentrations (MICs),[6−11] which promoted us to synthesize new compounds.

Benzo[*b*]thiophene moiety is a drug-like scaffold known to possess potential medicinal value in FDA approved drugs such as raloxifene, sertaconazole, zileuton, and benocyclidine. Therefore, in continuation of our program on the discovery of antitubercular compounds, various 1,3-diketones, flavones, pyrazoles, and carboxamides were synthesized from benzo[*b*]-thiophene carboxylic acid and demonstrated inhibitory activity against *MTB* H37Ra and *M. bovis* BCG. The cytotoxicity of compounds has been also tested.

In an effort to elucidate the possible mechanism by which the title compounds can induce antitubercular activity, molecular docking studies were performed to visualize the binding mode of the drug candidate at the molecular level. In the absence of resources to perform the target-based assays experimentally, the *in silico* approach of molecular docking has proved to be a very

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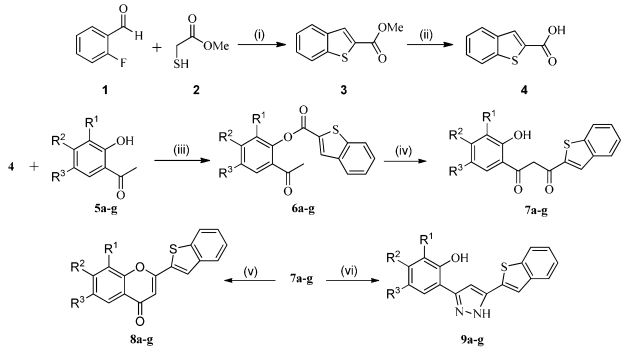
important tool for identifying the targets for different ligands and their associated thermodynamic intermolecular interactions with the target enzyme governing the inhibition of the pathogen. Molecular docking studies into the active site of DprE1 (decaprenylphosphoryl-β-D-ribose-2′-epimerase) enzyme revealed a similar binding mode to native ligand in the crystal structure thereby helping to understand the ligand−protein interactions and establish a structural basis for inhibition of *MTB*.

The synthetic sequences of benzo[*b*]thiophene derivatives are illustrated in Scheme 1. Commercially available 2-fluorobenzaldehyde **1** was reacted with methyl 2-mercaptoacetate **2** in *N*,*N*-dimethylformamide (DMF) under basic (K_2CO_3) conditions followed by base (LiOH) hydrolysis affording benzo[*b*]thiophene-2-carboxylic acid **4**. Further, the reaction between compound **4** and appropriate *o*-hydroxy acetophenones **5a−g** in pyridine and $POCl_3$ provided intermediate esters **6a−g**. In the next step, these intermediate undergoes Baker−Venkataraman rearrangement (BVR) under ultrasonication, affording 1-(benzo[*b*]thiophen-2-yl)-3-(2-hydroxyphenyl)propane-1,3-diones **7a−g**.

Scheme 1. Synthesis of Benzo[*b*]thiophene Derivatives[a]



7a, 8a, 9a; R_1 = -H, R_2 = -H, R_3 = -Br; **7b, 8b, 9b;** R_1 = -H, R_2 = -H, R_3 = -Cl; **7c, 8c, 9c;** R_1 = -H, R_2 = -H, R_3 = $-CH_3$; **7d, 8d, 9d;** R_1 = -H, R_2 = -H, R_3 = -H; **7e, 8e, 9e;** R_1 = -Cl, R_2 = -H, R_3 = -Cl; **7f, 8f, 9f;** R_1 = -H, R_2 = $-CH_3$, R_3 = -H; **7g, 8g, 9g;** R_1 = -H, R_2 = $-CH_3$, R_3 = -Cl

11a; R_1 = -H, R_2 = -H, R_3 = -Br; **11b;** R_1 = -H, R_2 = -H, R_3 = -H; **11c;** R_1 = -F, R_2 = -H, R_3 = -H; **11d;** R_1 = -H, R_2 = -H, R_3 = -F; **11e;** R_1 = -F, R_2 = -H, R_3 = -F; **11f;** R_1 = -H, R_2 = -Br, R_3 = -H; **11g;** R_1 = -H, R_2 = -H, R_3 = -Cl; **11h;** R_1 = -H, R_2 = -Cl, R_3 = -H; **11i;** R_1 = -Cl, R_2 = -H, R_3 = -H

[a]Reagents and conditions: (i) K_2CO_3, DMF; (ii) LiOH, THF/H_2O; (iii) pyridine, $POCl_3$, RT; (iv) KOH, pyridine. (v) PEG-400, cat. H_2SO_4; (vi) $NH_2NH_2{\cdot}H_2O$; (vii) EDC·HCl, HOBt, DMF.

