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Short communication

Development of antimycobacterial tetrahydrothieno[2,3-c]pyridine-3-carboxamides and hexahydrocycloocta[b]thiophene-3-carboxamides: Molecular modification from known antimycobacterial lead

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ABSTRACT

Twenty derivatives of 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide and ten of 2-substituted 4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide were synthesized by molecular modification of a known antimycobacterial molecule. Compounds were evaluated *in vitro* against *Mycobacterium tuberculosis* (MTB), and cytotoxicity against RAW 264.7 cell line. Among the compounds, 2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12f**) was found to be the most active compound against MTB with MIC of 3.70 μM and was more potent than Ethambutol (MIC of 7.64 μM), Ciprofloxacin (MIC of 9.41 μM) and standard lead compound SID 92097880 (MIC of 9.15 μM). Compound **12f** also showed MTB MIC of 1.23 μM in the presence of an efflux pump inhibitor verapamil, and showed no cytotoxicity at 50 μM.

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1. Introduction

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is one of the most lethal infectious agents affecting mankind [1]. The disease infects almost two billion people or one-third of the world's population, and accounts for an estimated 2 million deaths per year [2]. MTB infection is difficult to treat, requiring 6–9 months of chemotherapy with a cocktail of four antibiotics isoniazid, rifampin, pyrazinamide and ethambutol. In addition to toxic side effects, the lengthy treatment regime results in poor patient compliance and thus drug resistant strains are beginning to emerge [3]. The World Health Organization estimates that up to 50 million people worldwide are infected with multidrug resistant strains of MTB (MDR-TB) [4]. This number continues to grow as 300,000 new MDR-TB cases are diagnosed each year with 79 percent of individuals showing resistance to three or more frontline drugs [4]. Taken together, the growing problem of MDR-TB and the lack of drugs that effectively target persistent bacteria, there seem to be an urgent need for identification of new antimicrobial targets and inhibitors [5]. Target-based approaches are widely used in drug discovery; questions have been raised about the efficiency of this approach given the very high attrition rates that these projects have historically shown in the anti-infective field [6]. Compounds identified in whole-cell screens provide suitable chemical and biological starting points. The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established by the National Institute of Allergy and Infectious Diseases (NIAID) in 1994 to allow researchers access to high quality screening services in order to encourage antituberculosis drug discovery research. Recently TAACF reported anti-TB high-throughput results of large libraries of drug-like small molecules [7–9]. One among them was the molecule SID 92097880 6-acetyl-2-(thiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide that showed activity against *M. tuberculosis* H37Rv (MTB) with minimum inhibitory concentration (MIC) of 9.15 μM and selectivity index of 13 (Fig. 1). We took SID 92097880 as the starting point to design and synthesize various 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamides and 2-substituted 4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamides to study extensively their structure

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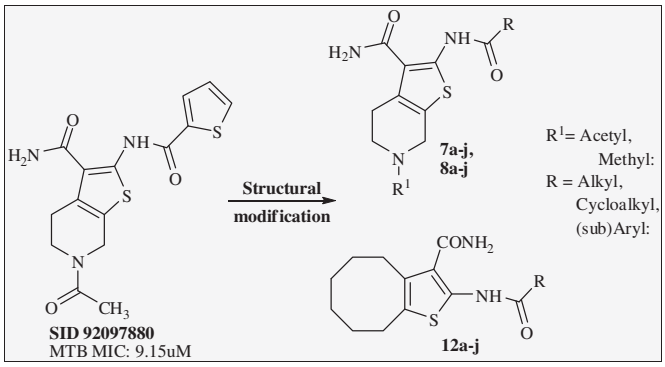


Fig. 1. Structural modification of lead compound.

activity relationship (SAR) (Fig. 1) and to develop a more potent compound.

