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Identification of thiophene-benzenesulfonamide derivatives for the treatment of multidrug-resistant tuberculosis

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Abstract

A series of thiophene-benzenesulfonamide derivatives was designed and synthesized by exploring the structure-activity relationship of lead compounds 2,3-disubstituted thiophenes **25a** and 297F as antituberculosis agents, which displayed potent antimycobacterial activity against clinical strains of drug-susceptible and drug-resistant tuberculosis. In particular, compound **17b**, which had improved activity (minimum inhibitory concentration of 0.023 μg/mL) compared with the lead compounds, displayed good intracellular antimycobacterial activity in macrophages with a reduction of 1.29 $\log_{10}$ CFU. A druggability evaluation indicated that compound **17b** had favorable hepatocyte stability, low cytotoxicity, and low hERG channel inhibition. Moreover, compound **17b** exhibited modest *in vivo* efficacy in an acute mouse model of tuberculosis. In addition, the molecular docking study elucidated the binding mode of compound **17b** in the active site of DprE1. Therefore, compound **17b** may be a promising antituberculosis lead for further research.

Keywords

Thiophene-benzenesulfonamide; drug-resistant tuberculosis; SAR exploration; druggability evaluation

1. Introduction

Tuberculosis (TB) is a communicable disease that results from infection with *Mycobacterium tuberculosis* (*M. tuberculosis*) [1]. TB remains a major cause of ill health and is one of the top 10 causes of death worldwide. Globally, TB caused the deaths of approximately 1.2 million HIV-negative people and 208,000 HIV-positive people in 2019. Moreover, 10 million new TB cases were identified and there were approximately half a million new cases of rifampicin-resistant TB (RR-TB), of which 78% were multidrug-resistant (MDR) TB [2]. The COVID-19 pandemic has had impeded access to TB treatment, and the current control efforts are now believed to be insufficient to achieve the goal of ending the TB epidemic by 2030 [3]. Therefore, there

is an urgent need for new drugs with new mechanisms of action to treat MDR-TB and extensively drug-resistant (XDR) TB [4].

Several structurally diverse chemical scaffolds with desirable pharmacological profiles against *M. tuberculosis* have been described in the literature [5-12]. Decaprenylphosphoryl-*β*-D-ribose 2′-epimerase (DprE1), an essential enzyme for biogenesis of the mycobacterial cell wall core component arabinogalactan, has become a target of research and development for TB treatments [13, 14]. This mechanism via DprE1 is unique to mycobacteria, so there is inherent biochemical selectivity over other bacterial species and human cells. A new carbostyril derivative, OPC-167832 (Figure 1), which is a DprE1 inhibitor with superior efficacy and the potential to shorten TB treatment, has been in the stage of phase II clinical trial [15, 16]. The DprE1 inhibitor, 1,4-azaindole TBA-7371 (Figure 1), showed good efficacy in *in vivo* animal models of TB and is also undergoing phase II clinical trial [17, 18]. The discovery of noncovalent DprE1 inhibitor TCA1 (Figure 1) prompted further research to identify potential antitubercular agents based on the 2,3-disubstituted thiophene core [19, 20].

We designed and synthesized a series of thiophene-arylamide compounds derived from the DprE1 inhibitor TCA1 through a scaffold-hopping strategy [21]. Among them, representative compound **25a** (Figure 1), which had potent DprE1 inhibition and good intracellular antimycobacterial activity, demonstrated potent *in vivo* efficacy with bactericidal activity in an acute mouse model of TB. In addition, we showed that sulfonamide **10d** (Figure 1) derived from antibacterial agent sulfaphenazole exhibited good *in vitro* activity against both drug-susceptible and drug-resistant TB [22].

The 2,3-disubstituted thiophene compound 297F containing a sulfonamide moiety (Figure 1), which was screened from a library of 20,000 compounds, inhibited guanosine triphosphatase (GTPase) activity of *M. tuberculosis* FtsZ. 297F displayed good antimycobacterial activity and showed no antibacterial activities toward other Gram-positive or Gram-negative strains [23]. We speculated that 297F may have another mode of action via DprE1 inhibition to exert antimycobacterial activity due to its structural similarity with TCA1 and **25a**. The subsequent assay showed that 297F displayed potent DprE1 inhibition and good antimycobacterial activity, but had some

cytotoxicity and moderate hERG channel inhibition. These results encouraged us to develop DprE1 inhibitors containing the benzenesulfonamide fragment as anti-TB agents with potent efficacy and good druggability.

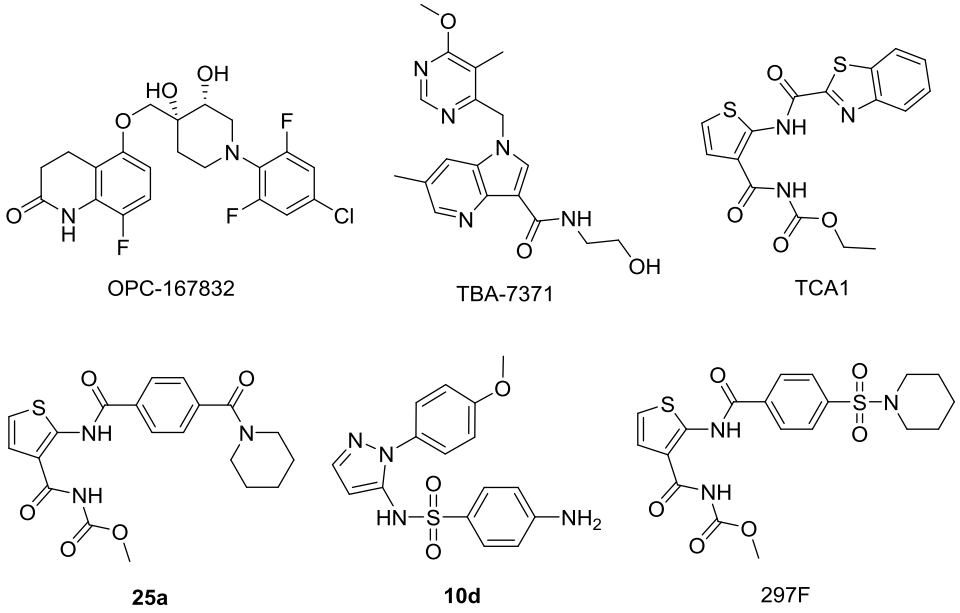


Figure 1. Structures of representative anti-TB agents.

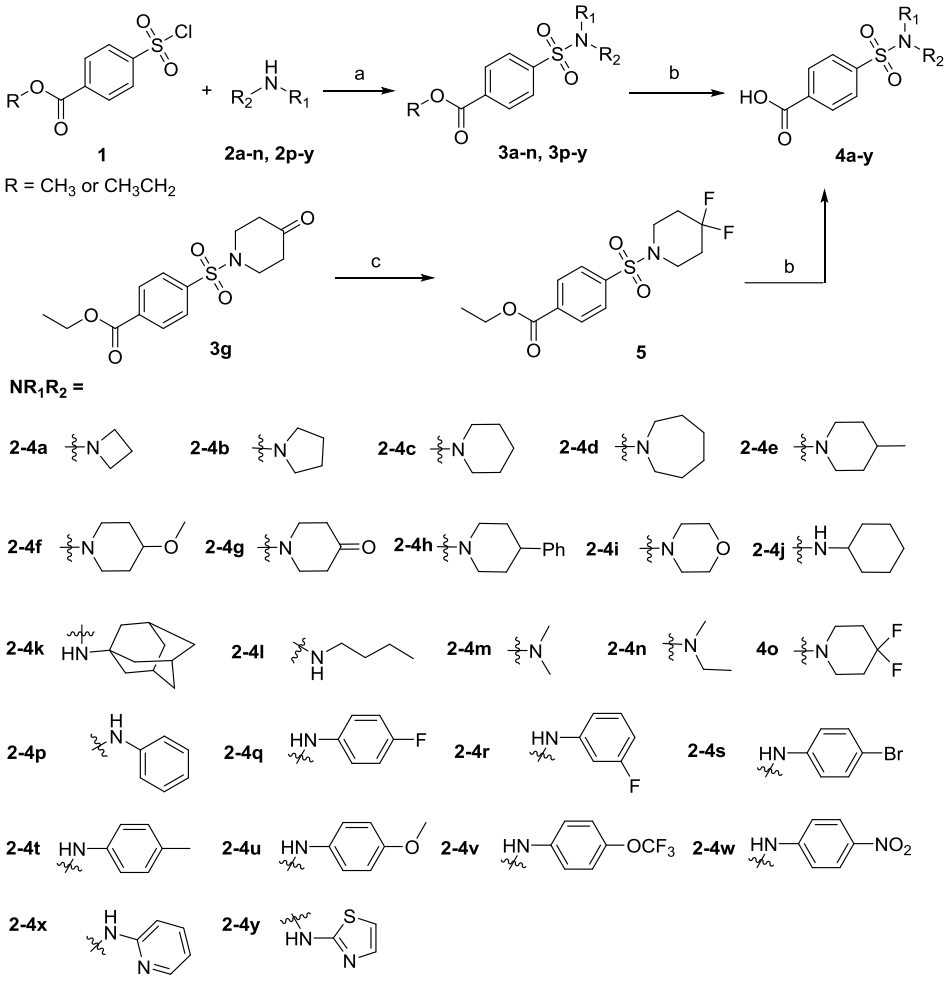
In this work, we designed and synthesized a series of benzenesulfonamide compounds derived from DprE1 inhibitors **25a** and 297F to explore their structure-activity relationships (SARs) and preliminary druggability profiles. Systematic optimization of the compounds on the two side chains flanking the thiophene core led to compound **17b** with improved *in vitro* activity and low cytotoxicity. Furthermore, the molecular docking study elucidated the binding mode of compound **17b** in the active site of DprE1. Compound **17b** with favorable druggability may be suitable as a lead compound to seek drug candidate for the treatment of multidrug-resistant TB.

2. Chemistry

The synthesis of benzoic acid intermediates with various sulfonamide motifs (**4a–y**) is outlined in Scheme 1. The amidation of commercially available alkyl 4-(chlorosulfonyl)benzoate (**1**) with the amines **2a–n** or **2p–y** in the presence of triethylamine produced the corresponding intermediates **3a–n** and **3p–y**, respectively. Difluorinated intermediate **5** was obtained from carbonyl intermediate **3g** via diethylaminosulfur trifluoride (DAST)-induced fluorination. Intermediates **3a–n**, **3p–**

y, and **5** were converted to benzoic acid intermediates **4a–y** via hydrolysis with aqueous lithium hydroxide solution.

Scheme 1. Synthesis of Benzoic Acid Intermediates **4a–y**.

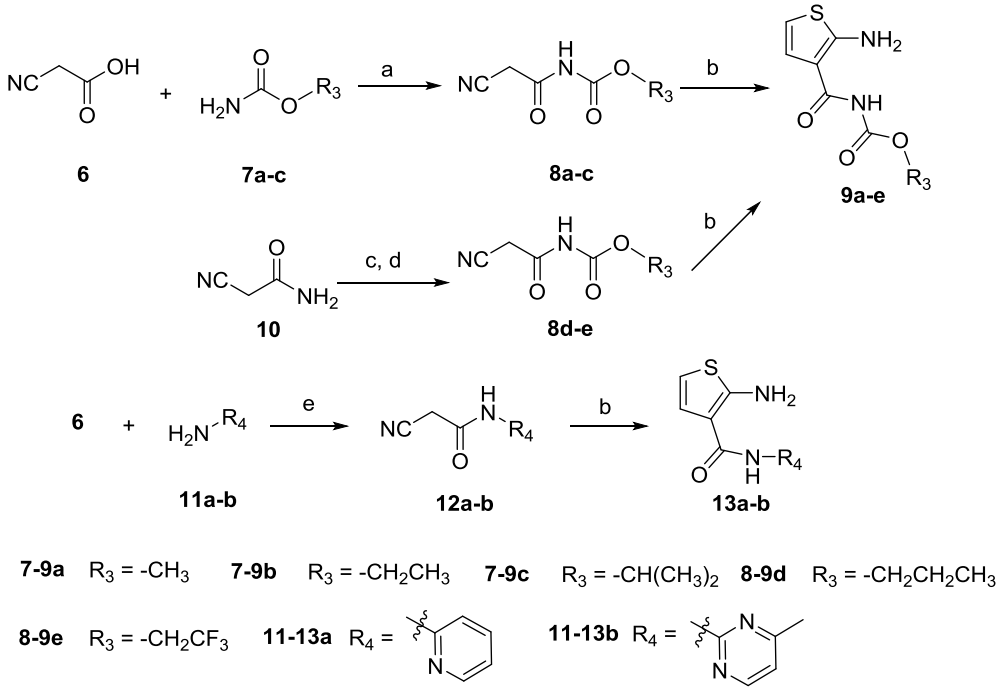


Reagents and conditions: (a) Et_3N, DCM, r.t, 5 h; (b) $LiOH{\cdot}H_2O$, CH_3OH, H_2O, r.t, 2 h; (c) DAST, DCM, r.t, 3 h.

Substituted aminothiophene intermediates **9a–e** and **13a–b** were synthesized via the Gewald reaction following the procedures shown in Scheme 2. Intermediates **8a–c** were obtained from 2-cyanoacetic acid (**6**) and carbamates **7a–c** in the presence of phosphorus oxychloride. 2-Cyanoacetamide (**10**) was reacted with oxalyl chloride in 1,2-dichloroethane under reflux to provide the isocyanate, and the subsequent reaction with the corresponding alcohols gave intermediates **8d–e**. Gewald heterocyclization of **8a–e** with 2,5-dihydroxy-1,4-dithiane produced aminothiophene intermediates **9a–e**. Treatment of **6** with aryl amines **11a–b** through condensation reactions in the presence

of DCC and DMAP produced intermediates **12a–b**. Corresponding aminothiophene intermediates **13a–b** were obtained from **12a–b** via the Gewald reaction.

Scheme 2. Synthesis of Aminothiophene Intermediates **9a–e** and **13a–b**.



