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Original article

Thiophene containing trisubstituted methanes [TRSMs] as identified lead against *Mycobacterium tuberculosis*

Priyanka Singh^a, Sudipta Kumar Manna^a, Amit Kumar Jana^a, Tiash Saha^a, Pankaj Mishra^a, Saurav Bera^a, Maloy Kumar Parai^a, Srinivas Lavanya Kumar M^a, Sankalan Mondal^a, Priyanka Trivedi^b, Vinita Chaturvedi^b, Shyam Singh^b, Sudhir Sinha^{b,*}, Gautam Panda^{a,*}

^a Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, B.S. 10/1, Jankipuram Extension, Sitapur Road, Lucknow-226031, UP, India

^b Biochemistry Division, CSIR-Central Drug Research Institute, B.S. 10/1, Jankipuram Extension, Sitapur Road, Lucknow-226031, UP, India

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ABSTRACT

Triarylmethanes (TRAMs) and thiophene containing trisubstituted methanes (TRSMs) have been reported by us, having potential against *Mycobacterium tuberculosis* and *Mycobacterium fortuitum* strains, respectively. Further, extension through synthesis and biological evaluation of novel TRSMs resulted into an identified lead 36 (S006-830) [(diisopropyl-(2-{4-[(4-methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy}-ethyl)-amine)] with MIC: 1.33 mg/L, non-toxic against Vero C-1008 cell line with selectivity index >10, *ex vivo* efficacy equivalent to first line TB drugs-isoniazid (INH), rifampicin (RFM) and pyrazinamide (PZA) in the mouse and human macrophages, and lung CFU count of 2.2×10^7 (approximately 15 fold lesser than untreated mice, 31×10^7) with efficacies comparable to ethambutol (EMB) (1.27×10^7) and PZA (1.9×10^7). Further, S006-830 also showed potent bactericidal activity against multi-drug resistant and single-drug resistant clinical isolates of *M. tuberculosis*.

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1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tb*) is not merely a disease rather it is an epidemic which is prevalent in modern era of mankind and is considered as the world's deadliest communicable disease by World Health Organization (WHO) [1,2]. Worldwide, according to WHO report 2014, about 9 million people were infected with TB in the year 2013 and an approximate of 1.4 million died in a single year of 2011 [1,2] and the scenario is being worsen by multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, often emerged from the patient's poor compliance to the standard drugs therapy regimen. Hence, discovery of newer inhibitors of *M. tb* is a global and urgent need. In our on-going campaign against TB, we previously reported triarylmethanes (TRAMs) [3–6] and thiophene containing trisubstituted methanes (TRSMs) [7] as novel cores having anti-tubercular activity (Fig. 1). In 2012, we reported the potential of few novel TRSMs

against acute and persistent infection of *Mycobacterium fortuitum* (responsible for the opportunist non-tubercular infection in humans) in a murine infection model and identified two promising lead compounds (Fig. 1) [8]. Further, optimization through synthetic and medicinal chemistry approach led us to present the current work with potential applicability of novel TRSMs as antimycobacterial compounds.

2. Results and discussion

2.1. Chemistry

The reaction scheme followed the similar protocol as outlined in our previous report [7,8]. We emphasized on the design, synthesis and anti-tubercular activity of thiophene containing TRSMs with alkylaminoethyl chains A.

In this work, we have emphasized on the design, synthesis and anti-tubercular activity of thiophene containing TRSMs with alkylaminoethyl chains A (Fig. 2). Initially, the Grignard reagent aryl magnesium bromide **1a–j** were reacted with various commercially available thiophene-2-aldehyde **2** to synthesize carbinol **3**. Then

* Corresponding authors.

E-mail addresses: sinha.sudhir@gmail.com, sudhir_sinha@cdri.res.in (S. Sinha), gautam.panda@gmail.com, gautam_panda@cdri.res.in (G. Panda).

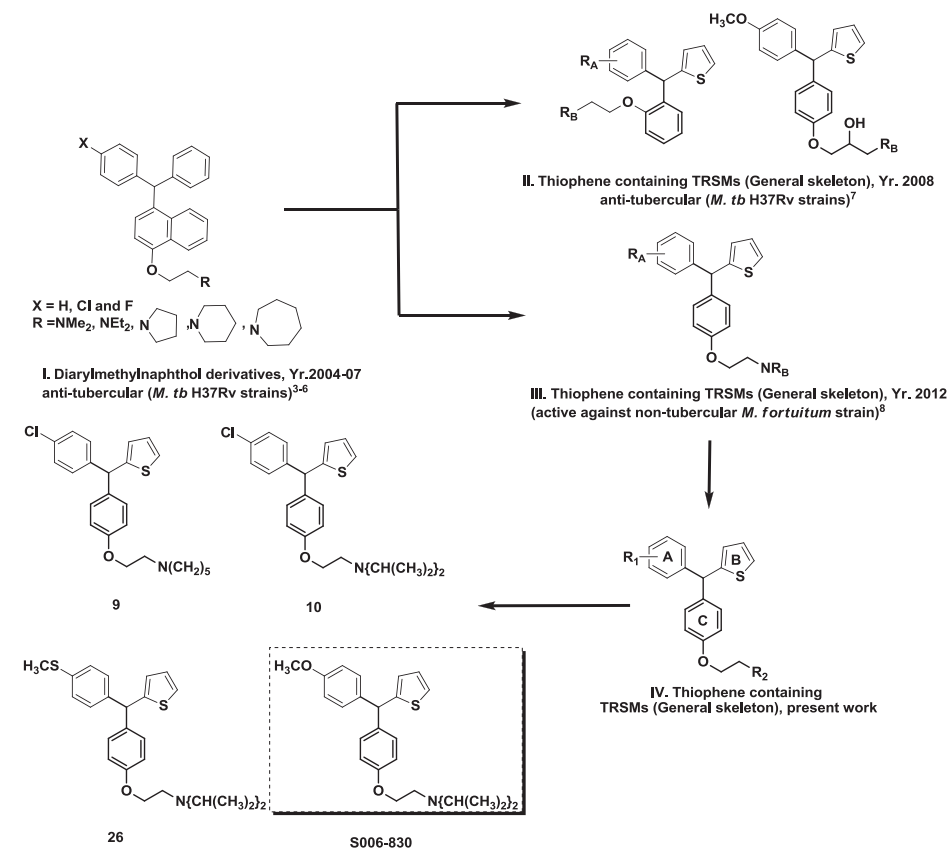


Fig. 1. Evolution of triarylmethanes into thiophene containing TRSMs identified potential lead **36** (S006-830) against *M. tuberculosis*.

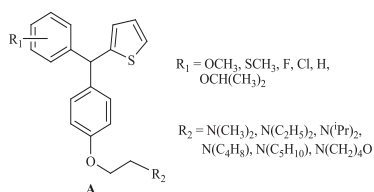


Fig. 2. General skeleton of designed molecules.

Friedel–Crafts alkylation of **3** with phenol in the presence of conc. H_2SO_4 in dry benzene furnished **4** in satisfactory yields. Alkylated product **4** was refluxed with various dialkylaminoethyl chloride hydrochloride chains in presence of the K_2CO_3 in acetone to obtain compounds **5–36** in good yields, (Schemes 1 and 2).

Later to analyze the activity of dithiophene compounds several compounds with 2-thiophene substitution were synthesized by taking commercially available 2-thiophene carboxaldehyde and 2-substituted thiophene bromide with magnesium in THF to prepare respective carbinol followed by refluxing it with various dialkylaminoethyl chloride hydrochloride chains with K_2CO_3 as base in acetone to obtain the final compounds **40–43**. Similarly, 3-thiophene substituted compounds were prepared from 3-thiophene carboxaldehyde and 3-thiophene bromide by the similar way as above to obtain final compounds **44–49**.

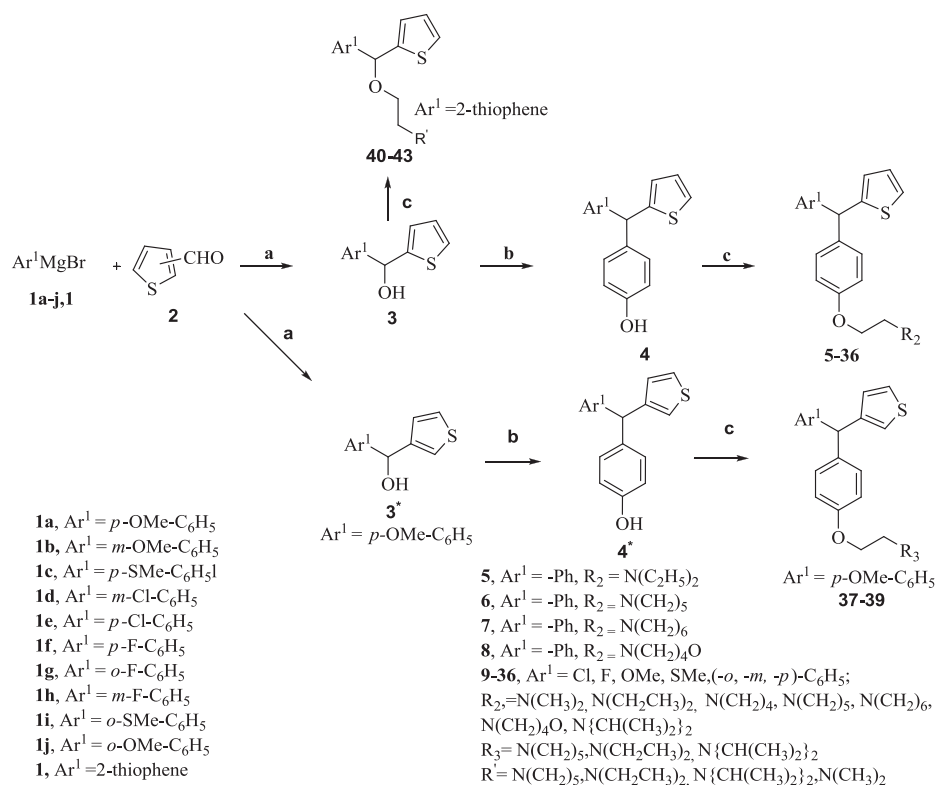
Initially, synthesis of compounds without any substitution on benzene ring were carried out by nucleophilic addition of phenylmagnesium bromide on 2-thiophene carbaldehyde to provide phenyl thiophen-2-yl carbinol. After this similar protocol was followed as above to synthesize compounds **5–8** (Table 1). To explore

the effect of substitution on benzene ring, various substitutions were incorporated in the basic framework like halogen, methoxy and thiomethoxy substituted arylbromide to synthesize the grignard reagent and then compounds **9–36** (Table 1) were synthesized by procedure stated above. Similarly, to understand the effect of substitution at 3 position of thiophene, 3-thiophene carboxaldehyde was also incorporated to provide *p*-methoxy substituted phenyl thiophen-3-yl carbinol which were used subsequently to synthesize compounds **37–39** by above mentioned procedure. Later dithiophene substituted compounds **40–49** were also synthesized by the same procedure.

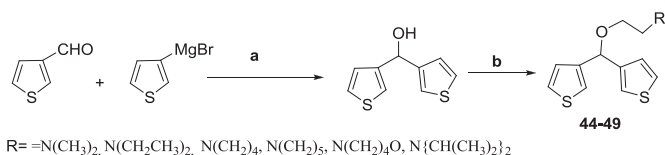
2.2. Biological evaluation

2.2.1. In vitro screening of compounds using virulent strain of *M. tuberculosis* to select 'hits'

This assay measures the capability of the test compound to kill or inhibit the multiplication of pathogenic *M. tuberculosis* H37Rv and is considered as 'primary screening method'. Compounds showing $\text{MIC} \leq 6.25 \text{ mg/L}$ were considered for further evaluation [11]. From our previous report [7], it was evident that the amino alkyl chain (Fig. 1II) exhibited the maximum impact on the anti-tubercular activity ($\text{MIC} < 12.5 \text{ mg/L}$) and *para*-substituted chain (R_B) over the ring C remains critical for the activity as the *ortho*-substituted derivatives demonstrated very poor activity (MIC : *para*-substituted = $< 12.5 \text{ mg/L}$; *ortho*-substituted = $> 12.5 \text{ mg/L}$). Surprisingly, both electron-donating group (EDG) and electron withdrawing group (EWG) substituted over the *para/meta*-position (R_B) of ring A exhibited good anti-tubercular activity. In line with the observations from the previous report, we decided to continue our



Scheme 1. Synthesis of Trisubstituted Methanes (TRSMs). Reagents and conditions (a) Dry THF, 0 °C-rt, 30 min; (b) Phenol, dry benzene, catalytic H₂SO₄, reflux, 1 h; (c) Alkylaminoethyl hydrochloride, dry acetone, anhyd. K₂CO₃, reflux, 6–7 h.



