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Article

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Diarylthiazole: an anti-mycobacterial scaffold potentially targeting PrrB-PrrA two component system

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Abstract

Diarylthiazole (DAT), a hit from diversity screening was found to have potent antimycobacterial activity against *Mycobacterium tuberculosis* (Mtb). In a systematic

medicinal chemistry exploration, we demonstrated chemical opportunities to optimize the potency and physico-chemical properties. The effort led to more than 10 compounds with sub-micromolar MICs and desirable physico-chemical properties. The potent anti-mycobacterial activity in conjunction with low molecular weight, made the series an attractive lead (antibacterial ligand efficiency >0.4). The series exhibited excellent bactericidal activity and was active against drug-sensitive and resistant Mtb. Mutational analysis showed that mutations in *prrB* impart resistance to DAT compounds, but not to reference drugs tested. The sensor kinase PrrB belongs to the PrrBA two component system and is potentially the target for DAT. PrrBA is a conserved, essential regulatory mechanism in Mtb and has been shown to have a role in virulence and metabolic adaptation to stress. Hence, DATs provide an opportunity to understand a completely new target system for antimycobacterial drug discovery.

Introduction

Tuberculosis (TB) remains a major global health challenge[1] and has been declared a public health emergency by WHO. Among various aspects of this disease, emergence and spread of drug resistant TB poses a significant threat to TB care and control worldwide. Reports of TB patients with severe drug resistance patterns, worse than multi- or, extreme- drug resistant TB (MDR-, XDR-) are increasing and is a cause of real concern (WHO meeting report).[2] It has created an urgent need to develop new drugs which can rapidly cure drug-resistant TB and prevent reccurrence of the disease. Current TB therapy is long and associated with various toxicities, hence, contributes to non-compliance and enhanced probability of emergence of resistance. A simplified, shortened combination therapy with significantly improved safety

profile will greatly enhance patient's compliance, improve therapy outcome and delay emergence of resistance.

A major focus of TB drug discovery is aimed at identifying antibiotics that act by novel mechanisms and are active against both drug-sensitive and resistant *Mycobacterium tuberculosis* (Mtb). Recently, approved new chemical entities, such as Sirturo and Delaminid for treatment of MDR-TB patients, have given hope for discovery of drugs with completely new mode-of-action.[3] Phenotypic whole-cell-based screens to identify anti-mycobacterial compounds followed by target identification is an attractive approach for lead generation against Mtb.[4] In continuation with our efforts to search for new drug candidates for TB including AZD5847[5], we pursued phenotypic screening of our in-house library using Mtb H37Rv. The screen identified multiple hits with potential to be developed into leads. In this report, we present optimization of one of the scaffolds; a diarylthiazole (DAT), from an initial hit to a lead chemical series. DAT compounds are active against drug sensitive and isoniazid/rifampicin resistant Mtb strains. Experiments to understand the mode of action of DATs led to the identification of PrrB, a histidine kinase belonging to PrrBA two component system (TCS), as a potential target.

Bacterial virulence factors and regulatory proteins are less explored target classes for drug discovery.[6,7] Bacterial two-component systems (TCSs) are ubiquitous regulatory mechanisms involved in transcriptional reprogramming in response to environmental changes, regulation of virulence determinants and antibiotic resistance.[8,9] Mtb has 11 paired TCSs; four are conserved across all mycobacterial species.[10] Investigations are required to understand the role of PrrBA in the physiology of Mtb, relationship of DAT to PrrBA and its translation to *in vivo* potency.

Results and Discussion

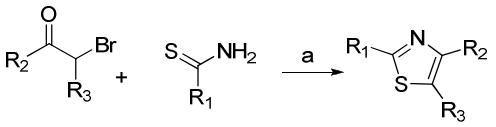
As part of a high-throughput screening program against the strain Mtb H37Rv, we used a representative set (~100,000) of the AstraZeneca corporate library. The screening cascade included activity against Mtb (minimum inhibitory concentration, MIC), bactericidality (minimum bactericidal concentration), selectivity index for cytotoxicity ($SI_{cytotox}$; ratio of Mtb MIC and IC_{50} against human A549 cell line) and confirmation of activity by resynthesis. The criteria for bactericidality and low human cytotoxicity played a critical role in this cascade. We observed a hit rate of 1% as primary actives (>80% growth inhibition at 20 μM). Compounds that exhibit an emerging MIC-based structure-activity relationship (SAR) from near neighbor screening were prioritized for further exploration.

A series of DATs emerged as promising hits from this screening campaign with good anti-Mtb properties. Compound 1 (Figure **1A**) was the most potent hit with an attractive antimycobacterial ligand efficiency[11] (ALE) of 0.37. In a recent disclosure of 177 antibacterial active compounds by GSK,[12] two compounds from the series were shown to be active against *Mycobacterium bovis* (BCG), with an MIC of 6 μM. However, no further work is reported with systematic SAR exploration and/or mechanism of action. In addition, the scaffold had been reported as inhibiting sterol regulatory element binding proteins (SREBPs), a target class involved in cholesterol and fatty acid biosynthesis in humans.[13] Fatostatin,[14] the lead candidate for anti-obesity therapy, is structurally close to our hit.

DAT compounds were synthesized using Hantzsch thiazole synthesis[15], for which the corresponding thioamides and substituted α–bromoketones were refluxed in ethanol as represented in **Scheme 1**. Individual thioamides were synthesized[16] from the corresponding nitriles using sodium hydrosulfide and magnesium chloride in DMF at room temperature. α–Bromination of acetophenone derivatives was carried out using cupric bromide[17] in ethyl

acetate/chloroform mixture (1:1) at reflux for 3 hrs, while α–bromination of heterocyclic acyl derivatives was carried out using bromine[18] with HBr in acetic acid at room temperature for 24 hrs. Detailed schematic representation and synthetic procedures are described as part of Supplementary Information.

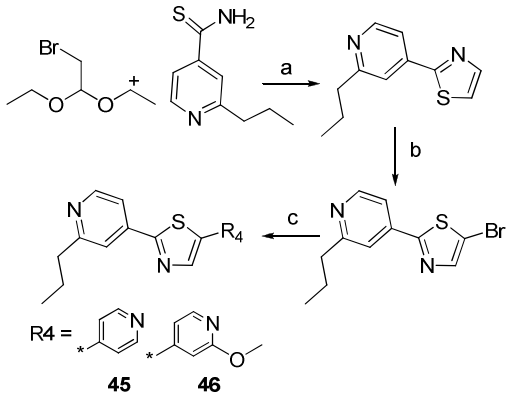
Scheme 1. General schematic representation of synthesis of diarylthiazole compounds[a]



[a]Reagents and conditions: (a) Ethanol, reflux, 8-12 h, 60-80%

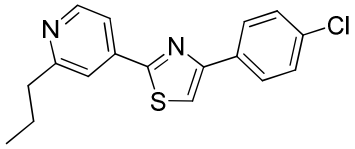
Synthesis of compounds **45**-**46** was carried out as per **Scheme 2**, in which 2-propylpyridine-4-carbothioamide and bromoacetaldehyde diethyl acetal were heated at 140 °C for 30 min in a microwave reactor to afford 2-(2-propylpyridin-4-yl)thiazole. This was brominated using NBS in acetic acid to 5-bromo-2-(2-propylpyridin-4-yl)thiazole, which was subjected to Suzuki coupling to produce the final molecule in moderate yields. Synthesis of thiadiazole **47** was carried out as described previously.[19]

Scheme 2: Synthesis of compounds **45**-**46**



[a]Reagents and conditions: (a) Ethanol, MW, 140 °C, 30 min 78% (b) NBS, AcOH, 70 °C, 24 h, 20-50% (c) R-B(OH)$_2$, $PdCl_2(PPh_3)_2$, $NaHCO_3$, DME:Water (4:1), MW, 130 °C, 45 min, 40-50%

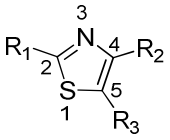
In our pursuit from 'hit to lead' we carried out a systematic exploration of the series replacing R_1 and R_2 on the thiazole with substituted aryl, heteroaryl and alicyclic rings (**Figure 1**). The prototype used for SAR exploration is shown in Figure 1B. Profiling of compound **1**'s *in vitro* DMPK properties suggested that the series will require optimization of physicochemical properties such as equilibrium aqueous solubility, logD and *in vitro* clearance.



1

Mtb MIC	= 1 µg/mL
Mtb MBC	= 2 µg/mL
ALE	= 0.37
Aq. Sol	< 1.5 µM
SI Index	> 50

A

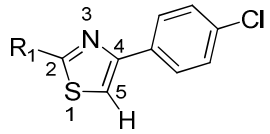


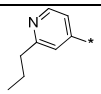
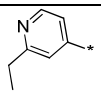
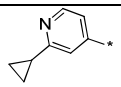
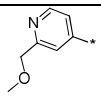
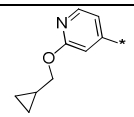
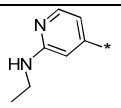
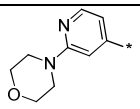
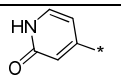
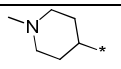
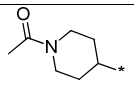
B

Figure 1: **A.** Compound **1**, its activity and calculated properties. B. Rings and diversification points of DAT used for SAR exploration. ALE = antibacterial ligand efficiency. SI index = (Mammalian MIC against A549 cell line) / Mtb MIC

Initial exploration was carried out by replacing the propyl group attached to the aryl ring at R_1 with polar and solubilizing groups such as alkoxy, amino and morpholine (**Table 1**, **4-7**). While the Mtb MIC were comparable, the improvement in solubility was modest. Similarly, replacing pyridine with pyridone and alicyclic rings (**Table 1**, **8-10**) resulted in the loss of activity to the highest concentration tested, which indicated that the 2-propyl/ethyl substituted pyridyl at R_1 is most effective.