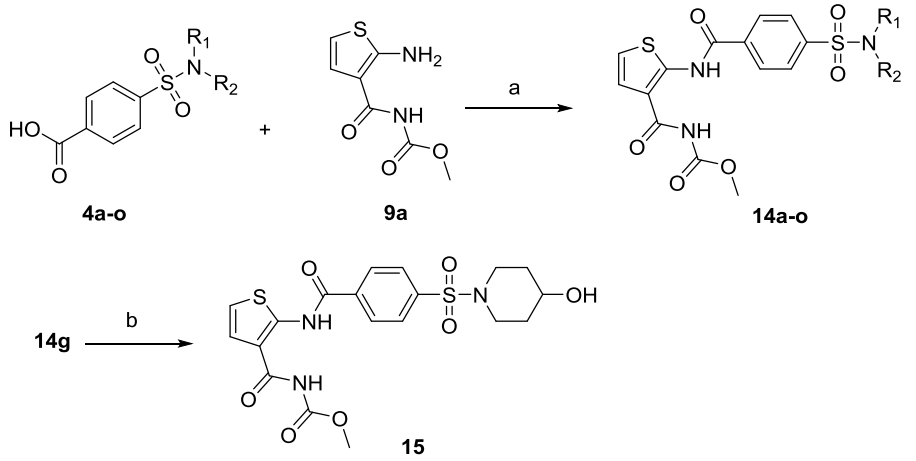
7-9a R_3 = -CH_3 **7-9b** R_3 = -CH_2CH_3 **7-9c** R_3 = -$CH(CH_3)_2$ **8-9d** R_3 = -$CH_2CH_2CH_3$

8-9e R_3 = -CH_2CF_3 **11-13a** R_4 = **11-13b** R_4 =

Reagents and conditions: (a) Phosphorus oxychloride, DMF, toluene, 80 °C, 3 h; (b) 2,5-Dihydroxy-1,4-dithiane, Et_3N, CH_3OH, 50 °C, 2.5–4.5 h; (c) Oxalyl chloride, 1,2-dichloroethane, reflux, 4 h; (d) R_3OH, CH_3CN, -10 °C, 3 h; (e) DCC, DMAP, DCM, rt, 5 h.

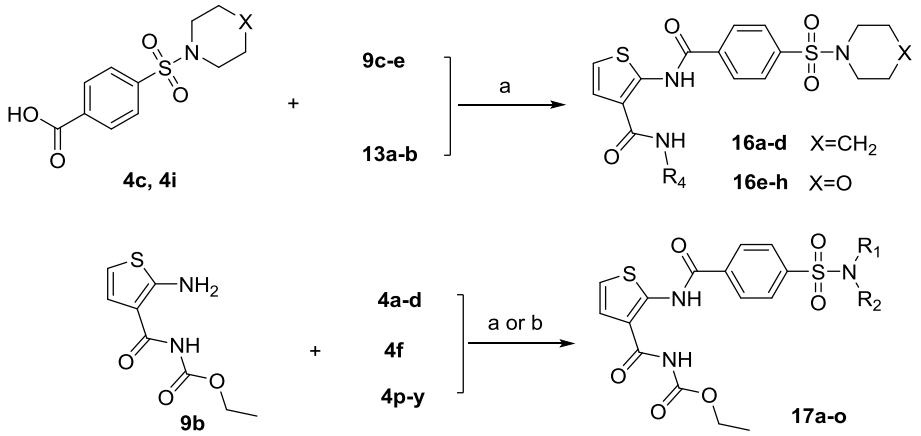
The target compounds **14a–o**, **16a–h**, and **17a–o** were obtained conveniently through the condensation reaction in the presence of HATU according to our previous work (Schemes 3 and 4) [21]. The subsequent reduction of intermediate **14g** with sodium borohydride afforded the target compound **15** (Scheme 3).

Scheme 3. Synthesis of the Target Products **14a-o** and **15**.



Reagents and conditions: (a) HATU, Et_3N, DMF, r.t, 12 h; (b) $NaBH_4$, CH_3OH, r.t, 12 h.

Scheme 4. Synthesis of the Target Products **16a-h** and **17a-o**.



Reagents and conditions: (a) HATU, Et_3N, DMF, r.t, overnight; (b) HATU, Et_3N, DMAP, DMF, r.t, overnight.

3. Results and discussion

3.1 Identification of anti-TB agent 297F as a DprE1 inhibitor

The anti-TB agent 297F, which has a 2,3-disubstituted thiophene core similar to TCA1 and **25a**, was screened against *M. tuberculosis* DprE1 and $H_{37}Rv$, Vero and HepG2 cells, and for hERG channel inhibition. 297F showed potent DprE1 inhibition with an IC_{50} of 0.1 μg/mL and good antimycobacterial activity with a minimum inhibitory concentration (MIC) of 0.24 μg/mL. These results indicated that 297F, which was previously reported to inhibit GTPase and polymerization of FtsZ, may be a dual-target

compound toward both FtsZ and DprE1 to exert antimycobacterial activity. However, 297F displayed some cytotoxicity against Vero and HepG2 cells with IC_{50} values of 3.62 and 36.79 μg/mL, respectively, and moderate hERG channel inhibition with an IC_{50} of 3.3 μM. To improve the efficacy and safety profiles, we subsequently explored the SARs of the 2,3-disubstituted thiophene compounds with the benzenesulfonamide fragment.

3.2 Optimization of thiophene-benzenesulfonamides as potent anti-TB agents

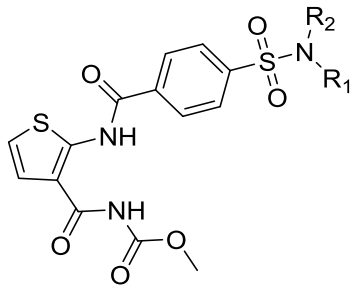
All the final compounds were evaluated for their inhibitory activity against *M. tuberculosis* $H_{37}Rv$ in a microplate Alamar Blue assay and cytotoxicity against the Vero cell line. Tables 1–3 summarize the biological data for benzenesulfonamide derivatives.

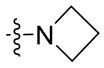
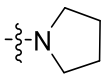
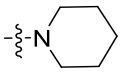
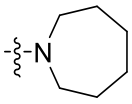
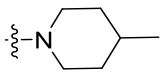
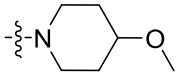
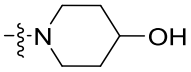
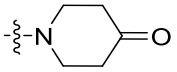
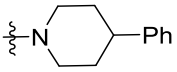
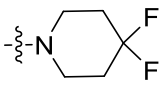
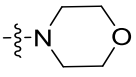
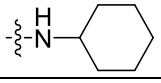
MIC-based SAR studies against *M. tuberculosis* for lead compounds **25a** and 297F were focused on optimizing the two side chains flanking the thiophene core.

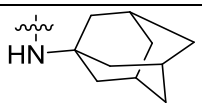
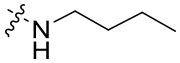
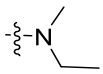
Firstly, various amines at the terminal of the side chain were investigated to explore the favorable occupancy of hydrophobic pockets of DprE1 (Table 1). Smaller substituents, such as azetidine (**14a**, MIC = 0.13 μg/mL) and pyrrolidine (**14b**, MIC = 0.10 μg/mL), improved the activity. In addition, compound **14b** showed a 26-fold increase in the selectivity index (SI) relative to 297F (**14c**) (SI = 395 vs 15). The compounds with bulky azepane (**14d**, MIC = 0.22 μg/mL) and 4-methylpiperidine moieties (**14e**, MIC = 0.23 μg/mL) exhibited antimycobacterial activities similar to 297F (MIC = 0.24 μg/mL), but also showed some cytotoxicity (IC_{50} < 10 μg/mL). Replacing the methyl group (**14e**) with a methoxy group (**14f**) improved the activity relative to 297F or **25a** eight- or six-fold, respectively (MIC = 0.03 μg/mL vs 0.24 and 0.19 μg/mL). The introduction of polar groups, such as hydroxyl (**15**) and carbonyl (**14g**) groups, decreased the activity (MIC = 3.67 and 3.52 μg/mL). The addition of a terminal phenyl group on the piperidine (**14h**) resulted in the dramatic loss of antimycobacterial activity (MIC > 32 μg/mL). The bioisosteric replacement strategy to replace methylene (297F, MIC = 0.24 μg/mL) with difluoromethylene (**14o**, MIC = 0.49 μg/mL) or oxygen (**14i**, MIC = 0.49 μg/mL) caused a two-fold decrease in antimycobacterial activity. Compounds **14j** and **14k** bearing amantadine and cyclohexylamine moieties, respectively, also decreased the

antimycobacterial activities relative to 297F and **25a** (MIC = 0.92 and 1.96 μg/mL vs 0.24 and 0.19 μg/mL, respectively), whereas compound **14j** displayed lower cytotoxicity against Vero cells (IC_{50} > 64 μg/mL). Finally, the compound with a linear amine (**14l**) (MIC = 0.28 μg/mL) had similar antimycobacterial activity to 297F. Ethyl(methyl) amine (**14n**, MIC = 0.49 μg/mL) was tolerated at the R_1 and R_2 positions, whereas the slightly smaller dimethyl amine (**14m**, MIC = 1.19 μg/mL) decreased the activity.

Table 1. SAR of benzenesulfonamides with imide methyl ester at the R_1 and R_2 sites



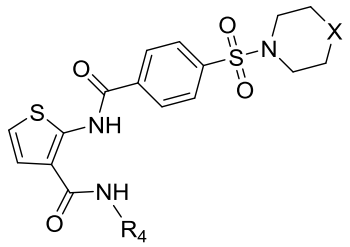
Compound	NR_1R_2	MIC[a] (μg/mL)	Vero IC_{50} (μg/mL)	SI[b]
14a		0.13	17.47	134
14b		0.10	39.45	395
14c (297F)		0.24	3.62	15
14d		0.22	9.13	42
14e		0.23	9.12	40
14f		0.03	18.02	601
15		3.67	-[c]	-
14g		3.52	-	-
14h		>32	-	-
14o		0.49	49.01	100
14i		0.49	35.65	73
14j		0.92	>64	>70

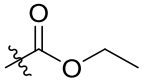
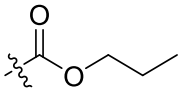
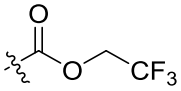
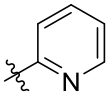
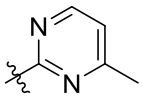
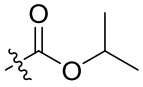
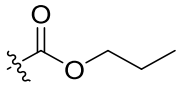
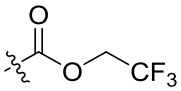
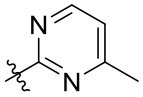
14k		1.96	-	-
14l		0.28	29.47	105
14m		1.19	16.17	14
14n		0.49	10.41	21
25a	N/A[d]	0.19	>64	>337

[a]MIC against *M. tuberculosis* $H_{37}Rv$. [b]SI = selectivity index, IC_{50}/MIC. [c]Not determined. [d]Not applicable.

Keeping the sulfonamide fragments at the 2-position side chain of thiophene, we focused on modifying the 3-position with substituents at the R_4 site (Table 2). Replacing the methyl ester in 297F with an ethyl ester moiety resulted in significantly improved activity (**17c**, MIC = 0.03 μg/mL). When we lengthened the terminal side chain to a propyl ester, compound **16a** showed slightly lower antimycobacterial activity than compound **17c**, although the cytotoxicity against Vero cells decreased (IC_{50} = 56.63 μg/mL). The replacement of the terminal methyl group in **17c** with a terminal trifluoromethyl group (**16b**, MIC = 29.74 μg/mL) caused a large decrease in activity. The introduction of a rigid *meta*-substituted pyrimidine (**16d**) showed similar antimycobacterial activity to **25a** (MIC = 0.24 vs 0.19 μg/mL), whereas compound **16c** with a pyridine moiety had a markedly lower activity (MIC = 29.61 μg/mL). Additionally, replacing piperidine (**16d**) with morpholine (**16h**) at the 2-position of thiophene resulted in a lower activity (MIC = 0.24 vs 0.87 μg/mL). Compound **16e** bearing an isopropyl ester moiety possessed good activity (MIC = 0.45 μg/mL), but showed some cytotoxicity (IC_{50} = 13.13 μg/mL). The introduction of propyl (**16f**) and trifluoroethyl (**16g**) groups decreased the activity. Therefore, the imide ethyl ester fragment was best tolerated at the 3-position side chain of thiophene core.

Table 2. SAR of benzenesulfonamides at the X and R_4 sites



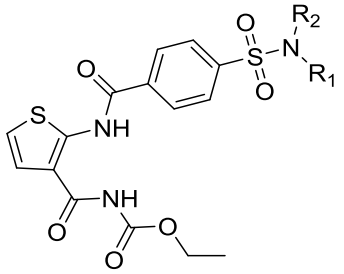
Compound	R_4	X	MIC[a] (μg/mL)	Vero IC_{50} (μg/mL)	SI[b]
17c		CH_2	0.03	18.57	619
16a		CH_2	0.051	56.63	1110
16b		CH_2	29.74	-[c]	-
16c		CH_2	29.61	-	-
16d		CH_2	0.24	32.76	137
16e		O	0.45	13.13	29
16f		O	2.45	-	-
16g		O	30.44	-	-
16h		O	0.87	42.73	49
25a	N/A[d]	N/A	0.19	>64	>337

[a]MIC against *M. tuberculosis* $H_{37}Rv$. [b]SI = selectivity index, IC_{50}/MIC. [c]Not determined. [d]Not applicable.

