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Synthesis, Biological and Molecular Docking Studies of Thiazole-Thiadiazole derivatives as potential Anti-Tuberculosis Agents

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Tuberculosis remains a global health threat, with increasing infection rates and mortality despite existing anti-TB drugs. The present work focuses on the research findings regarding the development and evaluation of thiadiazole-linked thiazole derivatives as potential anti-tuberculosis agents. We present the synthesis data and confirm the compound structures using spectroscopic techniques. The current study reports twelve thiazole-thiadiazole compounds (**5a**–**5l**) for their anti-tuberculosis and related bioactivities. This paper emphasizes compounds **5g**, **5i**, and **5l**, which exhibited promising MIC values, leading to further *in silico* and interaction analysis. Pharmacophore mapping data included in the present analysis identified tubercular ThyX as potential drug targets. The compounds were evaluated for anti-tubercular activity using standard methods, revealing significant MIC values, particularly compound **5l**, with the best MIC value of **7.1285 µg/ml**. Compounds **5g** and **5i** also demonstrated moderate to good MIC values against *M. tuberculosis* (H37Ra). Structural inspection of the docked poses revealed interactions such as hydrogen bonds, halogen bonds, and interactions containing Pi electron cloud, shedding light on conserved interactions with residues like Arg 95, Cys 43, His 69, and Arg 87 from the tubercular ThyX enzyme.

Introduction

■■Please provide academic titles (Prof., Dr.) for all authors■■

Tuberculosis (TB) remains one of the most contagious and life-threatening diseases worldwide. According to the World Health Organization (WHO), approximately 10.6 million people were infected with tuberculosis in 2021, which is higher than the 10.1 million cases reported in 2020.[1] The number of tuberculosis-related deaths in 2021 was about 1.6 million, compared to 1.5 million in 2020. Furthermore, it is alarming to note that the infection rate of tuberculosis experienced a 3.6% rise in 2021 compared to the previous year.[2] This highlights a potential deviation from the previous trend of nearly a 2% decrease over the last two decades. Despite the development of a large number of anti-tuberculosis drugs since 1944, including rifampicin, pyrazinamide, ethambutol, and amikacin, the increased TB infection rate is mainly due to the potential emergence and rapid spread of drug-resistant (XDR-TB) and multi-drug-resistant (MDR-TB) tuberculosis strains.[3] Thus, there is an urgent need and high demand to develop new broad-spectrum anti-tuberculosis drugs.[4] Figure 1 below depicts the

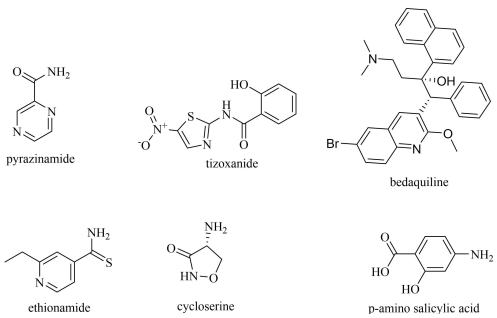

Figure 1. Chemical structures of few antituberculosis drugs.

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chemical structures of a few anti-tuberculosis drugs, namely p-amino salicylic acid, ethionamide, tizoxanide, bedaquiline, etc.

Thiazole and its derivatives have found great therapeutic applications in the field of medicinal chemistry since they display a broad range of biological activities. Namely, the 1,3,4-thiadiazole molecule, which comprises a sulphur pharmacophore, has been widely exploited for its biological activities such as antibacterial,[5] antifungal,[6] anticonvulsant,[7] antitumor,[8] and as a potential antituberculosis drug. As the literature suggests that thiazole and 1,3,4-dithiazole pharmacophores are active anti-tuberculosis agents, herein we attempted to combine both moieties through an –NH–CO–CH_2–S– linkage in a single molecule (Figure 2) and obtained a few novel derivatives (**5a**–**5l**). Next, the anti-tuberculosis and cytotoxic activities of these molecules were evaluated through biological studies, which were well corroborated by molecular docking studies.