Table 1: SAR exploration of substitutions at R_1

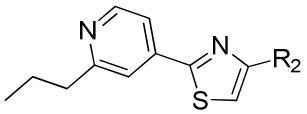


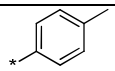
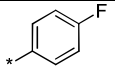
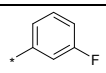
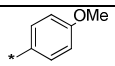
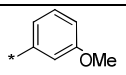
No.	R_1	MIC µg/mL	Solubility µM	ClogP
1		1	<1.56	5.5
2		2	< 6.6	5.0
3		4	<2.78	4.9
4		4	0	3.8
5		2	0.38	5.8
6		8	<3	5.1
7		> 32	0	4.0
8		> 32	<1	2.8
9		> 32	3393	3.9
10		> 32	3.6	2.5

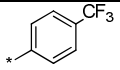
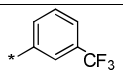
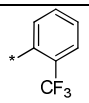
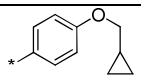
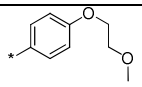
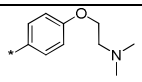
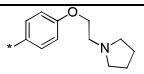
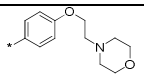
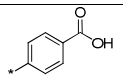
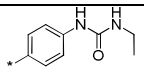
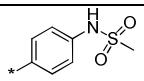
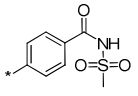
In the next design-make-test-analyze (DMTA) cycle, we explored R_2. A wide variety of substitutions on the phenyl ring were synthesised and tested for Mtb MIC as shown in **Table**

2 opening new avenues to improve potency. Interestingly, both electron-withdrawing and electron-donating groups were active. Furthermore, substituents at *ortho*, *meta*, and *para* positions were found to be equipotent from the limited set of electron-withdrawing and electron-donating groups explored (**Table 2**, **13-21**). Introduction of basic side chains with an ethylene glycol linker at the *para* position (**Table 2**, **23-26**) improved solubility, albeit with a decrease in potency. The side chain with morpholine (**Table 2**, **26**) offered a favorable balance between solubility and Mtb MIC. The pK_a of the terminal nitrogen played an important role in balancing MIC and solubility. Polar groups like carboxylic acid, urea, sulfonamide, acylsulfonamide (**Table 2**, **27-30**) did not improve solubility while maintaining a lower MIC.

Table 2: SAR exploration of aryl ring at R_2



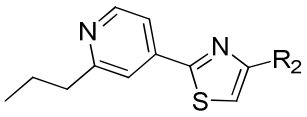
No.	R2	MIC µg/mL	Solubility µM	ClogP
11		1	<3.2	4.8
12		1	.4	5.3
13		1	0.2	5.0
14		1	1	5.0
15		2	1	5.0
16		1	<3.2	4.8
17		0.5	1	4.8

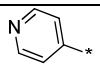
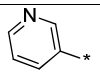
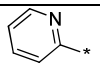
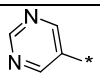
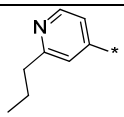
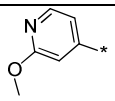
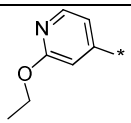
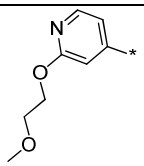
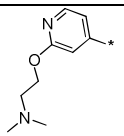
18		1	1	4.3
19		1	<2.9	5.7
20		0.5	0.15	5.7
21		> 32	0.37	5.7
22		0.25	<1	5.8
23		1	<1	4.7
24		16	278.8	4.9
25		8	154.6	5.6
26		1	29.8	5.0
27		16	21.6	4.6
28		1	0.2	4.5
29		0.4	1.3	3.8
30		32	>5000	3.5

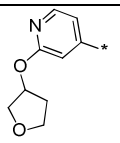
The results from this initial DMTA cycles (**Tables 1** & **2**) established the potential of the DAT class as anti-mycobacterial agents with opportunities to reach sub-micromolar Mtb MIC. However, the attempt to reduce logD and hence, improve solubility with polar side

chains led to weaker cellular activity. In subsequent DMTA cycles we replaced the phenyl group at R_2 with heteroaryl rings such as pyridine, pyrimidine as shown in **Table 3**.

Table 3: Substituted heterocylic rings at R_2



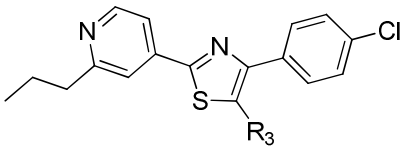
No.	R_2	MIC µg/mL	Solubility (µM)	ClogP
31		1.4	91.5	3.4
32		1.6	116.9	3.4
33		4	51.1	3.6
34		4	59.2	2.4
35		0.25	17.6	5.0
36		0.2	15.6	4.2
37		0.06	2.6	4.7
38		0.3	27.3	4.1
39		8	NA	4.3

40		2	9.0	4.0

The results from the simple pyridyl and pyrimidine derivatives (**Table 3**, **31-34**) were very encouraging. Interestingly, all the 3 possible pyridyl derivatives were active with, 3-pyridyl and 4-pyridyl being more potent than 2-pyridyl. The introduction of the heterocycles dramatically improved solubility for compounds showing Mtb MIC values of 4 μg/mL or lower. The alkyl or alkyl ether substituted pyridyl led to a significant improvement in potency (**Table 3**, **35-38**). Further extension of the side chain on the pyridyl group with a basic group (**39**) or carbocyclic ring (**40**) did not give any additional advantage for potency.

Further assuming planar nature of the molecules thus far may contribute to lower solubility, substitutents were added on the 4-position of the central thiazole ring to break the planarity and hence improve solubility. The polar groups such as amide, acid and ester (**Table 4**, **42-44**) led to weaker anti-Mtb activity whereas a methyl group which led to an MIC of 4 μg/mL (**Table 4**, **41**), however, did not improve solubility. Changing aryl substitution at thiazole ring from C-4 of thiazole to C-5, resulted in weaker Mtb MIC (**Figure 2**, **45-46**). Replacement of thiazole ring with relatively polar thiadiazole (**47**, **Figure 2**) also led to loss of potency.

Table 4: SAR exploration at Ring A.



No.	R_3	MIC μg/mL	Solubility μM	ClogP

41	-Me	4	<1	5.7
42	-COOEt	>32	<1	6.1
43	-$CONH_2$	> 32	0.5	4.2
44	-COOH	> 32	<1	4.9

The replacement of propyl with ethyl at R_1 aryl ring showed hints of improvement in solubility (matched pairs: **1** vs **2**) while retaining the Mtb MIC. The ethyl derivative **(Figure 3B, 48)** exhibited an optimal balance of physicochemical properties and Mtb MIC. The ALE for compound **48** was high (0.41) and improved by a margin of 0.5 log unit from the starting compound **1**.

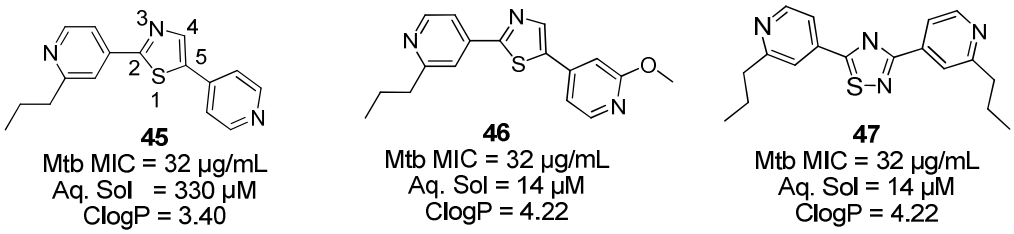
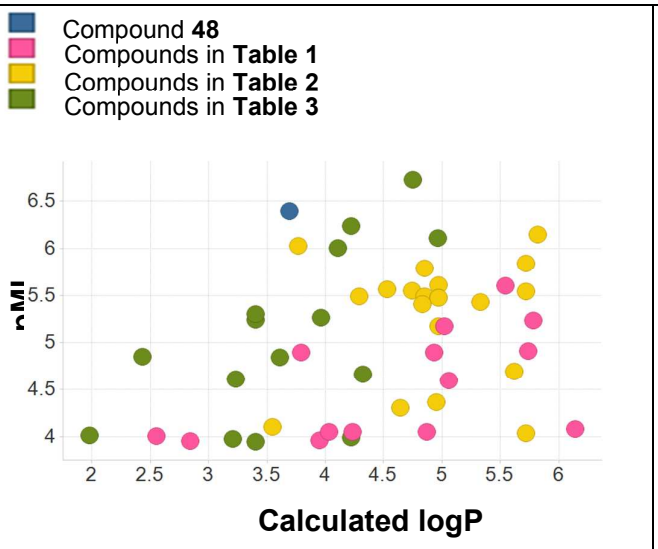


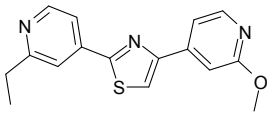
Figure 2: Limited SAR exploration at the central thiazole ring

The scatter plot of clogP and Mtb MIC (in log scale) is shown in **Figure 3A**. The distribution clearly indicates that the cellular activity was not linked with lipophilicity. Our medicinal chemistry efforts to decrease ClogP via introduction of polar groups (**Table 1-2**) and replacement of phenyl ring with heteroaromatic rings (**Table 3**) have culminated in finding the right balance. The last DMTA cycle (**Figure 3A**; green circles in the scatter plot) led us to compounds with lower lipophilicity, higher solubility (**Table 3**) and higher anti-

mycobacterial activity. These compounds were used for further microbiology and MoA experiments that are discussed in the following section.