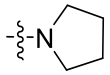
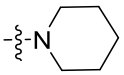
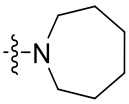
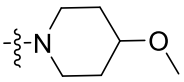
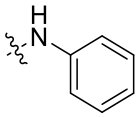
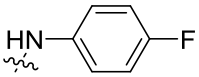
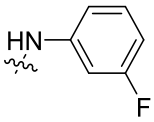
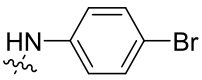
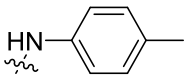
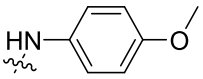
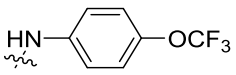
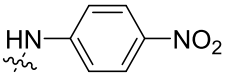
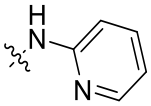
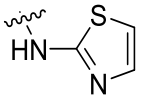
Guided by the above SAR results of the side chain at the 3-position of thiophene, further modification of the sulfonamide terminal amines was investigated (Table 3). To our delight, compounds **17a–e** with conformationally restricted cyclic amines demonstrated potent antimycobacterial activity with MICs of < 0.016–0.049 μg/mL. In particular, compound **17b** with a pyrrolidine moiety, which possessed low cytotoxicity (IC_{50} = 58.18 μg/mL), displayed a 242-fold increase in SI relative to 297F (SI > 3636 vs 15). Compound **17d** with a larger azepane moiety exhibited good activity (MIC = 0.024

μg/mL), but showed some cytotoxicity (IC_{50} = 3.42 μg/mL). Compounds **17c** and **17e** with piperidine moieties exhibited similar antimycobacterial activity and cytotoxicity and about a 40-fold increase in SI compared with 297F. Replacing the cyclic aliphatic amines with an aromatic aniline moiety (**17f**) resulted in good activity (MIC = 0.7 μg/mL). Inspired by this promising result, a series of substituted aromatic amine analogs with electron-donating and electron-withdrawing groups which may have the potential for forming additional π–π interactions in the hydrophobic pocket of DprE1 were evaluated (**17g–o**). Introducing a fluoro substituent onto the phenyl ring afforded compound **17g** (MIC = 0.98 μg/mL), which showed better activity than **17i** with a bromo substituent at the same position (MIC = 2.79 μg/mL). Compound **17h**, with a *meta*-fluoro group, demonstrated similar activity to **17g** with a *para*-fluoro group (MIC = 1.14 vs 0.98 μg/mL). The replacement of a methyl (**17j**) with a fluoro substituent (**17g**) decreased the antimycobacterial activity (MIC = 3.56 vs 0.98 μg/mL). Methoxy-substituted compound **17k** displayed moderate activity with a MIC of 3.64 μg/mL, whereas the introduction of a trifluoromethoxy group (**17l**) caused a sharp decrease in activity (MIC = 26.31 μg/mL). Compound **17m** with a nitro group demonstrated moderate activity (MIC = 3.61 μg/mL), which was similar to compound **17k** containing a methoxy group. We then turned our attention to replacing the phenyl scaffold with heteroaromatic rings. Pyridine (**17n**) and thiazole (**17o**) moieties gave moderate activities (MIC = 1.55 and 7.64 μg/mL) that were much lower than the reference compound **25a**.

Table 3. SAR of benzenesulfonamides with an imide ethyl ester at the R_1 and R_2 sites



Compound	NR_1R_2	MIC[a] (μg/mL)	Vero IC_{50} (μg/mL)	SI[b]
17a		0.049	>64	>1306

17b		<0.016	58.18	>3636
17c		0.03	18.57	619
17d		0.024	3.42	143
17e		0.03	21.03	701
17f		0.70	14.47	21
17g		0.98	20.53	21
17h		1.14	-[c]	-
17i		2.79	-	-
17j		3.56	-	-
17k		3.64	-	-
17l		26.31	-	-
17m		3.61	-	-
17n		1.55	-	-
17o		7.64	-	-
25a	N/A[d]	0.19	>64	>337

[a]MIC against *M. tuberculosis* $H_{37}Rv$. [b]SI = selectivity index, IC_{50}/MIC. [c]Not determined. [d]Not applicable.

3.3 Anti-XDR-TB activity of selected compounds

Based on the antimycobacterial activities against *M. tuberculosis* $H_{37}Rv$, representative compounds **14f**, **17a–c** and the reference compounds 297F, isoniazid (INH), rifampicin

(RFP), streptomycin (SM) and levofloxacin (LVFX) were evaluated further against two clinically isolated XDR-TB strains (Table 4). These selected compounds with diverse side chains displayed better anti-XDR-TB activities than 297F with MICs of < 0.016–0.44 μg/mL. In particular, benzenesulfonamide analogs **17a–c** with potent *in vitro* activities (MIC < 0.1 μg/mL) were identified as potential lead compounds for further investigation.

Table 4. Activity of representative compounds against clinical isolates of *M. tuberculosis*

Compound	MIC (μg/mL)		
	$H_{37}Rv$	13946[a]	14862[b]
14f	0.12	0.25	0.44
17a	0.027	0.060	0.062
17b	0.023	0.057	0.030
17c	<0.016	<0.016	<0.016
297F	0.10	0.48	0.51
INH	0.019	2.46	>10
RFP	0.015	>10	9.24
SM	0.49	>2	>2
LVFX	0.33	1.94	1.87

[a]Resistant to isoniazid (INH), streptomycin (SM), rifampicin (RFP), ethambutol (EMB), rifabutin, *para*-aminosalicylate (PAS), and ofloxacin. [b]Resistant to INH, SM, RFP, EMB, PAS, prothionamide, and capreomycin.

3.4 Preliminary ADME/T assay

The metabolic and toxic profiles of the selected benzenesulfonamide compounds from different series (**17b**, **17c**, **16d**, and **14f**), along with the reference compound 297F, were evaluated (Table 5). Compounds **17b** and **17c** displayed significantly higher stability in human hepatocytes compared with 297F ($t_{1/2}$ = 24.0–29.9 min vs 4.6 min). Compound **16d** with a pyrimidine motif exhibited similar stability to the other compounds in human hepatocytes, but low metabolic stability in mouse hepatocytes. Additionally, compounds **16d**, **14f**, and 297F showed moderate hERG channel inhibition (IC_{50} = 1.7–8.9 μM), which may cause QT prolongation. Because compound **17b** displayed high safety with very low hERG channel inhibition (IC_{50} > 30 μM) and cytotoxicity towards HepG2 and J774A.1 macrophage cells (IC_{50} > 60 μg/mL) alongside potent efficacy and good druggability, we investigated it further in a pharmacodynamic study.

Table 5. Hepatocyte stability, cytotoxicity, and hERG channel inhibition of selected compounds

Compound	Hepatocyte stability				Cytotoxicity IC_{50} (μg/mL)		hERG K^+ inhibition IC_{50} (μM)
	Mouse		Human				
	Remaining[a] (%)	$t_{1/2}$ (min)	Remaining[a] (%)	$t_{1/2}$ (min)	HepG2	J774A.1[b]	
17b	49.7	29.7	42.0	24.0	62.89	>64	>30
17c	66.3	50.6	49.8	29.9	38.77	34.33	-[c]
16d	7.3	7.9	41.2	23.4	-	-	1.7
14f	-	-	-	-	46.10	54.94	8.9
297F	22.7	14	1.0	4.6	36.80	36.88	3.3

[a]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [b]Mouse J774A.1 macrophage cell. [c]Not determined.

3.5 Intracellular antimycobacterial activity and *in vivo* efficacy study

We investigated the antimycobacterial activity of compound **17b** in an intracellular macrophage infection model because *M. tuberculosis* is an intracellular pathogen and survives in macrophages (Table 6). At a concentration of 5 μg/mL, compound **17b** exhibited a reduction of 1.19 $\log_{10}$ CFU, which was greater than those of TCA1 and **25a** (0.61 and 0.46 $\log_{10}$ CFU, respectively) and similar to the positive control of RFP [21]. However, treatment with 10 μg/mL compound **17b** for 3 days resulted in a reduction of 1.29 $\log_{10}$ CFU without obvious dose−response. It may be due to the permeability of the cell wall to the specific compounds.

Table 6. Activity of selected compounds in an intracellular macrophage infection model

Compound	Log_{10} CFU/macrophages[a]			
	10 μg/mL	ΔLog_{10} CFU[b]	5 μg/mL	ΔLog_{10} CFU
17b	4.23 ± 0.00	1.29	4.33 ± 0.04	1.19
RFP	-[c]	-	4.49	1.03
Untreated	5.52	-	5.52	-

[a]Log_{10} CFU against *M. tuberculosis* ($H_{37}Rv$) in infected mouse J774A.1 macrophages.
[b]ΔLog_{10} CFU = $\log_{10}$ CFU (untreated) - $\log_{10}$ CFU (treated with selected compounds).
[c]Not determined.

Subsequently, we evaluated the *in vivo* efficacy of compound **17b** in a murine model of acute infection with *M. tuberculosis* $H_{37}Rv$ (Table 7). The same formulation of 0.5% carboxymethylcellulose in water was used for all tested compounds. After three weeks of treatment, oral administration of 100 mg/kg compound **17b** showed modest activity, reducing the bacterial burden in the lungs by 0.54 $\log_{10}$ CFU compared with the

untreated control group. The inconsistency with the *in vitro* and *in vivo* activities may be due to the poor solubility and permeability profiles, which need to be explored in due course.

Table 7. Efficacy of compound **17b** after 3 weeks of treatment in Balb/c mice against *M. tuberculosis* H_{37}Rv infection (mean ± SD)

Compound	Dose (mg/kg)	Weight of mice (g)	Log_{10} CFU/lung
Untreated	-	21.32 ± 1.03	5.35 ± 0.17
17b	100	20.94 ± 0.63	4.81 ± 0.60
INH	25	20.90 ± 0.95	1.76 ± 0.49

3.6 Molecular docking study

Lastly, to explain the improved anti-TB activity of compound **17b** compared with the reference compounds 297F and **25a**, the binding mode of compound **17b** in the active site of DprE1 was investigated by using the CDOCKER protocol in Discovery Studio 2018 (Figure 2; PDB: 4KW5). There were key hydrogen-bonding interactions between the carbonyls in compound **17b** and Ser228 and Lys418. The sulfur atom of the thiophene moiety coupled with His132 deep in the binding pocket, similar to TCA1 and **25a** [21]. Additionally, the sulfonyl group of the sulfonamide moiety formed three critical hydrogen bonds with Tyr60, Gln334, and Arg325, whereas the terminal pyrrolidine and ethyl groups interacted with Leu317 and Pro316, respectively. These additional reinforcing interactions indicated that the combination of pyrrolidine and ethyl may result in a favorable molecular size for the optimal occupancy of the hydrophobic pockets of DprE1. We intend to perform further structural modifications around the binding domain to modulate the antimycobacterial activity and druggability.

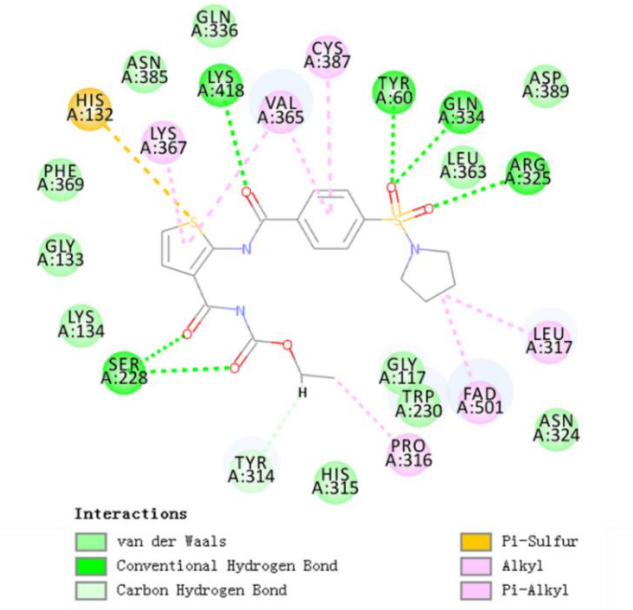


Figure 2. Molecular docking study of compound **17b** in DprE1.

4. Conclusion

We designed and synthesized a series of benzenesulfonamide compounds derived from lead compounds **25a** and 297F for treating multidrug-resistant TB. The SAR exploration indicated that structural modification of the two terminal side chains strongly affected antimycobacterial activity and cytotoxicity. The sulfonamide moiety was the key to maintaining the potent antimycobacterial activity. The conformationally restricted cyclic amines were a favorable size for optimal occupancy of the hydrophobic pockets of DprE1. The imide ethyl ester fragment at the 3-position of thiophene resulted in a significant increase in activity compared with other substituents at the R_4 site. Importantly, representative compound **17b** displayed potent *in vitro* activity against both drug-susceptible and drug-resistant TB clinical strains with MICs of < 0.1 μg/mL, as well as good intracellular antimycobacterial activity with a 1.29 $\log_{10}$ CFU reduction in macrophages. Preliminary druggability evaluation of **17b** revealed that this compound displayed superior mouse and human hepatocyte stability, very low cytotoxicity and hERG channel inhibition compared with the reference 297F. We will perform further structural optimization to improve the druggability of these benzenesulfonamide compounds toward developing a promising drug candidate for treating multidrug-resistant TB.

5. Experimental

5.1. General experimental information

All starting materials, reagents, and solvents were obtained from commercial sources and used without further purification. Flash column chromatography was carried out on silica gel (300 – 400 mesh). ^{1}H and ^{13}C NMR spectra were generated on Varian-400 and Mercury-500/600 spectrometers in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6. HR-MS (ESI) data were measured on Thermo Exactive Orbitrap plus spectrometer. Melting points were determined on a Yanaco MP-J3 microscope melting point apparatus. Chemical shifts values were referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (*J*) values were given in Hz.

5.2. General procedure for synthesis of compounds

*5.2.1. General procedure for the synthesis of intermediates **3a-n*** and ***3p-y***.

To a solution of sulphonyl chloride intermediate **1** (16.08 mmol) in anhydrous CH_2Cl_2 (300 mL) was added the corresponding amines **2a-n**, **3p-y** (24.58 mmol) and Et_3N (6.4 mL, 48.2 mmol) cooled with an ice bath. The reaction mixture was stirred at room temperature for 5 h under argon, then quenched with water (200 mL) and extracted with CH_2Cl_2 (150 mL×2). The combined organic phase was washed with 1 mol/L HCl (200 mL) and brine (200 mL) in turn. The obtained organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuum* to give compounds **3a-n** and **3p-y**.

*5.2.1.1. Ethyl 4-(azetidin-1-ylsulfonyl)benzoate (**3a**).* White solid; yield 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.24 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 3.83 – 3.80 (m, 4H), 2.13 – 2.06 (m, 2H), 1.43 (t, *J* = 7.2 Hz, 3H).

*5.2.1.2. Ethyl 4-(pyrrolidin-1-ylsulfonyl)benzoate (**3b**).* White solid; yield 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.4 Hz, 2H), 7.90 (d, *J* = 8.4 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.28 – 3.25 (m, 4H), 1.79 – 1.75 (m, 4H), 1.42 (t, *J* = 7.2 Hz, 3H).