Scheme 2. Synthesis of dithiophene substituted compounds. Reagents and conditions (a) Dry THF, 0 °C-rt, 30 min; 1 h; (b) Alkylaminoethyl hydrochloride, dry acetone, anhyd. K₂CO₃, reflux, 6–7 h.

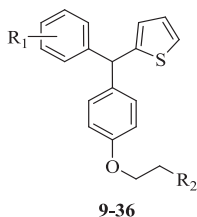
work by changing the best possible substitutions on the amino alkyl chain R₂ on ring C and observed their anti-tubercular effects. All thirty one compounds were found to be active against *M. tuberculosis* H37Rv with MIC ranging from 0.78 to 25.0 mg/L (Table 1). Nine compounds [MIC: **9** = 0.78 mg/L, **15** = 1.34 mg/L, **12** = 1.25 mg/L, **20** = 1.24 mg/L, **32** = 1.28 mg/L, **14** = 1.29 mg/L, **10** = 1.56 mg/L, **26** = 1.57 mg/L, and **S006-830** = 1.33 mg/L] demonstrated notable inhibitory capacity against *M. tuberculosis* H37Rv strain. While ring A substituted with *para*-chloro and N(CH₂)₅ as amino alkyl chain **9** showed the most potent inhibition with MIC of 0.78 mg/L, *meta*-substituted counterpart **14** showed somewhat diminished activity MIC = 1.29 mg/L. While *ortho*-fluoro substituted compound **20** with the similar alkyl chain [N(CH₂)₅] exhibited diminished inhibition [MIC = 1.24 mg/L] as compared to the chloro substituted derivative **9**, the *meta*-fluoro substituted compound **25** showed highly diminished activity [MIC = 19.8 mg/L]. Towards the observation with EDGs at ring A, somewhat comparable inhibition has been observed with compound **32** carrying *ortho*-OCH₃ with [N(CH₂)₅] side chain with MIC = 1.28 mg/L. Additionally, compound **36** (**S006-830**) with *para*-OCH₃ and N{CH(CH₃)₂}₂ demonstrated potent inhibition of *M. tuberculosis* H37Rv strain with MIC = 1.33 mg/L as compared to their *ortho*-

OCH₃ substituted counterpart **34** with very low activity (25.0 mg/L). Surprisingly, all 3-substituted compounds (**37–39**, **44–49**) exhibited low activity (25.0 mg/L). Moreover, 2-substituted dithiophene containing compounds also exhibited moderate activity (12.5 mg/L).

2.2.2. Cytotoxicity assessment through in vitro toxicity assay and ex vivo activity in the mouse and human macrophages model of tuberculosis

Initially, *in vitro* cytotoxicity of active compounds (having MIC ≤ 6.25 mg/L) was measured using Vero cells (Vero C-1008 cell line) and if found non-toxic, were then taken further for their testing by the *ex vivo* assay using mouse bone marrow derived (MBMD) or human monocyte derived macrophage models of tuberculosis. The macrophage model mimics growth environment of natural infection and demonstrates the ability of the candidate molecule to penetrate host cell membrane, phagocytic vacuole and bacilli residing within the vacuole, and hence gives the advantage to reach the desired drug targets. In addition, it also serves as a model for hypoxia induced latent TB infection, since tissue concentration of oxygen is considerably lower than that in the ambient air. Many latency marker proteins of the pathogen are known to get expressed during its intracellular regime. From Vero C-1008 cell line studies, four compounds viz. **9**, **10**, **26** and **36** (**S006-830**) could be evidenced to be non-toxic demonstrating SI of greater than 10 and taken further for their assessment in the MBMD macrophage model. All four compounds (at 4× MIC) killed greater than 90% of the intracellular *M. tuberculosis*, as judged by counting the viable bacilli (colony forming units or CFUs) in lysates of the drug-treated or untreated infected macrophages. As assessed from the statistical data, the *ex vivo* efficacy of **9**, **10**, **26** and **S006-830** were comparable to that of the first line TB drugs-INH, RFM and PZA (Fig. 2). Two

Table 1
Biological Activity of synthesized TRSMs (5–36).



Compd. no.	R ₁	R ₂	Mol. Weight	CLogP	MIC (mg/L)	Cytotoxic activity, IC ₅₀ (mg/L)	SI ^a
5	H	N(C ₂ H ₅) ₂	365.53	6.36	12.47	ND	ND ^b
6	H	N(CH ₂) ₅	377.54	6.44	6.23	ND	ND
7	H	N(CH ₂) ₆	391.57	6.97	3.10	13	<10
8	H	N(CH ₂) ₄ O	379.52	5.11	24.98	ND	ND
9	p-Cl	N(CH₂)₅	411.99	7.15	0.78	100	>10
10	p-Cl	N{CH(CH₃)₂}₂	428.03	7.69	1.56	100	>10
11	m-Cl	N(CH ₃) ₂	371.92	6.02	4.65	10.8	<10
12	m-Cl	N(CH ₂ CH ₃) ₂	399.98	7.08	1.25	10.1	<10
13	m-Cl	N(CH ₂) ₄	397.96	6.62	2.49	8.6	<10
14	m-Cl	N(CH ₂) ₅	411.99	7.15	1.29	8.7	<10
15	m-Cl	N{CH(CH ₃) ₂ } ₂	428.03	7.69	1.34	22.0	>10
16	m-Cl	N(CH ₂) ₄ O	413.96	5.83	5.18	23.4	<10
17	o-F	N(CH ₃) ₂	355.57	5.45	17.8	ND	ND
18	o-F	N(CH ₂ CH ₃) ₂	383.52	6.51	4.80	ND	ND
19	o-F	N(CH ₂) ₄	381.51	6.05	19.1	ND	ND
20	o-F	N(CH ₂) ₅	395.53	6.58	1.24	3.9	<10
21	o-F	N{CH(CH ₃) ₂ } ₂	411.58	7.12	10.3	ND	ND
22	o-F	N(CH ₂) ₄ O	397.51	5.26	19.9	ND	ND
23	m-F	N(CH ₃) ₂	355.47	5.45	17.8	ND	ND
24	m-F	N(CH ₂ CH ₃) ₂	383.52	6.51	2.40	5.8	<10
25	m-F	N(CH ₂) ₅	393.52	6.58	19.8	ND	ND
26	p-SCH₃	N{CH(CH₃)₂}₂	439.68	7.18	1.57	100	>10
27	o-SCH ₃	N(CH ₃) ₂	383.57	5.16	12.5	ND	ND
28	o-SCH ₃	N(CH ₂ CH ₃) ₂	411.62	6.22	25.0	ND	ND
29	o-OCH ₃	N(CH ₃) ₂	367.50	4.82	9.19	ND	ND
30	o-OCH ₃	N(CH ₂ CH ₃) ₂	395.56	5.88	25.0	ND	ND
31	o-OCH ₃	N(CH ₂) ₄	393.54	5.43	25.0	ND	ND
32	o-OCH ₃	N(CH ₂) ₅	407.57	5.96	1.28	9.2	<10
33	o-OCH ₃	N(CH ₂) ₆	421.59	6.49	2.64	12.7	<10
34	o-OCH ₃	N{CH(CH ₃) ₂ } ₂	423.61	6.50	25.0	ND	ND
35	o-OCH ₃	N(CH ₂) ₄ O	409.54	4.63	10.24	ND	ND
36 (S006-830)	p-OCH₃	N{CH(CH₃)₂}₂	423.61	6.90	1.33	100	>10

Compounds showing significant activity have been highlighted in bold.

^a SI = Selectivity Index.

^b ND = Not Determined.

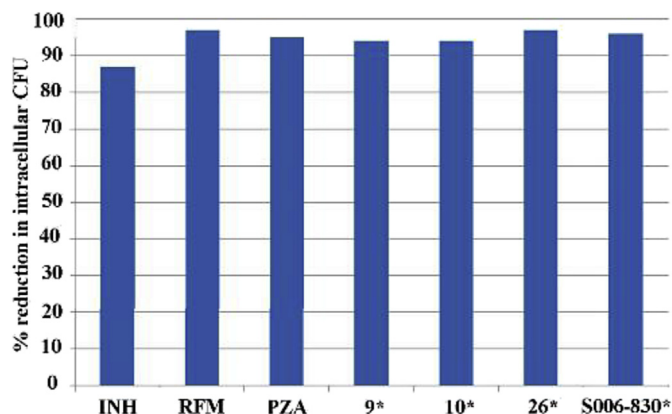


Fig. 3. Percent reduction in intracellular CFU of *M. tuberculosis* H37R_v in mouse bone marrow derived macrophages by **9**, **10**, **26** and **S006-830**. Isoniazid (INH), rifampicin (RFM) and pyrazinamide (PZA) were used as standard drugs.

compounds **26** and **S006-830** showed *ex vivo* activity comparable to that of the standard drug INH (INH) in human macrophages experimentally infected with *M. tuberculosis* H37R_v (Figs. 3 and 4).

2.2.3. In vivo assay against *M. tuberculosis* H37R_v infected mouse model

When infected with a high number of *M. tuberculosis* CFUs by the *i.v.* route, the mouse harbours a bacillary population that is similar in number and in metabolic state to that present in the lung cavity of human TB. Thus, the mouse model is able to reproduce bacteriologic conditions close to those present in the natural human disease and provide information on compound activity that can be extrapolated to humans. The most frequently used is the outbred 'Swiss' mouse. The differences in the immune status among outbred Swiss mice also exist among humans, and a drug or regimen that is active against the mycobacteria in Swiss mouse is likely to be active in humans. The strain of choice for tubercle bacilli in the mouse model is the H37R_v strain of *M. tuberculosis*, which maintains virulence through regular passages in the mouse. Efficacy of a compound is monitored by survival/mortality rate, evaluation of body weight, extent of gross lesions, and the enumeration of the CFUs in organs. Usually the CFU counts are performed in the spleen or lungs or both. Since spleen and lung display similar overall results, it is unnecessary to enumerate the CFU in both organs. In general, effective dosages of compounds (in mg/kg body weight) in the laboratory animals are larger than those in humans.

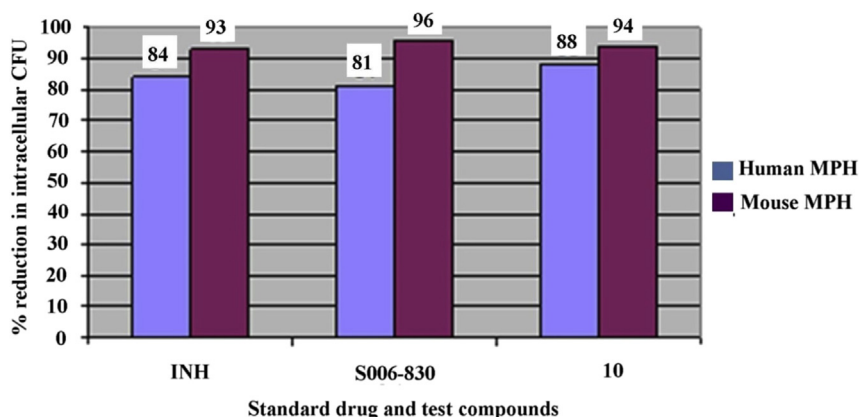


Fig. 4. Compounds **10** and **S006-830** were also able to kill intracellular *M. tuberculosis* H37Rv in human monocyte derived macrophages in a manner similar to the standard drug INH (corresponding mouse macrophage data are shown for comparison).

One possible reason for this is that drug metabolism correlates better with body surface area than body weight. The ratio of body surface area to body weight decreases sharply with increasing body weight. Thus, for most drugs the equipotent dosage is 12 times larger in the mouse than in humans. This assay was performed on outbred Swiss mice (obtained from CDRI-LAD) infected with *M. tuberculosis* H37Rv strain. All four *in vitro* and *ex vivo* active compounds **9**, **10**, **26** and **S006-830** were evaluated for their *in vivo* efficacy. Swiss mice were infected intravenously with *M. tuberculosis* H37Rv (10^7 CFU/mouse). The experimental groups received daily oral doses of test compounds (100 mg/kg body weight, for 28 days) dissolved in corn oil. Drug-treated control groups received INH (25 mg/kg body weight) or EMB (10 mg/kg body weight) or PZA (150 mg/kg body weight) which were dissolved in water. Untreated control groups received only the vehicles (corn oil or water). Mice were observed up to day-40 for determination of percentage survivors and mean survival time (MST). Maximum survivors (42% in treated and 0% in untreated) were seen in the **S006-830** treated groups, followed by **10** and **26** treated groups. No survivors were left in **9** treated mice. The highest enhancement in MST (7 days, $p < 0.05$) was also seen with **S006-830**. All dead animals were autopsied and their lungs were found full of typical TB lesions. 'Impression smears' of the cut surface of lungs were microscopically examined after staining with Ziehl-Neelsen stain and found full of Acid Fast Bacilli (AFB) confirming that the deaths were due to *M. tuberculosis* infection (Fig. 5).