After obtaining the compounds a suitable drug target was identified for the given series of compounds employing the pharmacophore screening method.[9,10] *In silico* molecular docking studies were performed to provide the structural rationale for the observed anti-tubercular effect. The binding free energy and comprehensive three-dimensional interaction analysis data obtained from the molecular docking are expected to provide further insights on the structural account of the anti-tubercular activities for the compounds (**5a**–**5l**).

Results and Discussion

Synthetic Chemistry

Acylation of 2-aminothiazoles by using chloroacetyl chloride to synthesize N-thiazol-2-yl-2-chloroacetamide derivatives **2a**–**2l**. Here, we report a series of novel thiadiazole linked thiazole derivatives (**5a**–**5l**) by clubbing compound **4** with compounds **2a**–**2l** (Scheme 1).

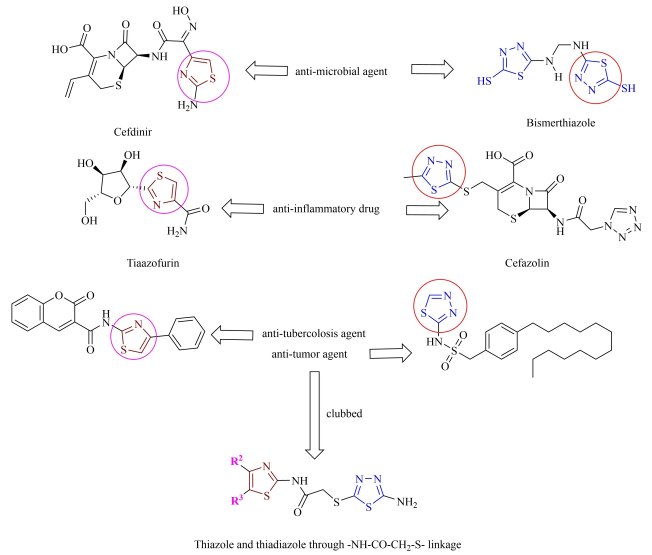


Figure 2. Drug molecules containing either thiazole or 1,3,4-thiadiazole moiety clubbed to obtain novel thiazole and dithiazole derivatives.

Thiadiazole linked thiazole derivatives (**5a**–**5l**) are readily prepared from **2a**–**2l** using potassium carbonate in the non-protic solvent THF at room temperature. For the substrates, both R^2 and R^3, being aliphatic, the yield of the product is higher, up to 92%. The reaction proceeded smoothly in the case of aromatic substituted R^1 and R^2. Both electron-donating groups and electron-withdrawing groups, such as *p*-Br, *p*-Cl, *p*-OMe, *p*-NO_2, *m*-Br, and *m*-NO_2, were well tolerated to afford the corresponding products as well as the clubbed final product. Further experiments showed that this method worked equally well for mono, di, and, tri substituted 2-amino thiazole derivatives as well as different moieties such as the coumarin ring.