*5.2.1.3. Methyl 4-(piperidin-1-ylsulfonyl)benzoate (**3c**).* White solid; yield 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 3.97 (s, 3H), 3.03 – 3.00 (m, 4H), 1.67 – 1.62 (m, 4H), 1.46 – 1.41 (m, 2H).

*5.2.1.4. Ethyl 4-(azepan-1-ylsulfonyl)benzoate (**3d**).* White solid; yield 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 2H), 4.42 (q, *J* = 7.2

Hz, 2H), 3.30 – 3.27 (m, 4H), 1.73 – 1.71 (m, 4H), 1.60 – 1.57 (m, 4H), 1.42 (t, *J* = 7.2 Hz, 3H).

*5.2.1.5. Ethyl 4-((4-methylpiperidin-1-yl)sulfonyl)benzoate (**3e**).* White solid; yield 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 8.4 Hz, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.79 – 3.76 (m, 2H), 2.29 – 2.23 (m, 2H), 1.69 – 1.66 (m, 2H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.32 – 1.26 (m, 3H), 0.9 (d, *J* = 5.6 Hz, 3H).

*5.2.1.6. Ethyl 4-((4-methoxypiperidin-1-yl)sulfonyl)benzoate (**3f**).* White solid; yield 97%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.5 Hz, 2H), 7.82 (d, *J* = 8.5 Hz, 2H), 4.43 (q, *J* = 7.0 Hz, 2H), 3.29 (br s, 1H), 3.25 (s, 3H), 3.15 – 3.11 (m, 2H), 3.06 – 3.05 (m, 2H), 1.89 – 1.85 (m, 2H), 1.75 – 1.72 (m, 2H), 1.42 (t, *J* = 7.0 Hz, 3H).

*5.2.1.7. Ethyl 4-((4-oxopiperidin-1-yl)sulfonyl)benzoate (**3g**).* White solid; yield 82%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.21 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H), 4.43 (q, *J* = 7.0 Hz, 2H), 3.45 – 3.42 (m, 4H), 2.57 – 2.54 (m, 4H), 1.42 (t, *J* = 7.0 Hz, 3H).

*5.2.1.8. Ethyl 4-((4-phenylpiperidin-1-yl)sulfonyl)benzoate (**3h**).* White solid; yield 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.32 – 7.26 (m, 2H), 7.24 – 7.19 (m, 1H), 7.15 – 7.14 (m, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 3.99 – 3.96 (m, 2H), 2.45 – 2.34 (m, 3H), 1.92 – 1.82 (m, 4H), 1.43 (t, *J* = 7.2 Hz, 3H).

*5.2.1.9. Methyl 4-(morpholinosulfonyl)benzoate (**3i**).* White solid; yield 91%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 3.98 (s, 3H), 3.76 – 3.74 (m, 4H), 3.04 – 3.02 (m, 4H).

*5.2.1.10. Ethyl 4-(N-cyclohexylsulfamoyl)benzoate (**3j**).* White solid; yield 87%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.17 (d, *J* = 8.0 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 2H), 4.78 (br s, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 3.17 (br s, 1H), 1.75 – 1.73 (m, 2H), 1.64 – 1.62 (m, 2H), 1.53 – 1.50 (m, 1H), 1.44 – 1.41 (m, 3H), 1.24 – 1.09 (m, 5H).

*5.2.1.11. Methyl 4-(N-((1s,3s)-adamantan-1-yl)sulfamoyl)benzoate (**3k**).* White solid; yield 74%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8.0 Hz, 2H), 7.98 (d, *J* = 8.0 Hz, 2H), 4.71 (br s, 1H), 3.96 (s, 3H), 2.01 (br s, 3H), 1.78 (br s, 6H), 1.61 – 1.54 (m, 6H).

*5.2.1.12. Ethyl 4-(N-butylsulfamoyl)benzoate (**3l**).* White solid; yield 95%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.0 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 2H), 5.10 (br s, 1H),

4.44 – 4.40 (m, 2H), 2.98 – 2.95 (m, 2H), 1.47 – 1.41 (m, 5H), 1.31 – 1.27 (m, 2H), 0.85 (t, *J* = 7.0 Hz, 3H).

*5.2.1.13. Ethyl 4-(N,N-dimethylsulfamoyl)benzoate (**3m**).* White solid; yield 93%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 2.74 (br s, 6H), 1.42 (t, *J* = 7.2 Hz, 3H).

*5.2.1.14. Ethyl 4-(N-ethyl-N-methylsulfamoyl)benzoate (**3n**).* White solid; yield 95%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 2H), 4.42 (q, *J* = 7.0 Hz, 2H), 3.14 (q, *J* = 6.5 Hz, 2H), 2.77 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H), 1.14 (t, *J* = 6.5 Hz, 3H).

*5.2.1.15. Methyl 4-(N-phenylsulfamoyl)benzoate (**3p**).* White solid; yield 78%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.43 (br s, 1H), 8.08 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.26 – 7.20 (m, 2H), 7.11 – 7.02 (m, 3H), 3.85 (s, 3H).

*5.2.1.16. Methyl 4-(N-(4-fluorophenyl)sulfamoyl)benzoate (**3q**).* Yellow solid; yield 90%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.38 (br s, 1H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.13 – 7.04 (m, 4H), 3.86 (s, 3H).

*5.2.1.17. Methyl 4-(N-(3-fluorophenyl)sulfamoyl)benzoate (**3r**).* Pink solid; yield 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.73 (br s, 1H), 8.12 – 8.03 (m, 2H), 7.85 – 7.77 (m, 2H), 7.21 – 7.29 (m, 1H), 7.16 – 7.12 (m, 1H), 6.91 – 6.84 (m, 2H), 3.87 (s, 3H).

*5.2.1.18. Methyl 4-(N-(4-bromophenyl)sulfamoyl)benzoate (**3s**).* Brown solid; yield 98%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.61 (br s, 1H), 8.14 – 8.04 (m, 2H), 7.93 – 7.81 (m, 2H), 7.48 – 7.38 (m, 2H), 7.09 – 6.97 (m, 2H), 3.86 (s, 3H).

*5.2.1.19. Methyl 4-(N-(p-tolyl)sulfamoyl)benzoate (**3t**).* Red solid; yield 85%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.26 (s, 1H), 8.10 – 8.05 (m, 2H), 7.86 – 7.81 (m, 2H), 7.06 – 7.00 (m, 2H), 6.98 – 6.92 (m, 2H), 3.86 (s, 3H), 2.18 (s, 3H).

*5.2.1.20. Methyl 4-(N-(4-methoxyphenyl)sulfamoyl)benzoate (**3u**).* Brown solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.07 (br s, 1H), 8.12 – 8.02 (m, 2H), 7.85 – 7.72 (m, 2H), 7.01 – 6.90 (m, 2H), 6.84 – 6.76 (m, 2H), 3.86 (s, 3H), 3.66 (s, 3H).

*5.2.1.21. Methyl 4-(N-(4-(trifluoromethoxy)phenyl)sulfamoyl)benzoate (**3v**).* White solid; yield 89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.69 (br s, 1H), 8.14 – 8.07 (m, 2H), 7.93 – 7.85 (m, 2H), 7.30 – 7.22 (m, 2H), 7.22 – 7.13 (m, 2H), 3.86 (s, 3H).

*5.2.1.22. Methyl 4-(N-(4-nitrophenyl)sulfamoyl)benzoate(**3w**)*. Brown solid; yield 99%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.50 (br s, 1H), 8.19 – 8.09 (m, 4H), 8.02 – 7.97 (m, 2H), 7.37 – 7.24 (m, 2H), 3.86 (s, 3H).

*5.2.1.23. Methyl 4-(N-(pyridin-2-yl)sulfamoyl)benzoate (**3x**)*. White solid; yield 67%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.12 – 8.04 (m, 2H), 8.03 – 7.91 (m, 3H), 7.82 – 7.71 (m, 1H), 7.25 – 7.17 (m, 1H), 6.90 – 6.80 (m, 1H), 3.86 (s, 3H).

*5.2.1.24. Methyl 4-(N-(thiazol-2-yl)sulfamoyl)benzoate (**3y**)*. White solid; yield 73%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.89 (br s, 1H), 8.12 – 8.07 (m, 2H), 7.97 – 7.89 (m, 2H), 7.29 (d, J = 4.6 Hz, 1H), 6.88 (d, J = 4.6 Hz, 1H), 3.87 (s, 3H).

*5.2.2. General procedure for the synthesis of intermediates **4a-y***.

To a solution of **3a-n, p-y** or **5** (14.12 mmol) in CH_3OH (100 mL) was added 1 mol/L LiOH aqueous solution (42.4 mL). The reaction mixture was stirred at room temperature for 2 h, then evaporated *in vacuo*. The residue was diluted with H_2O and the aqueous solution was acidified with 6 mol/L HCl to pH = 6 – 7. The precipitated solid was filtered to afford compounds **4a-y**.

*5.2.2.1. 4-(Azetidin-1-ylsulfonyl)benzoic acid (**4a**)*. White solid; yield 97%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.55 (br s, 1H), 8.21 (d, J = 8.0 Hz, 2H), 7.92 (d, J = 8.0 Hz, 2H), 3.71 (t, J = 7.5 Hz, 4H), 2.02 (t, J = 7.5 Hz, 2H).

*5.2.2.2. 4-(Pyrrolidin-1-ylsulfonyl)benzoic acid (**4b**)*. White solid; yield 88%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.51 (br s, 1H),8.14 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.4 Hz, 2H), 3.18 – 3.14 (m, 4H), 1.66 – 1.63 (m, 4H).

*5.2.2.3. 4-(Piperidin-1-ylsulfonyl)benzoic acid (**4c**)*. White solid; yield 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (br s, 1H), 8.15 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.4 Hz, 2H), 2.92 – 2.90 (m, 4H), 1.54 – 1.50 (m, 4H), 1.39 – 1.36 (m, 2H).

*5.2.2.4. 4-(Azepan-1-ylsulfonyl)benzoic acid (**4d**)*. White solid; yield 95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.51 (br s, 1H), 8.12 (d, J = 8.4 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 3.24 – 3.21 (m, 4H), 1.63 – 1.61 (m, 4H), 1.50 – 1.47 (m, 4H).

*5.2.2.5. 4-((4-Methylpiperidin-1-yl)sulfonyl)benzoic acid (**4e**)*. White solid; yield 84%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.54 (br s, 1H), 8.14 (d, J = 7.5 Hz, 2H), 7.84 (d, J

= 7.5 Hz, 2H), 3.63 – 3.61 (m, 2H), 2.26 – 2.21 (m, 2H), 1.65 – 1.63 (m, 2H), 1.30 (br s, 1H), 1.15 – 1.11 (m, 2H), 0.83 (d, *J* = 6.0 Hz, 3H).

*5.2.2.6. 4-((4-Methoxypiperidin-1-yl)sulfonyl)benzoic acid (**4f**)*. White solid; yield 84%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.52 (s, 1H) 8.16 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 3.24 – 3.20 (m, 1H), 3.19 (s, 3H), 3.09 – 3.05 (m, 2H), 2.86 – 2.8 (m, 2H), 1.84 – 1.77 (m, 2H), 1.55 – 1.47 (m, 2H).

*5.2.2.7. 4-((4-Oxopiperidin-1-yl)sulfonyl)benzoic acid (**4g**)*. White solid; yield 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.50 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 2H), 3.35 (t, *J* = 6.0 Hz, 4H), 2.42 (t, *J* = 6.0 Hz, 4H).

*5.2.2.8. 4-((4-Phenylpiperidin-1-yl)sulfonyl)benzoic acid (**4h**)*. White solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.52 (br s, 1H), 8.18 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.29 – 7.25 (m, 2H), 7.19 – 7.16 (m, 3H), 3.81 – 3.78 (m, 2H), 2.48 – 2.46 (m, 1H), 2.39 – 2.33 (m, 2H), 1.82 – 1.80 (m, 2H), 1.68 – 1.64 (m, 2H).

*5.2.2.9. 4-(Morpholinosulfonyl)benzoic acid (**4i**)*. White solid; yield 93%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.53 (br s, 1H), 8.18 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 2H), 3.63 (br s, 4H), 2.90 (br s, 4H).

*5.2.2.10. 4-(N-cyclohexylsulfamoyl)benzoic acid (**4j**)*. White solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.40 (br s, 1H), 8.10 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H),7.82 (d, *J* = 7.6 Hz, 1H), 2.97 – 2.96 (m, 1H), 1.56 – 1.54 (m, 4H), 1.45 – 1.42 (m, 1H), 1.17 – 1.00 (m, 5H).

*5.2.2.11. 4-(N-((1S,3s)-adamantan-1-yl)sulfamoyl)benzoic acid (**4k**)*. White solid; yield 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.40 (br s, 1H), 8.09 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.73 (br s, 1H), 1.69 – 1.67 (m, 15H).

*5.2.2.12. 4-(N-butylsulfamoyl)benzoic acid (**4l**)*. White solid; yield 89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.42 (br s, 1H), 8.12 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.74 (t, *J* = 6.0 Hz, 1H), 2.78 – 2.73 (m, 2H), 1.37 – 1.30 (m, 2H), 1.25 – 1.19 (m, 2H), 0.78 (t, *J* = 7.6 Hz, 3H).

*5.2.2.13. 4-(N,N-dimethylsulfamoyl)benzoic acid (**4m**)*. White solid; yield 85%;^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.49 (br s, 1H), 8.16 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 2.63 (br s, 6H).

*5.2.2.14. 4-(N-ethyl-N-methylsulfamoyl)benzoic acid (**4n**).* White solid; yield 86%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.47 (br s, 1H), 8.14 (d, J = 7.5 Hz, 2H), 7.88 (d, J = 7.5 Hz, 2H), 3.07 – 3.02 (m, 2H), 2.69 (s, 3H), 1.03 (t, J = 7.0 Hz, 3H).

*5.2.2.15. 4-((4,4-Difluoropiperidin-1-yl)sulfonyl)benzoic acid (**4o**).* White solid; yield 85%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.60 (br s, 1H), 8.17 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H), 3 3.16 – 3.06 (m, 4H), 2.11 – 2.01 (m, 4H).