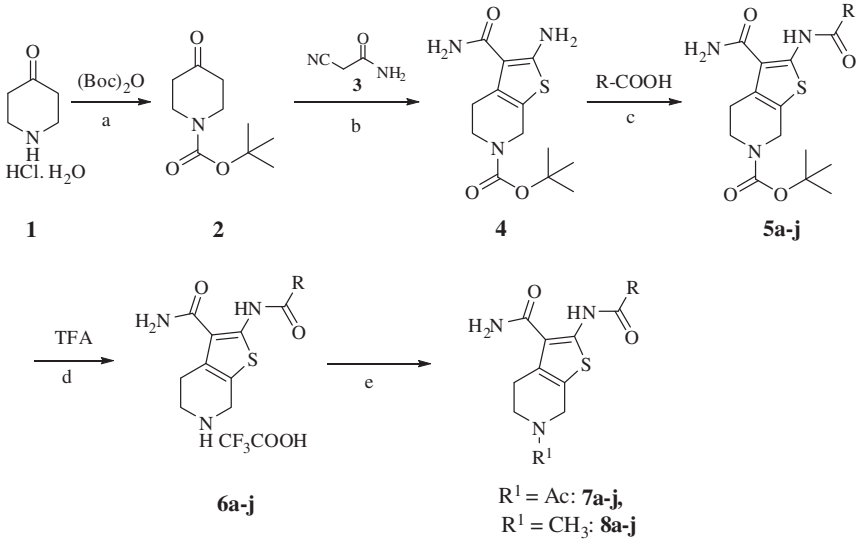
2. Results and discussion

2.1. Chemistry

In Scheme 1, we synthesised 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamides by following a five step synthetic protocol starting from the commercially available, less expensive 4-piperidone monohydrate hydrochloride (**1**). In first step we protected the secondary amine group by Boc-protection to get '*tert*-butyl 4-oxopiperidine-1-carboxylate' (**2**). In the next step, by following Gewald reaction conditions using morpholine, cyanoacetamide (**3**) and elemental sulphur achieved '*tert*-butyl 2-amino-3-carbamoyl-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate' (**4**). This conversion (**2**–**4**) was successful in both the conditions i.e. at room temperature and at 70 °C. But they differ in reaction time, purification process and yield. When reaction carried out at 70 °C we observed the complete conversion of starting material in less than 2 h and it has taken 6–9 h to complete the reaction at room temperature. Compared to reaction at 70 °C, we noticed good yields (nearly 20% more) when the reaction was carried out at room temperature, and in addition also observed that there was a good amount of solids formed in the reaction mixture which eased the purification process. The primary aromatic amine group of compound **4** was coupled with various alkyl, cycloalkyl, (sub)aryl carboxylic acids by employing peptide coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and HOBt to produce compounds **5a**–**j**. Due to the poor solubility of reaction products, we observed formation of desired product as solid in reaction mixture. Direct filtration of reaction mixture washing with distilled water, hexane produced pure products without any further purification. In next step deprotection of Boc-protection was carried out by using trifluoroacetic acid to get compounds **6a**–**j**. The conversion of compounds **6a**–**j** to target compounds **7a**–**j** was achieved by treating with acetyl chloride in dichloromethane using Et_3N as base.

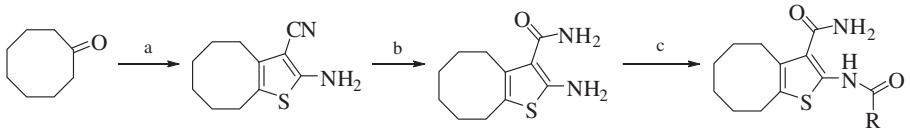
In order to achieve the synthesis of compound **8a**–**j**, we tried direct methylation using CH_3I and sodium hydride conditions, but the reaction was unsuccessful. Then we employed reductive amination conditions using formaldehyde and $Na(OAc)_3BH$ in MeOH with catalytic amount of acetic acid to produce compounds **8a**–**j**. We also tried to synthesize the final compounds **7a**–**j** and **8a**–**j** using 1-acetylpiperidin-4-one and 1-methylpiperidin-4-one respectively as the starting materials in order to achieve them in two steps. We succeeded in first step i.e. synthesis of 6-acetyl-2-amino-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide and 2-amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide. But in next step, due to very poor solubility, the coupling reaction was unsuccessful with various carboxylic acids.

In Scheme 2 (2-substituted 4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamides), for the preparation of intermediate compound **11**, we used the same reaction conditions which are followed for the conversion **2** to **4** (morpholine, cyanoacetamide and elemental sulphur), but the reaction was unsuccessful. Hence instead of cyanoacetamide we used malanonitrile under Gewald reaction conditions to get 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carbonitrile (**10**). Compound **10** was then treated with conc. H_2SO_4 to afford 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b]



Reagents & Conditions: a: $(Boc)_2O$, Et_3N, DCM, MeOH, rt; b: S_8, Morpholine, EtOH, rt, 6h; c: EDCI, HOBt, Et_3N, DCM, rt, 9h; d: TFA, DCM, rt, 2h; e: R^1 = Acetyl; Acetylchloride, Et_3N, CH_2Cl_2 and R_2 = Methyl; HCHO, $Na(OAc)_3BH$, MeOH, H_2O, rt, 2h:

Scheme 1. Synthetic protocol of tetrahydrothieno[2,3-c]pyridine-3-carboxamides.



Reagents & Conditions: a: Malanonitrile, S_8, Et_3N, DMF, rt; b: Concd H_2SO_4, rt, 6 h; c: RCOOH, EDCI, HOBt, Et_3N, DCM, rt, 9 h:

Scheme 2. Synthetic protocol of hexahydrocycloocta[b]thiophene-3-carboxamides.

thiophene-3-carboxamide (**11**). The free amino group of compound **11** on reaction with various carboxylic acids produced the target molecules **12a**–**j**. The purity of the synthesized compounds was checked by elemental analysis and TLC and the structures were identified by spectral analysis. In the nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR), the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The elemental analysis results were within ±0.4% of the theoretical values.

The structures of all final compounds were established from ^{1}H and ^{13}C NMR spectroscopic data as illustrated for a representative example **12f** (Fig. 2). The ^{1}H NMR spectrum of **12f** has a doublet at 7.89 ppm (J = 8.7 Hz) corresponds to aromatic H-1′, a doublet at 7.44 ppm (J = 7.8 Hz) is due to H-4′, a triplet at 7.24 ppm (J = 7.5 Hz) corresponds to H-5′ and the other doublet at 7.13 ppm (J = 8.4 Hz) corresponds to H-2′ and H-3′ protons. The two triplets at 2.84, 2.75 ppm corresponds to C-4 and C-9 protons respectively, the protons of C-5, C-6, C-7 and C-8 gave a multiplet (1.56–1.19) ppm.

2.2. In vitro MTB screening

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv (ATCC27294) using agar dilution method and drug concentrations from 50 μg/mL to 0.78 μg/mL in duplicates. The minimum inhibitory concentration (MIC) was determined for each compound. The MIC was taken as the minimum concentration of compound required to completely inhibit the bacterial growth. Isoniazid, Ethambutol, Ciprofloxacin and SID 92097880 were used as reference compounds for comparison. The MIC values of the synthesized compounds along with the standard drug for comparison are presented in Table 1. All the synthesized compounds showed activity against MTB with MIC ranging from 3.70 to 88.6 μM. Seven compounds (**8h**–**8j**, **12e**, **12f**, **12i** and **12j**) inhibited MTB with MIC <10 μM. Compound **12f** (2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide) was found to be the most active compound *in vitro*

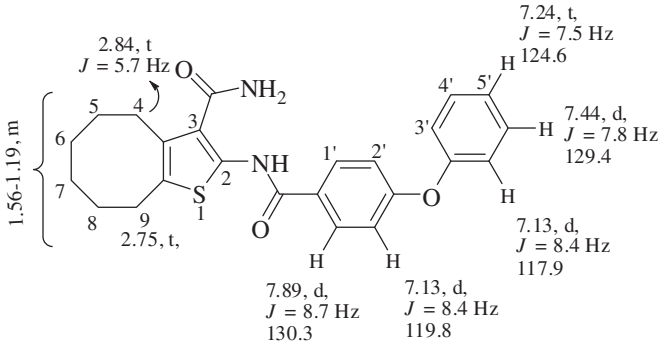


Fig. 2. ^{1}H and ^{13}C chemical shifts in compound **12f**.

with MIC of 3.70 μM against log-phase culture of MTB. All the synthesized compounds were less potent than standard first line antitubercular compound Isoniazid. Three compounds were found to be more potent than Ethambutol (MIC 7.64 μM). Five compounds were found to be more potent than lead compound SID 92097880 (MIC 9.15 μM).