A



48
Mtb MIC = 0.12 µg/mL
Mtb MBC = 0.5 µg/mL
ALE = 0.41
Aq. Solubility = 31 µM
$SI_{cytotox}$ > 50
hERG IC50 > 33 µM

B

Figure 3: **A:** Scatter plot of calculated logP and Mtb MIC (in logarithm scale). **B:** Structure and data for compound **48.**

The *in vitro* DMPK properties for a few selected compounds from the series are given in **Table 5**. DAT as a series showed low *in vitro* intrinsic human microsomal clearance (Cl_{int}) and high mouse microsomal clearance. The mouse plasma protein unbound fraction has improved significantly as we optimized the series, compound **48** being the best with 17 % free PPB (**Table 5**). This could largely be attributed to lowering of lipophilicity. Similarly, aqueous solubility of the compounds, which was one of major challenges during the early stage of lead generation (**Table 1**), improved as we progressed. Compound **48** turned out to be the best in terms of Mtb MIC and physico-chemical properties.

Table 5: *in vitro* DMPK properties of DAT

No.	Mtb MIC (μg/ml)	ClogP	Solubility (μM)	Mouse PPB (% free)	Mouse CLint (μl/min/mg)	Human CLint (μl/min/mg)
29	0.4	3.77	1.3	<1	28.84	4.3
32	1.6	3.4	120	0.56	174.2	55.6
33	4	3.61	51	1.2	279.3	30.2
36	0.2	4.22	16	1.3	297	14.3
37	0.06	4.75	2.6	0.09	171.7	13.6
38	0.3	4.11	27	1.6	170.4	23
48	0.12	3.69	31	17	170.4	ND

Microbiological properties

As discussed above, DAT as a series shows potent MICs against Mtb (best MIC observed 0.1 μg/ml, ~45 compounds with MIC ≤1 μg/ml). The minimum bactericidal concentration (MBC) was found to be excellent with an MBC/MIC ratio ~1-2 (**Table 6).**

Table 6: MIC vs MBC of representative compounds

No.	MIC (μg/mL)	MBC (μg/mL)	MBC / MIC
25	4.9	4.9	1
26	2.6	5.2	2
31	0.4	0.8	2
37	0.1	0.1	1
38	0.6	1.2	2
48	0.25	0.5	2

Treatment of ~10^6 Mtb cells/mL for 10 days with compound **31** led to a ~3 Log_{10} CFU/mL reduction. Compound **31** was also active in an *in vitro* intracellular THP-1 TB infection

model, a cell line model to assess activity on Mtb residing inside differentiated THP-1 monocytic cells (**Figure 4**). Compound **31** showed ~1.5 Log_{10}CFU/mL reduction in 7 days when treated with 2 fold of MIC.

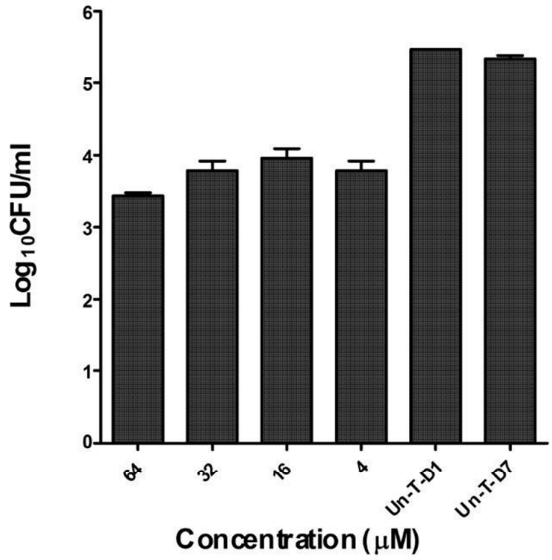


Figure 4: Potency of Compound **31** in intracellular THP1 macrophage model. Un-T-D1 and Un-T-D7 refers to bacterial counts on day 1 and day 7 with no drug treatment respectively.

Mode of action:

To understand if DAT compounds act by a mechanism distinct from INH and RIF, compounds were tested for inhibition of InhA and RNA polymerase, the respective targets of the two frontline drugs, using phenotypic and biochemical assays. Representative compounds including **31**, were tested for MIC modulation against H37Rv over-expressing InhA enzyme compared to wildtype. One of the evidences for target inhibition leading to cell growth arrest is by an upshift of MIC on expressing higher levels of the target protein in Mtb cells compared to wild type. For DAT compounds no MIC modulation was observed on overexpression of InhA (**Supplementary Table 1**). Whereas under similar experimental

conditions an upshift of MIC by 8-16 fold for H37Rv overexpressing InhA vs wildtype was observed for InhA inhibitors i.e. isoniazid, ethionamide and triclosan. Compound **31**, used as a representative compound of DAT series, also did not inhibit InhA enzymatic assay (IC_{50}s >50 μM). Hence, these observations ruled out InhA as the target for DATs. Further compound **31** was tested in an *in vitro* transcription assay and was found to be inactive at concentrations tested (IC_{50}s >50 μM), ruling out RNA polymerase as the target. Rifampicin, a frontline TB drug which acts by inhibiting RNA polymerase, was used as positive control in the assay. It inhibited the enzyme with a tight binding kinetics and showed IC_{50} of 30 nM under the experimental conditions. Taken together, the above results indicate that having a mechanism different from INH and RIF will enable the compounds to inhibit MDR TB strains, which are classified as TB isolates resistant to front line regimen primarily INH and RIF. In line with the above observations, the compounds were active against drug-sensitive and isoniazid (INH) and rifampicin (RIF) resistant Mtb clinical isolates, further strengthening the distinct mechanism for DAT (**Table 7**),

Table 7: MIC against clinical isolates

Mtb strains	**17**	**22**	**26**	**29**	**36**	**37**	**38**	**48**	INH	RIF
	μg / mL									
ATCC27294	0.25	0.5	2	0.25	0.13	0.13	0.25	0.25	0.06	0.007
ATCC25618	0.25	0.5	2	0.13	0.13	0.06	0.13	0.13	0.06	0.003
Erdman	0.25	0.5	1	0.13	0.25	0.5	0.5	0.13	0.06	0.003
Beijing	0.13	0.25	1	0.13	0.13	0.13	0.13	0.13	0.06	0.003
Harlingen	0.13	0.25	0.5	0.13	0.13	0.06	0.13	0.13	0.06	0.003
ATCC35811	0.5	1	1	0.5	0.5	0.25	0.5	0.5	0.06	0.003
DKU76	1	1	1	1	0.25	0.25	0.5	1	0.06	0.003
DKU97A	0.5	0.5	1	0.5	0.25	0.5	0.5	0.5	0.06	0.003
DKU211	1	0.5	1	0.5	0.5	0.5	2	0.5	0.06	0.003

DKU220	1	0.5	2	0.5	0.5	0.5	0.5	0.5	0.06	0.003
JAL	0.5	0.5	1	0.25	0.25	0.5	0.5	0.25	0.06	0.003
Inh^R	1	1	1	0.5	0.25	0.13	0.25	0.5	>16	0.003
Rif^R	0.5	0.13	0.5	0.13	0.25	0.13	0.13	0.13	0.03	>16

The MIC was determined following drug exposure for 7 days and growth was monitored by turbidometry. Inh: isoniazid; Rif: rifampin

To map the target of DAT compounds, resistant Mtb H37Rv mutants were raised to compound **31**. Mutants were isolated in the presence of 4, 8 and 16 fold of MIC of compound on solid media at an observed spontaneous resistant frequency of ~10^{-7}, 10^{-8} and 10^{-9} respectively. MIC of **31** for resistant strains was 8-32 fold higher than that of wildtype H37Rv (**Table 8**). Comparable levels of resistance was also observed with other compounds in the series, including compound **48**. This strongly suggests that the compounds in the series act by a common mechanism (**Table 8**). Whole genome sequencing of the representative resistant mutant strains revealed point mutations in the *prrB* gene (Rv0902c). *prrB* was the only gene which shows mutations across all resistant clones sequenced. The point mutations resulted in amino acid substitutions R148C, R148P, R149W, and L152P (**Table 9)**.

PrrBA, a two component system composed of PrrB histidine kinase and PrrA response regulator has been shown to be critical for viability of Mtb cells and is required for initial phase of macrophage infection by Mtb.[20, 21] It is conserved among all mycobacterial species pointing towards its critical function in mycobacterial physiology.[10] Moreover, PrrBA operon is transcribed during logarithmic growth of Mtb, while expression is decreased in stationary phase. Among various stress conditions, *prrBA* transcription is specifically induced under nitrogen limitation, indicating that the operon may be required for stress-induced metabolic adaptation.[20] Mtb PrrB partial structures have been solved, which included the HAMP linker

relative to the ATP binding and dimerization domains and the catalytic domain alone.[22] However the amino acids mutated in resistant strains of compound **31** were not part of the crystal structures reported. The residues are adjacent to the transmembrane domain spanning the membrane separating the C-terminal cytosolic region from N-terminal domain on the outside. Further investigations are required to understand how PrrB mutations impact the functioning of the two component system.