*5.2.2.16. 4-(N-phenylsulfamoyl)benzoic acid (**4p**).* White solid; yield 81%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.43 (br s, 1H), 10.43 (br s, 1H), 8.16 – 7.93 (m, 2H), 7.95 – 7.74 (m, 2H), 7.27 – 7.19 (m, 2H), 7.12 – 7.00 (m, 3H).

*5.2.2.17. 4-(N-(4-Fluorophenyl)sulfamoyl)benzoic acid (**4q**).* White solid; yield 97%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.44 (br s, 1H), 10.37 (br s, 1H), 8.13 – 7.99 (m, 2H), 7.86 – 7.78 (m, 2H), 7.14 – 7.01 (m, 4H).

*5.2.2.18. 4-(N-(3-Fluorophenyl)sulfamoyl)benzoic acid (**4r**).* Pink solid; yield 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.39 (br s, 1H), 10.74 (br s, 1H), 8.11 – 8.06 (m, 2H), 7.92 – 7.86 (m, 2H), 7.35 – 7.22 (m, 1H), 6.96 – 6.83 (m, 3H).

*5.2.2.19. 4-(N-(4-Bromophenyl)sulfamoyl)benzoic acid (**4s**).* Pink solid; yield 99%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45 (br s, 1H), 10.64 (br s, 1H), 8.13 – 8.00 (m, 2H), 7.91 – 7.80 (m, 2H), 7.48 – 7.36 (m, 2H), 7.10 – 6.97 (m, 2H).

*5.2.2.20. 4-(N-(p-tolyl)sulfamoyl)benzoic acid (**4t**).* Pink solid; yield 92%; ^{1}H NMR (400 MHz, DMSO- d_6) δ: 13.39 (br s, 1H), 10.28 (br s, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 2.18 (s, 3H).

*5.2.2.21. 4-(N-(4-Methoxyphenyl)sulfamoyl)benzoic acid (**4u**).* Grey solid; yield 89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.39 (br s, 1H), 10.06 (br s, 1H), 8.09 – 8.02 (m, 2H), 7.82 – 7.73 (m, 2H), 6.99 – 6.93 (m, 2H), 6.82 – 6.76 (m, 2H), 3.66 (s, 3H).

*5.2.2.22. 4-(N-(4-(Trifluoromethoxy)phenyl)sulfamoyl)benzoic acid (**4v**).* White solid; yield 99%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45 (br s, 1H), 10.68 (br s, 1H), 8.12 – 8.04 (m, 2H), 7.91 – 7.83 (m, 2H), 7.32 – 7.23 (m, 2H), 7.22 – 7.13 (m, 2H).

*5.2.2.23. 4-(N-(4-Nitrophenyl)sulfamoyl)benzoic acid (**4w**).* Pink solid; yield 83%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.45 (br s, 1H), 11.42 (br s, 1H), 8.19 – 8.08 (m, 4H), 8.02 – 7.94 (m, 2H), 7.38 – 7.28 (m, 2H).

*5.2.2.24. 4-(N-(Pyridin-2-yl)sulfamoyl)benzoic acid (**4x**).* White solid; yield 88%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.95 (br s, 2H), 8.14 – 7.98 (m, 2H), 8.03 – 7.90 (m, 3H), 7.83 – 7.71 (m, 1H), 7.22 – 7.20 (m, 1H), 6.91 – 6.78 (m, 1H).

*5.2.2.25. 4-(N-(Thiazol-2-yl)sulfamoyl)benzoic acid (**4y**).* Brown solid; yield 64%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (br s, 1H), 12.88 (br s, 1H), 8.18 – 8.02 (m, 2H), 7.97 – 7.86 (m, 2H), 7.28 (d, J = 4.4 Hz, 1H), 6.87 (d, J = 4.4 Hz, 1H).

*5.2.3. Synthesis of ethyl 4-((4,4-difluoropiperidin-1-yl)sulfonyl)benzoate (**5**).*

To a solution of **3g** (0.6 g, 1.93 mmol) in anhydrous CH_2Cl_2 (30 mL) was added DAST (0.78 mL, 5.78 mmol) cooled with an ice bath. The reaction mixture was stirred at room temperature for 3 h under argon, then quenched with water (50 mL) and extracted with CH_2Cl_2 (30 mL×2). The combined organic phase was washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuum* to give compound **5**. White solid; yield 89%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.21 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.5 Hz, 2H), 4.45 – 4.41 (m, 2H), 3.24 – 3.21 (m, 4H), 2.10 – 2.05 (m, 4H), 1.42 (t, J = 7.0 Hz, 3H).

*5.2.4. General procedure for the synthesis of intermediates **8a-c**.*

To a solution of 2-cyanoacetic acid **6** (15.0 g, 176.35 mmol) and carbamate **7a-c** (176.35 mmol) in the mixed solvents of toluene (90 mL) and DMF (5.4 mL) was added $POCl_3$ (8.22 mL, 88.18 mmol) cooled with an ice bath. The reaction mixture was stirred at 80 °C for 3 h under argon, then slowly poured into ice-cold water (500 mL). The precipitated solid was filtered and washed with saturated NH_4Cl and water to afford compounds **8a-c**.

*5.2.4.1. Methyl (2-cyanoacetyl)carbamate (**8a**).* White solid; yield 59%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.04 (brs, 1H), 4.11 (s, 2H), 3.67 (s, 3H).

*5.2.4.2. Ethyl (2-cyanoacetyl)carbamate (**8b**).* Light yellow solid; yield 64%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.98 (br s, 1H), 4.13 (q, J = 7.2 Hz, 2H), 4.09 (s, 2H), 1.21 (t, J = 7.2 Hz, 3H).

*5.2.4.3. Isopropyl (2-cyanoacetyl)carbamate (**8c**).* Light yellow solid; yield 49%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.90 (br s, 1H), 4.87 – 4.85 (m, 1H), 4.07 (s, 2H), 1.25 (d, J = 6.0 Hz, 6H).

*5.2.5. General procedure for the synthesis of intermediates **8d-e**.*

To a magnetically stirred solution of 2-cyanoacetamide **10** (2.0g, 23.79 mmol) in 1, 2-dichloroethane (40 mL) was added oxalyl chloride (3.4 mL, 40.44 mmol). The reaction mixture was heated to reflux for 4 h under an atmosphere of argon. The solvent was evaporated under reduced pressure. To a solution of the residue in anhydrous acetonitrile (30 mL) was added corresponding alcohols (15 mL) in anhydrous acetonitrile (30 mL) dropwise keeping the reaction under −10 °C. The reaction mixture was stirred for additional 3 h at −10 °C, then concentrated. The residue was washed with water, filtrated, dried and used to the next step without further purification.

*5.2.5.1. Propyl (2-cyanoacetyl)carbamate (**8d**).* White solid; yield 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.98 (br s, 1H), 4.09 (s, 2H), 4.04 (t, J = 6.8 Hz, 2H), 1.65 – 1.56 (m, 2H), 0.90 (t, J = 7.6 Hz, 3H).

*5.2.5.2. 2,2,2-Trifluoroethyl (2-cyanoacetyl)carbamate (**8e**).* White solid; yield 45%; mp 117-118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.46 (br s, 1H), 4.84 – 4.78 (m, 2H), 4.09 (s, 2H).

*5.2.6. General procedure for the synthesis of intermediates **9a-e**.*

To a solution of **8a-e** (102.3 mmol) in CH_3OH (150 mL) was added 2,5-dihydroxy-1,4-dithiane (7.77 g, 51.0 mmol) and Et_3N (15.6 mL, 112.3 mmol) in turn cooled with an ice bath. The reaction mixture was stirred at 50 °C for 2.5 h under argon, then concentrated. The residue was diluted with DCM (100 mL) and filtered. The obtained solid was washed with saturated NH_4Cl solution (100 mL) and water to afford compounds ***9a-e***.

*5.2.6.1. Methyl (2-aminothiophene-3-carbonyl)carbamate (**9a**).* Light Yellow solid; yield 59%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.07 (br s, 1H), 7.65 (br s, 2H), 7.23 (d, J = 6.0 Hz, 1H), 6.23 (d, J = 6.0 Hz, 1H), 3.67 (s, 3H).

*5.2.6.2. Ethyl (2-cyanoacetyl)carbamate (**9b**)*. Light yellow solid; yield 78%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.02 (br s, 1H), 7.65 (br s, 2H), 7.25 (d, J = 6.0 Hz, 1H), 6.22 (d, J = 6.0 Hz, 1H), 4.13 (q, J = 7.2 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H).

*5.2.6.3. Isopropyl (2-aminothiophene-3-carbonyl)carbamate (**9c**)*. Light yellow solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.98 (br s, 1H), 7.65 (br s, 2H), 7.24 (d, J = 6.0 Hz, 1H), 6.22 (d, J = 6.0 Hz, 1H), 4.91 – 4.88 (m, 1H), 1.25 (d, J = 6.4 Hz, 6H).

*5.2.6.4. Propyl (2-aminothiophene-3-carbonyl)carbamate (**9d**)*. Light yellow solid; yield 50%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.02 (br s, 1H), 7.64 (br s, 2H), 7.24 (d, J = 6.0 Hz, 1H), 6.23 (d, J = 6.0 Hz, 1H), 4.04 (t, J = 6.8 Hz, 2H), 1.66 – 1.60 (m, 2H), 0.93 (t, J = 7.6 Hz, 3H).

*5.2.6.5. 2,2,2-Trifluoroethyl (2-aminothiophene-3-carbonyl)carbamate (**9e**)*. Light yellow solid; yield 10%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.48 (br s, 1H), 7.75 (br s, 2H), 7.24 (d, J = 6.0 Hz, 1H), 6.25 (d, J = 6.0 Hz, 1H), 4.84 – 4.77 (m, 2H).

*5.2.7. General procedure for the synthesis of target compounds **12a-b***.

To a solution of the 2-cyanoacetic acid **6** (1.17 g, 13.81 mmol) and the corresponding amines **11a-b** (0.63mmol) in CH_2Cl_2 (100 mL) was added DCC (2.85 g, 13.81 mmol) and DMAP (65 mg, 0.53 mmol) in turn. The reaction mixture was stirred at room temperature for 5 h, then filtered. The filtrate was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1) to give compounds **12a-b**.

*5.2.7.1. 2-Cyano-N-(pyridin-2-yl)acetamide (**12a**)*. White solid; yield 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.81 (br s, 1H), 8.34 – 8.32 (m, 1H), 8.02 – 8.00 (m, 1H), 7.83 – 7.79 (m, 1H), 7.16 – 7.13 (m, 1H), 3.98 (s, 2H).

*5.2.7.2. 2-Cyano-N-(4-methylpyrimidin-2-yl)acetamide (**12b**)*. Light yellow solid; yield 38%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.88 (br s, 1H), 8.51 (d, J = 4.8 Hz, 1H), 7.10 (d, J = 4.8 Hz, 1H), 4.16 (s, 2H), 2.41 (s, 3H).

*5.2.8. General procedure for the synthesis of intermediates **13a-b***.

To a solution of **12a-b** (7.45 mmol) in CH_3OH (50 mL) was added 2,5-dihydroxy-1,4-dithiane (0.57 g, 3.72 mmol) and Et_3N (1.14 mL, 8.2 mmol) in turn cooled with an ice

bath. The reaction mixture was stirred at 50 °C for 4.5 h under argon, then concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1) to give compounds **13a-b**.

*5.2.8.1. 2-Amino-N-(pyridin-2-yl)thiophene-3-carboxamide (**13a**).* White solid; yield 70%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.85 (br s, 1H), 8.35 – 8.28 (m, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.80 – 7.71 (m, 1H), 7.57 – 7.42 (m, 3H), 7.12 – 7.03 (m, 1H), 6.29 (d, J = 5.6 Hz, 1H).

*5.2.8.2. 2-Amino-N-(4-methylpyrimidin-2-yl)thiophene-3-carboxamide (**13b**).* White solid; yield 30%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.49 (d, J = 5.2 Hz, 1H), 8.12 (br s, 1H), 6.89 (d, J = 5.6 Hz, 1H), 6.86 (d, J = 5.2 Hz, 1H), 6.44 (br s, 2H), 6.25 (d, J = 5.6 Hz, 1H), 2.49 (s, 3H).

*5.2.9. General procedure for the synthesis of target compounds **14a-o**.*

To a solution of sulfonamide benzoic acids **4a-o** (1.5 mmol) and 2-aminothiophene **9a** (200 mg, 1 mmol) in DMF (4 mL) was added HATU (760.5 mg, 2 mmol) and Et_3N (0.42 mL, 3 mmol) in turn. The reaction mixture was stirred at room temperature for 12 h, then quenched with water (30 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined organic phase was washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1) to give compounds **14a-o**.

*5.2.9.1. Methyl (2-(4-(azetidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14a**).* White solid; yield 45%; mp 190-191 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.68 (br s, 1H), 10.88 (br s, 1H), 8.22 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H) 7.74 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 3.80 – 3.69 (m, 7H), 2.05 – 1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.8, 137.4, 136.1, 129.1, 128.5, 123.3, 117.3, 115.7, 52.5, 51.1, 14.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{18}N_3O_6S_2$, 424.0632; found, 424.0608.

*5.2.9.2. Methyl (2-(4-(pyrrolidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14b**).* White solid; yield 14%; mp 179-180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.87 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H) 7.74 (d, J = 5.6 Hz, 1H), 7.13 (d, J = 5.6 Hz, 1H), 3.76 (s, 3H), 3.22 –

3.17 (m, 4H), 1.68 – 1.66 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.0, 151.8, 148.8, 140.0, 135.6, 128.4, 128.2, 123.4, 117.2, 115.6, 52.5, 48.0, 24.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{20}N_3O_6S_2$, 438.0788; found, 438.0769.

*5.2.9.3. Methyl (2-(4-(piperidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14c, 297F**)*. White solid; yield 42%; mp 178-179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.87 (br s, 1H), 8.16 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 2.96 – 2.94 (m, 4H), 1.56 – 1.52 (m, 4H), 1.38 – 1.37 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.8, 139.4, 135.8, 128.8, 128.3, 123.4, 117.2, 115.6, 52.5, 46.6, 24.8, 22.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{22}N_3O_6S_2$, 452.0945; found, 452.0958.