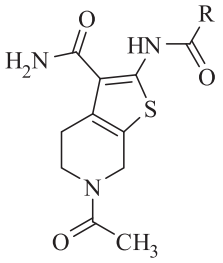
With respect to SAR of 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamides (**7a**–**j** and **8a**–**j**); we have prepared various alkyl, cycloalkyl and (sub)aryl amide at 2nd position and at N-6 position we have prepared acetyl (**7a**–**j**) and methyl (**8a**–**j**) derivatives. At N-6 position, compounds with N-methyl derivatives showed better activity (MIC ranging 5.06–39.43 μM) than N-acetyl derivatives (MIC ranging 57.30–88.60 μM). These results indicated that alkyl substituent favour bioactivity than the acyl derivative. Among compounds **8a**–**j**; hydrophobic substituent at C-2 position increased the activity. Cycloalkyl derivatives (**8h**–**8j**) showed promising activity with MIC less than 10 μM; followed by naphthyl derivative (**8g**) with MIC of 17.07 μM. When compared to phenyl derivative (**8b**), naphthyl derivative showed two times more potency. Among (sub)phenyl derivatives (**8b**–**8f**), not much difference were found between electron withdrawing and donating groups at phenyl ring. With respect to SAR of 4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamides (**12a**–**j**), where we replaced N-acetyl tetrahydropyridine moiety of lead compound SID 92097880 with highly hydrophobic cyclooctyl moiety showed better activity with MIC ranging from 3.70 to 18.72 μM. In this series some of substituted phenyl derivatives at C-2 position (**12e** and **12f**) showed better activity (less than 5 μM) than cycloalkyl (**12h**–**12j**) and naphthyl derivative (**12g**).

When compared to lead compound SID 92097880, many of the synthesized compounds showed less activity. It could be due to the involvement of wide array of efflux mechanisms mediated by several ABC (ATP-binding cassette) transporters and major facilitator superfamily (MFS) proteins, or antibiotic-modifying and-degrading enzymes, to name a few possibilities [10]. Multiple drugs like verapamil, reserpine, phenothiazines such as thioridazine, and piperine have been shown to inhibit bacterial efflux pumps *in vitro* [11]. In general, the mechanisms by which these agents act were poorly understood. Several models have been proposed [12], such as: (1) direct binding and inhibition of pump assembly or function; (2) disruption of the transmembrane gradients utilized by secondary transporters; (3) inhibitor binding to the antimicrobial compound; and (4) competition for efflux. We have tested some selected compounds [MIC of <20 μM], in presence of reported efflux pump inhibitor verapamil; and in most of the cases MIC was decreased 2–3 fold when compared to absence of efflux pump inhibitor. Most active compound **12f** showed MIC of 1.23 μM in this study.

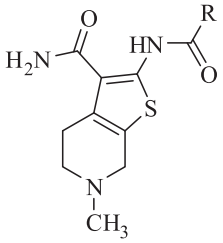
2.3. Cytotoxicity study

Compounds with MIC less than 20 μM were further examined for cytotoxicity in a RAW 264.7 cell line (mouse leukaemic

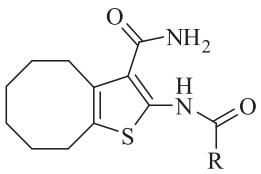
Table 1
Biological activities of synthesized compounds.



7a-j



8a-j



12a-j

Compound	R	Yield (%)	MP (°C)	MTB MIC in μM	MTB MIC in μM [in presence of verapamil]	Cytotoxicity at 50 μM (RAW 264.7 cells) % inhibition
7a	Methyl	81	223–224	88.6	NT	NT
7b	Phenyl	63	231–232	72.65	NT	NT
7c	2-Methoxyphenyl	70	202–204	66.84	NT	NT
7d	3-Nitrophenyl	84	251–252	64.26	NT	NT
7e	4-Tolyl	79	204–205	69.80	NT	NT
7f	4-Phenoxyphenyl	72	190–191	57.30	NT	NT
7g	1-Napthyl	61	270–271	63.45	NT	NT
7h	Cyclopropyl	74	204–205	81.15	NT	NT
7i	Cyclopentyl	64	280–281	74.40	NT	NT
7j	Cyclohexyl	78	208–209	71.40	NT	NT
8a	Methyl	58	222–223	39.43	NT	NT
8b	Phenyl	66	182–183	37.87	NT	NT
8c	2-Methoxyphenyl	62	205–206	36.12	NT	NT
8d	3-Nitrophenyl	81	224–225	34.62	NT	NT
8e	4-Tolyl	73	133–135	37.87	NT	NT
8f	4-Phenoxyphenyl	73	207–208	30.60	NT	NT
8g	1-Napthyl	56	180–181	17.07	8.53	28.42
8h	Cyclopropyl	72	231–232	5.57	2.78	22.16
8i	Cyclopentyl	62	260–261	5.06	5.06	18.16
8j	Cyclohexyl	72	206–207	7.76	2.58	18.28
12a	Methyl	79	186–187	18.72	9.36	24.56
12b	Phenyl	88	213–214	15.19	5.06	26.62
12c	2-Methoxyphenyl	60	243–244	13.92	6.96	24.80
12d	3-Nitrophenyl	69	228–229	16.71	16.71	36.14
12e	4-Tolyl	63	216–217	4.54	2.27	20.52
12f	4-Phenoxyphenyl	69	210–211	3.70	1.23	22.16
12g	1-Napthyl	70	190–191	13.19	3.29	28.32
12h	Cyclopropyl	66	145–146	17.06	8.53	16.86
12i	Cyclopentyl	54	175–176	9.73	9.73	18.34
12j	Cyclohexyl	79	223–224	9.33	3.11	16.64
Isoniazid				0.72	0.72	NT
Ethambutol				7.64	3.82	NT
Ciproflaxacin				9.41	9.41	NT
SID 92097880				9.15	4.57	NT