Fig. 6 shows results of treatment with **S006-830** and the anti-TB drugs-ethambutol (EMB) and pyrazinamide (PZA) in terms of viable bacilli (CFU) in the lungs of mice. **S006-830** treated mice showed CFU count of 2.2×10^7 (mean of 3 animals) which was approximately 15 fold lesser than that of the untreated mice (31×10^7), and comparable with the efficacies of EMB (1.27×10^7) and PZA (1.9×10^7). Based on the result of this and prior studies, compound **S006-830** was chosen as the lead molecule and taken forward for the further assessment.

2.2.4. Minimum bactericidal concentration (MBC) determination of the lead compound **36** (S006-830) through *in vitro* and *ex vivo* studies and potency assessment on multi-drug and single-drug resistant *M. tuberculosis*

These experiments were aimed at determining the capacity of test compounds to kill the bacilli, in addition to inhibiting their multiplication. The assays were thus designed to record killing of the seed culture (inoculum) and in principle, if a compound in *in vitro* study reduces the CFU of the inoculum; it can be considered as bactericidal and corresponding concentration as its MBC. If a

compound does not kill the inoculum but only prevents its multiplication (i.e., reduction in day-14 CFU), it can be considered as bacteriostatic (as per method described in the experimental section). For *ex vivo*, if a compound reduces the day-0 CFU (as per method described in the experimental section) of macrophages infected with *M. tuberculosis* it can be considered as bactericidal. If it reduces only the day-5 CFU of such macrophages, it can be considered as bacteriostatic [15]. The lead compound **36** (**S006830**) produced 100% killing (zero CFU) *in vitro* and 75% killing (1200 CFU) *ex vivo* of the inoculum of *M. tuberculosis* H37Rv. The bactericidal potency was comparable with standard drug RFM (Fig. 7). S006-830 also showed potent bactericidal activity against multi-drug resistant (i.e., resistant to RFM and INH) and single-drug resistant (to RFM) clinical isolates of *M. tuberculosis* (Fig. 8).

Further, in order to develop S006-830 as identified lead, pharmacokinetics (PK) studies were conducted at Pharmacokinetics & Metabolism Division of our institute CSIR-CDRI using newly developed and validated methods on HPLC and LC-MS/MS for quantitative estimation in rat plasma and demonstrated good PK properties with fast intestinal absorption, peak plasma concentration one hour post oral dose, optimum elimination half-life of 9–13 h, plasma protein binding (PPB) of ~60%, mean residence time of around 18–20 h and favourable bioavailability in the range of 45–50%. As the plasma protein binding was not too high, it will lead to tissue redistribution as well as fast clearance of the unbound fraction of S006-830 from the body. Further, S006-830 was found stable in rat plasma under various operating conditions [9,10].

S006-830 is racemic and after separation through chiral column chromatography, we are separately evaluating the anti-TB activity of both enantiomers and results will be communicated in future.

3. Conclusion

While it was evident from our earlier report that TRSMs can have significant potential against *M. tuberculosis*, this series led us to identify a highly potent and viable lead S006-830. Collectively, from earlier reported compounds [7,8] and the current work, a preliminary structure activity relationship could be drawn and directed to the subsequent consideration: (a) presence of benzene ring with amino alkyl chains is important for potent anti-tubercular activity; (b) TRSMs without any substituent on benzene ring (**5–8**) showed moderate anti-tubercular activity; (c) Among TRSMs with electron withdrawing group, *para*-substituted chloro (**9**, **10**) containing compounds showed promising *in vitro* activity than their *meta* analogues **11–16**. On the other hand, TRSMs with electron donating group i.e., methoxy and thiomethoxy at *para* position of

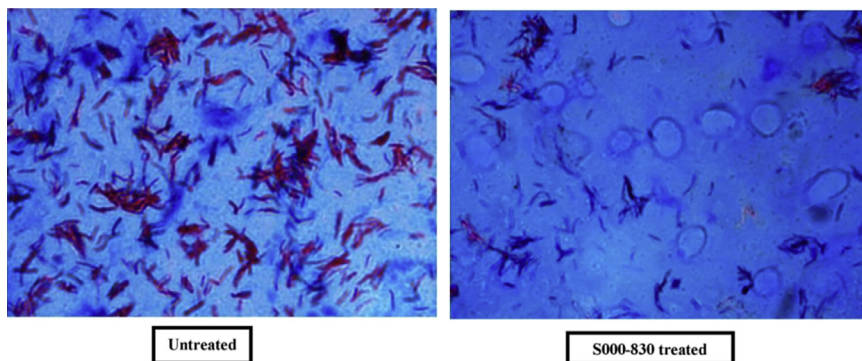


Fig. 5. Ziehl–Neelsen staining of lung tissue smears of mice untreated and treated with **S006-830**.

benzene ring **26**, **36** showed good anti-tubercular activities than *ortho* isomers **27–35**. Anti-tubercular activity of four TRSMs, which showed MIC of <6.25 mg/L against *M. tuberculosis* H37R_v and lacked cytotoxicity towards mammalian cells showed >90% inhibition in multiplication of *M. tuberculosis* within mouse and human macrophages (*ex vivo*), which was comparable to *ex vivo* efficacy of standard TB drugs-isoniazid, rifampicin and pyrazinamide. The lead molecule **S006-830** brought approximately 15 fold reduction in the viable bacilli (CFUs) in lungs of the infected mice, which was comparable to the *in vivo* efficacy of ethambutol and pyrazinamide. **S006-830** with noticeable microbiological profile and encouraging PK properties can be considered as potential lead against *M. tuberculosis*.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

All reagents and solvents were purchased from commercial sources and used without further purification. Organic solvents were dried by standard methods. Analytical TLC was performed using 2.5 × 5 cm aluminium plates coated with a 0.25 mm thickness of silica gel (60F-254), visualization was accomplished with iodine and under UV lamp. The spots on TLC were also visualized by warming ceric sulphate (2% CeSO₄ in 2 N H₂SO₄) sprayed plates in hot plate or in oven at about 100 °C. Column chromatography was performed using silica gel (60–120 and 100–200 mesh). ¹H NMR spectra were recorded on 200 and 300 MHz spectrometer in CDCl₃ (all signals are reported in ppm with the internal chloroform signal at 7.26 ppm as standard) at 25 °C. ¹³C NMR spectra were recorded on 50 and 75 MHz spectrometer in CDCl₃ (all signals are reported in

ppm with the internal chloroform signal at 77.00 ppm as standard) at 25 °C. In a few cases tetramethylsilane (TMS) at 0.00 ppm was used as the reference standard. Coupling constants (*J* values) are given in hertz (Hz). ¹H NMR splitting patterns are designated as singlet (s), doublet (d), double doublet (dd), triplet (t), quartet (q) or multiplet (m). IR spectra were recorded using a FTIR spectrophotometer in cm^{−1}.

4.1.1.1. General procedure for the preparation of heteroarylcarbinols (3) through Grignard reaction. To a suspension of Mg (1.50 g, 61.7 mmol) in dry THF (40 mL) was added drop wise a solution of 4-bromoanisole (6.52 mL, 52.1 mmol) in dry THF (40 mL). After stirring the mixture for 30 min a solution of thiophenylcarbaldehyde (4.21 mL, 45 mmol) in dry THF (30 mL) was added drop wise and the resulting solution was allowed to stir for further 30 min. After quenching by adding a saturated solution of NH₄Cl (20 mL), the reaction mixture was extracted with ethyl acetate (100 mL), washed with water (100 mL), brine (2 × 50 mL) and then dried over Na₂SO₄. The organic layer was dried under reduced pressure. The crude product was purified by column chromatography.

4.1.1.2. General procedure for the preparation of thiophene containing trisubstituted methanes through Friedel Crafts alkylation reaction. Phenol, benzene, 1–2 drops of sulphuric acid along with the carbinol **3** was refluxed at 60–75 °C for 2–3 h. Completion of reaction was confirmed by checking TLC by which compound **4** was obtained. After completion, reaction was quenched with water, reaction mixture was extracted with ethyl acetate (100 mL), washed with water and brine and dried over Na₂SO₄. Solvent was removed under reduced pressure and purified by column chromatography.

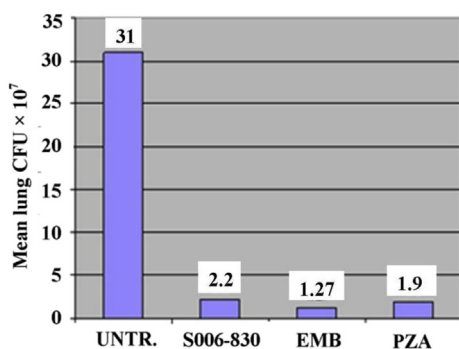


Fig. 6. CFU in the lungs of mice treated with the **S006-830** and standard drugs ethambutol (EMB) and pyrazinamide (PZA). UNTR stands for untreated.

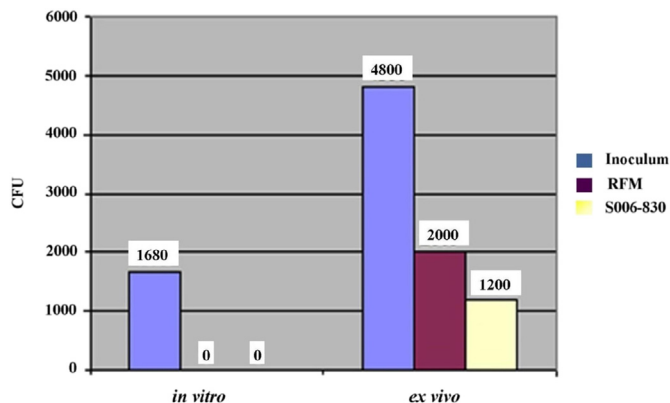


Fig. 7. *In vitro* and *ex vivo* bactericidal property of **36** (**S006-830**) in comparison with rifampicin (RFM).

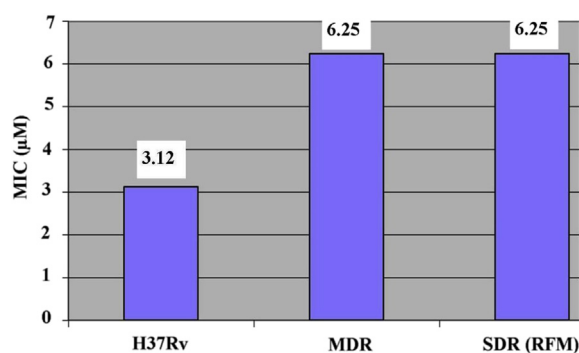


Fig. 8. Activity of **36** (**S006-830**) against sensitive (H37R_v), single- and multi-drug resistant *M. tuberculosis*.

4.1.1.3. Diethyl-{2-[4-(phenyl-thiophen-2-yl-methyl)-phenoxy]-ethyl}-amine (5**).** Compound **4** (0.2 g, 0.8 mmol) refluxed with K₂CO₃ (0.2 g, 1.9 mmol), 1-(2-chloroethyl)-diethyl amine hydrochloride (0.2 g, 0.7 mmol) in acetone furnished **5** (0.2 g, 72.8%) as brown viscous oil. ¹H NMR (300 MHz, CDCl₃): δ 7.18–7.15 (m, 8H), 6.91–6.78 (m, 3H), 6.64–6.63 (m, 1H), 5.59 (s, 1H), 4.02 (t, *J* = 6.22, 2H), 2.87 (t, *J* = 6.2, 2H), 2.65 (q, *J* = 7.1, 4H), 1.08 (t, *J* = 7.0, 6H); ¹³C NMR (CDCl₃, 50 MHz): δ 155.8, 147.9, 143.7, 137.7, 130.1, 129.8, 128.7 (2C), 128.4 (2C), 128.3 (2C), 126.7, 126.6, 126.3, 124.6, 114.5 (2C), 65.6, 51.2, 48.8, 47.7, 11.2; IR (Neat): 3019, 2928, 2855, 2362, 1612, 1509, 1217, 761 cm⁻¹; MS (ESI): *m/z* 366 (M+H)⁺; Anal. Calcd for C₂₃H₂₇NOS: C, 75.57; H, 7.45; N, 3.83. Found: C, 75.61; H, 7.52; N, 3.72.