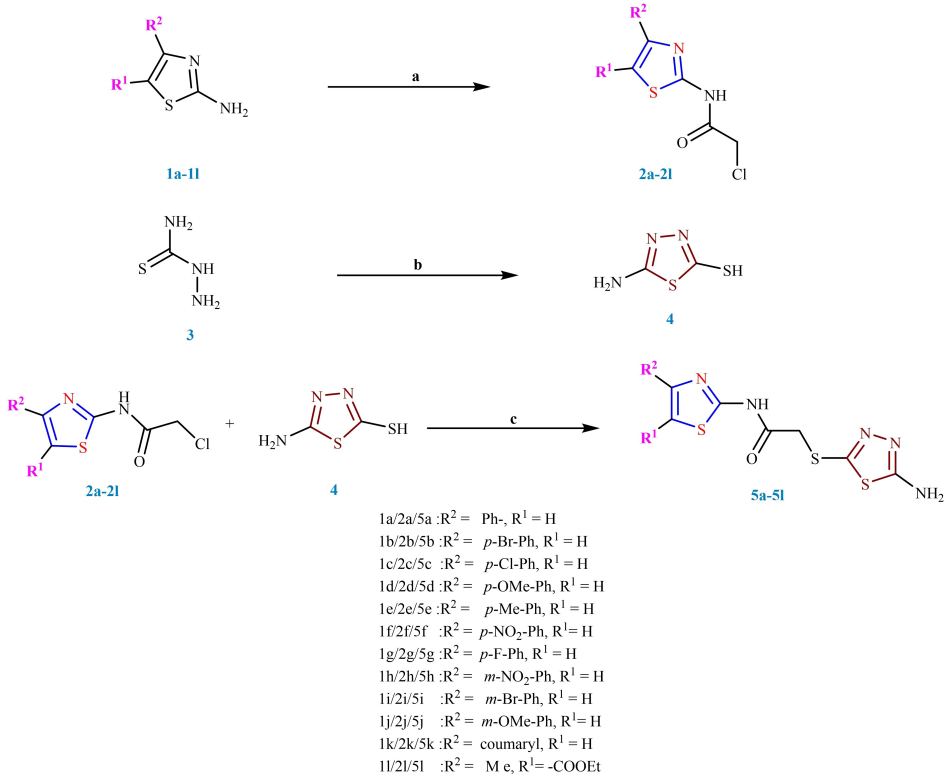
Spectral Analysis

The structures of the synthesized compounds were characterized by different spectroscopic techniques such as FTIR, ^{1}H-NMR, ^{13}C-NMR and MS. The FT-IR spectra of the compounds display characteristic four bands, the first band at ~3400 cm^{-1} assigned to νN–H, the second band at 610–690 cm^{-1}. The third band at ~2000 cm^{-1} is assigned to νS–C=N- whereas fourth band at 1600 cm^{-1} which is ascribed to νCO of amide linkage. The one located at 1500–1300 cm^{-1} due to the stretching band of νC=N and νC=C confirming the presence of imine and aromatic skeleton. The 1H-NMR spectra of all the compounds show clear and recognizable singlet peak for NH proton at ~8–12.5 δ whereas –NH2 signal appear as singlet at 6–8 δ. The proton of thiazole and methylene groups appears as a singlet. The aromatic protons present at 6.5–8.5 δ exhibit ortho coupling (J = ~8 Hz) and meta coupling (J = ~2 Hz). Other signals appear as expected at the respective chemical shift values. The ^{13}C-NMR of all compounds showed all the signals at the chemical shift values. There is at least one aliphatic carbon signal in all compounds from 35 to 60 δ. The important peak in all compounds is at ~170 δ which is the characteristic peak of amidic carbonyl carbon (O=**C**–N). The MS spectra of all compounds exhibit molecular ion peak (M^+) peaks with high accuracy.

Antitubercular Activities

The antituberculosis assay was performed by disc diffusion method and the results are summarized in Table 1. The novel thiadiazol linked thiazole derivatives (**5a**–**5l**) by screening them for antituberculosis activity against *M. tuberculosis* (H37Ra) and MIC values were determined using standard Rifampicin.

The MIC values of synthesized compounds were assessed. Compounds **5l** showed best MIC value of **7.1285 *μg/ml***. Compounds **5g** & **5i** also showed moderate to good MIC towards studied microorganisms as compared with the standards used. The results are expressed as the mean values of three independent experiments.



Reaction conditions: **a**. Acylation: ClCOCH2Cl (CAC), Et3N (TEA), DMF, 0-5°C, 0.5-2.5 h; **b**. anhydrous Na2CO3 (1 equiv.), CS2 (1 equiv.), absolute ethanol; **c**. **2a-2l** (1.0 equiv.), **4** (1.0 equiv.), K2CO3 (1 equiv.), THF.