Table 8: MIC modulation for resistant strains

Fold shift in MIC (first mutant generation experiment)			**Fold shift in MIC for resistant strains (Second mutant generation experiment)**				
Compounds	**1024_1**	**1024_18**	Compounds	**1024_18**	**1024_8.12**	**1024_16.5**	**1024_16.6**
Isoniazid	1	1	Isoniazid	1	1	1	1
Rifampicin	0.5	0.5	Moxifloxacin	0.5	1	1	1
Moxifloxacin	1	1	TMC-207	0.25	0.25	0.5	0.5
Linezolid	1	1	**31**	8	8	16	16
TMC-207	1	0.5	**26**	8	8	4	>8
31	16	8	**12** (fatostatin)	16	16	16	>32
12 (fatostatin)	8	16	**34**	>8	>8	>8	>8
34	>8	>8	**48**	8	8	8	64

Table 9: Mutations in Compound **31** resistant strains

Resistant Clone	***prrB* nucleotide mutation**	**PrrB amino acid mutation**
1024_1	444, 445 CC -> TT	R149W
1024_18	442 C -> T	R148C
1024_8.12	455 T -> C	L152P

1024_16.5	455 T -> C	L152P
1024_16.6	443 G -> C	R148P

Fatostatin

Fatostatin (125B11), initially identified as anti-obesity molecule has been also shown to have anticancer and antidiabetic properties.[23,24, 25] The molecule in preclinical development has been shown to inhibit activation of sterol regulatory element-binding protein (SREBP), a key regulator that controls the biosynthesis of fatty acids and sterols in human cells.[14] Transcriptome analysis reveals that fatostatin treatment reduces activity of 63 genes, 34 involved in fatty acid and cholesterol biosynthesis in the cells.[25] The compound inhibits SREBs by binding to SCAP (SREBP cleavage-activating protein) and preventing it from interacting with SREB.[14,25] As mentioned above, fatostatin, a DAT series compound (**12**) has antimycobacterial activity (MIC ~1 μg/mL). Hence, here we report a novel activity of fatostatin (**12).** To understand if **12** behaves similarly to other DAT molecules, it was tested against compound **31** resistant strains containing the PrrB point mutation. An increase in MIC of **12** (fatostatin) was observed against resistant strains compared to wildtype Mtb. Thus, **12** behaved like other DAT compounds (**Table 8**). Since, it is already known that fatostatin binds to SCAP in humans cells, we searched for Mtb homologues of SCAP, however, no proteins with significant homology could be identified. Sequence comparison of PrrB and SCAP does not show significant similarity.

Transcriptome analysis

The absence of SCAP in Mtb and mapping SNPs in the *prrB* gene suggested that DAT compound **31** has a different target in Mtb. In order to further understand the mechanism of action and the potential downstream effects this compound may exert on the metabolism of

Mtb, we undertook transcriptomic analysis of Mtb exposed to compound **31**. Changes in the cellular transcriptome upon treatment with compound **31** were mapped using DNA microarray analysis. Mtb cells were exposed for 4 hours to 5X MIC of the compound. Total RNA isolated from these cells were subjected to DNA microarray analysis using RNA from untreated Mtb culture as control. Genespring GX analysis of the microarray data identified 379 up-regulated genes and 552 down-regulated genes. Cells responded to depletion in the levels of PrrB by significantly inducing the expression of a number of genes involved in fatty acid metabolism and genes required for maintenance of electron transport chain (ETC) in an anaerobic state (genes encoding subunits of cytochrome ubiquinol oxidase, fumarate reductase and nitrate reductase). Induced genes also included many oxido-reductases along with a few genes involved in iron transport (**Supplementary Table 2**). *prrA* and *prrB* genes were moderately induced with a mean fold change of ~0.7 as compared to untreated control (therefore doesn't figure in the top 50 induced genes list – **Supplementary Table 2**). Since the operon is autoregulated[21], one could expect an elevated expression from this operon when the functional protein levels deplete in the cell. However, the moderate induction observed could be due to the experimental conditions used, i.e., 4 hours of compound exposure which is probably not sufficient enough to exert the auto-regulatory effect. This is similar to an observation made earlier where in no induction of *prrA* and *prrB* expression was seen 4 hours post shifting cells to nitrogen limiting conditions, however, 5-7 fold induction was observed after 4 days.[20] Induction of genes involved in lipid metabolism and ETC of the anaerobic state indicate the cellular response to modulation in the functional PrrB levels as found in intracellular bacteria in the initial phases of infection.[21] Most genes belonging to RNA metabolism and protein synthesis were down regulated. Genes encoding RNA polymerase subunits, sigma factors with the exception of *sigJ*, ribosomal protein synthesis, aminoacyl t-RNA synthetases were among the genes that were significantly down regulated

(**Supplementary Table 2**) and thus, would result in overall reduction in cellular metabolism and energy requirement in the cell.

Conclusion

Diarylthiazole is reported as a lead series with potent anti-mycobacterial activity and has the potential to be developed for treatment of drug sensitive and drug resistant TB. The systematic medicinal chemistry exploration has resulted in compounds with desired physico-chemical properties. The resistance mapping indicates that DAT may be acting through the PrrBA two component system. Understanding the underlying mechanism of *prrB* mutations imparting resistance to DAT compounds, could uncover a completely new target class for discovery of antimycobacterials and build new knowledge around role of PrrBA in Mtb physiology.

EXPERIMENTAL SECTION

Chemical reagents and synthesis

All commercial reagents and solvents were used without further purification. Flash column chromatography was performed using the Combiflash Isco purification system with SiO_2 60-120 for loading and ready-made silica columns form Isco. Analytical thin-layer chromatography (TLC) was performed on SiO_2 plates on alumina. Visualization was by UV irradiation at 254 and 220 nm. The purity of all final derivatives for biological testing was confirmed to be >95%, as determined using the following conditions: a Shimadzu HPLC instrument with a Hamilton reversedphase column (HxSil, C18, 3 μm, 2.1 mm × 50 mm (H_2)). Eluent A, 5% CH_3CN in H_2O; eluent B, 90% CH_3CN in H_2O. A flow rate of 0.2 mL/min was used with UV detection at 254 and 214 nm. The structure of the intermediates and end products was confirmed by 1H NMR and mass spectroscopy. ^{1}H NMR spectra were

recorded on dilute solutions in $CDCl_3$ or DMSO-d_6 or MeOD-d_4 using Bruker DRX- 300 or Bruker DRX-400 spectrometers operating at 300 or 400 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS, and coupling constants (J) are reported in Hz. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). LCMS data was acquired using Agilent LCMS VL series. Source: ES ionization coupled with an Agilent 1100 series HPLC system and an Agilent 1100 series PDA as the front end. HRMS data was acquired using an Agilent 6520, quadrupole time-of-flight tandem mass spectrometer (Q-TOF MS/MS) coupled with an Agilent 1200 series HPLC system.

General Procedure for Synthesis of DAT Compounds

A mixture of 2-substituted pyridine-4-carbothioamide (0.65 mmol) and substituted heteroarylacyl bromide (0.65 mmol) in ethanol (10 mL) was stirred for overnight at 80 ^{0}C. The volatiles were removed by Rota evaporator at low pressure, the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate. The combined extracts were washed with water, and dried over anhydrous sodium sulphate and concentrated to produce the crude compound, which was purified by combiflash column chromatography to get 4-aryl-2-(2-substitutedpyridin-4-yl)thiazole.

4-(4-Chlorophenyl)-2-(propylpyridin-4-yl)thiazole (**1**).Yield : 42%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.00 (t, *J*=7.28 Hz, 3H), 1.74 (sxt, *J*=14.68, 7.49 Hz, 2H), 2.95 (t, *J*=7.47 Hz, 2H), 7.47 (t, *J*=8.78 Hz, 2H), 7.75 (d, *J*=5.02 Hz, 1H), 7.83 (s, 1H), 8.13 (dd, *J*=8.78, 5.77 Hz, 2H), 8.38 (s, 1H), 8.67 (d, *J*=5.52 Hz, 1H). HRMS: *m/z* (ES+) = 315.07016 (MH^+) for $C_{17}H_{15}ClN_2S$. HPLC purity: 98.00%

4-(4-Chlorophenyl)-2-(ethylpyridin-4-yl)thiazole (**2**).Yield : 82%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.37 (t, *J*=7.54 Hz, 3H), 3.06 (d, *J*=7.54 Hz, 2H), 7.59 (d, *J*=8.67 Hz, 2H), 8.15-8.22 (m, *J*=8.48

Hz, 2H), 8.29 (s, 1H), 8.36 (s, 1H), 8.62 (s, 1H), 8.85 (d, *J*=5.84 Hz, 1H). HRMS: *m/z* (ES+) = 300.04878 (MH^+) for $C_{16}H_{13}ClN_2S$. HPLC purity: 98.00%

4-(4-Chlorophenyl)-2-(cyclopropylpyridin-4-yl)thiazole (**3**).Yield : 76%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.92 - 1.09 (m, 4H), 2.28 (q, *J*=6.40 Hz, 1H), 7.57 (m, *J*=8.67 Hz, 2H), 7.71 (d, *J*=5.27 Hz, 1H), 7.91 (s, 1H), 8.11 (d, *J*=8.48 Hz, 2H), 8.42 (s, 1H), 8.55 (d, *J*=5.09 Hz, 1H). HRMS: *m/z* (ES+) = 313.05684 (MH^+) for $C_{17}H_{13}ClN_2S$. HPLC purity: 98.50%