*5.2.9.4. Methyl (2-(4-(azepan-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14d**)*. White solid; yield 6%; mp 173-174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.64 (br s, 1H), 10.87 (br s, 1H), 8.13 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 3.27 – 3.24 (m, 4H), 1.64 (br s, 4H), 1.52 – 1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.9, 142.7, 135.4, 128.5, 127.6, 123.4, 117.2, 115.6, 52.5, 47.9, 28.6, 26.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_6S_2$, 466.1101; found, 466.1104.

*5.2.9.5. Methyl (2-(4-((4-methylpiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14e**)*. White solid; yield 19%; mp 140-141 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.87 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 5.6 Hz, 1H), 7.12 (d, J = 5.6 Hz, 1H), 3.76 (s, 3H), 3.66 – 3.63 (m, 2H), 2.31 – 2.25 (m, 2H), 1.66 – 1.63 (m, 2H), 1.32 – 1.30 (m, 1H), 1.18 – 1.11 (m, 2H), 0.84 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.8, 139.5, 138.8, 135.8, 128.4, 128.3, 123.4, 117.2, 52.5, 46.1, 32.9, 29.3, 21.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_6S_2$, 466.1101; found, 466.1072.

*5.2.9.6. Methyl (2-(4-((4-methoxypiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14f**)*. White solid; yield 23%; mp 184-185 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.88 (br s, 1H), 8.16 (d, J = 7.5 Hz, 2H), 7.99 (d, J = 7.5 Hz, 2H) 7.73 (d, J = 5.5 Hz, 1H), 7.14 (d, J = 5.5 Hz, 1H), 3.76 (s, 3H), 3.25 (br s,

1H), 3.14 (br s, 5H), 2.89 – 2.87 (m, 2H), 1.82 (br s, 2H), 1.54 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.0, 151.8, 148.8, 139.3, 135.9, 128.5, 128.4, 123.4, 117.2, 115.6, 73.3, 55.1, 52.5, 43.2, 29.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_7S_2$, 482.1050; found, 482.1042.

*5.2.9.7. Methyl (2-(4-((4-oxopiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14g**)*. White solid; yield 27%; mp 199-200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (br s, 1H), 10.87 (br s, 1H), 8.17 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 3.41 – 3.33 (m, 4H), 2.45 – 2.41 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 205.8, 164.4, 162.4, 152.2, 149.2, 140.3, 135.4, 129.0, 128.6, 123.8, 117.6, 116.0, 52.9, 45.4, 40.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{20}N_3O_7S_2$, 466.0737; found, 466.0763.

*5.2.9.8. Methyl (2-(4-((4-phenylpiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14h**)*. White solid; yield 27%; mp 175-176 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (br s, 1H), 10.89 (br s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 5.6 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.19 – 7.14 (m, 4H), 3.83 – 3.80 (m, 2H), 3.76 (s, 3H), 2.43 – 2.37 (m, 2H), 1.72 – 1.66 (m, 2H), 1.72 – 1.66 (m, 2H), 1.23 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.4, 162.5, 152.2, 149.2, 145.6, 139.6, 136.3, 128.8, 127.2, 126.8, 123.8, 117.6, 116.0, 52.9, 47.0, 40.8, 32.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{25}H_{26}N_3O_6S_2$, 528.1258; found, 528.1297.

*5.2.9.9. Methyl (2-(4-(morpholinosulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14i**)*. White solid; yield 18%; mp 205-207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (br s, 1H), 10.88 (br s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 3.65 – 3.63 (m, 4H), 2.95 – 2.93 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.3, 162.4, 152.1, 149.1, 138.6, 136.5, 128.9, 128.9, 123.8, 117.6, 116.0, 65.7, 52.9, 46.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{20}N_3O_7S_2$, 454.0737; found, 454.0751.

*5.2.9.10. Methyl (2-(4-(N-cyclohexylsulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**14j**)*. White solid; yield 15%; mp 151-152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.62 (br s, 1H), 10.87 (br s, 1H), 8.12 (d, J = 8.4 Hz, 2H), 8.05 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 7.2 Hz, 1H), 7.72 (d, J = 6.0 Hz, 1H), 7.12 (d, J = 6.0 Hz, 1H),

3.76 (s, 3H), 3.00 (br s, 1H), 1.59 – 1.57 (m, 4H), 1.23 (br s, 2H), 1.16 – 1.11 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.9, 146.1, 135.0, 128.3, 127.2, 123.4, 117.2, 115.6, 52.5, 52.3, 33.3, 24.9, 24.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_6S_2$, 466.1101; found, 466.1095.

*5.2.9.11. Methyl (2-(4-(N-((1S,3s)-adamantan-1-yl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**14k**)*. White solid; yield 7%; mp 183-184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.62 (br s, 1H), 10.87 (br s, 1H), 8.14 – 8.04 (m, 4H), 7.81 (br s, 1H), 7.73 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 1.93 (br s, 3H), 1.72 (br s, 6H), 1.55 – 1.46 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 162.2, 151.8, 148.8, 148.7, 134.6, 128.2, 127.0, 123.4, 117.1, 115.5, 54.2, 52.5, 42.5, 35.5, 28.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{28}N_3O_6S_2$, 518.1414; found, 518.1392.

*5.2.9.12. Methyl (2-(4-(N-butylsulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**14l**)*. White solid; yield 7%; mp 183-184 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.62 (br s, 1H), 10.87 (br s, 1H), 8.13 (d, J = 8.0 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 7.71 (br s, 1H), 7.73 (d, J = 5.5 Hz, 1H), 7.13 (d, J = 5.5 Hz, 1H), 3.76 (s, 3H), 2.80 – 2.78 (m, 2H), 1.37 – 1.34 (m, 2H), 1.26 – 1.23 (m, 2H), 0.80 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 162.1, 151.8, 148.8, 144.4, 135.1, 128.3, 127.4, 123.4, 117.2, 115.6, 52.5, 42.3, 31.2, 19.2, 13.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{22}N_3O_6S_2$, 440.0945; found, 440.0941.

*5.2.9.13. Methyl (2-(4-(N,N-dimethylsulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**14m**)*. White solid; yield 5%; mp 176-166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (br s, 1H), 10.88 (br s, 1H), 8.17 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 2.67 (br s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 162.0, 151.7, 148.7, 138.5, 135.7, 128.4, 128.3, 123.3, 117.1, 115.5, 52.4, 37.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{18}N_3O_6S_2$, 412.0632; found, 412.0630.

*5.2.9.14. Methyl (2-(4-(N-ethyl-N-methylsulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**14n**)*. White solid; yield 14%; mp 177-178 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.87 (br s, 1H), 8.15 (d, J = 7.5 Hz, 2H), 8.02 (d, J = 7.5 Hz, 2H), 7.73 (d, J = 5.5 Hz, 1H), 7.13 (d, J = 5.5 Hz, 1H), 3.76 (s, 3H), 3.10 –

3.08 (m, 2H), 2.72 (s, 3H), 1.05 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 162.1, 151.8, 148.8, 141.0, 135.6, 128.5, 128.0, 123.4, 117.2, 115.6, 52.5, 44.6, 34.0, 12.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_6S_2$, 426.0788; found, 426.0788.

*5.2.9.15. Methyl (2-(4-((4,4-difluoropiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**14o**)*. White solid; yield 30%; mp 195-196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (br s, 1H), 10.87 (br s, 1H), 8.19 – 8.15 (m, 2H), 8.07 – 8.03 (m, 2H), 7.73 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 3.76 (s, 3H), 3.17 – 3.15 (m, 4H), 2.09 – 2.06 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 163.8, 161.8, 151.6, 148.6, 139.3, 136.0, 128.5, 128.1, 123.3, 121.6 (J = 239.9 Hz), 117.1, 115.5, 52.4, 43.2 (J = 57.8 Hz), 32.7 (J = 23.4 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{20}N_3O_6S_2F_2$, 488.0756; found, 488.0735.

*5.2.10. Synthesis of target compound methyl (2-(4-((4-hydroxypiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**15**)*.

To a solution of compound **14g** (60 mg, 0.128 mmol) in anhydrous CH_3OH (50 mL) was added $NaBH_4$ (15 mg, 0.387 mmol) cooled with an ice bath. The reaction mixture was stirred at room temperature for 12 h under argon, then concentrated. The residue was diluted with CH_2Cl_2, washed with 1 mol/L HCl aqueous solution (15 mL), brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/3) to give the compound **15**. White solid; yield 87%; mp 224-225 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.87 (br s, 1H), 8.16 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 4.68 (br s, 1H), 3.76 (s, 3H), 3.54 (br s, 1H), 3.21 (br s, 2H), 2.81 – 2.78 (m, 2H), 1.75 (br s, 2H), 1.44 – 1.42 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 162.1, 151.8, 148.8, 139.4, 135.8, 128.4, 128.3, 123.4, 117.2, 115.6, 63.8, 52.5, 43.3, 33.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{22}N_3O_7S_2$, 468.0894; found, 468.0895.

*5.2.11. General procedure for the synthesis of target compounds **16a-h**.*

Compounds **16a-d** were prepared from **4c** (0.44 mmol) and **9d-e**, **13a-b** (0.29 mmol), while compounds **16e-h** were prepared from **4i** (0.57 mmol) and **9c-e**, **13b** (0.44 mmol) in the same manner as described for **14a-o**.

*5.2.11.1. Propyl (2-(4-(piperidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**16a**)*. White solid; yield 24%; mp 189-190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.83 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 4.13 (t, J = 6.8 Hz, 2H), 2.96 – 2.94 (m, 4H), 1.69 – 1.67 (m, 2H), 1.55 – 1.52 (m, 4H), 1.38 – 1.37 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.1, 151.3, 148.7, 139.4, 135.8, 128.4, 128.3, 123.5, 117.2, 115.7, 66.8, 46.7, 24.8, 22.8, 21.8, 10.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_6S_2$, 480.1258; found, 480.1252.

*5.2.11.2. 2,2,2-Trifluoroethyl (2-(4-(piperidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**16b**)*. White solid; yield 32%; mp 195-196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.51 (br s, 1H), 11.27 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.15 (d, J = 6.0 Hz, 1H), 4.93 – 4.86 (m, 2H), 2.96 – 2.94 (m, 4H), 1.58 – 1.52 (m, 4H), 1.39 – 1.37 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 162.2, 149.8, 149.2, 139.4, 135.8, 128.5, 128.3, 123.6, 126.5 (J = 276 Hz), 127.3, 115.6, 60.4 (J = 35 Hz), 46.6, 24.8, 22.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{21}N_3O_6F_3S_2$, 520.0818; found, 520.0864.

*5.2.11.3. 2-(4-(Piperidin-1-ylsulfonyl)benzamido)-N-(pyridin-2-yl)thiophene-3-carboxamide (**16c**)*. White solid; yield 35%; mp 236-237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.07 (br s, 1H), 10.76 (br s, 1H), 8.44 (br s, 1H), 8.18 – 8.16 (m, 3H), 8.00 – 7.98 (m, 3H), 7.90 – 7.80 (m, 1H), 7.24 – 7.16 (m, 2H), 2.95 (br s, 4H), 1.55 (br s, 4H), 1.38 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.5, 161.8, 151.5, 148.1, 147.4, 139.3, 138.2, 136.0, 128.4, 128.3, 123.5, 120.4, 117.2, 116.1, 116.0, 46.7, 24.8, 22.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{23}N_4O_4S_2$, 471.1155; found, 471.1185.

*5.2.11.4. N-(4-Methylpyrimidin-2-yl)-2-(4-(piperidin-1-ylsulfonyl)benzamido)thiophene-3-carboxamide (**16d**)*. White solid; yield 18%; mp 216-217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.03 (brs, 1H), 10.93 (brs, 1H), 8.63 (d, J = 4.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 7.0 Hz, 1H), 7.21 (d, J = 5.2 Hz, 1H), 7.16 (d, J = 5.2 Hz, 1H), 2.96-2.93 (m, 4H), 2.49 (s, 3H),1.57-1.52 (m, 4H), 1.40-1.36 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.5, 164.1, 161.9, 158.1, 157.3, 147.7, 139.3, 137.0, 128.4, 123.6, 117.4, 117.1, 116.2, 46.6,

24.8, 23.6, 22.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{24}N_5O_4S_2$, 486.1264; found, 486.1248.

*5.2.11.5. Isopropyl (2-(4-(morpholinosulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**16e**)*. White solid; yield 38%; mp 210-211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (br s, 1H), 10.75 (br s, 1H), 8.14 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 5.6 Hz, 1H), 7.09 (d, J = 5.6 Hz, 1H), 4.95 – 4.92 (m, 1H), 3.61 – 3.59 (m, 4H), 2.91 – 2.89 (m, 4H), 1.26 (d, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.2, 162.0, 150.8, 148.7, 138.3, 136.2, 128.6, 128.5, 123.5, 117.2, 115.8, 69.2, 65.4, 46.0, 21.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_7S_2$, 482.1050; found, 482.1030.

*5.2.11.6. Propyl (2-(4-(morpholinosulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**16f**)*. White solid; yield 60%; mp 219-220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (br s, 1H), 10.84 (br s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 4.13 (t, J = 6.8 Hz, 2H), 3.65 – 3.63 (m, 4H), 2.95 – 2.93 (m, 4H), 1.69 – 1.65 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.1, 151.4, 148.7, 138.3, 136.2, 128.6, 128.5, 123.5, 117.2, 115.8, 66.8, 65.4, 46.0, 21.7, 10.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_7S_2$, 482.1050; found, 482.1028.

*5.2.11.7. 2,2,2-Trifluoroethyl (2-(4-(morpholinosulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**16g**)*. White solid; yield 10%; mp 201-202 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.52 (br s, 1H), 11.27 (br s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.15 (d, J = 6.0 Hz, 1H), 4.93 – 4.86 (m, 2H), 3.65 – 3.63 (m, 4H), 2.95 – 2.93 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.3, 162.6, 150.2, 149.5, 138.7, 136.5, 128.9, 124.0, 123.9 (J = 276 Hz), 117.7, 116.0, 65.8, 60.83 (J = 35 Hz), 46.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{19}N_3O_7F_3S_2$, 522.0611; found, 522.0626.