4.1.1.4. 1-[2-[4-(Phenyl-thiophen-2-yl-methyl)-phenoxy]-ethyl]-piperidine (6**).** Compound **4** (200 mg, 0.75 mmol) refluxed with K₂CO₃ (259.42 mg, 1.87 mmol), 1-(2-chloroethyl)-piperidine hydrochloride (138.23 mg, 0.75 mmol) in acetone furnished **6** (203.8 mg, 71.9%) as brown viscous oil. ¹H NMR (300 MHz, CDCl₃): δ 7.28–7.15 (m, 6H), 7.09–7.06 (m, 2H), 6.90–6.87 (m, 1H), 6.81–6.78 (m, 2H), 6.64–6.63 (m, 1H), 5.58 (s, 1H), 4.07 (t, *J* = 6.0, 2H), 2.76 (t, *J* = 6.0, 2H), 2.52 (t, *J* = 4.9, 4H), 1.65–1.58 (m, 4H), 1.48–1.42 (m, 2H); ¹³C NMR (CDCl₃, 50 MHz): δ 157.2, 156.6, 148.5, 136.1, 132.8, 129.8 (2C), 129.6 (2C), 127.7, 126.3, 126.0, 123.9, 120.4, 114.1, 110.5, 96.1, 65.5, 57.8, 55.5, 54.9, 43.8, 25.6, 24.0; IR (Neat): 3020, 2936, 2362, 1610, 1508, 1217, 761 cm⁻¹; MS (ESI): *m/z* 378 (M+H)⁺; Anal. Calcd for C₂₄H₂₇NOS: C, 76.35; H, 7.21; N, 3.71. Found: C, 76.40; H, 7.15; N, 3.69.

4.1.1.5. 1-[2-[4-(Phenyl-thiophen-2-yl-methyl)-phenoxy]-ethyl]-azepane (7**).** Compound **4** (200 mg, 0.75 mmol) refluxed with K₂CO₃ (259.42 mg, 1.87 mmol), 1-(2-Chloro-ethyl)-azepane hydrochloride (148.58 mg, 0.75 mmol) in acetone furnished **7** (220 mg, 75%) as brown viscous oil. ¹H NMR (300 MHz, CDCl₃): δ 7.21–7.06 (m, 8H), 6.91–6.78 (m, 3H), 6.64–6.63 (m, 1H), 5.59 (s, 1H), 4.07 (t, *J* = 6.0, 2H), 2.97 (t, *J* = 6.1, 2H), 2.81 (t, *J* = 5.6, 4H), 1.69–1.61 (m, 8H); ¹³C NMR (CDCl₃, 50 MHz): δ 157.5, 148.4, 144.1, 136.2, 129.8 (2C), 128.7 (2C), 128.4 (2C), 126.6, 126.5, 126.2, 124.4, 114.4 (2C), 65.7, 57.8, 54.9 (2C), 51.3, 25.7 (3C), 24.1; IR (Neat): 3019, 2938, 2362, 1610, 1509, 1218, 763 cm⁻¹; MS (ESI): *m/z* 392 (M+H)⁺; Anal. Calcd for C₂₅H₂₉NOS: C, 76.68; H, 7.46; N, 3.58. Found: C, 76.72; H, 7.39; N, 3.61.

4.1.1.6. 4-[2-[4-(Phenyl-thiophen-2-yl-methyl)-phenoxy]-ethyl]-morpholine (8**).** Compound **4** (200 mg, 0.75 mmol) refluxed with K₂CO₃ (259.42 mg, 1.87 mmol), 4-(2-Chloro-ethyl)-morpholine hydrochloride (258.45 mg, 0.75 mmol) in acetone furnished **8** (205.1 mg, 72%) as brown viscous oil. ¹H NMR (300 MHz, CDCl₃): δ 7.29–7.15

(m, 6H), 7.10–7.06 (m, 2H), 6.90–6.78 (m, 3H), 6.64–6.63 (m, 1H), 5.59 (s, 1H), 4.06 (t, *J* = 5.7, 2H), 3.70 (t, *J* = 4.4, 4H), 2.77 (t, *J* = 5.6, 2H), 2.55 (t, *J* = 4.6, 4H); ¹³C NMR (CDCl₃, 50 MHz): δ 157.4, 148.3, 144.0, 136.3, 129.8, 128.7, 128.7 (2C), 128.5 (2C), 128.4, 126.6, 126.5, 126.2, 124.4, 114.4 (2C), 66.7, 65.5, 57.6, 54.0 (2C), 51.3; IR (Neat): 3018, 2927, 2862, 1609, 1509, 1217, 753 cm⁻¹; MS (ESI): *m/z* 378 (M–H)⁺, 379 (M⁺), 380 (M+H)⁺; Anal. Calcd for C₂₃H₂₅NO₂S: C, 72.79; H, 6.64; N, 3.69. Found: C, 72.82; H, 6.70; N, 3.55.

4.1.1.7. 1-(2-[4-[(4-Chloro-phenyl)-thiophen-2-yl-methyl]-phenoxy]-ethyl)-piperidine (9**).** Compound **4** *p*-Cl substituted at Ar¹ (0.20 g, 0.667 mmol) refluxed with K₂CO₃ (0.280 g, 2.00 mmol), 1-(2-Chloroethyl)-piperidine hydrochloride (0.135 g, 0.734 mmol) in acetone furnished **9** (0.205 g, 75%) as wine red viscous liquid. ¹H NMR (200 MHz, CDCl₃): δ 7.15–6.94 (m, 7H), 6.82–6.52 (m, 4H), 5.47 (s, 1H), 3.99 (t, *J* = 4.0, 2H), 2.67 (t, *J* = 4.0, 2H), 2.45–2.41 (m, 4H), 1.57–1.37 (m, 6H); ¹³C NMR (CDCl₃, 50 MHz): δ 158.1, 148.1, 143.0, 135.8, 132.8, 130.4 (2C), 130.0 (2C), 128.9 (2C), 126.9, 126.6, 125.0, 114.8 (2C), 77.4, 66.2, 58.3, 55.4, 51.0, 30.1, 26.3, 24.6; IR (Neat) 3438, 2990, 1712, 1623, 775, 664 cm⁻¹; MS (ESI): *m/z* 412 (M+H)⁺; Anal. Calcd for C₂₄H₂₆ClNOS: C, 69.97; H, 6.36; N, 3.40. Found: C, 70.04; H, 6.34; N, 3.46.

4.1.1.8. (2-[4-[(4-Chloro-phenyl)-thiophen-2-yl-methyl]-phenoxy]-ethyl)-diisopropyl-amine (10**).** Compound **4** *p*-Cl substituted at Ar¹ (0.20 g, 0.67 mmol) refluxed K₂CO₃ (0.28 g, 2.00 mmol), (2-Chloro-ethyl)-diisopropyl-amine hydrochloride (0.15 g, 0.74 mmol) in acetone furnished **10** as yellow viscous oil (0.21 g, 73%). ¹H NMR (200 MHz, CDCl₃): δ 7.29–7.19 (m, 4H), 7.14–7.05 (m, 3H), 6.94–6.64 (m, 4H), 5.58 (s, 1H), 3.87 (t, *J* = 7.4, 2H), 3.07–3.00 (m, 2H), 2.80 (t, *J* = 7.4, 2H), 1.03 (d, *J* = 6.5, 12H); ¹³C NMR (CDCl₃, 50 MHz): δ 156.6, 146.5, 141.4, 134.0, 131.1, 128.8 (2C), 128.4 (2C), 127.2 (2C), 125.3 (2C), 124.9, 123.3, 113.1 (2C), 67.9, 49.4, 48.2, 43.1, 19.5 (4C); IR (Neat): 3312, 2967, 1602, 1446, 1353, 1023, 759 cm⁻¹; MS (ESI): *m/z* 428 (M+H)⁺; Anal. Calcd for C₂₅H₃₀ClNOS: C, 70.15; H, 7.06; N, 3.27. Found: C, 70.13; H, 7.13; N, 3.31.

4.1.1.9. 2-(4-[(3-Chlorophenyl) (thiophen-2-yl)methyl]phenoxy)-N,N-dimethylethanamine (11**).** Yield, 72%; ¹H NMR (300 MHz, CDCl₃) δ 7.19–7.18 (m, 4H), 7.09–7.07 (m, 3H), 6.93–6.90 (m, 1H), 6.86–6.84 (m, 2H), 6.66–6.65 (m, 1H), 5.57 (s, 1H), 4.04 (t, *J* = 5.7, 2H), 2.72 (t, *J* = 5.7, 2H), 2.33 (s, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 157.6, 147.3, 146.0, 135.2, 134.1, 129.6, 129.5 (2C), 128.7, 126.9, 126.7, 126.5, 126.3, 124.6, 114.3 (2C), 65.7, 58.1, 50.8, 45.8 (2C); IR (Neat) 3471, 2980, 1748, 1643, 768 cm⁻¹; MS (ESI) *m/z* 372 (M+H)⁺; Anal. Calcd for C₂₁H₂₂ClNOS: C, 67.82; H, 5.96; Cl, 9.53; N, 3.77. Found: C, 67.77; H, 5.92; N, 3.71.

4.1.1.10. 2-(4-[(3-Chlorophenyl) (thiophen-2-yl)methyl]phenoxy)-N,N-diethylethanamine (12**).** Yield, 75%; ¹H NMR (300 MHz, CDCl₃) δ 7.21–7.18 (m, 4H), 7.09–7.07 (m, 3H), 6.94–6.91 (m, 1H), 6.85–6.82 (m, 2H), 6.67–6.66 (m, 1H), 5.58 (s, 1H), 4.02 (t, *J* = 6.4, 2H), 2.86 (t, *J* = 6.4, 2H), 2.63 (q, *J* = 7.1, 4H), 1.06 (t, *J* = 7.1, 6H); ¹³C NMR (50 MHz, CDCl₃): δ 157.4, 146.1, 135.5, 134.2, 129.8, 129.6 (2C), 128.8 (2C), 126.9, 126.8, 126.6, 126.4, 124.7, 114.4 (2C), 65.7, 51.5, 50.9, 47.6 (2C), 11.1 (2C); IR (Neat) 3433, 2985, 1747, 1611, 779 cm⁻¹; MS (ESI) *m/z* 400 (M+H)⁺; Anal. Calcd for C₂₃H₂₆ClNOS: C, 69.07; H, 6.55; N, 3.50. Found: C, 69.13; H, 6.64; N, 3.55.

4.1.1.11. 1-(2-(4-[(3-Chlorophenyl) (thiophen-2-yl)methyl]phenoxy)ethyl)pyrrolidine (13**).** Yield, 68%; ¹H NMR (300 MHz, CDCl₃) δ 7.19–7.18 (m, 4H), 7.10–7.07 (m, 3H), 6.93–6.90 (m, 1H), 6.86–6.83 (m, 2H), 6.66–6.65 (m, 1H), 5.57 (s, 1H), 4.12 (t, *J* = 5.8, 2H), 2.94 (t, *J* = 5.8, 2H), 2.69 (s, 4H), 1.82 (s, 4H); ¹³C NMR (50 MHz, CDCl₃): δ 157.5, 147.2, 146.0, 135.3, 134.1, 129.6, 129.5, 128.7 (2C),

126.8, 126.7, 126.5, 126.2, 124.6, 114.4 (2C), 66.5, 54.8, 54.5 (2C), 50.8, 23.3 (2C); IR (Neat) 3451, 2962, 1745, 1611, 871 cm^{-1} ; MS (ESI) m/z 398 (M+H)⁺; Anal. Calcd for C₂₃H₂₄ClNOS: C, 69.42; H, 6.08; N, 3.52. Found: C, 69.33; H, 6.04; N, 3.55.