4-(4-Chlorophenyl)-2-(methoxymethylpyridin-4-yl)thiazole (**4**).Yield : 37%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.45 (s, 3H), 4.61 (s, 2H), 7.58-7.65 (m, 2H), 7.88 - 7.92 (m, 1H), 7.97 (s, 1H), 8.11 (m, *J*=9.03 Hz, 2H), 8.45 (s, 1H), 8.69 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES^+) = 317.05185 (MH^+) for $C_{16}H_{13}ClN_2OS$. HPLC purity: 97.80%

4-(4-Chlorophenyl)-2-(ethylaminopyridin-4-yl)thiazole (**5**).Yield : 80%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.18-1.13 (t, *J*=7.16 Hz, 3H), 3.29 - 3.36 (m, 2H), 6.84 (t, *J*=5.27 Hz, 1H), 6.98 - 7.11 (m, 2 H), 7.56 (d, *J*=8.48 Hz, 2H), 8.02 - 8.14 (m, 3H), 8.34 (s, 1H). HRMS: *m/z* (ES^+) = 316.06753 (MH^+) for $C_{16}H_{14}ClN_3S$. HPLC purity: 99.40%

4-(4-Chlorophenyl)-2-(morpholinopyridin-4-yl)thiazole (**6**).Yield : 47%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.56-3.59 (m, 4H), 3.74-3.79 (m, 4H), 7.17 - 7.30 (m, 1H), 7.34 (s, 1H), 7.56 (d, *J*=7.72 Hz, 2H), 8.11 (d, *J*=7.54 Hz, 2H), 8.20 - 8.33 (m, 1H), 8.37 (s, 1H). HRMS: *m/z* (ES^+) = 358.07846 (MH^+) for $C_{18}H_{16}ClN_3OS$. HPLC purity: 98.00%

4-(4-Chlorophenyl)-2-(cyclopropylmethoxypyridin-4-yl)thiazole (**7**).Yield : 51%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.30-0.40 (m, 2H), 0.50-0.60 (m, 2H), 1.20-1.30 (m, 1H), 4.15 (d, *J*=21 Hz, 2H), 7.40 (s, 1H), 7.50-7.60 (m, 3H), 8.10 (d, *J*=30 Hz, 2H), 8.30 (d, *J*=21 Hz, 1H), 8.45 (s, 1H). HRMS: *m/z* (ES+) = 343.06698 (MH^+) for $C_{18}H_{15}ClN_2OS$. HPLC purity: 98.00%

4-(4-(4-Chlorophenyl)thiazol-2-yl)pyridin-2-ol (**8**).Yield : 19.42%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.80 (d, *J*=6.02 Hz, 1H), 6.92 (s, 1H), 7.56 (d, *J*=8.53 Hz, 3H), 8.08 (d, *J*=8.53 Hz, 2H), 8.44 (s, 1H), 11.86 (br. s., 1H). HRMS: *m/z* (ES^+) = 289.02001 (MH^+) for $C_{14}H_{19}ClN_2OS$. HPLC purity: 98.00%

4-(4-Chlorophenyl)-2-(1-methylpiperidin-4-yl)thiazole (**9**).Yield : 29.4%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.72 - 1.82 (m, 2H), 2.08 (d, *J*=10.54 Hz, 4H), 2.23 (s, 3H), 2.87 (d, *J*=11.04 Hz, 2H), 3.01 (m, 1H), 7.51 (d, *J*=8.53 Hz, 2H), 7.98 (d, *J*=8.53 Hz, 2H), 8.07 (s, 1H). HRMS: *m/z* (ES^+) = 293.08842 (MH^+) for $C_{15}H_{17}ClN_2S$. HPLC purity: 99.00%

1-(4-(4-(4-Chlorophenyl)thiazol-2-yl)piperidin-1-yl)ethanone (**10**).Yield : 57.9%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.55 (qd, *J*=12.13, 4.27 Hz, 1H), 1.65 - 1.78 (m, 1H), 2.01 - 2.16 (m, 5H), 2.65 -

2.80 (m, 1H), 3.15 - 3.28 (m, 2H), 3.91 (d, *J*=14.05 Hz, 1H), 4.44 (d, *J*=13.05 Hz, 1H), 7.50 (m, *J*=8.53 Hz, 2H), 7.98 (m, *J*=8.53 Hz, 2H), 8.08 (s, 1H). HRMS: *m/z* (ES^+) = 321.08338 (MH^+) for $C_{16}H_{17}ClN_2OS$. HPLC purity: 99.80%

4-Phenyl-2-(2-propylpyridin-4-yl)thiazole (**11**).Yield: 62.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.00 (t, *J*=7.26 Hz, 3H), 1.70-1.90 (m, 2H), 2.95-3-05 (m, 2H), 7.40-7.50 (m, 1H), 7.55-7.60 (m, 2H), 8.05-8.10 (m, 2H), 8.35 (d, *J*=8.53 Hz, 1H), 8.45 (s, 1H), 8.60 (s, 1H), 8.90 (d, *J*=8.65 Hz, 1H). HRMS: *m/z* (ES+) = 280.10339 (MH^+) for $C_{17}H_{16}N_2S$. HPLC purity: 99.60%

2-(2-Propylpyridin-4-yl)-4-p-tolylthiazole (**12**). Yield: 28.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.76 (sxt, *J*=7.42 Hz, 2H), 2.37 (s, 3H), 2.83 (t, *J*=7.54 Hz, 2H), 7.31-7.35 (m, 2H), 7.74 - 7.86 (m, 2H), 7.97-8.05 (m, 2H), 8.26 (s, 1H), 8.63 (d, *J*=5.09 Hz, 1H). HRMS: *m/z* (ES^+) = 295.12529 (MH^+) for $C_{18}H_{18}N_2S$. HPLC purity: 99.60%

4-(4-Fluorophenyl)-2-(2-propylpyridin-4-yl)thiazole (**13**).Yield: 42.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.28 Hz, 3H), 1.76 (dq, *J*=14.68, 7.49 Hz, 2H), 2.83 (t, *J*=7.53 Hz, 2H), 7.34 (t, *J*=8.78 Hz, 2H), 7.79 (d, *J*=5.02 Hz, 1H), 7.84 (s, 1H), 8.13 (dd, *J*=8.78, 5.77 Hz, 2H), 8.35 (s, 1H), 8.64 (d, *J*=5.52 Hz, 1H). HRMS: *m/z* (ES+) = 299.10155 (MH^+) for $C_{17}H_{15}FN_2S$. HPLC purity: 98.00%

4-(3-Fluorophenyl)-2-(2-propylpyridin-4-yl)thiazole (**14**).Yield: 72.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.28 Hz, 3H), 1.76 (sxt, *J*=7.43 Hz, 2H), 2.83 (t, *J*=7.53 Hz, 2H), 7.21 - 7.33 (m, 1H), 7.49 - 7.63 (m, 1H), 7.80 (d, *J*=5.52 Hz, 1H), 7.83 - 8.03 (m, 3H), 8.48 (s, 1H), 8.65 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 299.10137 (MH^+) for $C_{17}H_{15}FN_2S$. HPLC purity: 99.00%

4-(2-Fluorophenyl)-2-(2-propylpyridin-4-yl)thiazole (**15**).Yield: 30.00%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.28 Hz, 3H), 1.76 (dq, *J*=15.00, 7.38 Hz, 2H), 2.83 (t, *J*=7.53 Hz, 2H), 7.34 - 7.53 (m, 3H), 7.78 - 7.89 (m, 2H), 8.20 - 8.33 (m, 2H), 8.65 (d, *J*=5.02 Hz,1H). HRMS: *m/z* (ES+) = 299.10153 (MH^+) for $C_{17}H_{15}FN_2S$. HPLC purity: 96.00%

4-(4-Methoxyphenyl)-2-(2-propylpyridin-4-yl)thiazole (**16**).Yield: 42.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.97 (t, *J*=7.35 Hz, 3H), 1.80 (sxt, *J*=7.42 Hz, 2H), 2.83 (t, *J*=7.54 Hz, 2H), 3.80 (s, 3H), 7.35 (m, *J*=8.10 Hz, 2H), 7.78 - 7.88(m, 2H), 7.99 (m, *J*=8.10 Hz, 2H), 8.30 (s, 1H), 8.70 (d, *J*=5.09 Hz, 1H): HRMS: *m/z* (ES+) = 310.11396 (MH^+) for $C_{18}H_{18}N_2OS$. HPLC purity: 96.30%

4-(3-Methoxyphenyl)-2-(2-propylpyridin-4-yl)thiazole (**17**). Yield: 58.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (t, *J*=7.28 Hz, 3H), 1.75 (sxt, *J*=7.43 Hz, 2H), 2.82 (t, *J*=7.53 Hz, 2H), 3.85 (s, 3H), 6.98 (dd, *J*=8.03, 2.51 Hz, 1H), 7.41 (t, *J*=8.03 Hz, 1H), 7.59 - 7.69 (m, 2H), 7.76 - 7.87 (m, 2H),

8.38 (s, 1H), 8.63 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 310.11399 (MH^+) for $C_{18}H_{18}N_2OS$. HPLC purity: 99.00%