*5.2.11.8. N-(4-Methylpyrimidin-2-yl)-2-(4-(morpholinosulfonyl)benzamido)thiophene-3-carboxamide (**16h**)*. White solid; yield 27%; mp > 250 °C. ^{1}H NMR (500 MHz, DMSO) δ: 13.02 (br s, 1H), 10.91 (br s, 1H), 8.63 (d, J = 5.5 Hz, 1H), 8.18 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 2H), 7.86 (d, J = 6.0 Hz, 1H), 7.21 (d, J = 5.0 Hz, 1H),

7.16 (d, J = 6.0 Hz, 1H), 3.63 (t, J = 4.5 Hz, 4H), 2.94 (t, J = 4.5 Hz, 4H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.5, 164.1, 161.8, 158.1, 157.3, 147.7, 138.2, 136.3, 128.6, 128.5, 123.6, 117.4, 117.2, 116.3, 65.4, 45.9, 23.6. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{22}N_5O_5S_2$, 488.1057; found, 488.1040.

*5.2.12. General procedure for the synthesis of target compounds **17a-o**.*

Compounds **17a-e** were prepared from **9b** (0.75 mmol) and **4a-d**, **f** (1.13 mmol), while compounds **17f-o** were prepared from **9b** (0.70 mmol) and **4p-y** (0.84 mmol) in the presence of DMAP (0.07 mmol) in the same manner as described for **14a-o.**

*5.2.12.1. Ethyl (2-(4-(azetidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**17a**)*. White solid; yield 32%; mp 189-190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.69 (br s, 1H), 10.85 (br s, 1H), 8.22 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.76 – 3.72 (m, 4H), 2.05 – 1.98 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.0, 151.2, 148.8, 137.4, 136.1, 129.1, 128.5, 123.5, 117.2, 115.7, 61.4, 51.1, 14.9, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{20}N_3O_6S_2$, 438.0788; found, 438.0772.

*5.2.12.2. Ethyl (2-(4-(pyrrolidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**17b**)*. White solid; yield 22%; mp 179-180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (br s, 1H), 10.84 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.12 (d, J = 6.0 Hz, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.22 – 3.18 (m, 4H), 1.69 – 1.65 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.1, 151.2, 148.8, 140.0, 135.7, 128.4, 128.2, 123.5, 117.2, 115.7, 61.4, 48.0, 24.9, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{22}N_3O_6S_2$, 452.0945; found, 452.0936.

*5.2.12.3. Ethyl (2-(4-(piperidin-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**17c**)*. White solid; yield 41%; mp 179-180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.69 (br s, 1H), 10.87 (br s, 1H), 8.19 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 6.0 Hz, 1H), 7.16 (d, J = 6.0 Hz, 1H), 4.25 (q, J = 7.0 Hz, 2H), 2.98 (br s, 4H), 1.58 (br s, 4H), 1.40 (br s, 2H), 1.31 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.1, 151.2, 148.8, 139.4, 135.8, 128.4, 128.3, 123.5,

117.2, 115.7, 61.4, 46.6, 24.8, 22.8, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_6S_2$, 466.1101; found, 466.1087.

*5.2.12.4. Ethyl (2-(4-(azepan-1-ylsulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**17d**)*. White solid; yield 27%; mp 182-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (br s, 1H), 10.84 (br s, 1H), 8.13 (d, J = 8.4 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 5.6 Hz, 1H), 7.12 (d, J = 5.6 Hz, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.27 – 3.24 (m, 4H), 1.64 (br s, 4H), 1.52 – 1.50 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.0, 151.2, 148.8, 142.7, 135.4, 128.4, 127.6, 123.4, 117.1, 115.6, 61.4, 47.9, 28.6, 26.4, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_6S_2$, 480.1258; found, 480.1261.

*5.2.12.5. Ethyl (2-(4-((4-methoxypiperidin-1-yl)sulfonyl)benzamido)thiophene-3-carbonyl)carbamate (**17e**)*. White solid; yield 8%; mp 184-185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (br s, 1H), 10.85 (br s, 1H), 8.16 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 6.0 Hz, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.27 – 3.23 (m, 1H), 3.14 (s, 3H), 3.13 – 3.11 (m, 2H), 2.89 – 2.85 (m, 2H), 1.86-1.81 (m, 2H), 1.55 – 1.51 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.1, 162.1, 151.2, 148.7, 139.3, 135.9, 128.5, 128.4, 123.5, 117.2, 115.7, 73.3, 61.4, 55.1, 43.2, 29.4, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_7S_2$, 496.1207; found, 496.1190.

*5.2.12.6. Ethyl (2-(4-(N-phenylsulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17f**)*. Yellow solid; yield 16%; mp 184-185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.57 (br s, 1H), 10.82 (br s, 1H), 10.51 (br s, 1H), 8.09 – 8.06 (m, 2H), 8.00 – 7.97 (m, 2H), 7.72 (d, J = 6.0 Hz, 1H), 7.27 – 7.23 (m, 2H), 7.13 – 7.10 (m, 3H), 7.07 – 7.03 (m, 1H), 4.21 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.9, 161.8, 151.1, 148.6, 143.0, 137.2, 135.6, 129.3, 128.3, 127.5, 124.4, 123.4, 120.4, 117.0, 115.6, 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{20}N_3O_6S_2$, 474.0788; found, 474.0778.

*5.2.12.7. Ethyl (2-(4-(N-(4-fluorophenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17g**)*. White solid; yield 30%; mp 169-170 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.58 (br s, 1H), 10.84 (br s, 1H), 10.47 (br s, 1H), 8.10 – 8.07 (m,

2H), 7.96 – 7.95 (m, 2H), 7.76 – 7.67 (m, 1H), 7.18 – 7.06 (m, 5H), 4.24 – 4.19 (m, 2H), 1.30 – 1.26 (m, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 163.9, 161.8, 159.3 (*J* = 240 Hz), 151.1, 148.6, 142.7, 135.6, 133.4 (*J* = 3 Hz), 128.3, 127.5, 123.4, 123.2 (*J* = 8 Hz), 117.1, 116.0 (*J* = 23 Hz), 115.6, 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{19}N_3O_6S_2F$, 492.0694; found, 492.0685.

*5.2.12.8. Ethyl (2-(4-(N-(3-fluorophenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17h**)*. White solid; yield 19%; mp 184-186 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 12.58 (br s, 1H), 10.82 (br s, 2H), 8.11 – 8.09 (m, 2H), 8.07 – 8.01 (m, 2H), 7.72 (d, *J* = 6.0 Hz, 1H), 7.38 – 7.28 (m, 1H), 7.11 (d, *J* = 6.0 Hz, 1H), 7.00 – 6.84 (m, 3H), 4.21 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO) δ: 163.9, 162.3 (*J* = 242 Hz), 161.8, 151.2, 148.6, 142.6, 139.2 (*J* = 10 Hz), 135.9, 131.2 (*J* = 9 Hz), 128.5, 127.6, 123.4, 117.1, 115.7, 115.6 (*J* = 3 Hz), 110.9 (*J* = 21 Hz), 106.6 (*J* = 25 Hz), 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{19}N_3O_6S_2F$, 492.0694; found, 492.0686.

*5.2.12.9. Ethyl (2-(4-(N-(4-bromophenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17i**)*. Grey solid; yield 14%; mp 185-187 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 12.58 (br s, 1H), 10.82 (br s, 1H), 10.68 (br s, 1H), 8.09 (d, *J* = 8.0 Hz, 2H), 7.99 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 6.0 Hz, 1H), 7.46 – 7.41 (m, 2H), 7.14 – 7.04 (m, 3H), 4.21 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.9, 161.8, 151.1, 148.6, 142.6, 136.4, 135.8, 132.2, 128.4, 127.5, 123.4, 122.2, 117.1, 116.6, 115.6, 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{19}N_3O_6S_2Br$, 551.9893; found, 551.9886.

*5.2.12.10. Ethyl (2-(4-(N-(p-tolyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17j**)*. Yellow solid; yield 16%; mp 130-131 °C. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 12.56 (br s, 1H), 10.82 (br s, 1H), 10.33 (br s, 1H), 8.10 – 8.03 (m, 2H), 7.99 – 7.92 (m, 2H), 7.72 (d, *J* = 6.0 Hz, 1H), 7.11 (d, *J* = 6.0 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 7.00 – 6.98 (m, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.18 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ: 163.9, 161.9, 151.1, 148.6, 143.0, 135.5, 134.5, 133.8, 129.7, 128.2, 127.5, 123.4, 120.9, 117.1, 115.6, 61.3, 20.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{22}N_3O_6S_2$, 488.0945; found, 488.0933.

*5.2.12.11. Ethyl (2-(4-(N-(4-methoxyphenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17k**)*. Yellow solid; yield 8%; mp 149-151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.58 (br s, 1H), 10.83 (br s, 1H), 10.14 (br s, 1H), 8.12 – 8.02 (m, 2H), 7.96 – 7.87 (m, 2H), 7.72 (d, J = 6.0 Hz, 1H), 7.11 (d, J = 6.0 Hz, 1H), 7.05 – 6.95 (m, 2H), 6.86 – 6.76 (m, 2H), 4.21 (q, J = 7.2 Hz, 2H), 3.66 (s, 3H), 1.28 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.0, 161.9, 156.8, 151.2, 148.6, 143.0, 135.4, 129.6, 128.2, 127.6, 123.8, 123.4, 117.1, 115.6, 114.4, 61.3, 55.2, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{22}N_3O_7S_2$, 504.0894; found, 504.0884.

*5.2.12.12. Ethyl (2-(4-(N-(4-(trifluoromethoxy)phenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17l**)*. Yellow solid; yield 26%; mp 180-181 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.58 (brs, 1H), 10.82 (brs, 1H), 10.77 (brs, 1H), 8.15 – 8.06 (m, 2H), 8.05 – 7.98 (m, 2H), 7.72 (d, J = 6.0 Hz, 1H), 7.29 – 7.27 (m, 2H), 7.24 – 7.18 (m, 2H), 7.11 (d, J = 5.6 Hz, 1H), 4.21 (q, J = 6.8 Hz, 2H), 1.28 (t, J = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.5, 161.3, 150.7, 148.1, 144.2, 142.3, 136.0, 135.4, 128.0, 127.1, 122.9, 121.8, 121.2, 119.5 (J = 255 Hz), 116.6, 115.2, 60.9, 13.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{19}N_3O_7S_2F_3$, 558.0611; found, 558.0603.

*5.2.12.13. Ethyl (2-(4-(N-(4-nitrophenyl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17m**)*. Yellow solid; yield 19%; mp 181-182°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.58 (br s, 1H), 11.50 (br s, 1H), 10.83 (br s, 1H), 8.20 – 8.07 (m, 6H), 7.71 (d, J = 6.0 Hz, 1H), 7.40 – 7.30 (m, 2H), 7.11 (d, J = 6.0 Hz, 1H), 4.24 – 4.18 (m, 2H), 1.30 – 1.26 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.0, 161.8, 151.2, 148.6, 143.8, 142.9, 142.4, 136.2, 128.7, 127.7, 125.5, 123.4, 118.3, 117.2, 115.7, 61.4, 14.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{19}N_4O_8S_2$, 519.0639; found, 519.0635.

*5.2.12.14. Ethyl (2-(4-(N-(pyridin-2-yl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17n**)*. Grey solid; yield 31%; mp 158-160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.60 (br s, 1H), 10.82 (br s, 1H), 8.11 – 8.05 (m, 4H), 7.96 – 7.95 (m, 1H), 7.82 – 7.75 (m, 1H), 7.72 (d, J = 6.0 Hz, 1H), 7.25 – 7.24 (m, 1H), 7.11 (d, J = 6.0 Hz, 1H), 6.87 – 6.84 (m, 1H), 4.21 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0 162.1, 153.9, 151.1, 148.8, 146.4, 141.8, 139.1,

134.6, 128.0, 127.2, 123.3, 117.0, 115.5, 114.8, 106.9, 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{20}H_{19}N_4O_6S_2$, 475.0741; found, 475.0730.

*5.2.12.15. Ethyl (2-(4-(N-(thiazol-2-yl)sulfamoyl)benzamido)thiophene-3-carbonyl)carbamate (**17o**)*. Yellow solid; yield 40%; mp 210-211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.91 (br s, 1H), 12.61 (br s, 1H), 10.82 (br s, 1H), 8.12 – 7.97 (m, 4H), 7.73 (d, J = 6.0 Hz, 1H), 7.29 (d, J = 4.4 Hz, 1H), 7.11 (d, J = 6.0 Hz, 1H), 6.88 (d, J = 4.4 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.2, 164.0, 162.0, 151.1, 148.7, 145.8, 134.8, 128.1, 126.6, 124.7, 123.3, 117.0, 115.5, 108.7, 61.3, 14.2. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{17}N_4O_6S_3$, 481.0305; found, 481.0296.

5.3. Biological evaluation

5.3.1. DprE1 inhibition assay

DprE1 assays were performed as described previously [7]. Briefly, assays were performed at 30 °C in 384-well black plates in buffer containing 50 mM Hepes, pH 7.5, 100 mM NaCl, 1.5% (v/v) DMSO, 100 μM Tween-20, 50 μM resazurin, and 2 μM FAD, with variable concentrations of FPR and DprE1. Reactions were monitored by following an increase in fluorescence intensity (λ_{em} = 595 nm, λ_{ex} = 530 nm) associated with the formation of resorufin. For inhibition assays, DprE1 (1 μM) was measured with the resazurin assay with 1 mM FPR in the presence of different inhibitor concentrations. The IC_{50} values were obtained by plotting the initial velocities with inhibitor concentration and calculated using the software program GraphPad Prism.

5.3.2. MIC determination

MICs against replicating *M. tuberculosis* H_{37}Rv or clinical isolates were determined by the microplate Alamar Blue assay (MABA) following the protocol as described previously [21, 24]. RFP, INH, 297F and **25a** were included as the positive controls. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacterium-only controls.

5.3.3. Cytotoxicity assay

Vero, HepG2 and mouse J774A.1 macrophage cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum

(FBS). Cytotoxicity testing was performed in a transparent 96-well microplate using Vero, HepG2 and mouse J774A.1 macrophage cells by methyl-thiazolyldiphenyl-tetrazolium bromide (MTT) assay according to the protocol as described previously [21, 24].