4.1.1.12. 1-(2-(4-((3-Chlorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl) piperidine (14). Yield, 79%; ¹H NMR (300 MHz, CDCl₃) δ 7.19–7.17 (m, 4H), 7.09–7.06 (m, 3H), 6.93–6.90 (m, 1H), 6.85–6.82 (m, 2H), 6.67–6.66 (m, 1H), 5.57 (s, 1H), 4.08 (t, J = 6.6, 2H), 2.77 (t, J = 5.9, 2H), 2.53–2.50 (m, 4H), 1.64–1.57 (m, 4H), 1.47–1.43 (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ 157.5, 147.2, 146.0, 135.1, 134.0, 129.6, 129.4, 128.6, 126.8 (2C), 126.7, 126.5, 126.2, 124.6, 114.3 (2C), 65.6, 57.7, 54.8 (2C), 50.8, 25.6 (2C), 23.9; MS (ESI) m/z 412 (M+H)⁺; Anal. Calcd for C₂₄H₂₆ClNOS: C, 69.97; H, 6.36; N, 3.40. Found: C, 69.89; H, 6.34; N, 3.45.

4.1.1.13. N-(2-(4-((3-chlorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl)-N-isopropylpropan-2-amine (15). Yield, 63%; ¹H NMR (300 MHz, CDCl₃) δ 7.19–7.18 (m, 4H), 7.09–7.06 (m, 3H), 6.92–6.89 (m, 1H), 6.84–6.81 (m, 2H), 6.66 (d, J = 3.3, 1H), 5.57 (s, 1H), 3.87 (t, J = 7.4, 2H), 3.07–2.98 (m, 2H), 2.80 (t, J = 7.5, 2H), 1.02 (d, J = 6.5, 12H); ¹³C NMR (75 MHz, CDCl₃): δ 157.4, 147.4, 146.1, 135.0, 134.2, 129.6, 129.5, 128.8, 126.9 (2C), 126.8, 126.6, 126.3, 124.7, 114.5 (2C), 66.9 (2C), 69.2, 50.9, 49.6 (2C), 44.4, 20.8 (4C); IR (Neat) 3432, 2990, 1742, 1631 cm^{-1} ; MS (ESI) m/z 428 (M+H)⁺; Anal. Calcd for C₂₅H₃₀ClNOS: C, 70.15; H, 7.06; N, 3.27. Found: C, 69.99; H, 7.14; N, 3.30.

4.1.1.14. 4-(2-(4-((3-Chlorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl) morpholine (16). Yield, 68%; ¹H NMR (300 MHz, CDCl₃) δ 7.20–7.18 (m, 4H), 7.10–7.08 (m, 3H), 6.94–6.92 (m, 1H), 6.85–6.83 (m, 2H), 6.67–6.66 (m, 1H), 5.58 (s, 1H), 4.09 (t, J = 4.5, 2H), 3.72 (t, J = 3.5, 4H), 2.79 (t, J = 4.4, 2H), 2.57 (d, J = 3.0, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 157.6, 147.3, 146.1, 135.4, 134.2, 129.7, 129.5, 128.8, 126.9 (2C), 126.8, 126.6, 126.3, 124.7, 114.5 (2C), 66.9 (2C), 65.7, 57.6, 54.0 (2C), 50.9; IR (Neat) 3435, 2980, 1732, 1630 cm^{-1} . MS (ESI) m/z 414 (M+H)⁺; Anal. Calcd for C₂₃H₂₄ClNO₂S: C, 66.73; H, 5.84; N, 3.38. Found: C, 66.79; H, 5.88; N, 3.31.

4.1.1.15. 2-(4-((2-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy)-N,N-dimethylethanamine (17). Isolated as light brownish oily liquid (yield 71%). ¹H NMR (300 MHz, CDCl₃) δ 7.25–7.22 (m, 1H), 7.21–7.12 (m, 1H), 7.09–7.02 (m, 5H), 6.94–6.91 (m, 1H), 6.87–6.67 (m, 2H), 6.66 (d, J = 2.5, 1H), 5.93 (s, 1H), 4.05 (t, J = 5.7, 2H), 2.73 (t, J = 5.7, 2H), 2.33 (s, 6H); ¹³C NMR (50 MHz, CDCl₃): δ 162.7, 157.6, 146.9, 134.8, 130.1, 129.6, 128.5, 126.6 (2C), 126.3, 124.5, 124.0, 115.5, 115.1, 114.4 (2C), 65.8, 58.2, 45.8 (2C), 43.7; IR (Neat): 3432, 2904, 1611, 1445, 1249, 1176, 1023, 823, 778, 661 cm^{-1} ; MS (ESI): m/z 356 (M+H)⁺, Anal. Calcd for C₂₁H₂₂FNOS: C, 70.96; H, 6.24; N, 3.94. Found: C, 70.91; H, 6.23; N, 3.97.

4.1.1.16. N, N-diethyl-2-(4-((2-fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethanamine (18). Isolated as light blackish oily liquid (yield 64%). ¹H NMR (300 MHz, CDCl₃) δ 7.24–7.12 (m, 2H), 7.09–7.01 (m, 5H), 6.93–6.90 (m, 1H), 6.84–6.81 (m, 2H), 6.66 (d, J = 3.4, 1H), 5.93 (s, 1H), 4.04 (t, J = 6.3, 2H), 2.88 (t, J = 6.3, 2H), 2.65 (q, J = 7.1, 4H), 1.07 (d, J = 7.1, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 162.7, 157.6, 146.9, 134.7, 130.1, 129.6, 128.4, 128.3, 126.5, 126.2 (2C), 124.5, 123.9, 115.5, 114.3 (2C), 66.2, 51.7, 47.7 (2C), 43.7, 11.6 (2C); IR (Neat): 3433, 2904, 1617, 1445, 1254, 1176, 1033, 823, 779, 662 cm^{-1} ; MS (ESI): m/z 384 (M+H)⁺, Anal. Calcd for C₂₃H₂₆FNOS: C, 72.03; H, 6.83; N, 3.65. Found: C, 72.09; H, 6.80; N, 3.67.

4.1.1.17. 1-(2-(4-((2-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl) pyrrolidine (19). Isolated as brownish oily liquid (yield 68%). ¹H NMR (300 MHz, CDCl₃): δ 7.25–7.12 (m, 2H), 7.09–6.99 (m, 5H),

6.94–6.91 (m, 1H), 6.86–6.83 (m, 2H), 6.66 (d, J = 0.9, 1H), 5.93 (s, 1H), 4.09 (t, J = 5.9, 2H), 2.90 (t, J = 5.9, 2H), 2.63 (s, 4H), 1.80 (d, J = 3.8, 4H); ¹³C NMR (50 MHz, CDCl₃): δ 157.7, 146.9, 139.2, 134.7, 130.1, 129.6, 128.5, 128.3, 126.5 (2C), 126.3, 124.5, 124.0, 123.9, 115.5, 114.4 (2C), 66.8, 54.6 (2C), 43.7, 23.4 (2C); IR (Neat): 3433, 2909, 1617, 1445, 1235, 1176, 1043, 823, 779, 660 cm^{-1} ; MS (ESI): m/z 382 (M+H)⁺, Anal. Calcd for C₂₃H₂₄FNOS: C, 72.41; H, 6.34; N, 3.67. Found: C, 72.42; H, 6.39; N, 3.71.

4.1.1.18. 1-(2-(4-((2-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl) piperidine (20). Isolated as light brownish oily liquid (yield 75%). ¹H NMR (300 MHz, CDCl₃): δ 7.24–7.12 (m, 2H), 7.09–6.98 (m, 5H), 6.93–6.90 (m, 1H), 6.84–6.81 (m, 2H), 6.66 (d, J = 3.4, 1H), 5.92 (s, 1H), 4.09 (t, J = 6.0, 2H), 2.78 (t, J = 6.0, 2H), 2.52 (t, J = 4.6, 4H), 1.64–1.57 (m, 4H), 1.47–1.40 (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ 159.1, 157.6, 146.9, 134.8, 131.3, 129.7 (2C), 128.5, 128.4, 126.6, 124.5, 115.4, 114.4 (2C), 65.6, 57.8, 54.9 (2C), 43.8, 43.7, 37.9, 25.7 (2C), 24.0; IR (Neat): 3423, 2914, 1617, 1425, 1254, 1176, 1031, 823, 774, 661 cm^{-1} ; MS (ESI): m/z 396 (M+H)⁺, Anal. Calcd for C₂₄H₂₆FNOS: C, 72.88; H, 6.63; N, 3.54. Found: C, 72.83; H, 6.57; N, 3.59.

4.1.1.19. N-(2-(4-((2-fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl)-N-isopropylpropan-2-amine (21). Isolated as light brownish oily liquid (yield 72%). ¹H NMR (300 MHz, CDCl₃): δ 7.22–7.11 (m, 2H), 7.08–6.97 (m, 5H), 6.92–6.89 (m, 1H), 6.83–6.81 (m, 2H), 6.66 (d, J = 2.5, 1H), 5.92 (s, 1H), 3.86 (t, J = 7.3, 2H), 3.07–2.98 (m, 2H), 2.80 (t, J = 7.3, 2H), 1.02 (d, J = 6.5, 12H); ¹³C NMR (75 MHz, CDCl₃): δ 162.7, 157.8, 147.0, 134.4, 130.1, 129.6, 128.4, 126.5, 126.2 (2C), 124.4, 123.9, 115.5, 115.0, 114.2 (2C), 69.1, 49.6 (2C), 44.4, 43.7, 20.8 (4C); IR (Neat): 3432, 2924, 1621, 1458, 1249, 1166, 1033, 833, 768, 663 cm^{-1} ; MS (ESI): m/z 412 (M+H)⁺, Anal. Calcd for C₂₅H₃₀FNOS: C, 72.96; H, 7.35; N, 3.40. Found: C, 72.92; H, 7.27; N, 3.46.

4.1.1.20. 4-(2-(4-((2-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethyl) morpholine (22). Isolated as brownish oily liquid (yield 71%). ¹H NMR (300 MHz, CDCl₃): δ 7.24–7.21 (m, 1H), 7.19–7.12 (m, 1H), 7.09–6.98 (m, 5H), 6.93–6.90 (m, 1H), 6.84–6.81 (m, 2H), 6.66 (d, J = 3.3, 1H), 5.92 (s, 1H), 4.09 (s, 2H), 3.72 (t, J = 4.5, 4H), 2.80 (s, 2H), 2.60 (d, J = 4.5, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 161.5, 159.1, 146.6, 135.0, 129.7, 128.5 (2C), 128.4 (2C), 126.5 (2C), 124.6 (2C), 115.2, 114.4 (2C), 66.8 (2C), 65.6, 57.6, 54.0 (2C), 37.9; IR (Neat): 3453, 2904, 1617, 1445, 1224, 1176, 1033, 823, 770, 662 cm^{-1} ; MS (ESI): m/z 398 (M+H)⁺, Anal. Calcd for C₂₃H₂₄FNOS₂: C, 69.49; H, 6.09; N, 3.52. Found: C, 69.51; H, 6.13; N, 3.48.

4.1.1.21. 2-(4-((3-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy)-N,N-dimethylethanamine (23). Colourless oil; yield, 78%; ¹H NMR (300 MHz, CDCl₃) δ 7.20–7.17 (m, 3H), 7.00–6.86 (m, 7H), 6.65 (s, 1H), 6.07 (s, 1H), 4.02–3.96 (m, 2H), 2.55 (t, J = 5.7, 2H), 2.23 (s, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 157.6, 147.3, 146.0, 135.2, 129.5, 128.7 (2C), 126.9, 126.7, 126.5, 125.9, 125.4, 125.0, 124.6, 114.3 (2C), 65.7, 58.1, 50.8, 45.8 (2C); IR (Neat): 3015, 2871, 2121, 1580, 1434, 1251, 1013, 729 cm^{-1} ; MS (ESI): m/z 356 [M+H]⁺; Anal. Calcd for C₂₁H₂₂FNOS: C, 70.96; H, 6.24; N, 3.94. Found: C, 70.90; H, 6.30; N, 3.90.

4.1.1.22. N, N-diethyl-2-(4-((3-fluorophenyl) (thiophen-2-yl)methyl)phenoxy) ethanamine (24). Colourless oil; yield, 75%; ¹H NMR (300 MHz, CDCl₃) δ 7.14–7.10 (m, 3H), 7.02–6.99 (m, 2H), 6.86–6.59 (m, 5H), 6.59 (s, 1H), 5.52 (s, 1H), 3.94 (t, J = 6.3, 2H), 2.78 (t, J = 6.3, 2H), 2.58–2.51 (m, 4H), 0.98 (t, J = 2.3, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 164.5, 157.7, 147.5, 135.3, 129.7, 129.7, 126.6, 126.3 (2C), 124.6 (2C), 115.8, 115.5, 114.4 (2C), 113.6, 113.3, 66.4, 51.7, 50.9, 47.8 (2C), 11.8 (2C); IR (neat): 3076, 2811, 2190, 1581, 1446, 1242, 1039,

777 cm⁻¹; MS (ESI): *m/z* 384 (M+H)⁺; Anal. Calcd for C₂₃H₂₆FNOS: C, 72.03; H, 6.83; N, 3.65. Found: C, 72.06; H, 6.89; N, 3.71.