Scheme 1. Synthesis of 2-(5-amino-1,3,4-thiadiazole-2-ylthio)-N-(thiazole-2-yl) acetamide derivatives (**5a**–**5l**).

Table 1. Anti-tuberculosis activities of synthesized compounds against *M. tuberculosis* H37Ra.

Compound	Inhibition zone	MIC
5a	NZ	>500
5b	++	250
5c	+	250
5d	++	62.5
5e	++	62.5
5f	++	62.5
5g	+++	**15.625**
5h	++	250
5i	+++	**15.625**
5j	+	250
5k	+	250
5l	+++	**7.1285**
Rifampicin	+++	**1.9531**

+ = <5 mm, ++ = >5 & <10 mm, +++ = >10 & <18 mm, NZ=No zone

DPPH and OH Radical Scavenging Activity

The results for radical scavenging activity are presented in Table 2.

The compound 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH) and Hydroxyl radical (OH) were calculated. Compound, **5i** exhibited best antioxidant activity. It was observed that the screened compounds have moderate to good DPPH and OH radical scavenging activities as compared to standard ascorbic acid and α-tocopherol.

Haemolytic Activity

The haemolytic potential of synthesized compounds towards host RBCs was calculated by *in vitro* haemolytic assay. The results revealed that, all the synthesized compounds exhibit negligible to maximum 1% haemolytic activity as compared to positive control Triton ×100 (12%) and all were within the permissible limit of 5% haemolysis. (Table 3)

Table 2. Antioxidant activities.

Compound	DPPH	OH
5a	64.78 ± 0.53	68.16 ± 0.40
5b	63.01 ± 0.46	64.8 ± 0.88
5c	62.31 ± 0.60	60.06 ± 0.65
5d	58.4 ± 0.26	55.2 ± 0.52
5e	51.24 ± 0.52	52.33 ± 0.25
5f	64.3 ± 0.55	66.66 ± 0.73
5g	65.88 ± 0.88	63.46 ± 0.35
5h	57.98 ± 0.62	67.56 ± 0.20
5i	**71.28 ± 1.22**	**88 ± 0.45**
5j	62.53 ± 0.05	64.73 ± 0.58
5k	62.11 ± 0.47	61.43 ± 0.46
5l	61.8 ± 0.87	58.13 ± 0.64
Ascorbic acid	**84.53 ± 0.11**	NA
α Tocopherol	NA	**82.9 ± 0.5**

Table 3. Cytotoxicity towards red blood cells.

Compound	% Cytotoxicity
5a	0.24 ± 0.005
5b	0.66 ± 0.005
5c	1.04 ± 0.020
5d	0.86 ± 0.005
5e	0.93 ± 0.015
5f	0.59 ± 0.020
5g	1.06 ± 0.020
5h	0.66 ± 0.020
5i	0.37 ± 0.025
5j	0.95 ± 0.026
5k	0.65 ± 0.03
5l	0.45 ± 0.032
Triton X 100	11.26 ± 0.971