4-(2-Methoxyphenyl)-2-(2-propylpyridin-4-yl)thiazole (**18**). Yield: 43.3%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.28 Hz, 3H), 1.76 (sxt, *J*=7.43 Hz, 2H), 2.76 - 2.87 (m, 2H), 3.96 (s, 3H), 7.03 - 7.16 (m, 1H), 7.20 (d, *J*=8.53 Hz, 1H), 7.34 - 7.46 (m, 1H), 7.75 - 7.87 (m, 2H), 8.25 - 8.36 (m, 2H), 8.63 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 310.11397(MH^+) for $C_{18}H_{18}N_2OS$. HPLC purity: 99.00%

2-(2-Propylpyridin-4-yl)-4-(4-(trifluoromethyl)phenyl)thiazole (**19**). Yield: 42.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.96 (t, *J*=7.35 Hz, 3H), 1.81 (sxt, *J*=7.46 Hz, 2H), 3.03 (t, *J*=7.63 Hz, 2H), 8.08 - 8.12 (m, 2H), 8.25 - 8.32 (m, 2H), 8.38 (d, *J*=5.65 Hz, 1H), 8.46 (br. s., 1H), 8.80 (s, 1H), 8.92 (d, *J*=6.03 Hz, 1H). HRMS: *m/z* (ES+) = 348.09077 (MH^+) for $C_{18}H_{15}F_3N_2S$. HPLC purity: 97.50%

2-(2-Propylpyridin-4-yl)-4-(3-(trifluoromethyl)phenyl)thiazole (**20**).Yield: 48.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.53 Hz, 3H), 1.69 - 1.85 (m, 2H), 2.83 (t, *J*=7.53 Hz, 2H), 7.72 - 7.89 (m, 4H), 8.38 - 8.44 (m, 2H), 8.60 - 8.69 (m, 2H). HRMS: *m/z* (ES+) = 348.09081 (MH^+) for $C_{18}H_{15}F_3N_2S$. HPLC purity: 98.00%

2-(2-Propylpyridin-4-yl)-4-(2-(trifluoromethyl)phenyl)thiazole (**21**). Yield: 30.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.92 (t, *J*=7.28 Hz, 3H), 1.73 (sxt, *J*=7.43 Hz, 2H), 2.80 (t, *J*=7.53 Hz, 2H), 7.65 - 7.83 (m, 5H), 7.90 (d, *J*=7.53 Hz, 1H), 8.04 (s, 1H), 8.62 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 348.09078 (MH^+) for $C_{18}H_{15}F_3N_2S$. HPLC purity: 99.30%

4-(4-(Cyclopropylmethoxy)phenyl)-2-(2-propylpyridin-4-yl)thiazole (**22**).Yield: 50.0%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm 0.33 - 0.37 (m, 2H), 0.56 - 0.61 (m, 2H), 0.94 (t, *J*=7.53 Hz, 3H), 1.23-1.26 (m, 1H), 1.75 (sxt, *J*=7.43 Hz, 2H), 2.82 (t, *J*=7.53 Hz, 2H), 3.87 - 3.88 (m, 2H), 7.03 (d, *J*=8.53 Hz, 2H), 7.75 - 7.77 (m, 2H), 8.01 (d, *J*=9.03 Hz, 2H), 8.20 (s, 1H), 8.63 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 351.14529 (MH^+) for $C_{21}H_{22}N_2OS$. HPLC purity: 98.00%

4-(4-(2-Methoxyethoxy)phenyl)-2-(2-propylpyridin-4-yl)thiazole (**23**). Yield: 57.8 %. ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 0.95 (t, *J*=7.53 Hz, 3H), 1.76 (sxt, *J*=7.43 Hz, 2H), 2.82 (t, *J*=7.53 Hz, 2H), 3.33 (s, 3H), 3.66 - 3.74 (m, 2H), 4.11 - 4.20 (m, 2H), 7.07 (d, *J*=8.53 Hz, 2H), 7.75 - 7.86 (m, 2H), 8.01 (d, *J*=9.03 Hz, 2H), 8.20 (s, 1H), 8.63 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 355.1477 (MH^+) for $C_{20}H_{22}N_2O_2S$. HPLC purity: 98.90%

N,N-Dimethyl-2-(4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)phenoxy)ethanamine (**24**). Yield: 81.0%. ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 0.94 (t, *J*=7.35 Hz, 3H), 1.75 (sxt, *J*=7.38 Hz, 2H), 2.24 (s, 6H), 2.66 (t, *J*=5.56 Hz, 2H), 2.82 (t, *J*=7.54 Hz, 2H), 4.11 (t, *J*=5.75 Hz, 2H), 7.06 - 7.15 (m, 2H), 7.72 -

7.87 (m, 2H), 8.00-8.05 (m, 2H), 8.19 (s, 1H), 8.63 (d, *J*=5.09 Hz, 1H). HRMS: *m/z* (ES+) = 368.17946 (MH^+) for $C_{21}H_{25}N_3OS$. HPLC purity: 96.80%

2-(2-Propylpyridin-4-yl)-4-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)thiazole (**25**). Yield: 55.0%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm 0.95 (t, *J*=7.53 Hz, 3H), 1.68 - 1.72 (m, 4H), 1.77 (sxt, *J*=7.43 Hz, 2H), 2.53 - 2.58 (m, 4H), 2.79 - 2.83 (m, 4H), 4.11 - 4.14 (m, 2H), 7.06 (d, *J*=8.53 Hz, 2H), 7.76 - 7.82 (m, 2H), 7.95 (d, *J*=9.03 Hz, 2H), 8.18 (s, 1 H), 8.68 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 394.18785(MH^+) for $C_{23}H_{27}N_3OS$. HPLC purity: 99.00%

4-(2-(4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenoxy)ethyl)morpholine (**26**). Yield: 60.0%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm 0.95 (t, *J*=7.53 Hz, 3H), 1.76 (sxt, *J*=7.43 Hz, 2H), 2.48 - 2.49 (m, 4H), 2.69 - 2.72 (m, 2H), 2.79 (t, *J*=7.53 Hz, 2H), 3.32 - 3.64 (m, 4H), 4.11 - 4.15 (m, 2H), 7.05 (d, *J*=8.53 Hz, 2H), 7.75 - 7.60 (m, 2H), 8.10 (d, *J*=9.03 Hz, 2H), 8.17 (s, 1H), 8.63 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 410.18295 (MH^+) for $C_{23}H_{27}N_3O_2S$. HPLC purity: 98.00%

4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)benzoic acid (**27**). Yield: 50 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.98 (t, *J*=7.35 Hz, 3H), 1.81 (sxt, *J*=7.46 Hz, 2H), 3.03 (t, *J*=7.63 Hz, 2H), 8.08 (m, *J*=8.48 Hz, 2H), 8.25 (m, *J*=8.48 Hz, 2H), 8.36 (d, *J*=5.65 Hz, 1H), 8.46 (br. s., 1H), 8.78 (s, 1H), 8.90 (d, *J*=6.03 Hz, 1H), 12.80 (s, 1H). HRMS: *m/z* (ES+) = 325.09964 (MH^+) for $C_{18}H_{16}N_2O_2S$. HPLC purity: 99.00%

1-Ethyl-3-(4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)phenyl)urea (**28**). Yield : 54.8%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.88 - 1.02 (m, 3H), 1.07 (t, *J*=7.16 Hz, 3H), 1.66 - 1.86 (m, 2H), 2.82 (t, *J*=7.63 Hz, 2H), 3.06 - 3.21 (m, 2H), 6.16 (t, *J*=5.65 Hz, 1H), 7.51-7.55 (m, *J*=8.67 Hz, 2H), 7.77 (d, *J*=5.27 Hz, 1H), 7.82 (s, 1H), 7.93 - 7.97 (m, 2H), 8.15 (s, 1H), 8.58 - 8.70 (m, 2H). HRMS: *m/z* (ES+) = 367.15884 MH^+) for $C_{20}H_{22}N_4OS$. HPLC purity: 98.00%

N-(4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenyl)methanesulfonamide (**29**). Yield : 55.4%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.94 (t, *J*=7.35 Hz, 3H), 1.75 (sxt, *J*=7.38 Hz, 2H), 2.82 (t, *J*=7.63 Hz, 2H), 3.05 (s, 3H), 7.32 -7.35 (m, 2H), 7.70 - 7.96 (m, 2H), 8.04 - 8.08 (m, 2H), 8.26 (s, 1H), 8.64 (d, *J*=5.27 Hz, 1H), 9.96 (s, 1H). HRMS: *m/z* (ES+) = 374.09150 (MH^+) for $C_{18}H_{19}N_3O_2S_2$. HPLC purity: 99.00%

N-(Methylsulfonyl)-4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)benzamide (**30**). Yield : 16%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.67 - 1.84 (m, 2H), 2.76 - 2.94 (m, 5H), 7.41 (br. s, 1H), 7.80 (d, *J*=4.90 Hz, 1H), 7.86 (s, 1H), 8.04 (s, 4H), 8.40 (s, 1H), 8.64 (d, *J*=5.09 Hz, 1H). HRMS: *m/z* (ES+) = 402.08696 (MH^+) for $C_{19}H_{19}N_3O_3S_2$. HPLC purity: 96.00%

2-(2-Propylpyridin-4-yl)-4-(pyridin-4-yl)thiazole (**31**). Yield: 49.9 %, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (t, *J*=7.28 Hz, 3H) 1.75 (sxt, *J*=7.43 Hz, 2H) 2.82 (t, *J*=7.53 Hz, 2H) 7.70 - 7.84 (m, 4H) 8.61 - 8.76 (m, 4H), HRMS: *m/z* (ES+) = 282.10506 (MH^+) for $C_{16}H_{15}N_3S$. HPLC purity: 99.20%