The intramolecular cyclization of 1,3-diketones **7a−g** yielded novel 2-(benzo[*b*]thiophen-2-yl)-4*H*-chromen-4-ones **8a−g** in good yields. The formation of the chromen-4-ones was accomplished through a different protocol, PEG-400 as a green solvent with a nonconventional approach, and ultrasound irradiation was successfully utilized. Moreover, 2-(5-(benzo[*b*]-thiophen-2-yl)-1*H*-pyrazol-3-yl)phenols **9a−g** synthesis proceeds via a condensation reaction between substituted 1-(benzo[*b*]thiophen-2-yl)-3-(2-hydroxyphenyl)propane-1,3-diones **7a−g** and hydrazine hydrate in ethanol under ultrasound irradiation. Further, the condensation reaction between compound **4** and anilines **10a−i** using EDC·HCl and HOBt as coupling reagents under ultrasound irradiation within 1−2 h afforded carboxamides **11a−i**. The structures of the compounds were chemically characterized by thin layer chromatography (TLC), spectroscopic data, and elemental analyses.

All 31 synthesized compounds were tested for their *in vitro* anti-TB activity.[9,12] The primary screen results showed that four compounds **7a**, **7b**, **7d**, and **7f** in particular exhibit >92% inhibitory activity against *MTB* H37Ra and nine compounds **7a−d**, **7f**, **7g**, and **9b−d** exhibit >97% against *M. bovis* BCG strain (Supporting Information). Compounds **7a−f**, **8c** and **8g** have been selected for further evaluation to dose response by both the IC_{50} (Supporting Information) and MIC_{90} (minimum concentration bringing 90% inhibition) values presented in Table 1.

The 1,3-diketone **7d** with no substitution appears to provide strong activity against dormant *MTB* H37Ra (MIC = 2.05 μg/mL). Compounds **7a** and **7b** having bromo substituent showed good activity against active as well as dormant *MTB* H37Ra with MIC value of 2.87 and 2.63 μg/mL, respectively. However, it was observed that the compounds are more susceptible to *M. bovis* BCG than *MTB* H37Ra, providing significantly enhanced biological activities against these mycobacteria. Compounds **7g**, **8g**, **8c**, **7f**, **7d**, and **7a** exhibited notable inhibitory activity, having MIC values in the 0.56−1.90 μg/mL range against active *M. bovis* BCG, and compounds **8g**, **8c**, **7g**, **7a**, **7d**, **7b** and **7c** with 0.60−1.37 μg/mL range showed excellent activity against dormant *M. bovis* BCG. We observed that compounds with methyl (**7c**, **7f** and **8c**) or both methyl and chloro groups (**7g** and **8g**) are more effective than other derivatives. Importantly, the flavone **8g** exhibited superior activity against both active

Table 1. Antitubercular Activity of Compounds 7a−b, 7d, 7f, 8c, and 8g

	MIC_{90}								
	ex vivo		*in vitro*						
	MTB H37Ra		drug resistant H37Ra		MTB H37Ra		*M. bovis* BCG		
compd	active	dormant	active	dormant	active	dormant	active	dormant	cLogP[a]
7a	4.94	3.91	8.04	7.11	2.87	2.63	1.16	1.77	5.12
7b	2.83	0.91	5.3	2.73	3.29	2.79	2.22	2.7	4.97
7d	2.96	1.82	12.41	2.87	25.17	2.05	2.34	1.36	4.13
7f	25.84	2.61	8.97	9.42	5.45	4.41	1.68	1.61	4.62
8c	8.39	2.76	22.86	7.22	28.87	29.98	0.62	0.61	5.23
8g	7.67	2.18	9.58	9.1	25.36	24.34	0.56	0.60	5.94
RP	0.5	0.8	>10	>10	0.048	0.043	0.004	0.0042	
INH	0.05	0.075	>10	>10	0.074	0.075	0.075	0.0078	

[a]cLogP calculated using ChemDraw Ultra 12.0 software by Cambridge Soft.

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Table 2. Cytotoxicity of Compounds 7a−g, 8c, and 8g

	HeLa		Panc-1		THP-1	
compd	GI_{50}	GI_{90}	GI_{50}	GI_{90}	GI_{50}	GI_{90}
7a	6.75	20.28	18.33	>30	>100	>100
7b	4.42	8.24	8.44	26.03	7.48	>30
7c	7.12	>30	15.83	>30	>100	>100
7d	7.86	>30	7.77	>30	>100	>100
7e	>100	>100	26.22	>30	>100	>100
7f	23.11	>30	9.24	>30	>100	>100
7g	>100	>100	20.36	>30	>100	>100
8c	15.55	27.8	12.87	29.75	>100	>100
8g	21.22	>30	>100	>100	>100	>100
paclitaxel[a]	0.004	0.075	0.127	5.715	0.133	5.81

[a]Standard anticancer drug and also positive control.