Structure Activity Relationship

The thiadiazole-linked thiazole derivatives (**5a**–**5l**) exhibit a broad spectrum of anti-tubercular activities. Compound **5l**, containing both R^2 and R^3 substituents as aliphatic, is found to be more active as an anti-tubercular agent than other compounds containing coumaryl and aromatic substitutes. The synthesized compounds containing strong electron-withdrawing substituents (**5g** and **5h**) exhibit more anti-tubercular activity than those containing moderate to weak electron-donating substituents like Br (**5b**), –Cl (**5c**), and –CH3 (**5e**). In meta-substituted compounds, a strong electron-withdrawing group such as $–NO_2$ (**5h**) shows more anti-tubercular activity than moderate electron-withdrawing substituents like –Br and –OMe (compounds **5i** and **5j**). Conversely, the strong electron-donating –OMe substituent at the C-4 position (**5d**) shows more anti-tubercular potential compared to the C-3 position (**5j**). Among halogens, the DPPH scavenging activity increases from chloro to fluoro substituents. Interestingly, it is observed that the OH radical scavenging activity increases from chloro to fluoro substituents. The 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(thiazol-2-yl) acetamide derivatives **5d**, **5l**, **5k**, and **5h** have been found to exhibit potent anti-tubercular activity. The presence of a coumarin ring (**5k**) diminishes the anti-tubercular potential compared to the phenyl ring (**5a**). The MIC values are lower for compounds **5g**, **5i**, and **5l** compared to the other derivatives. This study suggests that enhancing electron-withdrawing effects results in enhanced anti-tubercular activities. From the above observations, it is inferred that compounds having substituents with moderate electron-donating properties are the most suitable for achieving the best anti-tubercular activities. It has been accounted that electron-withdrawing substituents decrease the electron density, making the compounds efficient anti-tubercular agents and augmenting their anti-tubercular activity. Thus, compounds having methoxy substituents show less anti-tubercular potential. The radical scavenging study demonstrated that **5a**–**5l** exhibit promising antioxidant characteristics, as evidenced by their inhibitory effect on DPPH and OH radicals (Table 2). All the synthesized compounds **5a**–**5l** show strong antioxidant activity, implying that all compounds may minimize DPPH and OH radicals.

Structural Studies through Molecular Modelling

In silico *Target Identification*

To account for the observed anti-tubercular activities of the novel compounds, a plausible target protein in *Mycobacterium tuberculosis* needs to be identified. To identify the target, pharmacophore mapping was performed with the help of the Pharm Mapper web server. A pharmacophore is the spatial arrangement of molecular features responsible for the interaction of a chemical compound with its target protein.[11] Pharm Mapper identifies drug targets by pharmacophore mapping against the in-house library of the pharmacophore database named Pharm Target DB, which includes annotations from Target Bank, Binding DB, Drug Bank, and potential drug target databases.[12] Pharm Mapper identifies the target protein by determining the best mapping poses of query molecules against all the pharmacophore models in Pharm Target DB. The output is provided in the form of numerous best-fitted hits with the necessary target annotations, along with aligned conformers.

Based on the common pharmacophore properties, the software assigns a 'fit score,' which is a direct indicator of the compatibility and binding affinity of the ligand and its target receptor. A pharmacophore search with the core compound of 5l identified thymidylate synthase (ThyX) as a potent drug target, yielding a fit score of **7.1285**, with a total of 12 pharmacophore features matched between the ligand and the receptor. Therefore, flavin-dependent thymidylate synthase (ThyX) of *Mycobacterium tuberculosis*, with the PDB identifier 2AF6, was selected as the target protein.

Thymidylate synthase is a potential therapeutic target since humans and most eukaryotes lack the ThyX gene.[13] In the case of eukaryotic systems, the conventional thymidylate synthase (TS) gene is expressed for the synthesis of dTMP.[14] The thymidylate synthase enzyme catalyzes the biosynthesis of dTMP (deoxythymidine monophosphate), which is an essential component of the DNA replication and repair system. Apart from that, ThyX contributes to some biologically significant processes essential for the survival of the pathogen.[15] Mycobacterium tuberculosis codes for both conventional thymidylate synthase (ThyA) and ThyX, and it was experimentally demonstrated that the pathogen can survive without the expression of ThyA, but ThyX is absolutely essential for the *in vivo* growth of the pathogen, even in the presence of conventional TS and supplied thymidine exogenously.[16] Unlike ThyA, ThyX methylates the dUMP using dUMP and methylenetetrahydrofolate as reactants without oxidizing the tetrahydrofolate, following an alternative pathway. Due to these reasons, ThyX can be used as a potential drug target.