2-(2-Propylpyridin-4-yl)-4-(pyridin-3-yl)thiazole (**32**). Yield: 59.9 %, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.76 (sxt, *J*=7.42 Hz, 2H), 2.83 (t, *J*=7.54 Hz, 2H), 7.54 (dd, *J*=8.01, 4.80 Hz, 1H), 7.77 - 7.90 (m, 2H), 8.43 (dt, *J*=8.01, 1.84 Hz, 1H), 8.52 (s, 1H), 8.58 - 8.69 (m, 2H), 9.29 (d, *J*=1.51 Hz, 1H). HRMS: *m/z* (ES+) = 282.10489 (MH^+) for $C_{16}H_{15}N_3S$. HPLC purity: 99.00%

2-(2-Propylpyridin-4-yl)-4-(pyridin-2-yl)thiazole (**33**). Yield: 80.0 %, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.77 (sxt, *J*=7.42 Hz, 2H), 2.78 - 2.89 (m, 2H), 7.42 (ddd, *J*=7.54, 4.80, 1.04 Hz, 1H), 7.77 - 7.89 (m, 2H), 7.96 (td, *J*=7.72, 1.70 Hz, 1H), 8.24 (d, *J*=7.91 Hz, 1H), 8.49 (s, 1H), 8.63 - 8.71 (m, 2H). HRMS: *m/z* (ES+) = 282.10477 (MH^+) for $C_{16}H_{15}N_3S$. HPLC purity: 99.00%

2-(2-Propylpyridin-4-yl)-4-(pyrimidin-5-yl)thiazole (**34**). Yield: 46.3 %, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H) 1.77 (sxt, *J*=7.46 Hz, 2H) 2.83 (t, *J*=7.63 Hz, 2H) 7.79 - 7.94 (m, 2H) 8.61 - 8.72 (m, 2H) 9.23 (s, 1H) 9.47 (s, 2H), HRMS: *m/z* (ES+) = 283.09961 (MH^+) for $C_{15}H_{14}N_4S$. HPLC purity: 99.00%

2,4-Bis(2-propylpyridin-4-yl)thiazole (**35**). Yield: 22.29 %, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.28 Hz, 6H), 1.76 (sxt, *J*=7.43 Hz, 4H), 2.81 (dt, *J*=14.56, 7.28 Hz, 4H), 7.79 - 7.87 (m, 3H), 7.90 (s, 1H), 8.59 (d, *J*=5.02 Hz, 1H), 8.63 - 8.67 (m, 2H). HRMS: *m/z* (ES+) = 324.14588 (MH^+) for $C_{19}H_{21}N_3S$. HPLC purity: 99.00%

4-(2-Methoxypyridin-4-yl)-2-(2-propylpyridin-4-yl)thiazole (**36**). Yield: 45.6%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.76 (sxt, *J*=7.46 Hz, 2H), 2.83 (t, *J*=7.54 Hz, 2H), 3.92 (s, 3H), 7.47 (s, 1H), 7.64 (dd, *J*=5.37, 1.22 Hz, 1H), 7.80 (dd, *J*=5.09, 1.51 Hz, 1H), 7.86 (s, 1H), 8.28 (d, *J*=5.27 Hz, 1H), 8.62 - 8.69 (m, 2H). HRMS: *m/z* (ES+) = 312.10950 (MH^+) for $C_{17}H_{17}N_3OS$. HPLC purity: 99.50%

4-(2-Ethoxypyridin-4-yl)-2-(2-propylpyridin-4-yl)thiazole (**37**). Yield: 62.3%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.36 (t, *J*=7.06 Hz, 3H), 1.76 (sxt, *J*=7.42 Hz, 2H), 2.83 (t, *J*=7.54 Hz, 2H), 4.37 (q, *J*=6.97 Hz, 2H), 7.45 (s, 1H), 7.62 (dd, *J*=5.37, 1.22 Hz, 1H), 7.76 - 7.90 (m, 2H), 8.26 (d, *J*=5.46 Hz, 1H), 8.62 - 8.69 (m, 2H). HRMS: *m/z* (ES+) = 326.13073 (MH^+) for $C_{18}H_{19}N_3OS$. HPLC purity: 98.70%

4-(2-(2-Methoxyethoxy)pyridin-4-yl)-2-(2-propylpyridin-4-yl)thiazole (**38**). Yield: 65.2%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.35 Hz, 3H), 1.76 (sxt, *J*=7.42 Hz, 2H), 2.83 (t, *J*=7.54 Hz,

2H), 3.32 (s, 3H), 3.64 - 3.76 (m, 2H), 4.37 - 4.52 (m, 2H), 7.50 (s, 1H), 7.64 (dd, *J*=5.27, 1.13 Hz, 1H), 7.76 - 7.90 (m, 2H), 8.26 (d, *J*=5.27 Hz, 1H), 8.61 - 8.71 (m, 2H). HRMS: *m/z* (ES+) = 356.13560 (MH^+) for $C_{19}H_{21}N_3O_2S$. HPLC purity: 97.90%

N,N-Dimethyl-2-(4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)pyridin-2-yloxy)ethanamine (**39**). Yield: 28.6%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (t, J=7.28 Hz, 3H), 1.76 (sxt, J=7.43 Hz, 2H), 2.23 (s, 6H), 2.65 (t, *J*=5.77 Hz, 2H), 2.83 (t, *J*=7.53 Hz, 2H), 4.40 (t, *J*=5.77 Hz, 2H), 7.47 (s, 1H), 7.63 (d, *J*=5.02 Hz, 1H), 7.81 (dd, *J*=5.02, 1.00 Hz, 1H), 7.87 (s, 1H), 8.27 (d, *J*=5.52 Hz, 1H), 8.63 - 8.71 (m, 2H). HRMS: *m/z* (ES+) = 369.1746 (MH^+) for $C_{20}H_{24}N_4OS$. HPLC purity: 95.30%

(S)-2-(2-Propylpyridin-4-yl)-4-(2-(tetrahydrofuran-3-yloxy)pyridin-4-yl)thiazole (**40**). Yield: 45.9%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (t, *J*=7.53 Hz, 3H), 1.76 (sxt, *J*=7.43 Hz, 2H), 2.00 - 2.10 (m, 1H), 2.20 - 2.32 (m, 1H), 2.83 (t, J=7.53 Hz, 2H), 3.76 - 4.00 (m, 4H), 5.55 - 5.61 (m, 1H), 7.50 (s, 1H), 7.66 (dd, *J*=5.52, 1.00 Hz, 1H), 7.81 (dd, *J*=5.27, 1.76 Hz, 1H), 7.88 (s, 1H), 8.28 (d, *J*=5.52 Hz, 1H), 8.63 - 8.71 (m, 2H). HRMS: *m/z* (ES+) = 368.14291 (MH^+) for $C_{20}H_{21}N_3O_2S$. HPLC purity: 97.00%

4-(4-Chlorophenyl)-5-methyl-2-(2-propylpyridin-4-yl)thiazole (**41**). Yield: 33.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.93 (t, *J*=7.28 Hz, 3H), 1.74 (sxt, *J*=7.43 Hz, 2H), 2.65 (s, 3H), 2.80 (t, J=7.53 Hz, 2H), 7.58 - 7.62 (m, *J*=8.53 Hz, 2H), 7.68 (d, *J*=5.02 Hz, 1H), 7.74 (s, 1H), 7.81 (m, *J*=8.53 Hz, 2H), 8.61 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 329.08747 (MH^+) for $C_{18}H_{17}ClN_2S$. HPLC purity: 96.00%

Ethyl 4-(4-chlorophenyl)-2-(2-propylpyridin-4-yl)thiazole-5-carboxylate (**42**). Yield: 63.2%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.93 (t, *J*=7.35 Hz, 3H), 1.26 (t, *J*=7.06 Hz, 3H), 1.75 (sxt, *J*=7.42 Hz, 2H), 2.82 (t, *J*=7.54 Hz, 2H), 4.28 (q, *J*=7.10 Hz, 2H), 7.52 - 7.60 (m, 2H), 7.78 - 7.91 (m, 4H), 8.67 (d, *J*=5.09 Hz, 1H). HRMS: *m/z* (ES+) = 387.09305 (MH^+) for $C_{20}H_{19}ClN_2O_2S$. HPLC purity: 98.60%

4-(4-Chlorophenyl)-2-(2-propylpyridin-4-yl)thiazole-5-carboxamide (**43**). Yield : 38.6%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.93 (t, *J*=7.28 Hz, 3H), 1.74 (sxt, *J*=7.43 Hz, 2H), 2.82 (t, *J*=7.53 Hz, 2H), 7.57 (d, *J*=8.53 Hz, 2H), 7.76 (dd, *J*=5.02, 1.51 Hz, 1H), 7.79 - 7.87 (m, 3H), 7.91 (br. s., 1H), 8.06 (br. s., 1H), 8.65 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 358.07615 (MH^+) for $C_{18}H_{16}ClN_3OS$. HPLC purity: 98.30%

4-(4-Chlorophenyl)-2-(2-propylpyridin-4-yl)thiazole-5-carboxylic acid (**44**). Yield : 46.2%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.92 (t, *J*=7.28 Hz, 3H), 1.74 (sxt, *J*=7.43 Hz, 2H), 2.81 (t, *J*=7.53 Hz, 2H), 6.55 (s, 1H), 7.53 (d, *J*=8.53 Hz, 2H), 7.77 (d, *J*=5.02 Hz, 1H), 7.83 (s, 1H), 7.91 (d, *J*=7.53 Hz, 2H), 8.65 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 359.06093 (MH^+) for $C_{18}H_{15}ClN_2O_2S$. HPLC purity: 98.60%