4.1.1.23. 1-(2-(4-((3-Fluorophenyl) (thiophen-2-yl)methyl)phenoxy)ethyl) piperidine (25). Colourless oil; yield, 77%; ¹H NMR (300 MHz, CDCl₃) δ 7.11–7.06 (m, 3H), 6.92–6.56 (m, 7H), 6.55 (s, 1H), 5.98 (s, 1H), 3.97–3.92 (m, 2H), 2.53 (t, *J* = 5.7, 2H), 2.28 (t, *J* = 4.3, 4H), 1.45–1.17 (m, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 164.0, 155.8, 147.0, 139.2, 131.9, 129.5, 128.2, 126.5 (2C), 126.4 (2C), 124.3 (2C), 115.9, 115.7, 114.6 (2C), 66.5, 57.8 (2C), 44.8, 25.8 (3C); IR (Neat): 3042, 2862, 2115, 1587, 1491, 1211, 1047, 769 cm⁻¹; MS (ESI): *m/z* 396 (M+H)⁺; Anal. Calcd for C₂₄H₂₆FNOS: C, 72.88; H, 6.63; N, 3.54. Found: C, 72.82; H, 6.69; N, 3.50.

4.1.1.24. N-isopropyl-N-(2-(4-((4-(methylthio)phenyl) (thiophen-2-yl)methyl)phenoxy)ethyl)propan-2-amine (26). Compound **4** *p*-SMe substituted at Ar¹ (0.10 g, 0.35 mmol) refluxed with K₂CO₃ (0.135 g, 0.96 mmol), (2-Chloro-ethyl)-diethyl-amine hydrochloride (0.07 g, 0.35 mmol) in acetone furnished **26** as a light yellow viscous oil (0.105 g, 74%); ¹H NMR (200 MHz, CDCl₃): δ 7.18–6.99 (m, 7H), 6.86–6.85 (m, 1H), 6.74 (d, *J* = 8.1, 2H), 6.60 (d, *J* = 3.6, 1H), 5.49 (s, 1H), 3.80 (t, *J* = 7.4, 2H), 2.99–2.93 (m, 2H), 2.73 (t, *J* = 7.4, 2H), 2.38 (s, 3H), 0.96 (d, *J* = 6.4, 12H); ¹³C NMR (75 MHz, CDCl₃): δ 158.1, 148.7, 141.6, 136.9, 136.1, 130.1 (2C), 129.6 (2C), 127.0 (2C), 126.6 (2C), 124.8, 114.7 (2C), 69.5, 51.2, 50.1 (2C), 44.9, 21.2 (4C), 16.3; IR (Neat): 2964, 1608, 1505, 1248 cm⁻¹; MS (ESI): *m/z* 440 (M+H)⁺, Anal. Calcd for C₂₆H₃₃NOS₂: C, 71.02; H, 7.57; N, 3.19. Found: C, 71.04; H, 7.63; N, 3.24.

4.1.1.25. Dimethyl-(2-(4-[(2-methylsulfanyl-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-amine (27). Compound **4** *o*-SMe substituted at Ar¹ (200 mg, 0.64 mmol) refluxed with K₂CO₃ (221.17 mg, 1.6 mmol), 1-(2-chloroethyl)-dimethyl amine hydrochloride (92.20 mg, 0.64 mmol) in acetone furnished **27** (174 mg, 70.8%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.22–7.14 (m, 3H), 7.05–7.01 (m, 4H), 6.89–6.78 (m, 3H), 6.59–6.57 (m, 1H), 6.07 (s, 1H), 4.02 (t, *J* = 5.8, 2H), 2.70 (t, *J* = 5.7, 2H), 2.36 (s, 3H), 2.32 (s, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 157.7, 156.7, 136.1, 129.8 (3C), 127.8, 126.4, 126, 124 (2C), 120 (2C), 114 (2C), 110, 65.8, 58.3, 55.6, 45.8 (2C), 43.8; IR (Neat): 3017, 2926, 2361, 2338, 1609, 1508, 1465, 1217, 1040, 762 cm⁻¹; MS (ESI): *m/z* 384 (M+H)⁺; Anal. Calcd for C₂₂H₂₅NOS₂: C, 68.89; H, 6.57; N, 3.65. Found: C, 68.95; H, 6.62; N, 4.09.

4.1.1.26. Diethyl-(2-(4-[(2-methylsulfanyl-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-amine (28). Compound **4** *o*-SMe substituted at Ar¹ (200 mg, 0.64 mmol) refluxed with K₂CO₃ (221.17 mg, 1.6 mmol), 1-(2-chloroethyl)-diethyl amine hydrochloride (110.16 mg, 0.64 mmol) in acetone furnished **28** (191.7 mg, 72.7%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.21–7.14 (m, 4H), 7.08–6.99 (m, 4H), 6.89–6.76 (m, 2H), 6.59–6.58 (m, 1H), 6.06 (s, 1H), 4.01 (t, *J* = 6.3, 2H), 2.86 (t, *J* = 6.2, 2H), 2.67–2.60 (m, 4H), 2.36 (s, 3H), 1.07 (t, *J* = 7.0, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 157.4, 137.5, 135.4, 130.1 (2C), 129.1 (2C), 127.3, 126.6 (3C), 125.1 (2C), 124.5, 114.3 (2C), 65.8, 51.5, 47.7, 47.6, 42.3, 16.5, 11.3 (2C); IR (Neat): 2967, 2358, 1617, 1222, 1040, 767 cm⁻¹; MS (ESI): *m/z* 412 (M+H)⁺, Anal. Calcd for C₂₄H₂₉NOS₂: C, 70.03; H, 7.10; N, 3.40. Found: C, 70.10; H, 7.19; N, 3.30.

4.1.1.27. (2-(4-[(2-Methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-dimethyl-amine (29). Compound **4** *o*-OMe substituted at Ar¹ (400 mg, 1.349 mmol) refluxed with K₂CO₃ (466.32 mg, 3.374 mmol), 1-(2-chloroethyl)-dimethyl amine hydrochloride (194.32 mg, 1.349 mmol) in acetone furnished **29** (350 mg, 70.5%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.21–6.99 (m, 5H),

6.89–6.78 (m, 5H), 6.61–6.60 (m, 1H), 6.00 (s, 1H), 4.02 (t, *J* = 5.8, 2H), 3.74 (s, 3H), 2.71 (t, *J* = 5.7, 2H), 2.32 (s, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 157.3, 156.7, 136.1, 129.8 (3C), 127.8, 126.4 (2C), 126.0 (2C), 124.0, 120.4, 114.2 (2C), 110.7, 65.5, 58.1, 55.4, 45.6 (2C), 43.8; IR (Neat): 3011, 2933, 26 21, 1608, 1509, 1463, 1243, 1218, 1032, 758 cm⁻¹; MS (ESI): *m/z* 368 (M+H)⁺, 295 (C₁₈H₁₅O₂S)⁺, 72 (C₄H₁₀N)⁺; Anal. Calcd for C₂₂H₂₅NO₂S: C, 71.90; H, 6.86; N, 3.81. Found: C, 71.92; H, 6.83; N, 3.85.

4.1.1.28. Diethyl-(2-(4-[(2-methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-amine (30). As described above, compound **4** *o*-OMe substituted at Ar¹ (400 mg, 1.349 mmol) refluxed with K₂CO₃ (466.32 mg, 3.37 mmol), 1-(2-chloroethyl)-diethyl amine hydrochloride (232.16 mg, 1.349 mmol) in acetone furnished **30** (360 mg, 67.5%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.24–7.02 (m, 5H), 6.91–6.79 (m, 5H), 6.63–6.62 (m, 1H), 6.03 (s, 1H), 4.02 (t, *J* = 6.3, 2H), 3.73 (s, 3H), 2.85 (t, *J* = 6.3, 2H), 2.66–2.59 (m, 4H), 1.05 (t, *J* = 7.1, 6H); ¹³C NMR (300 MHz, CDCl₃): δ 157.3, 156.6, 148.6, 135.9, 132.8, 129.7 (2C), 129.5, 127.7, 126.3, 125.9, 123.9, 120.3, 114.1 (2C), 110.6, 66.3, 55.5, 51.7, 47.7 (2C), 43.8, 11.7 (2C); IR (Neat): 3017, 2971, 2935, 2836, 2362, 1607, 1509, 1244, 1217, 764 cm⁻¹; MS (ESI): *m/z* 396 (M+H)⁺; Anal. Calcd for C₂₄H₂₉NO₂S: C, 72.87; H, 7.39; N, 3.54. Found: C, 72.95; H, 7.36; N, 3.58.

4.1.1.29. 1-(2-(4-[(2-Methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-pyrrolidine (31). Compound **4** *o*-OMe substituted at Ar¹ (500 mg, 1.687 mmol) refluxed with K₂CO₃ (582.83 mg, 4.217 mmol), 1-(2-chloroethyl)-pyrrolidine hydrochloride (286.92 mg, 1.687 mmol) in acetone furnished **31** (478.6 mg, 72.1%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.24–6.98 (m, 5H), 6.88–6.77 (m, 5H), 6.60–6.59 (m, 1H), 5.99 (s, 1H), 4.09 (t, *J* = 5.9, 2H), 3.73 (s, 3H), 2.92 (t, *J* = 5.9, 2H), 2.68 (bs, 4H), 1.84–1.80 (m, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 157.0, 156.5, 148.4, 136.1, 132.6, 129.7 (2C), 129.5, 127.7, 126.3, 125.9, 123.9, 120.3, 114.1 (2C), 110.4, 66.1, 55.4, 54.7, 54.5 (2C), 43.8, 23.4 (2C); IR (Neat): 3017, 2964, 2601, 1607, 1509, 1224, 1217, 769 cm⁻¹; MS (ESI): *m/z* 394 (M+H)⁺; Anal. Calcd for C₂₄H₂₇NO₂S: C, 73.25; H, 6.92; N, 3.56. Found: C, 73.18; H, 6.95; N, 3.54.

4.1.1.30. 1-(2-(4-[(2-Methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-piperidine (32). Compound **4** *o*-OMe substituted at Ar¹ (500 mg, 1.687 mmol) refluxed with K₂CO₃ (582.83 mg, 4.217 mmol), 1-(2-chloroethyl)-piperidine hydrochloride (310.59 mg, 1.687 mmol) in acetone furnished **32** (630 mg, 91.62%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.11–6.99 (m, 5H), 6.88–6.76 (m, 5H), 6.61–6.59 (m, 1H), 5.99 (s, 1H), 4.06 (t, *J* = 6.0, 2H), 3.73 (s, 3H), 2.76 (t, *J* = 5.9, 2H), 2.52 (t, *J* = 5.2, 4H), 1.61 (q, *J* = 5.6, 4H), 1.47–1.42 (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ 157.2, 156.6, 148.5, 136.0, 132.8, 129.8, 129.6, 127.7, 126.3, 126.0, 123.9, 120.4, 114.2 (2C), 110.5, 96.2 65.5, 57.8, 55.5, 54.9 (2C), 43.8, 25.6 (2C), 24.1; IR (Neat): 3020, 2962, 260, 1610, 1217, 1091, 929, 761 cm⁻¹; MS (ESI): *m/z* 408 (M+H)⁺; Anal. Calcd for C₂₅H₂₉NO₂S: C, 73.67; H, 7.17; N, 3.44. Found: C, 73.76; H, 7.21; N, 3.34.