Molecular Docking Study

According to the experimental results, compounds **5 g**, **5 i**, and **5 l** demonstrated very effective MIC values; hence, further interaction analysis was focused on these compounds. The comparative summary of binding free energy with the observed MIC values is presented in Table 4. A careful observation of the interaction data from table 10 ■■Dear Author, please check (Table 10?■■ reveals that Arg 95 is involved in various conventional and non-conventional polar interactions. Similarly, Cys 43 can be involved in Pi-sulfur and Pi-donor hydrogen bonds. Additionally, His 69 is observed to support the ligand via Pi–Pi stacking interaction.

Table 4. Comparative account of the MIC and binding free energy values.

Compound	Minimum Inhibitory concentration (μg/mL)	Binding Free Energy (kcal/mol)
5 a	>500	−5.09
5 b	250	−5.15
5 c	250	−5.59
5 d	62.5	−5.32
5 e	62.5	−4.93
5 f	62.5	−6.74
5 g	**15.625**	−4.78
5 h	250	−5.03
5 i	**15.625**	−5.16
5 j	250	−4.58
5 k	250	−5.79
5 l	**7.1285**	−5.44
Rifampicin	**1.9531**	–
BrdUMP (substrate analog 5-Bromo-2′-Deoxyuridine-5′-Monophosphate, co-crystallized inhibitor)	–	**−7.39**

Interaction Analysis of Compound 5 g

Careful observation of the docking results reveals that residues like Arg 87, Gln 106, and Arg 172 forms a binding pocket where the cyclohexane ring of the **5 g** is stabilized by a network of hydrogen bond (Figure 3B).

Similarly, residues like Glu 92, Arg 97, Arg 95, Arg 107 and Arg 199 are observed to stabilize the ligands Pi electron cloud by Pi-cation, Pi-anion, and Pi-alkyl interactions (Figure 3A). The sulphur containing functional group of the ligand appears to be stabilized by the Arg 95 by the sulphur-X interaction (Figure 3C).

Interaction Analysis of Compound 5 i

Visual inspection of the docking poses identified His 69 to be in an effective Pi stacking interaction with the aromatic functional group of the ligand **5 i**. Similarly, the terminal guanidine group of Arg 87 appears to stabilize the ligand by offering the Pi-cation interaction (Figure 4A).

A portion of the D chain consisting of the residue 103 to 107 forms strong hydrogen bond with the ligand **5 i** (Figure 4B). Other interactions like alkyl interactions appears to aid the strong binding of the ligand **5 i** in the receptor binding pocket (Figure 4D).

Interaction Analysis of Compound 5 l

Interaction data observed in the study reveals that the Pi-sulphur interaction with Cys 43 and Tyr 108 might hold the compounds aromatic ring structure thereby providing a very complex and stable conformation (Figure 5A). Moreover, important residues, shown in panel **B** of Figure 5 forms a network of hydrogen bonds for providing stability to the protein ligand

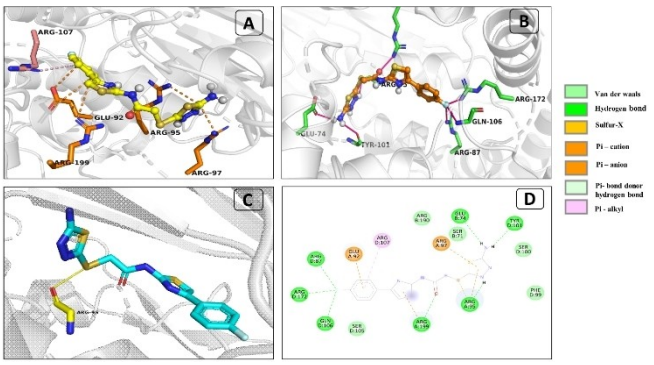


Figure 3. Summary of various interactions between compound **5 g** and residues from the MTB ThyX. **(A)** Interactions with Pi electron cloud of the ligand. **(B)** Conventional hydrogen bonds **(C)** Sulphur X interaction and **(D)** 2D plot of the interactions.