(MIC = 0.56 μg/mL) and dormant (MIC = 0.60 μg/mL) *M. bovis* BCG. In other derivatives, the flavone **8c** with MIC value of 0.62 and 0.61 μg/mL against active and dormant *M. bovis* BCG, respectively, and 1,3-diketone **7g** with MIC value of 0.71 and 0.90 μg/mL against active and dormant *M. bovis* BCG, respectively, also exhibited excellent anti-TB activity. Compounds with hydrogen (**7d**), one chloro group (**7b**), and two chloro groups (**7e**) showed significant reduction of *M. bovis* BCG growth. Replacement of the chloro group by bromo group (**7a**) offers an advantage in the inhibition of *M. bovis* BCG growth, resulting in a dramatic enhancement of activity.

The activity of benzo[*b*]thiophene-2-carboxylic acid derivatives against multidrug-resistant H37Ra was determined by a "microdilution" 96-well plate assay.[13,14] On every microtiter plate containing one MDR *MTB* H37Ra strain, a dilution series of RIF and INH was included as positive control compounds. The results for all the compounds **7a−f**, **8c**, and **8g** at concentrations of 30−0.001 μg/mL (one-third dilutions) were scored by using an established XTT Reduction Menadione assay (XRMA),[12] and the results are presented in Table 1. The results were also cross checked by taking a final spectrophotometric reading of the microtiter plates at 600 nm. The most active compounds **7a−f**, **8c**, and **8g** were highly active against multidrug-resistant *M. tuberculosis* H37Ra (MICs range, 2.73 to 22.86 μg/mL against active and dormant stages). Compound **7b** having one bromo and one chloro substituent was highly active against multidrug-resistant *MTB* H37Ra (MIC = 2.73 μg/mL against dormant stage). In the same experiment, the activity of the compounds was compared to those of clinically available compounds RIF and INH and were found to be slightly better than that of RIF and INH.

These synthetic compounds **7a−f**, **8c**, and **8g** were further tested to determine their MIC against mycobacteria within THP-1 host macrophages (Table 1). *Ex vivo* studies against *MTB* revealed the strong anti-TB activity of benzo[*b*]-thiophene-2-carboxylic acid derivatives (**7a−f**, **8c**, and **8g**).

Among derivatives, **7b** was found to be highly effective and inhibited both active and dormant mycobacteria with MIC ranging from 0.91 to 2.83 μg/mL, corroborating its antimicrobial nature. However, the activity is not as profound as that of RIF and INH. The pattern was similar in *ex vivo* THP-1 infection model assay with MDR *MTB* H37Ra (*in vitro*) with compounds having the highest efficiency to inhibit *MTB*.

The cytotoxicity is important to find out potent antibiotics and to get insight into potential toxicity of identified inhibitor molecules. The cytotoxicity of most potent anti-TB agents **7a−g**, **8c**, and **8g** was further assessed against a panel of three human cancer cell lines HeLa (human epithelial cervical cancer), Panc-1 (pancreas carcinoma), and THP-1 (acute monocytic leukemia) using modified MTT cell viability assay.[15−17] The cytotoxic effect of these compounds was checked to determine the growth inhibition (GI), GI_{50} (Supporting Information) and GI_{90}, and paclitaxel was used as positive control (Table 2).

Except **7a**, **7b**, and **8c**, all compounds showed GI_{90} values >30 μg/mL in HeLa cell line. In the case of Panc-1 cell line, except **7b** and **8c**, all other compounds showed GI_{90} values >30 μg/mL. Notably, all compounds showed cytotoxic GI_{90} > 30 μg/mL on THP-1 cell line. Overall, all the tested compounds had low cytotoxic effect on these three human cell lines. The ratios between IC_{50} for human cancer cell lines (cytotoxicity) and MIC (antimycobacterial activity) *in vitro* against both active and dormant *MTB* H37Ra and *M. bovis* BCG[18,19] enabled the determination of selectivity index (SI) (Supporting Information). According to drug susceptibility study of TB, compounds that exhibited SI values >10 in all three cell lines were considered nontoxic.[20] For simultaneous detection of active and dormant stage inhibitors against tubercular bacilli, XRMA assay protocol was used, which follows a similar principle of hypoxia model of dormancy.[21]