NT indicates not tested.

monocyte macrophage) at single concentration of 50 μM. We have selected this macrophage cell line to test the toxicity as generally MTB reside inside the macrophages and we expected the new molecules not to show any toxicity against macrophages. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay. Most of the tested compounds were not cytotoxic to RAW 264.7 cells based on their percentage growth inhibitions (less than 30%) as reported in Table 1. The most active anti-TB compound **12f** showed 22.6% cytotoxicity at 50 μM with selectivity index of >10 for MTB.

3. Conclusion

In this study we have designed, synthesized and studied SAR of various inhibitors of MTB based on the lead compound SID 92097880 earlier reported by TAACF. Among the compounds, 2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12f**) was found to be the most active compound *in vitro* with MIC of 3.70 μM against log-phase culture of MTB and also non-toxic up to 50 μM. Compound **12f** was ~3 times more potent than lead compound SID: 92097880. Compound **12f** showed MIC of 1.23 μM in the presence of efflux pump inhibitor. Further structural optimization can be performed to get compounds with better potency than the lead compound and also to explore the possible mechanism of action against various MTB essential enzymes. In addition, efflux could be the rate-limiting step in the discovery of novel anti-TB compounds, as has already been recognized in the discovery of drugs for Gram-negative bacterial infections. The discovery of new pumps with multiple specificities in MTB and the impact of these pumps on antitubercular therapy by conferring resistance to many of the new molecules discovered necessitate the study of efflux mechanism as an important therapeutic target.

4. Experimental

4.1. Chemistry

4.1.1. Preparation of tert-butyl 4-oxopiperidine-1-carboxylate (**2**)

Et_3N (26.45 mL, 185.17 mmol) was added drop-wise to a stirred solution of compound (**1**) (4.0 g, 29.60 mmol) in CH_2Cl_2 (20 mL) and MeOH (4 mL) at 0 °C, then $(Boc)_2O$ (7.74 mL, 35.52 mmol) was added drop wise to the reaction mixture at same temperature and allowed to stir at room temperature for 16 h. The reaction mixture was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 60 mL). The separated organic layer was concentrated under reduced pressure and the crude residue was washed with hexanes to get compound **2** (4.30 g, 72%) as an off-white solid. MS (ESI) 200 $[M + H]^+$. Anal. calcd for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03% Found C, 60.34; H, 8.64; N, 7.11%.

4.1.2. Preparation of tert-butyl 2-amino-3-carbamoyl-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (**4**)

To the stirred solution of compound **2** (6.0 g, 30.14 mmol), cyanoacetamide (3.01 g, 37.18 mmol), sulphur powder (2.13 g, 30.14 mmol) in ethanol (60 mL) was added morpholine (6.01 mL, 60.30 mmol) and stirred the reaction mixture at room temperature for 12 h the reaction mixture was concentrated, and the crude solid was filtered, washed with H_2O and dried in vacuum oven for 2 h. The obtained dried compound was purified by column chromatography to yield (3.67 g, 82%) as a light yellow solid. MS (ESI) 298 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.25 (s, 2H), 7.90 (s, 2H), 4.32 (s, 2H), 3.51 (t, J = 6.8 Hz, 2H), 3.04 (t, J = 6.8 Hz, 2H); Anal. calcd for $C_{13}H_{19}N_3O_3S$: C, 52.51; H, 6.44; N, 14.13% Found C, 52.64; H, 6.55; N, 14.11%.

4.1.3. General procedure for the preparation of **5a–j**

To the stirred solution of R–COOH (1.20 equiv) in CH_2Cl_2 was added EDCI (1.30 equiv), HOBt (1.30 equiv) and Et_3N (2.50 equiv) allowed the reaction mixture to stir for few minutes, then added compound **4** (1.00 equiv). The reaction mixture was stirred at room temperature for 3 h. Concentrated and triturated with H_2O to get solid compound. The solids were given cold ethanol, diethyl ether and hexane washings to get pure products.

4.1.4. Preparation of **6a–j**

To the stirred solution of Compound **5a–j** in CH_2Cl_2 at 0 °C under N_2 atm, was added TFA (2 volumes) and allowed to stir the reaction mixture at room temperature for 2 h. The reaction mixture was concentrated to dryness and the obtained solids were washed with hexanes to afford respective compounds **6a–j**.

4.1.5. General procedure for the preparation of **7a–j**

To the stirred solution of compounds **6a–j** in CH_2Cl_2 at 0 °C under N_2 atm, was added Et_3N (2.0 equiv), followed by acetyl chloride (1.2 equiv) and allowed to stir the reaction mixture at room temperature for 4 h. The reaction mixture was concentrated to dryness and the obtained solids were washed with excess water, cold ethanol and hexanes to afford respective final compounds **7a–j**.