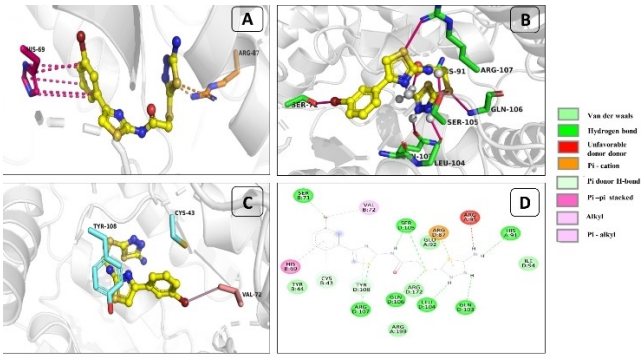


Figure 4. Various interactions between compound **5 i** and amino acid residues of target protein. **(A)** Pi–Pi stacked interaction of His 69 and pi-cation interaction of Arg 87 with the aromatic ring of the ligand. **(B)** Hydrogen interactions of the **5 i** compound with its neighbour amino acid residues. **(C)** Alkyl interaction along with other non-conventional interaction and **(D)** 2D interaction plot for **5 i** compound.

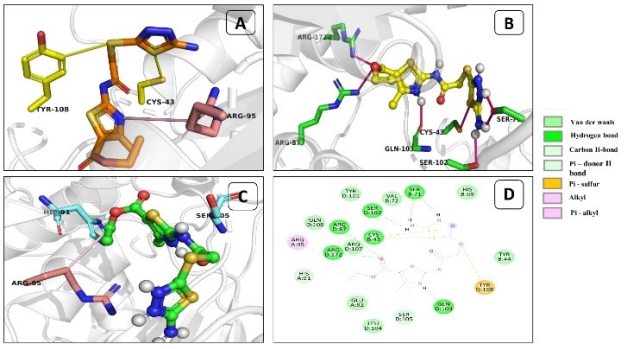


Figure 5. Summary of various interactions between compound **5 l** and its neighbouring amino acid residues from Mtb ThyX. **(A)** Pi-sulphur bonds of Tyr 108 and Cys 43 with the sulphur of ligand and pi-alkyl interaction of Arg 95. **(B)** Conventional hydrogen bonds of **5 l** compound formed with amino acid residues. **(C)** Alkyl interaction and other non-conventional hydrogen bond of **5 l** ligand and **(D)** 2D interaction image of **5 l** ligand.

complex. Arg 95 forms an effective alkyl interaction with the terminal alkyl group of compounds **5 l**.

Interestingly, two carbon-hydrogen bonds with residues His **5 l** and Ser 105 were observed to stabilize the protein ligand complex (Figure 5C). 2D plot revealed that the common surface residues like Tyr 101, Leu 104, Ser 105, Gln 106, Arg 107 might stabilize the structure with Van der Waals interactions (Figure 5D).

Conclusions

In conclusion a novel series of thiadiazole linked thiazole derivatives (**5 a**–**5 l**) were synthesized and subsequently evaluated for their biological activities. The *in vitro* assays reveals that the series of compounds possess excellent antitubercular activities. Among the series of compounds **5 g**, **5 i** **(MIC = 15.625 μg/mL)**, and **5 l** **(MIC = 7.1285 μg/mL)** exhibited a marked zone of inhibition against Mycobacterium tuberculosis H37Ra. Additionally, these compounds possess a good radical scavenging potential. Molecular modelling and docking studies support the anti-tubercular activities of the compounds in consistent to their experimental data. For instance, the experimental results of this study emphasize the significant MIC values of compounds **5 g**, **5 i**, and **5 l**, which lead to a focused analysis of their interactions with the conserved residues from the ThyX enzyme. A comparative summary of binding free energy alongside observed MIC values, revealed some pivotal interactions with the conserved residues from the tubercular ThyX protein. For example, Arg 95 engages in diverse polar interactions, while Cys 43 contributes to Pi-sulphur and Pi-donor, hydrogen bonds, and His 69 supports ligands via Pi–Pi stacking. Compound **5 g**'**s** interaction analysis reveals stabilizing interactions with residues like Arg 87, Gln 106, and Arg 172 forming a binding pocket, and interactions with Glu 92, Arg 97, and Arg 199 stabilizing the ligand's Pi electron cloud. Compound 5 i exhibits effective Pi stacking with His 69 and Pi-cation interaction with Arg 87, with additional strong hydrogen bonds and alkyl interactions. Interaction data for compound **5 l** suggests complex stabilization mechanisms involving Pi-sulphur interaction, hydrogen bonds, and alkyl interactions, with notable contributions from residues like Arg 95, His 51, and Ser 105. The present studies are expected to further lead the development of efficient anti-tubercular agents comprising the thiadiazole-thiazole derivatives.