4.1.1.31. 1-(2-(4-[(2-Methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy)-ethyl)-azepane (33). Compound **4** *o*-OMe substituted at Ar¹ (200 mg, 0.674 mmol) refluxed with K₂CO₃ (233.16 mg, 1.68 mmol), 1-(2-Chloro-ethyl)-azepane hydrochloride (265.47 mg, 0.674 mmol) in acetone furnished **33** (197 mg, 69.2%) as brown viscous oil; ¹H NMR (300 MHz, CDCl₃): δ 7.19–6.98 (m, 5H), 6.87–6.75 (m, 5H), 6.60–6.58 (m, 1H), 5.99 (s, 1H), 4.04 (t, *J* = 5.8, 2H), 3.73 (s, 3H), 2.95 (t, *J* = 5.5, 2H), 2.79 (t, *J* = 4.2, 4H), 1.67 (bs, 4H), 1.60 (bs, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 157.2, 156.6, 148.5, 136.1, 132.8, 129.8 (2C), 129.6, 127.7, 126.3, 125.9, 123.9, 120.4, 114.2 (2C), 110.5, 65.8, 56.5, 55.7, 55.4 (2C), 43.8, 27.3 (2C), 27.0 (2C); IR

(Neat): 3017, 2937, 1609, 1509, 1242, 1217, 767 cm^{-1} ; MS (ESI): m/z 422 ($\text{M}+\text{H}^+$); 126 ($\text{C}_8\text{H}_{16}\text{N}^+$); Calcd for $\text{C}_{26}\text{H}_{31}\text{NO}_2\text{S}$: C, 74.07; H, 7.41; N, 3.32. Found: C, 74.02; H, 7.39; N, 3.33.

4.1.1.32. Diisopropyl-(2-{4-[(2-methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy}-ethyl)-amine (34). Compound **4** *o*-OMe substituted at Ar^1 (500 mg, 1.687 mmol) refluxed with K_2CO_3 (582.83 mg, 4.217 mmol, (2-Chloro-ethyl)-diisopropyl-amine hydrochloride (337.65 mg, 1.687 mmol) in acetone furnished **34** (520 mg, 72.7%) as yellow viscous oil; ^1H NMR (300 MHz, CDCl_3): δ 7.17–6.98 (m, 5H), 6.88–6.78 (m, 5H), 6.60–6.59 (m, 1H), 5.99 (s, 1H), 3.87 (bs, 2H), 3.74 (s, 3H), 3.06 (bs, 2H), 2.82 (t, $J = 6.7$, 2H), 1.05 (d, $J = 6.2$, 12H); ^{13}C NMR (75 MHz, CDCl_3): δ 157.7, 156.8, 135.9, 133.0, 129.9 (2C), 129.7 (2C), 127.9, 126.5, 126.1, 124.1, 120.5, 114.2 (2C), 110.8, 69.3, 55.6, 49.8 (2C), 44.6, 44.0, 21.0 (4C); IR (Neat): 3018, 2968, 2930, 2361, 1605, 1508, 1217, 764 cm^{-1} ; MS (ESI): m/z 424 ($\text{M}+\text{H}^+$); Anal. $\text{C}_{26}\text{H}_{33}\text{NO}_2\text{S}$: C, 73.72; H, 7.85; N, 3.31. Found: C, 73.81; H, 7.90; N, 3.25.

4.1.1.33. 4-(2-{4-[(2-Methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy}-ethyl)-morpholine (35). Compound **4** *o*-OMe substituted at Ar^1 (400 mg, 1.349 mmol), K_2CO_3 (466.32 mg, 3.37 mmol), 4-(2-Chloro-ethyl)-morpholine hydrochloride (251.02 g, 1.349 mmol) furnished **35** (510 mg, 92.2%) as brown viscous oil. ^1H NMR (200 MHz, CDCl_3): δ 7.23–6.98 (m, 5H), 6.88–6.75 (m, 5H), 6.60–6.59 (m, 1H), 5.99 (s, 1H), 4.05 (t, $J = 5.7$, 2H), 3.73 (s, 3H), 3.69 (t, $J = 4.5$, 4H), 2.75 (t, $J = 5.7$, 2H), 2.53 (t, $J = 4.6$, 4H); ^{13}C NMR (75 MHz, CDCl_3): δ 157.2, 156.6, 148.4, 136.1, 132.7, 129.8 (2C), 129.6, 127.8, 126.3, 126.0, 123.9, 120.4, 114.1 (2C), 110.5, 66.8 (2C), 65.6, 57.7, 55.4, 54.3 (2C), 43.8; IR (Neat): 3012, 2936, 2863, 1605, 1508, 1243, 1220, 767 cm^{-1} . MS (ESI): m/z 410 ($\text{M}+\text{H}^+$); Anal. Calcd for $\text{C}_{24}\text{H}_{27}\text{NO}_3\text{S}$: C, 70.39; H, 6.65; N, 3.42. Found: C, 70.43; H, 6.72; N, 3.43.

4.1.1.34. Diisopropyl-(2-{4-[(4-methoxy-phenyl)-thiophen-2-yl-methyl]-phenoxy}-ethyl)-amine (36). Compound **4** *p*-OMe substituted at Ar^1 (0.10 g, 0.33 mmol), K_2CO_3 (0.14 g, 1.01 mmol), (2-Chloro-ethyl)-diisopropyl-amine hydrochloride (0.07 g, 0.37 mmol) furnished **36** as yellow viscous oil (0.11 g, 75%). ^1H NMR (300 MHz, CDCl_3): δ 7.17 (d, $J = 4.5$, 1H), 7.12–7.07 (m, 4H), 6.92–6.89 (m, 1H), 6.84–6.80 (m, 4H), 6.67–6.64 (d, $J = 3.0$, 1H), 5.56 (s, 1H), 3.86 (t, $J = 7.3$, 2H), 3.76 (s, 3H), 3.07–2.98 (m, 2H), 2.80 (t, $J = 7.5$, 2H), 1.03 (d, $J = 6.3$, 12H); ^{13}C NMR (75 MHz, CDCl_3): δ 158.2, 157.5, 148.9, 136.3, 136.2, 129.6 (4C), 126.4, 125.9, 124.2, 114.2 (2C), 113.6 (2C), 68.8, 55.1 (2C), 50.5, 49.9, 44.5, 20.6 (4C); IR (Neat): 2964, 1654, 1611, 1509 cm^{-1} ; MS (ESI): m/z 424 ($\text{M}+\text{H}^+$); Anal. Calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_2\text{S}$: C, 73.72; H, 7.85; N, 3.31. Found: C, 73.79; H, 7.79; N, 3.29.

4.1.1.35. 1-(2-(4-((4-Methoxyphenyl) (thiophen-3-yl)methyl)phenoxy)ethyl)piperidine (37). Compound **1a** reacted with **2*** to gave corresponding **3*** which on refluxing with phenol and two drops of H_2SO_4 in benzene provided with **4*** which was refluxed with K_2CO_3 1-(2-chloroethyl)-piperidine hydrochloride in acetone furnished compound **37**. ^1H NMR (400 MHz, CDCl_3): δ 7.00 (d, $J = 8$ Hz, 1H), 6.95 (m, 4H), 6.77–6.63 (m, 5H), 6.62 (s, 1H), 7.32 (s, 1H), 4.32 (t, $J = 4$ Hz, 2H), 3.7 (s, 3H), 3.27 (t, $J = 4$ Hz, 2H), 3.09 (m, 4H), 1.88 (m, 4H), 1.59 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3): δ 158.11, 155.7, 145.3, 137.7, 136.0, 130.0 (2C), 129.7 (2C), 125.5 (2C), 122.4, 114.3 (2C), 113.7 (2C), 63.0, 56.3, 55.2 (2C), 54.1, 50.9, 23.0 (2C), 21.9.

4.1.1.36. N, N –diethyl-2-(4-((4-methoxyphenyl) (thiophen-3-yl)methyl)phenoxy)ethanamine (38). R_f : 0.4 (20% ethyl acetate in hexane). Isolated as light yellow oily liquid (yield 65%) by elution with 2% methanol in dichloromethane from silica gel. ^1H NMR

(400 MHz, CDCl_3): δ 7.19 (t, $J = 4$ Hz, 1H), 7.0 (m, 4H), 6.97 (m, 5H), 6.76–6.62 (m, 1H), 5.32 (s, 1H), 4.29 (t, $J = 4$ Hz, 2H), 3.70 (s, 3H), 3.43 (t, $J = 4$ Hz, 2H), 3.23 (m, 4H), 1.31 (t, $J = 8$ Hz, 6H).

4.1.1.37. N-isopropyl-N-(2-(4-((4-methoxyphenyl) (thiophen-3-yl)methyl)phenoxy)ethyl)propan-2-amine (39). R_f : 0.43 (20% ethyl acetate in hexane). Isolated as light yellow oily liquid (yield 62%) by elution with 2% methanol in dichloromethane from silica gel. ^1H NMR (300 MHz, CDCl_3): δ 7.23 (s, 1H), 7.03 (m, 4H), 6.85 (m, 5H), 6.69 (m, 1H), 5.39 (s, 1H), 3.87 (t, $J = 6$ Hz, 2H), 3.76 (s, 3H), 3.03 (t, $J = 6$ Hz, 2H), 2.80 (m, 2H), 1.04 (d, $J = 6$ Hz, 12H).

4.1.1.38. 2-(Di(thiophen-2-yl)methoxy)-N,N-diethylethanamine (40). To a solution of 2-thiophene carboxaldehyde, thiophene 2-magnesium bromide (*in situ* generation of 2-bromo thiophene in presence of Mg in THF for 1hr) was added and was stirred for another 1hr to furnish corresponding carbinol. It was then refluxed with diethyl aminoethyl chloride hydrochloride chain in presence of K_2CO_3 in acetone at 60–75 $^\circ\text{C}$ to furnish compound **40**. Quenched with NH_4Cl soln, extracted with ethyl acetate and dried over Na_2SO_4 . The solvent was removed under reduced pressure. Product was purified by column chromatography. R_f : 0.3 (2% methanol in DCM). Isolated as pale yellow, oily liquid, (yield 52%), elution with 1.5% methanol in DCM from silica gel. ^1H NMR (400 MHz, CDCl_3): δ 7.23 (s, 2H), 6.90 (s, 4H), 5.83 (s, 1H), 3.72 (s, 2H), 3.00–2.90 (m, 6H), 1.16 (d, $J = 6.48$ Hz, 6H)ppm. ^{13}C NMR (75 MHz, CDCl_3): δ 145.1 (2C), 126.3 (2C), 125.7 (2C), 125.6 (2C), 75.9, 66.5, 51.8, 47.6 (2C), 11.1 (2C) ppm. IR (Neat cm^{-1}): 3442, 2924, 1630, 1218, 772. Anal. calcd. for $\text{C}_{13}\text{H}_{17}\text{NOS}_2$: C 58.39; H, 6.41; N, 5.24%; found C, 58.42; H, 6.34; N, 5.15%.

4.1.1.39. N-(2-(dithiophen-2-ylmethoxy)ethyl)-N-isopropylpropan-2-amine (41). R_f : 0.6 (40% ethyl acetate in hexane). Isolated as pale brown, oily liquid, (yield 70%) by elution with 0.5% methanol in DCM from silica gel. ^1H NMR (300 MHz, CDCl_3): δ 7.21 (d, $J = 4.89$ Hz, 2H), 6.88 (t, $J = 5.07$ Hz, 4H), 5.85 (s, 1H), 3.64 (s, 2H), 3.29 (s, 2H), 2.87 (s, 2H), 1.10 (d, $J = 6.09$ Hz, 12H)ppm. IR (Neat cm^{-1}): 3440, 2928, 1642, 1218, 770. Anal. calcd. for $\text{C}_{17}\text{H}_{25}\text{NOS}_2$: C, 63.11; H, 7.79; N, 4.33%; found C, 63.24; H, 7.65; N, 4.41%.

4.1.1.40. 2-(Di(thiophen-2-yl)methoxy)-N,N-dimethylethanamine (42). R_f : 0.35 (2% methanol in DCM). Isolated as pale brown, oily liquid, (yield 35%) by elution with 2% methanol in DCM from silica gel. ^1H NMR (300 MHz, CDCl_3): δ 7.25 (d, $J = 4.5$ Hz, 2H), 6.91 (d, $J = 4.44$ Hz, 4H), 5.85 (s, 1H), 3.87 (t, $J = 3.69$ Hz, 2H), 3.25 (d, $J = 3.75$ Hz, 2H), 2.85 (s, 6H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ 143.7 (2C), 126.6 (2C), 126.3 (2C), 126.1 (2C), 76.3, 63.8, 57.3, 44.3 (2C) ppm. IR (Neat cm^{-1}): 3435, 2924, 1634, 1217, 770. Anal. calcd. for $\text{C}_{15}\text{H}_{21}\text{NOS}_2$: C, 60.98; H, 7.16; N, 4.74%; found C, 60.77; H, 7.32; N, 4.71%.