4.1.6. 2-Acetamido-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7a**)

MS (ESI) 282 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 2H), 7.90 (s, 1H), 4.61(s, 2H), 3.91(t, J = 7.6 Hz, 2H), 3.18 (t, J = 8.0 Hz, 2H), 2.19 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 169.8, 161.6, 133.1, 129.8, 128.5, 112.2, 51.9, 42.8, 26.3, 23.4, 22.5. Anal. calcd for $C_{12}H_{15}N_3O_3S$: C, 51.23; H, 5.37; N, 14.94% Found C, 51.32; H, 5.39; N, 15.01%.

4.1.7. 6-Acetyl-2-benzamido-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7b**)

MS (ESI) 344 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 2H), 7.81–7.63 (m, 6H), 4.50 (s, 2H), 3.81 (t, J = 7.6 Hz, 2H), 3.10 (t, J = 7.6 Hz, 2H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.3, 172.4, 167.9, 139.4, 133.8, 131.4(2C), 130.8, 128.4(2C), 126.3, 123.8, 118.9, 43.8, 39.7, 25.0, 22.5. Anal. calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24% Found C, 59.52; H, 5.12; N, 12.32%.

4.1.8. 6-Acetyl-2-(2-methoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7c**)

MS (ESI) 374 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 12.45 (s, 1H), 9.97 (s, 2H), 7.74–7.63 (m, 2H), 7.56 (d, J = 7.6 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 4.58 (s, 2H), 3.94 (s, 3H), 3.72 (t, J = 7.2 Hz, 2H), 3.15 (t, J = 7.6 Hz, 2H), 2.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.3, 176.3, 171.9, 148.9, 141.8, 139.4, 133.8, 132.9, 130.3, 128.7, 127.4, 122.4, 114.8, 55.8, 41.6, 38.9, 22.5, 21.6. Anal. calcd for $C_{18}H_{19}N_3O_4S$: C, 57.89; H, 5.13; N, 11.25% Found C, 57.92; H, 5.22; N, 11.32%.

4.1.9. 6-Acetyl-2-(3-nitrobenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7d**)

MS (ESI) 389 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 9.21 (s, 1H), 8.31 (s, 1H), 8.10 (s, 2H), 7.99–7.90 (m, 2H), 7.72 (t, J = 8.4 Hz, 1H), 4.59 (s, 2H), 3.79 (t, J = 7.6 Hz, 2H), 3.09 (t, J = 8.0 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.9, 175.3, 168.4, 141.9, 139.8, 132.5, 130.5, 128.4, 126.9, 124.8, 122.4, 120.4, 116.3, 45.2, 38.4, 24.9, 22.5. Anal. calcd for $C_{17}H_{16}N_4O_5S$: C, 52.57; H, 4.15; N, 14.43% Found C, 52.62; H, 4.22; N, 14.52%.

4.1.10. 6-Acetyl-2-(4-methylbenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7e**)

MS (ESI) 358 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 10.27 (s, 2H), 7.81 (s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.0 Hz, 2H), 4.60 (s, 2H), 3.81 (t, J = 7.6 Hz, 2H), 3.18 (t, J = 7.2 Hz, 2H), 2.41 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.7, 169.3, 166.2, 134.0, 132.9, 130.3(2C), 129.3(2C), 127.4, 123.4, 121.4, 116.3, 51.8, 42.6, 26.2, 24.5, 23.7. Anal. calcd for $C_{18}H_{19}N_3O_3S$: C, 60.49; H, 5.36; N, 11.76% Found C, 60.52; H, 5.39; N, 11.91%.

4.1.11. 6-Acetyl-2-(4-phenoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7f**)

MS (ESI) 436 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H), 7.92 (s, 2H), 7.81 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 7.69–7.54 (m, 5H), 4.70 (s, 2H), 3.80 (t, J = 8.4 Hz, 2H), 3.13 (t, J = 8.0 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 172.7, 169.6, 146.3, 143.7, 132.3(2C), 129.0(2C), 128.4, 127.4(2C), 125.9, 125.1(2C), 122.6, 123.5, 121.8, 115.9, 46.8, 41.8, 23.6, 20.4. Anal. calcd for $C_{23}H_{21}N_3O_4S$: C, 63.43; H, 4.86; N, 9.65% Found C, 63.52; H, 4.99; N, 9.72%.

4.1.12. 2-(1-Naphthamido)-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7g**)

MS (ESI) 394 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 10.29 (s, 1H), 8.19–8.10 (m, 4H), 7.92–7.74 (m, 5H), 4.57 (s, 2H), 3.90 (t, J = 8.0 Hz, 2H), 3.14 (t, J = 7.2 Hz, 2H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 176.2, 168.2, 141.3, 140.7, 138.4, 137.9, 136.2, 135.4, 132.9, 128.7, 126.0, 125.3, 123.3, 122.6, 121.8, 118.1, 44.4, 39.8, 26.2, 21.7. Anal. calcd for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68% Found C, 64.12; H, 5.02; N, 10.72%.

4.1.13. 6-Acetyl-2-(cyclopropanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7h**)

MS (ESI) 308 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H), 6.93 (s, 1H), 4.66 (s, 2H), 3.97(t, J = 8.0 Hz, 2H), 3.21

(t, J = 8.0 Hz, 2H), 2.25 (s, 3H), 1.92–1.86 (m, 1H), 1.28–1.03 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 172.9, 166.4, 133.3, 131.3, 129.4, 119.1, 43.4, 41.6, 27.4, 24.5, 22.3, 20.7(2C). Anal. calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67% Found C, 54.82; H, 5.64; N, 13.71%.

4.1.14. 6-Acetyl-2-(cyclopentanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7i**)

MS (ESI) 336 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 2H), 7.69 (s, 1H), 4.71 (s, 2H), 3.94 (t, J = 7.6 Hz, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.18 (s, 3H), 2.16–2.10 (m, 1H), 1.89–1.60 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 167.8, 161.6, 133.2, 132.7, 124.5, 114.9, 47.9, 46.4, 41.2(2C), 36.9, 31.5(2C), 26.5, 23.4. Anal. calcd for $C_{16}H_{21}N_3O_3S$: C, 57.29; H, 6.31; N, 12.53% Found C, 57.32; H, 6.42; N, 12.61%.