When comparing the toxicity exhibited by these potent antitubercular 1,3-diketones and flavones, one compound **8g** exhibited comparatively higher selectivity index (SI > 30) at active and dormant state of THP-1 cell line of both *MTB* H37Ra and *M. bovis* BCG. Compounds **7f** (>13) and **8g** (>35) against both active and dormant HeLa cell line of *M. bovis* BCG possesses superior selectivity index. Importantly, 1,3-diketone derivative **8c** possessed a more favorable selectivity index (SI > 20) against all active and dormant three cell lines of *M. bovis* BCG. In addition, compound **7a** (>10) against active and dormant Panc-1 cell line, compounds **7c** and **7e** (>10) against dormant Panc-1 cell line, and compounds **7g** and **8c** (>22) against active and dormant state of THP-1 cell line exhibited better selectivity index for *M. bovis* BCG. These results demonstrated that compounds **8c** and **8g** have been found to have potential against *MTB* H37Ra and *M. bovis* BCG.

Mycobacteria can induce ROS (reactive oxygen species) production by activating phagocytes.[22] Although these are an important part of the host defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation, which may further contribute to immunosuppression,[23] particularly in HIV infected patients.[24] Therefore, the antioxidant activity of the antitubercular compounds was assessed by DPPH (1,1-diphenyl-2-picryl-hydrazil) radical

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scavenging method[25,26] (Supporting Information). The interaction of all tested compounds with the stable free radical DPPH indicates their radical scavenging activity by EC_{50} (effective concentration). These results indicate that the antioxidant activity of the 1,3-diketone and flavone derivatives could not simply be attributed to their antitubercular activity. The 1,3-diketone **7f** (EC_{50} = 20.00 μg/mL) was the best synthetic antioxidant as compared to standard antioxidant drug BHT.

Promising antitubercular activities demonstrated by benzo[*b*]thiophene-2-carboxylic acid derivatives motivated us to perform molecular docking studies to identify a potential target for them and thereby gaining an insight into the key molecular mechanisms in exerting inhibitory activity against *MTB*.

After scanning through the crystal structures available for *mycobacterium* targets in the Protein Data Bank (PDB), we obtained a fairly good agreement between experimental antitubercular data and the docking results against DprE1 enzyme (Figure 1). It is a key enzyme involved in the

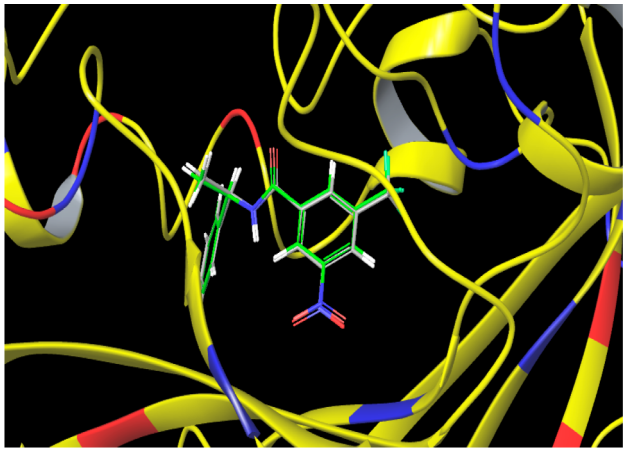

Figure 1. Overlay of the crystallographically observed binding mode of the native ligand on its best docked conformation.

biosynthesis of decaprenylphosphoryl-D-arabinose (DPA), which is the only known donor of D-arabinofuranosyl residues for the synthesis of arabinogalactan, a basic precursor for the mycobacterial cell wall core.[27,28] Therefore, DprE1 is also essential for the cell growth and survival making it a potential target for antimycobacterial drug design strategy.[29−32] Molecular docking study was performed using the standard protocol implemented in the GLIDE (Grid-based Ligand Docking with Energetics) module of the Schrodinger Molecular modeling package (Schrodinger, LLC, New York, NY, 2015).[33,34]

In order to rationalize the observed antitubercular results and to get more insight into the inhibition pattern, interactions of benzo[*b*]thiophene-2-carboxylic acid derivatives with the binding pocket of mycobacterial (DprE1) were analyzed and depicted using molecular docking studies.[28] Visual inspection of the minimum energy docked poses revealed that these derivatives snuggly fit into the binding pocket, making close contacts with the surrounding residues.