4.1.1.41. 1-(2-(Di(thiophen-2-yl)methoxy)ethyl)piperidine (43). R_f : 0.4 (5% methanol in DCM). Isolated as pale orange, oily liquid, (yield 33%) by elution with 1.5% methanol in DCM from silica gel. ^1H NMR (400 MHz, CDCl_3): δ 7.28 (dd, $J_1 = 1.68$, $J_2 = 2.88$ Hz, 2H), 6.88 (t, $J = 4.64$ Hz, 4H), 5.82 (s, 1H), 3.65 (t, $J = 5.88$ Hz, 2H), 2.66 (t, $J = 5.84$ Hz, 2H), 2.51 (s, 4H), 1.61–1.55 (m, 4H), 1.39 (d, $J = 4.96$ Hz, 2H)ppm. IR (Neat cm^{-1}): 3450, 2918, 1620, 1212, 775. ($\text{C}_7\text{H}_{14}\text{NO}$). Anal. calcd. for $\text{C}_{16}\text{H}_{21}\text{NOS}_2$: C, 62.50; H, 6.88; N, 4.56%; found C, 62.63; H, 6.78; N, 4.56%.

4.1.1.42. N-(2-(di(thiophen-3-yl)methoxy)ethyl)-N-isopropylpropan-2-amine (44). R_f : 0.38 (20% ethyl acetate in hexane). Isolated as yellow oily liquid (yield 64%) by elution with 2% methanol in dichloromethane from silica gel. ^1H NMR (400 MHz, CDCl_3):

δ 7.20–7.17 (m, 4H), 7.01 (q, J = 1 Hz, 1H), 6.83 (d, J = 5.28 Hz, 1H), 5.7 (s, 1H), 3.5 (s, 2H), 3.05 (s, 2H), 2.70 (s, 2H), 0.99 (d, J = 5.84 Hz, 12H).

4.1.1.43. 1-(2-(Di(thiophen-3-yl)methoxy)ethyl)piperidine (45).

R_f: 0.20 (30% ethyl acetate in hexane). Isolated as yellow oily liquid (yield 67%) by elution with 5% methanol in dichloromethane from silica gel. ¹H NMR (400 MHz, CDCl₃): δ 7.22–7.19 (m, 4H), 6.99 (q, J = 1.6 Hz, 1H), 6.86 (d, J = 5.28 Hz, 1H), 5.76 (s, 1H), 3.75 (t, J = 5.32 Hz, 2H), 2.39 (t, J = 5.36 Hz, 2H), 2.78 (s, 4H), 1.75–1.69 (m, 4H), 1.52 (s, 2H).

4.1.1.44. 2-(Di(thiophen-3-yl)methoxy)-N,N-diethylethanamine (46).

R_f: 0.30 (20% ethyl acetate in hexane). Isolated as yellow oily liquid (yield 68%) by elution with 6% methanol in dichloromethane from silica gel. ¹H NMR (400 MHz, CDCl₃): δ 7.18 (t, J = 4.56 Hz, 4H), 6.99 (q, J = 1.24 Hz, 1H), 6.83 (d, J = 5.28 Hz, 1H), 5.76 (s, 1H), 3.57 (t, J = 6.16 Hz, 2H), 2.72 (t, J = 6.2 Hz, 2H), 2.57 (q, J = 7.16 Hz, 4H), 0.99 (t, J = 7.16 Hz, 6H).

4.1.1.45. 2-(Di(thiophen-3-yl)methoxy)-N,N-dimethylethanamine (47).

R_f: 0.30 (20% ethyl acetate in hexane). Isolated as Brown yellow oily liquid (yield 72%) by elution with 10% methanol in dichloromethane from silica gel. ¹H NMR (400 MHz, CDCl₃): δ 7.20 (t, J = 3.24 Hz, 4H), 7.00 (d, J = 4.72 Hz, 1H), 6.852 (d, J = 5.28 Hz, 1H), 5.75 (s, 1H), 3.63 (t, J = 5.52 Hz, 2H), 2.69 (t, J = 5.52 Hz, 2H), 2.35 (s, 6H).

4.1.1.46. 1-(2-(Di(thiophen-3-yl)methoxy)ethyl)pyrrolidine (48).

R_f: 0.30 (30% ethyl acetate in hexane). Isolated as pale yellow oily liquid (yield 68%) by elution with 4% methanol in dichloromethane from silica gel. ¹H NMR (300 MHz, CDCl₃): δ 7.25–7.20 (m, 4H), 7.00 (q, J = 1.48 Hz, 1H), 6.86 (d, J = 5.28 Hz, 1H), 5.77 (s, 1H), 3.82 (q, J = 1.92 Hz, 2H), 3.14 (t, J = 4.28 Hz, 6H), 1.95 (t, J = 6.6 Hz, 4H).

4.1.1.47. 4-(2-(Di(thiophen-3-yl)methoxy)ethyl)morpholine (49).

R_f: 0.36 (10% ethyl acetate in hexane). Isolated as yellow oily liquid (yield 67%) by elution with 2% methanol in dichloromethane from silica gel. ¹H NMR (400 MHz, CDCl₃): δ 7.20 (q, J = 1.52 Hz, 4H), 6.99 (q, J = 1.72 Hz, 1H), 6.86 (d, J = 5.28 Hz, 1H), 5.76 (s, 1H), 3.72 (t, J = 4.64 Hz, 4H), 3.68 (t, J = 5.48 Hz, 2H), 2.74 (t, J = 5.36 Hz, 2H), 2.64 (s, 4H).

4.2. Biological evaluation

4.2.1. Assay for in vitro activity against *M. tuberculosis* H37R_v [11]

The compounds were dissolved in dimethyl sulfoxide (DMSO) to make stocks. Serial dilutions from stocks were also made in DMSO. To 1.9 mL Middlebrook (MB) 7H10 agar supplemented with OADC (in tubes, held at 45–50 °C), 0.1 mL of test compound or DMSO (negative control) or INH (positive control) was added. The contents were mixed and allowed to solidify (by cooling) as slants. Three-week old culture of *M. tuberculosis* H37R_v was harvested from L-J medium and its suspension (1 mg/mL, equivalent to 10⁸ bacilli approximately) was made in normal saline containing 0.05% Tween-80. 10 μ L of 1:10 dilution of this suspension (~10⁵ bacilli) was inoculated into each tube and incubated at 37 °C for 4 weeks. The lowest concentration of a compound up to which there was no visible growth of bacilli was its minimal inhibitory concentration (MIC). Compounds having MIC of \leq 6.25 mg/L were selected as 'secondary hits' for further development.

4.2.2. Assays for in vitro cytotoxicity [12]

In vitro cytotoxicity of active compounds (having MIC \leq 6.25 mg/L) was measured using Vero cells and mouse bone-

marrow derived macrophages. Vero C-1008 cell line was procured from Laboratory Animals Division (LAD) of CDRI. For preparation of macrophages, a Swiss mouse (bred in LAD) was euthanized by exposure to CO₂ and femurs dissected out. The bones were trimmed at each end and marrow flushed out with Dulbecco's Minimal Essential Medium containing 10% foetal bovine serum (DMEM-FBS) and antibiotics. The medium was also supplemented with 15% (v/v) L929 fibroblast conditioned medium and non-essential amino acids. The cell suspension (Vero/macrophage) was plated in 96-well tissue culture plates (20,000 cells/200 μ L/well) and incubated overnight (37 °C, 5% CO₂) to allow their adherence. Compounds at different concentrations were added to the wells. A known toxic compound (Staurosporine) was used as a positive control and DMSO was used as negative control. After 24 h of incubation, 20 μ L of MTS solution (tetrazolium compound, Owen's reagent) was added to each well and incubated for further 2 h (37 °C, 5% CO₂). O. D. was read at 490 nm using a plate reader. A compound was considered as potentially toxic if its IC₅₀ (concentration causing 50% loss in cell viability) was \leq 10 times its MIC for *M. tuberculosis* H37R_v.

4.2.3. Assay for ex vivo activity in the macrophage model of tuberculosis [13]

The effect of selected compounds (MIC \leq 6.25 mg/L and SI \geq 10, i.e., non-toxic) on the survival and multiplication of *M. tuberculosis* H37R_v within mouse bone-marrow derived as well as human blood monocyte derived macrophages was evaluated by light microscopy and counting of colony-forming units (CFU). Mouse bone-marrow derived macrophages were prepared as above and the human blood monocyte derived macrophages were prepared as follows: 10 mL blood from healthy volunteers was collected in anti-coagulant solution (acid-citrate-dextrose, ACD), centrifuged at 2500 rpm (to remove plasma) and reconstituted with RPMI 1640 tissue culture medium containing ACD. This suspension was layered over Ficoll-Isopaque (Sigma Chemical Company, USA) and centrifuged at 600 g for 20 min. The mononuclear cells on the top of Ficoll layer were collected, washed twice with RPMI medium, and counted using hemocytometer. The cells were finally suspended at a density of 10⁶ cells/mL in RPMI containing heat inactivated pooled normal human serum (PNS, 5%).

For evaluation by microscopy, macrophages (human/mouse) were seeded in 8-chambered slides at a density of 5 \times 10⁵ cells (in 0.5 mL RPMI-PNS/DMEM-FBS media) per chamber. The cells were incubated at 37 °C with 5% CO₂. On 6th day, washed adherent macrophages in each chamber were infected for 3 h with 0.5 mL suspension containing 2.5 \times 10⁶ *M. tuberculosis* H37R_v in antibiotic-free medium. Later, the chambers were thoroughly washed to remove extracellular bacteria and replenished with fresh 0.5 mL medium containing 4 \times MIC of standard drugs (INH, RFM and PZA) or test compounds. After further 4 days incubation in the CO₂ incubator, each chamber was gently washed and medium aspirated off. After removing the chambers, infected macrophages on slide were heat-fixed, stained with Ziehl-Neelsen stain, counter stained with methylene blue, and viewed under oil immersion lens (100 \times) of a microscope.

For evaluation by counting colony forming units (CFU), 5-day old cultures of adherent macrophages (10⁶ cells/well, in 24-well plates) were infected for 3 h with 5 \times 10⁶ *M. tuberculosis* H37R_v in antibiotic-free medium. Later, the wells were washed to remove extracellular bacteria and replenished with fresh 1 mL antibiotic-free medium containing 4 \times MIC of standard drugs or test compounds. In order to determine the number of bacilli phagocytosed during the 3 h infection period (0 day count), one well was lysed with 0.1% saponin (15 min) and lysate (50 μ L of 1:100 dilution) plated on MB 7H11 agar (in petri dishes) for colony counting. Other

wells, after further 4 days of incubation, were gently washed, cells lysed and lysates plated on MB 7H11 agar plates. CFUs were counted after 4 weeks of incubation at 37 °C.

4.2.4. Assay for *in vivo* activity in the mouse model of tuberculosis [14]

Outbred Swiss mice (obtained from CDRI-LAD) were infected with *M. tuberculosis* H37R_v (10⁷ CFU/mouse, i.v.) and divided into groups of 8–10 animals. Each experimental group received daily oral dose of the test compound (100 mg/kg body weight, for 28 days) dissolved in a suitable vehicle (water/corn oil, depending on its solubility profile). The drug-treated control groups received INH (25 mg/kg) or EMB (10 mg/kg) or PZA (150 mg/kg body weight) for 28 days. The untreated control groups received only the vehicle. At least 3 mice from each group were sacrificed on day 30 for determination of viable bacilli in the lungs. Serial dilutions of lung homogenates were placed on MB 7H11 agar medium and colonies (CFU) were counted after 4 weeks of incubation at 37 °C. Remaining mice, if survived, were observed for further 15 days for determination of mean survival time (MST) and % survivors.

4.2.5. Determination of minimum bactericidal concentration (MBC) [15]

For *in vitro* MBC determinations, to each culture tube having 2.5 mL MB7H9 broth, 50 µL inoculum containing 10⁵ *M. tuberculosis* H37R_v was added and incubated (37 °C) with or without 1× and 4× MIC of test compounds or standard *anti*-TB drugs. CFU in a tube containing inoculum alone was determined on day 0 and in others it was determined on day 14. The same principle was applied to the determination of *ex vivo* MBC.

4.2.6. Activity of selected compounds against drug-resistant strains of *M. tuberculosis* [11]

The drug sensitive and drug-resistant Indian clinical isolates of *M. tuberculosis* were obtained from the DBT Mycobacterium Repository at NJIL-OMD, Agra (Courtesy of Dr VM Katoch, DG-ICMR) and propagated on L-J medium. The cultures thus obtained were used for testing *in vitro* sensitivity against selected active compounds using agar dilution method.

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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.2015.03.036>.

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