4.1.15. 6-Acetyl-2-(cyclohexanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**7j**)

ESI-MS showed 350 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H), 7.72 (s, 1H), 4.66 (s, 2H), 3.97(t, J = 8.0 Hz, 2H), 3.21 (t, J = 8.0 Hz, 2H), 2.14–2.09 (m, 4H), 1.98–1.53 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.7, 171.8, 166.6, 136.1, 130.8, 122.5, 116.6, 49.9, 44.8, 38.6(2C), 30.4(2C), 26.3, 24.4, 23.5, 21.4. Anal. calcd for $C_{17}H_{23}N_3O_3S$: C, 58.43; H, 6.63; N, 12.02% Found C, 58.52; H, 6.73; N, 12.13%.

4.1.16. General procedure for the preparation of **8a–j**

40% formaldehyde solution (6 vol) was added to the stirred solution of Compound **6a**–j in MeOH/H_2O (9:1) and stirred for 1 h, then Na(OAc)$_3$BH (2.0 equiv) was added. The reaction mixture was stirred at room temperature for 6 h. The product was extracted with EtOAc and purified by triturating with CH_2Cl_2, Diethyl ether and Hexanes to get title compounds **8a–j**.

4.1.17. 2-acetamido-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8a**)

MS (ESI) 254 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H), 8.01 (s, 2H), 4.12 (s, 2H), 3.44 (t, J = 7.6 Hz, 2H), 3.15 (t, J = 8.0 Hz, 2H), 2.41 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.7, 167.4, 158.7, 133.4, 129.8, 127.6, 51.3, 49.8, 42.7, 25.2, 22.5. Anal. calcd for $C_{11}H_{15}N_3O_2S$: C, 52.15; H, 5.97; N, 16.59% Found C, 52.22; H, 6.02; N, 16.72%.

4.1.18. 2-Benzamido-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8b**)

MS (ESI) 316 [M + H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.63 (s, 1H), 9.97 (s, 2H), 7.92–7.63 (m, 5H), 4.54 (s, 2H), 3.71 (t, J = 8.4 Hz, 2H), 3.13 (t, J = 7.6 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.4, 170.5, 136.2, 129.5, 127.2(2C), 125.8(2C), 125.1, 124.4, 123.6, 115.4, 48.3, 42.6, 39.0, 21.8. Anal. calcd for $C_{16}H_{17}N_3O_2S$: C, 60.93; H, 5.43; N, 13.32% Found C, 61.02; H, 5.52; N, 13.42%.

4.1.19. 2-(2-Methoxybenzamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8c**)

MS (ESI) 346 [M + H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.45 (s, 2H), 7.81 (d, J = 7.6 Hz, 1H), 7.72–7.54 (m, 4H), 4.61 (s, 2H), 3.90 (s, 3H), 3.69 (t, J = 7.6 Hz, 2H), 3.21 (t, J = 8.0 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 172.3, 146.9, 142.8, 134.4, 133.9, 132.3, 131.4, 128.4, 126.9, 125.1, 120.0, 60.1, 56.6, 52.9, 39.9, 23.6. Anal. calcd for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.17% Found C, 59.22; H, 5.61; N, 12.16%.

4.1.20. 6-Methyl-2-(3-nitrobenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8d**)

MS (ESI) 361 [M + H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.21 (s, 2H), 9.45 (s, 1H), 8.40 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.81–7.72 (m, 2H), 4.61 (s, 2H), 3.81 (t, J = 8.0 Hz, 2H), 3.12 (t, J = 8.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.8, 173.3, 149.8, 144.6, 139.4, 137.4, 136.4, 133.4, 129.9, 126.8, 124.4, 118.3, 51.2, 46.4, 38.9, 26.5. Anal. calcd for $C_{16}H_{16}N_4O_4S$: C, 53.32; H, 4.47; N, 15.55% Found C, 53.42; H, 4.52; N, 15.64%.

4.1.21. 6-Methyl-2-(4-methylbenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8e**)

MS (ESI) 330 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 1H), 7.84–7.72 (m, 4H), 7.54 (d, J = 8.0 Hz, 2H), 4.31 (s, 2H), 3.61 (t, J = 7.6 Hz, 2H), 3.04 (t, J = 7.6 Hz, 2H), 2.43 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.3, 148.2, 146.5, 139.0, 136.5, 130.6(2C), 128.4(2C), 126.3, 118.8, 49.8, 43.6, 38.2, 24.5, 21.9. Anal. calcd for $C_{17}H_{19}N_3O_2S$: C, 61.98; H, 5.81; N, 12.76% Found C, 62.02; H, 5.89; N, 12.81%.

4.1.22. 6-Methyl-2-(4-phenoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8f**)

MS (ESI) 408 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.72–7.63 (m, 3H), 7.54–7.45 (m, 5H), 4.84 (s, 2H), 3.72 (t, J = 8.4 Hz, 2H), 3.21 (t, J = 8.4 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 178.7, 148.2, 146.9, 136.4(2C), 136.0, 134.3, 132.0(2C), 129.5, 128.2(2C), 126.9(2C), 125.3, 124.6, 119.9, 52.9, 46.8, 36.9, 24.1. Anal. calcd for $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31% Found C, 64.92; H, 5.21; N, 10.52%.

4.1.23. 2-(1-Naphthamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8g**)

MS (ESI) 366 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 2H), 8.22–8.10 (m, 2H), 7.89–7.72 (m, 6H), 4.54 (s, 2H), 3.79 (t, J = 8.0 Hz, 2H), 3.13 (t, J = 7.6 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 168.4, 148.2, 139.3, 138.6, 138.0, 137.4, 135.8, 135.2, 134.5, 132.1, 129.8, 127.2, 126.3, 124.6, 121.1, 51.3, 44.8, 37.8, 22.7. Anal. calcd for $C_{20}H_{19}N_3O_2S$: C, 65.73; H, 5.24; N, 11.50% Found C, 65.82; H, 5.32; N, 11.62%.