Their docking score varied from −9.198 for the most active analogue **7a** to −6.995 for the least active **7e**, while the docking score for the native ligand was found to be −7.953. This trend of docking scores corroborated well with the observed antitubercular activity where the active compounds showed higher scores, while those with relatively low inhibition were also predicted to have a lower docking score. Quantitative measures of the docking scores along with binding energy and noncovalent interactions observed for these derivatives are summarized in the Table S4 (Supporting Information). A detailed per-residue interaction analysis between the protein and the most active analogue **7a** only is elucidated in the next section for the sake of brevity through which we can speculate regarding the binding patterns in the cavity.

The binding mode of **7a** is presented in Figure 2. Though it showed multiple interactions with the residues in the active site,

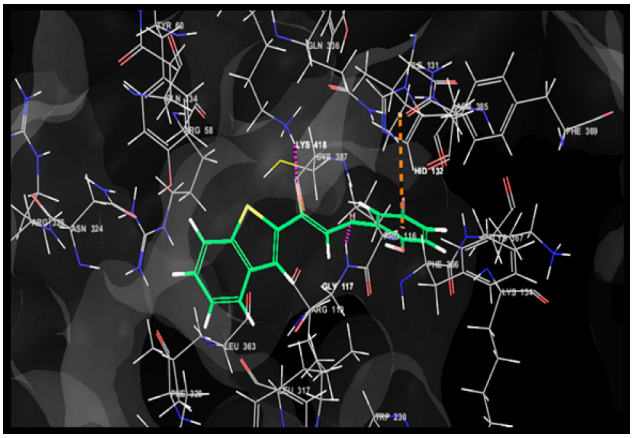

Figure 2. Binding mode of the most active compound **7a** into active site of DprE1.

however, for visibility and clarity only selected interacting residue are exhibited. Analysis of the docked complex showed very strong binding affinity (−50.095 kcal/mol) with the receptor wherein the contribution of the van der Waals interactions (−45.951 kcal/mol) was found to be more than the Coulombic interactions (−4.144 kcal/mol). The compound was found to be stabilized within the active site through an extensive chain of favorable van der Waals interactions observed with Cys387(−3.463), Asn385(−1.651), Phe369(−1.374), Lys367(−2.704), Phe366(−1.268), Val365(−4.669), Leu363(−2.049), Gln336(−1.763), Gln334(−1.44), Arg325(−10.823), Asn324(−1.039), Phe320(−2.084), Leu317(−2.551), Trp230(−1.012), His132(−3.631), Ile131(−1.393), Gly117(−1.577), Pro116(−2.175), and Tyr60(−1.545). Analysis of polar contacts such as electrostatic and pi−pi stacking interactions displayed that **7a** formed multiple closed interactions with Lys418(−2.185), Tyr415(−1.565), Arg413(−9.938), Lys367(−1.418), Lys134(−1.73), Arg119(−1.872), Gly117(−1.463), Pro116(−1.49) and , Arg58(−1.037) residues of DprE1. Strong binding of **7a** with active site of DprE1 is also contributed by its position in the pi-interaction in space of His 132 and through significant hydrogen bonding interactions with Lys418 and Gly117. Although all benzo[*b*]thiophene-2-carboxylic acid derivatives were observed to show similar binding patterns, the presence of relatively stronger noncovalent interactions in terms of van der Waals, electrostatic and pi−pi interactions and the higher number of intermolecular hydrogen bonds augments the stronger binding of the compound **7a** to the active site of DprE1.

In summary, our work demonstrates benzo[*b*]thiophenes as a new family of inhibitors with potent antimycobacterial properties. The most promising compounds in the present

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series, **8c** and **8g**, showed excellent activity and inhibited the growth of both active and dormant *M. bovis* BCG with MIC_{90} of 0.56−0.62 μg/mL range. Molecular docking studies of these benzo[*b*]thiophene-2-carboxylic acid derivatives have shown a high binding affinity toward the active site of DprE1 enzyme, which provides a strong platform for development of the lead molecules for this series forming potent antitubercular agents. Overall, our *ex vivo* infection model indicates that there is significant potential for **7b** for the treatment of tuberculosis. Meanwhile, diverse structural modifications are currently being investigated through iterative synthesis in conjugation with computer modeling, and the results will be given in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00077.

Experimental procedures for the synthesis of **4**, **7a−g**, **8a−g**, **9a−g**, and **11a−i**, representative ^{1}H NMR, ^{13}C NMR, FT-IR, and HRMS spectra for final compounds, and details of *in vitro* and *ex vivo* assay (PDF)

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Author Contributions

P.S.M. performed the chemical syntheses. V.M.K. performed the molecular docking study. L.U.N. and D.S. performed *in vitro* and *ex vivo* biological screenings. C.H.G. participated in the design and execution of this study.

Notes

The authors declare no competing financial interest.

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