5.3.4. Hepatocyte stability assay

The assay was performed with hepatocytes from mixed human (BioIVT) and male mice (BioIVT) following the protocol as described previously [10]. Briefly, selected compounds were tested at 1 μM with a final hepatocyte concentration of 1 million cells/mL. The reaction was initiated by addition of pre-warmed hepatocyte working solution (2 million cells/mL) to the compound working solution (2 μM). Reaction mixtures were incubated for up to 120 min at 37 °C in a CO_2 incubator at ~ 100 rpm. At the pre-determined time points (0, 15, 30, 60, 90 and 120 min), 30 μL of the reaction mixtures was removed and reaction was terminated by addition of 200 μL ice-cold ACN/MeOH (50:50) spiked with internal standard. Samples were mixed well and then were centrifuged at 4,000 rpm at 4 °C for 15 min. Supernatants were removed and analysed by LC-MS/MS. The assay evaluated the metabolic stability of compounds in hepatocytes by measuring amount of parent remaining of the test compounds.

5.3.5. Inhibition Evaluation on hERG K^+ Channel

The electrophysiology recording of hERG channel current was carried out following the standard protocol as described previously [25]. hERG current inhibition in presence of 5 concentrations, including 30, 10, 3.0, 1.0 and 0.3 μM, was tested for IC_{50} determination. All experiments were performed in duplicate for IC_{50} determination. The compound with $IC_{50} > 30$ μM was generally considered to have a lower potential for hERG K^+ channel inhibition.

5.3.6. Anti-tuberculosis activity in macrophages

The assays were performed as described previously using mouse J774A.1 macrophages [26]. The final concentrations of compound **17b** was 10 μg/mL and 5 μg/mL. The concentration of rifampicin (RFP) as the positive control was 5 μg/mL. All assays were performed in triplicate in at least three separate experiments.

5.3.7. In vivo TB infection assay

All animal protocols were approved by the Institute Animal Care and Welfare Committee of Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University. SPF Balb/c mice (female, 18−20 g) were used in this assay [21, 27]. Each treated group was composed of 6 mice. Mice were infected via aerosol with a suspension of 5×10^6 CFU/mL *M. tuberculosis* $H_{37}Rv$ using a Glas-Col inhalation system, to deposit 50-100 bacilli into the lungs of each animal. INH and **17b** were dissolved or suspended in 0.5% CMC and administered by oral gavage in a maximum volume of 200 μL such that a dose of 25 and 100 mg/kg body weight was achieved. The control group received only 0.5% CMC. Mice were treated 5 days per week during the acute phase of infection, from day 10 until day 30. Mice were sacrificed the day after the last day of treatment, lungs removed, homogenized, and serially diluted in 10-fold steps in HBSS. 100μL was spread on 7H11 agar in duplicate. The plates were incubated at 37 °C for 3 weeks. Data are expressed as the $\log_{10}$ (and as $\log_{10}$ reduction) provided by a given dose of the compound against the growth of the organism in the untreated control group. Mean $\log_{10}$ values were calculated from bacterial burden counts. Student's *t* test was used to compare means between the test and control groups. A *P* value of $\leqslant 0.05$ was considered significant.

Notes

The authors declare no competing financial interest.

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References

[1] J. Furin, H. Cox, M. Pai, Tuberculosis, The Lancet, 393 (2019) 1642-1656.

[2] Word Health Organization, Global tuberculosis report 2020.

[3] E. Harding, WHO global progress report on tuberculosis elimination, Lancet Resp. Med., 8

(2020) 19.

[4] N.R. Gandhi, P. Nunn, K. Dheda, H.S. Schaaf, M. Zignol, D. van Soolingen, P. Jensen, J. Bayona, Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis, The Lancet, 375 (2010) 1830-1843.

[5] D.T. Hoagland, J. Liu, R.B. Lee, R.E. Lee, New agents for the treatment of drug-resistant *Mycobacterium tuberculosis,* Adv. Drug Deliver. Rev., 102 (2016) 55-72.

[6] R.V. Chikhale, M.A. Barmade, P.R. Murumkar, M.R. Yadav, Overview of the development of DprE1 inhibitors for combating the menace of tuberculosis, J. Med. Chem., 61 (2018) 8563-8593.

[7] S.M. Batt, M. Cacho Izquierdo, J. Castro Pichel, C.J. Stubbs, L. Vela-Glez Del Peral, E. Pérez-Herrán, N. Dhar, B. Mouzon, M. Rees, J.P. Hutchinson, R.J. Young, J.D. McKinney, D. Barros Aguirre, L. Ballell, G.S. Besra, A. Argyrou, Whole cell target engagement identifies novel inhibitors of *Mycobacterium tuberculosis* decaprenylphosphoryl-β-D-ribose oxidase, ACS Infect. Dis., 1 (2015) 615-626.

[8] V. Makarov, G. Manina, K. Mikusova, U. Möllmann, O. Ryabova, B. Saint-Joanis, N. Dhar, M.R. Pasca, S. Buroni, A.P. Lucarelli, A. Milano, E. De Rossi, M. Belanova, A. Bobovska, P. Dianiskova, J. Kordulakova, C. Sala, E. Fullam, P. Schneider, J.D. McKinney, P. Brodin, T. Christophe, S. Waddell, P. Butcher, J. Albrethsen, I. Rosenkrands, R. Brosch, V. Nandi, S. Bharath, S. Gaonkar, R.K. Shandil, V. Balasubramanian, T. Balganesh, S. Tyagi, J. Grosset, G. Riccardi, S.T. Cole, Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis, Science, 324 (2009) 801-804.

[9] V. Makarov, B. Lechartier, M. Zhang, J. Neres, A.M. van der Sar, S.A. Raadsen, R.C. Hartkoorn, O.B. Ryabova, A. Vocat, L.A. Decosterd, N. Widmer, T. Buclin, W. Bitter, K. Andries, F. Pojer, P.J. Dyson, S.T. Cole, Towards a new combination therapy for tuberculosis with next generation benzothiazinones, EMBO Mol. Med., 6 (2014) 372-383.

[10] P. Li, B. Wang, X. Zhang, S.M. Batt, G.S. Besra, T. Zhang, C. Ma, D. Zhang, Z. Lin, G. Li, H. Huang, Y. Lu, Identification of novel benzothiopyranone compounds against *Mycobacterium tuberculosis* through scaffold morphing from benzothiazinones, Eur. J. Med. Chem., 160 (2018) 157-170.

[11] P. Li, B. Wang, L. Fu, K. Guo, C. Ma, B. Wang, Z. Lin, G. Li, H. Huang, Y. Lu, Identification of novel benzothiopyranones with ester and amide motifs derived from active metabolite as

promising leads against *Mycobacterium tuberculosis*, Eur. J. Med. Chem., 222 (2021) 113603.

[12] L.P. Khonde, R. Müller, G.A. Boyle, V. Reddy, A.T. Nchinda, C.J. Eyermann, S. Fienberg, V. Singh, A. Myrick, E. Abay, M. Njoroge, N. Lawrence, Q. Su, T.G. Myers, H.I.M. Boshoff, C.E. Barry, F.A. Sirgel, P.D. van Helden, L.M. Massoudi, G.T. Robertson, A.J. Lenaerts, G.S. Basarab, S.R. Ghorpade, K. Chibale, 1,3-Diarylpyrazolyl-acylsulfonamides as potent anti-tuberculosis agents targeting cell wall biosynthesis in *Mycobacterium tuberculosis*, J. Med. Chem., 64 (2021) 12790-12807.

[13] G. Riccardi, M.R. Pasca, L.R. Chiarelli, G. Manina, A. Mattevi, C. Binda, The DprE1 enzyme, one of the most vulnerable targets of *Mycobacterium tuberculosis*, Appl. Microbiol. Biot., 97 (2013) 8841-8848.

[14] M. Brecik, I. Centárová, R. Mukherjee, G.S. Kolly, S. Huszár, A. Bobovská, E. Kilacsková, V. Mokošová, Z. Svetlíková, M. Šarkan, J. Neres, J. Korduláková, S.T. Cole, K. Mikušová, DprE1 is a vulnerable tuberculosis drug target due to its cell wall localization, ACS Chem. Biol., 10 (2015) 1631-1636.

[15] N. Hariguchi, X. Chen, Y. Hayashi, Y. Kawano, M. Fujiwara, M. Matsuba, H. Shimizu, Y. Ohba, I. Nakamura, R. Kitamoto, T. Shinohara, Y. Uematsu, S. Ishikawa, M. Itotani, Y. Haraguchi, I. Takemura, M. Matsumoto, OPC-167832, a novel carbostyril derivative with potent antituberculosis activity as a DprE1 inhibitor, Antimicrob. Agents Chemother., 64 (2020) e02020-19.

[16] G.T. Robertson, M.E. Ramey, L.M. Massoudi, C.L. Carter, M. Zimmerman, F. Kaya, B.G. Graham, V. Gruppo, C. Hastings, L.K. Woolhiser, D.W.L. Scott, B.C. Asay, F. Eshun-Wilson, E. Maidj, B.K. Podell, J.J. Vásquez, M.A. Lyons, V. Dartois, A.J. Lenaerts, Comparative analysis of pharmacodynamics in the C3HeB/FeJ mouse tuberculosis model for DprE1 inhibitors TBA-7371, PBTZ169, and OPC-167832, Antimicrob. Agents Chemother., 65 (2021) e00583-21.

[17] P.S. Shirude, R. Shandil, C. Sadler, M. Naik, V. Hosagrahara, S. Hameed, V. Shinde, C. Bathula, V. Humnabadkar, N. Kumar, J. Reddy, V. Panduga, S. Sharma, A. Ambady, N. Hegde, J. Whiteaker, R.E. McLaughlin, H. Gardner, P. Madhavapeddi, V. Ramachandran, P. Kaur, A. Narayan, S. Guptha, D. Awasthy, C. Narayan, J. Mahadevaswamy, K.G. Vishwas, V. Ahuja, A. Srivastava, K.R. Prabhakar, S. Bharath, R. Kale, M. Ramaiah, N.R. Choudhury, V.K. Sambandamurthy, S. Solapure, P.S. Iyer, S. Narayanan, M. Chatterji, Azaindoles: noncovalent DprE1 inhibitors from scaffold morphing efforts, kill *Mycobacterium tuberculosis* and are efficacious *in vivo*, J. Med. Chem., 56

(2013) 9701-9708.

[18] M. Chatterji, R. Shandil, M.R. Manjunatha, S. Solapure, V. Ramachandran, N. Kumar, R. Saralaya, V. Panduga, J. Reddy, P. KR, S. Sharma, C. Sadler, C.B. Cooper, K. Mdluli, P.S. Iyer, S. Narayanan, P.S. Shirude, 1,4-Azaindole, a potential drug candidate for treatment of tuberculosis, Antimicrob. Agents Chemother., 58 (2014) 5325-5331.

[19] F. Wang, D. Sambandan, R. Halder, J. Wang, S.M. Batt, B. Weinrick, I. Ahmad, P. Yang, Y. Zhang, J. Kim, M. Hassani, S. Huszar, C. Trefzer, Z. Ma, T. Kaneko, K.E. Mdluli, S. Franzblau, A.K. Chatterjee, K. Johnsson, K. Mikusova, G.S. Besra, K. Fütterer, S.H. Robbins, S.W. Barnes, J.R. Walker, W.R. Jacobs, P.G. Schultz, Identification of a small molecule with activity against drug-resistant and persistent tuberculosis, Proc. Natl. Acad. Sci. U. S. A., 110 (2013) E2510-E2517.

[20] R. Liu, X. Lyu, S.M. Batt, M.H. Hsu, M.B. Harbut, C. Vilchèze, B. Cheng, K. Ajayi, B. Yang, Y. Yang, H. Guo, C. Lin, F. Gan, C. Wang, S.G. Franzblau, W.R. Jacobs, G.S. Besra, E.F. Johnson, M. Petrassi, A.K. Chatterjee, K. Fütterer, F. Wang, Determinants of the inhibition of DprE1 and CYP2C9 by antitubercular thiophenes, Angew. Chem., Int. Ed., 56 (2017) 13011-13015.

[21] P. Wang, S.M. Batt, B. Wang, L. Fu, R. Qin, Y. Lu, G. Li, G.S. Besra, H. Huang, Discovery of novel thiophene-arylamide derivatives as DprE1 inhibitors with potent antimycobacterial activities, J. Med. Chem., 64 (2021) 6241-6261.

[22] H. Chen, B. Wang, P. Li, H. Yan, G. Li, H. Huang, Y. Lu, The optimization and characterization of functionalized sulfonamides derived from sulfaphenazole against *Mycobacterium tuberculosis* with reduced CYP 2C9 inhibition, Bioorg. Med. Chem. Lett., 40 (2021) 127924.

[23] Y. Lin, N. Zhu, Y. Han, J. Jiang, S. Si, Identification of anti-tuberculosis agents that target the cell-division protein FtsZ, J. Antibiot., 67 (2014) 671-676.

[24] Y. Lu, M. Zheng, B. Wang, L. Fu, W. Zhao, P. Li, J. Xu, H. Zhu, H. Jin, D. Yin, H. Huang, A.M. Upton, Z. Ma, Clofazimine analogs with efficacy against experimental tuberculosis and reduced potential for accumulation, Antimicrob. Agents Chemother., 55 (2011) 5185-5193.

[25] M.C. Trudeau, J.W. Warmke, B. Ganetzky, G.A. Robertson, HERG, a human inward rectifier in the voltage-gated potassium channel family, Science, 269 (1995) 92-95.

[26] D. Zhang, Y. Lin, X. Chen, W. Zhao, D. Chen, M. Gao, Q. Wang, B. Wang, H. Huang, Y. Lu, Y. Lu, Docking- and pharmacophore-based virtual screening for the identification of novel *Mycobacterium tuberculosis* protein tyrosine phosphatase B (MptpB) inhibitor with a

thiobarbiturate scaffold, Bioorg. Chem., 85 (2019) 229-239.

[27] H.Y. Zhao, B. Wang, L. Fu, G. Li, H.J. Lu, Y.K. Liu, L. Sheng, Y. Li, B.X. Zhang, Y. Lu, C. Ma, H.H. Huang, D.F. Zhang, Y. Lu, Discovery of a conformationally constrained oxazolidinone with improved safety and efficacy profiles for the treatment of multidrug-resistant tuberculosis, J. Med. Chem., 63 (2020) 9316-9339.