4.1.24. 2-(Cyclopropanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8h**)

MS (ESI) 280 [M + H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.11 (s, 1H), 8.22 (s, 2H), 4.45 (s, 2H), 3.81 (t, J = 8.4 Hz, 2H), 3.18 (t, J = 8.0 Hz, 2H), 2.45 (s, 3H), 2.14–2.10 (m, 1H), 1.54–1.12 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.4, 171.9, 144.4, 134.3, 132.3, 120.9, 48.4, 43.6, 38.4, 22.5, 19.3, 18.7(2C). Anal calcd for $C_{13}H_{17}N_3O_2S$: C, 55.89; H, 6.13; N, 15.04% Found C, 55.92; H, 6.24; N, 15.11%.

4.1.25. 2-(Cyclopentanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8i**)

MS (ESI) 308 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 2H), 8.67 (s, 1H), 4.64 (s, 2H), 3.86 (t, J = 8.0 Hz, 2H), 3.12 (t, J = 7.6 Hz, 2H), 2.38 (s, 3H), 2.11–2.0 (m, 1H), 1.69–1.34 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.9, 169.8, 149.6, 132.2, 130.7, 122.5, 52.9, 49.4, 46.2, 39.9, 31.6(2C), 26.3(2C), 21.4. Anal. calcd for $C_{15}H_{21}N_3O_2S$: C, 58.61; H, 6.89; N, 13.67% Found C, 58.72; H, 7.02; N, 13.71%.

4.1.26. 2-(Cyclohexanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide (**8h**)

MS (ESI) 322 [M + H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H), 7.74 (s, 2H), 4.58 (s, 2H), 3.87 (t, J = 8.0 Hz, 2H), 3.19 (t, J = 8.0 Hz, 2H), 2.61 (s, 3H), 2.16–2.09 (m, 1H), 1.89–1.44

(m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 169.9, 132.1, 129.8, 128.4, 119.7, 61.0, 56.5, 47.8, 43.5, 31.6(2C), 28.4, 23.3(2C), 22.4. Anal. calcd for $C_{16}H_{23}N_3O_2S$: C, 59.78; H, 7.21; N, 13.07% Found C, 59.82; H, 7.29; N, 13.11%.

*4.1.27. Preparation of 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carbonitrile (**10**)*

To the stirred solution of cyclooctanone (**9**) (2.5 mL, 19.0 mmol), malanonitrile (1.38 g, 20.9 mmol) and elemental sulphur (670 mg, 20.9 mmol) in DMF (10 mL) was added Et_3N (4.0 mL, 28.5 mmol) and stirred at room temperature for 9 h. The reaction mixture was diluted with EtOAc (60 mL), the organic layer was washed with 1 M HCl (2 × 20 mL) and brine (2 × 30 mL). The separated organic layer was dried over anhyd. Na_2SO_4 and concentrated under *vacuo* to get crude compound. The crude compound was triturated with CH_2Cl_2, diethyl ether and hexanes to get compound **10** (2.70 g, 66%) as an off-white solid. ESI-MS showed 207 $[M + H]^+$. Anal. calcd for $C_{11}H_{14}N_2S$: C, 64.04; H, 6.84; N, 13.58% Found C, 64.12; H, 6.89; N, 13.64%.

*4.1.28. Preparation of 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (**11**)*

Compound **10** (2.70 g) was suspended in conc. H_2SO_4 (10 mL) and stirred for 12 h. The reaction mixture was quenched with ice, neutralised with solid Na_2CO_3, and the product was extracted with EtOAc, and the combined organic layer was concentrated under reduced pressure to get compound **11** (1.6 g, 54%) as an off-white solid. MS (ESI) 225 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.55 (bs, 2H), 6.10 (bs, 2H), 2.77–2.62 (m, 4H), 1.70–1.35 (m, 8H); Anal. calcd for $C_{11}H_{16}N_2OS$: C, 58.90; H, 7.19; N, 12.49% Found C, 58.94; H, 7.29; N, 12.60%.

*4.1.29. General procedure for the preparation of **12a–j***

To the stirred solution of R–COOH (1.20 equiv) in CH_2Cl_2 was added EDCI (1.30 equiv), HOBt (1.30 equiv) and Et_3N (2.50 equiv) while stirred the mixture for few minutes, then added compound **11** (1.0 equiv). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated and triturated with H_2O to get solid compound. The solids were given cold Ethanol, diethyl ether and hexane washings to get pure products.

*4.1.30. 2-Acetamido-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12a**)*

MS (ESI) 267 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 2H), 7.47 (s, 1H), 2.73–2.50 (m, 4H), 2.23 (s, 3H), 1.60–1.35 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 166.9, 149.4, 135.3, 133.3, 123.4, 29.7, 27.3, 25.4, 24.8(2C), 23.6, 22.5. Anal. calcd for $C_{13}H_{18}N_2O_2S$: C, 58.62; H, 6.81; N, 10.52% Found C, 58.69; H, 6.84; N, 10.61%.

*4.1.31. 2-Benzamido-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12b**)*

MS (ESI) 329 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 2H), 7.81–7.62 (m, 6H), 2.64–2.36 (m, 4H), 1.71–1.41 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 169.9, 146.2, 139.7, 136.3(2C), 134.4(2C), 129.3, 127.6, 126.7, 120.8, 27.8, 26.9, 26.3, 24.5(2C), 22.9. Anal. calcd for $C_{18}H_{20}N_2O_2S$: C, 65.83; H, 6.14; N, 8.53% Found C, 65.99; H, 6.24; N, 8.71%.