2-(2-Propylpyridin-4-yl)-5-(pyridin-4-yl)thiazole (**45**). Yield: 31.2%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (t, *J*=7.28 Hz, 3H), 1.75 (sxt, *J*=7.43 Hz, 2H), 2.82 (t, *J*=7.53 Hz, 2H), 7.70 - 7.84 (m, 4H), 8.61 - 8.76 (m, 4H). HRMS: *m/z* (ES+) = 282.10438 (MH^+) for $C_{16}H_{15}N_3S$. HPLC purity: 98.70%

5-(6-Methoxypyridin-3-yl)-2-(2-propylpyridin-4-yl)thiazole (**46**). Yield: 47.3%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.93 (t, *J*=7.53 Hz, 3H), 1.67 - 1.78 (m, 2H), 2.80 (t, *J*=7.53 Hz, 2H), 3.90 (s, 3H), 7.19 (s, 1H), 7.35 (dd, *J*=5.52, 1.51 Hz, 1H), 7.69 - 7.79 (m, 2H), 8.25 (d, *J*=5.52 Hz, 1H), 8.64 (d, *J*=5.52 Hz, 1H), 8.69 (s, 1H). HRMS: *m/z* (ES+) = 312.11691 (MH^+) for $C_{17}H_{17}N_3OS$. HPLC purity: 99.00%

3,5-Bis(2-propylpyridin-4-yl)-1,2,4-thiadiazole (**47**). Yield : 69.9%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (t, *J*=7.28 Hz, 6H), 1.77 (sxt, *J*=7.43 Hz, 4H), 2.79 - 2.91 (m, 4H), 7.90 (dd, *J*=5.02, 1.51 Hz, 1H), 7.97 (s, 1H), 8.03 (d, *J*=5.02 Hz, 1H), 8.08 (s, 1H), 8.76 (d, *J*=5.02 Hz, 1H), 8.72 (d, *J*=5.02 Hz, 1H). HRMS: *m/z* (ES+) = 324.14087 (MH^+) for $C_{18}H_{20}N_4S$. HPLC purity: 98.00%

2-(2-Ethylpyridin-4-yl)-4-(2-methoxypyridin-4-yl)thiazole (**48**). Yield: 55.3%, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.30 (t, *J*=7.53 Hz, 3H), 2.88 (q, *J*=7.53 Hz, 2H), 3.92 (s, 3H), 7.48 (s, 1H), 7.65 (dd, *J*=5.27, 1.25 Hz, 1H), 7.81 (dd, *J*=5.02, 1.51 Hz, 1H), 7.87 (s, 1H), 8.29 (d, *J*=5.52 Hz, 1H), 8.60 - 8.72 (m, 2H). HRMS: *m/z* (ES+) = 298.09370 (MH^+) for $C_{16}H_{15}N_3OS$. HPLC purity: 99.00%

Microbiological assays:

Assays to determine MIC for H37Rv and clinical isolates, MBC under aerobic and hypoxic conditions, killing kinetics and intracellular THP-1 (human lung adenocarcinoma epithelial cell line) potency was carried out as described before.[26] Minimal inhibitory concentration (MIC) is defined as compound concentration that shows ≥80% growth inhibition compared to untreated controls. MIC variation within four fold is considered as acceptable variation between different experiments using various compound batches. Minimal bactericidal concentration (MBC) is defined as compound concentration that shows ≥2 Log_{10}CFU/mL reduction compared to untreated controls.

InhA assay

InhA enzymatic reaction was set up in a 25 µl volume containing 30 mM PIPES pH 6.8, 50 mM NaCl, 0.05% CHAPS, 2 mM DTT and 0.1 mM EDTA. 10 µl of InhA enzyme (0.3 nM final) pre-incubated with NADH (50 µM final) for 15 min. After 10 min of incubation, reaction was started by the addition of 15 µl of dodecyl coA (100 µM final) and allowed to continue for 45 min at 25 ºC. 50 µl Acetonitrile quench containing 100 ng/ml Carbamazepine as internal standard was dispensed at the end of 45 min to stop the reaction. Substrate ddcoA and product rddcoA were quantified by LC-MS/MS method. The area under curve (AUC) of ddcoA and rddcoA peaks calculated by Quantlynx software were considered for % product conversion calculations.

RNA Polymerase assay

RNA Polymerase assay buffer contains 50 mM Tris-Cl pH=8.0, 12.5 mM MgCl2, 0.1 mM DTT, 50 mM NaCl, 0.05 mM EDTA, 2% Glycerol, 50 mM Potassium Glutamate and 0.002% Brij-35. 30 µl assay contains 15 µl of enzyme mix (66 nM of RNA polymerase enzyme, 66 nM of SigmaA, 0.01 U/ml of pyrophosphatase) and 10 µg/ml of T4 phage DNA in assay buffer. The reaction was started by the addition of 15 µl of substrate mix in assay buffer containing 100 µM of each of the following nucleotide-ATP, GTP, CTP and UTP. The reaction was carried out for 2 hrs at 25 °C. At the end of the reaction, 30 µL of Baykov's reagent[27] was added and mixed well and incubated for 30 minutes at room temperature. The amount of Pi released is measured by monitoring the absorbance at 630 nm using SpectraMax (Molecular Devices).

Resistant mutant generation & whole genome sequencing

M. tuberculosis H37Rv was grown to mid-logarithmic phase in 7H9 broth supplemented with 10% ADC. The cells were centrifuged and concentrated 100-fold to achieve a bacterial

number of ~10^{10} CFU/mL. Varying dilutions of the bacterial culture were plated onto compound containing plates. Appropriate dilutions of the bacterial culture were also plated on drug-free Middlebrook 7H11 agar to enumerate the bacterial numbers in the culture. Plates were incubated for 4 to 6 weeks at 37 ^{o}C and the CFUs were enumerated. The spontaneous rate of resistance was calculated by dividing the number of colonies on drug -containing plates (at a given concentration) divided by the total number of viable bacteria estimated on drug-free plates. Resistant colonies were randomly picked from the drug containing plates and grown in complete 7H9 broth. The colonies were characterized by MIC modulation to determine their level of resistance against parent compound, as well as, other standard TB drugs with different mechanisms of action. Genomic DNA was isolated by phenol chloroform extraction method and sent for whole genome sequencing.[26]

Microarray analysis

10 mL culture of Mtb H37Rv ATCC 27294 at A600 ~0.2 was exposed to 7.5 μg/mL (5X MIC) of compound **31** for 4 hours. A culture of Mtb H37Rv without the compound treatment was used as control. Cells harvested were re-suspended in 1mL trizol (GIBCO-BRL) and transferred to 2 mL screw cap tubes (Biospec Products) which had a pinch of 0.1 mm Zirconia beads (Biospec Products). Cells were lysed by bead beating 3 times at 5000 rpm for 20 sec each in a mini bead beater (Biospec products), total RNA was extracted and purified using the Qiagenasy kit (Qiagen) and used for labelling as per Agilent manual. The labelled material was hybridized to custom designed Mtb Agilent microarray chip following manufacturers manual. The data was analyzed using Genespring GX tool and the fold change in expression levels were reported with p value for statistical significance.

ASSOCIATED CONTENT

Details of the synthesis of all compounds, results from biological experiments are provided in supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

Author Contributions

E. B., M. N., M. P. and B.S.B were responsible for medicinal chemistry design and analyses. E.B., M.N. and V.V. performed the synthesis of the compounds. A. A., S. R., R. M., J. W., S. M. and D. A. performed the experiments related to resistant mutant generation and analyses. A.A. and S. R. performed transcriptome analysis. V.R. was responsible for design and analyses of microbiological experiments. S. S., A. N., S. G., and P. K. performed the microbiological experiments. P. V. was responsible for design and analyses of *in vitro* DMPK experiments. A. R. and M.C. were responsible for hit evaluation and analysis related to Fatostatin. V. A. was responsible for compound management for the high throughput screening. M.C.was responsible for design and analyses of MoA experiments and for driving the overall biology. M. P., M.C. and E. B. wrote the manuscript.

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ABBREVIATIONS

TB, Tuberculosis; Mtb, *Mycobacterium tuberculosis*; MoA, Mode of action; CFU, Colony forming unit; DAT, Diarylthiazole;

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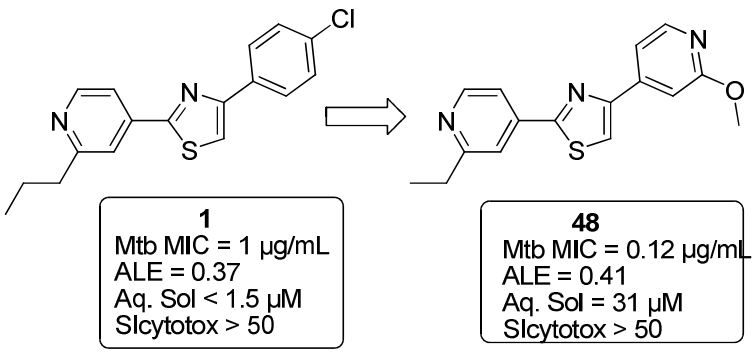
SYNOPSIS (Graphical abstract for Table of Contents)



1
Mtb MIC = 1 µg/mL
ALE = 0.37
Aq. Sol < 1.5 µM
SIcytotox > 50

48
Mtb MIC = 0.12 µg/mL
ALE = 0.41
Aq. Sol = 31 µM
SIcytotox > 50