*4.1.32. 2-(2-Methoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta [b]thiophene-3-carboxamide (**12c**)*

MS (ESI) 359 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.25 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.81 (s, 1H), 7.72–7.63 (m, 4H), 3.81 (s, 3H), 2.81–2.49 (m, 4H), 1.62–1.39 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.9, 152.2, 144.7, 138.3, 136.5, 134.0, 133.2, 130.5, 128.7, 126.9, 123.6, 54.9, 26.8, 25.4, 24.2(2C), 22.9, 22.3. Anal. calcd for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.82% Found C, 63.72; H, 6.21; N, 8.01%.

*4.1.33. 2-(3-Nitrobenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (**12d**)*

MS (ESI) 374 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 2H), 9.00 (s, 1H), 8.39 (s, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.83–7.69 (m, 2H), 2.81–2.39 (m, 4H), 1.59–1.19 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 183.8, 175.6, 148.6, 143.5, 138.8, 133.6, 132.6, 130.5, 128.6, 126.7, 125.3, 121.4, 27.9, 27.2, 25.6(2C), 24.3, 22.5. Anal. calcd for $C_{18}H_{19}N_3O_4S$: C, 57.89; H, 5.13; N, 11.25% Found C, 57.92; H, 5.22; N, 11.34%.

*4.1.34. 2-(4-Methylbenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (**12e**)*

MS (ESI) 343 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 2H), 8.01 (d, J = 8.0 Hz, 2H), 7.84 (s, 1H), 7.72 (d, J = 8.0 Hz, 2H), 2.52 (s, 3H), 2.70–2.29 (m, 4H), 1.87–1.36 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 173.9, 147.2, 138.4, 137.7(2C), 136.4, 135.3(2C), 127.3, 125.4, 121.3, 26.8, 25.2, 24.9, 24.3(2C), 22.5, 21.4. Anal. calcd for $C_{19}H_{22}N_2O_2S$: C, 66.64; H, 6.48; N, 8.18% Found C, 66.72; H, 6.59; N, 8.31%.

*4.1.35. 2-(4-Phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (**12f**)*

MS (ESI) 421 $[M + H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.40 (s, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.48 (s, 2H), 7.44 (t, J = 7.8 Hz, 2H), 7.24 (t, J = 7.5 Hz, 1H), 7.13 (d, J = 8.4 Hz, 4H), 2.84–2.75 (m, 4H), 1.56–1.42 (m, 6H), 1.19–1.17 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 162.0, 160.4, 155.2, 141.4, 131.9, 130.3(2C), 130.2(2C), 129.4, 127.1, 124.6, 119.8(2C), 117.9(2C), 117.7, 32.2, 29.8, 26.2, 25.6, 25.0, 24.3. Anal. calcd for $C_{24}H_{24}N_2O_3S$: C, 68.55; H, 5.75; N, 6.66% Found C, 68.62; H, 5.81; N, 6.72%.

*4.1.36. 2-(1-Naphthamido)-4,5,6,7,8,9-hexahydrocycloocta[b] thiophene-3-carboxamide (**12g**)*

MS (ESI) 379 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34–8.19 (m, 4H), 7.92–7.81 (m, 4H), 7.72–7.63 (m, 2H), 2.79–2.38 (m, 4H), 1.53–1.20 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.2, 166.3, 146.3, 139.6, 138.2, 137.6, 137.2, 136.0, 135.3, 133.9, 133.2, 130.5, 129.4, 126.3, 122.6, 118.7, 28.9, 27.6, 26.2(2C), 24.2, 19.6. Anal calcd for $C_{22}H_{22}N_2O_2S$: C, 69.81; H, 5.86; N, 7.40% Found C, 69.92; H, 5.94; N, 7.62%.

*4.1.37. 2-(Cyclopropanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12h**)*

MS (ESI) 293 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 2H), 6.77 (s, 1H), 2.79–2.46 (m, 4H), 2.20–2.07 (m, 1H), 1.69–1.12 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.4, 171.9, 156.4, 136.3, 133.3, 121.1, 28.7, 27.2, 26.4(2C), 24.8, 22.3, 21.4, 18.7(2C). Anal. calcd for $C_{15}H_{20}N_2O_2S$: C, 61.62; H, 6.89; N, 9.58% Found C, 61.69; H, 6.94; N, 9.71%.

*4.1.38. 2-(Cyclopentanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12i**)*

MS (ESI) 321 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 2H), 7.27 (s, 1H), 2.73–2.49 (m, 4H), 2.01–1.93 (m, 1H), 1.80–1.33 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 166.5, 152.1, 136.6, 128.7, 120.4, 44.8, 33.3(2C), 29.5, 27.2(2C), 25.2, 24.6(2C), 23.4, 22.5. Anal. calcd for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74% Found C, 63.82; H, 7.72; N, 8.82%.

*4.1.39. 2-(Cyclohexanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**12j**)*

MS (ESI) 335 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 7.27 (s, 2H), 6.73 (s, 1H), 2.74–2.41 (m, 4H), 2.18–2.10 (m, 1H), 1.92–1.24 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 171.9, 149.2, 133.3, 129.6, 119.6, 48.9, 29.8, 27.4, 25.2, 24.9(2C), 22.9(2C), 22.0(2C), 21.6, 20.5. Anal. calcd for $C_{18}H_{26}N_2O_2S$: C, 64.64; H, 7.84; N, 8.38% Found C, 64.72; H, 7.99; N, 8.52%.

4.2. Biological evaluation

4.2.1. In vitro MTB screening

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middle-brook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μg/mL with or without efflux pump inhibitor. Inoculum of *M. tuberculosis* H37Rv ATCC 27294 was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of $\sim 10^7$ cfu/mL. Five microlitres of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in μg/mL) were determined after 28 days. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

4.2.2. In vitro cytotoxicity screening

Some compounds were further examined for toxicity in a RAW 264.7 cell line at the concentration of 50 μM. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.028.

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