Experimental Section

Synthesis of 2-chloro-N-(thiazol-2-yl) acetamide derivatives (2 a–2 l)

Compounds **2 a**–**2 l** were synthesized following a known literature report.[17] The mixture of thiazole-2-amino derivatives **1 a**–**1 l** (1.0 mmol) and triethylamine (1.3 mmol) was taken in a round-bottom flask. The reaction mass was stirred at room temperature for 30 minutes. Next, chloroacetyl chloride (2.2 mmol) was added to the reaction at 0–5 °C. The reaction was completed within 1–4 hours as monitored on TLC. The reaction mixture was washed with brine solution and then with water. A 1 : 1 (HCl: H_2O) was used to neutralize the solution. The products were filtered and washed with water. (TLC mobile phase n-hexane: ethyl acetate (6 : 4).

Synthesis of 5-Amino-1,3,4-thiadiazole-2-thiol (4)

Compound **4** was synthesized following a known literature report.[18] A mixture of thiosemicarbazide **(3)** (10.0 mmol) anhydrous Na_2CO_3 (10.0 mmol) and carbon disulfide (10.0 mmol) was suspended in absolute ethanol. Next, the reaction mixture was refluxed for 1 h. After the reaction completion the solvent was evaporated under vacuum and the residue was dissolved in water (20 ml) and acidified with conc. HCl to yield the compound **4**.

Synthesis of novel 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(thiazol-2-yl) acetamide derivatives (5 a–5 l)

A solution of **4** (1 mmol), potassium carbonate (1.1 mmol) in THF (10 ml) was stirred at ambient temperature for 30 minutes. Next, a solution of **2 a**–**2 l** (1 mmol) in THF (5 ml) was added dropwise to the reaction mass. Stirred the reaction mass at room temperature and monitored the reaction progress on TLC. After the reaction

completion, the reaction mixture was poured into crushed ice. The product was filtered, washed with water, and recrystallized from ethyl acetate to yield pure product **5 a**–**5 l**.

General Information

All reagents and solvents were commercially available of analytical grade and used as received. The solvents used were dried using standard techniques. Liquide Chromatography Mass Spectra (LCMS) of compounds were obtained on Waters Micromass Q-Tof Micro spectrometer. The instrument is a hybrid quadrupole time of flight mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APcI) sources having mass Range of 4000 amu in quadruple and 20000 amu in ToF. Mass Spectra (M.S.) of compounds were obtained on an FTICR-MS (Ionspec 7.0T) spectrometer. Fourier-transform infrared spectroscopy (FTIR) (cm^{-1}) was recorded on Spectrum RX-IFTIR. The ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solvents on a Bruker Avance Neo 500 MHz NMR Spectrometer. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (7.27 ppm) for ^{1}H-NMR data and $CDCl_3$ (77.0 ppm) for ^{13}C-NMR data or the peak of DMSO-d_6, defined at 2.50 (^{1}H-NMR) or 39.5 (^{13}C-NMR). The following abbreviations explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dt = double triplet and br = broad.

2-(5-amino-1,3,4-thiadiazole-2-ylthio)-N-(4-phenylthiazole-2-yl) acetamide (5 a)

Color: Off White; Yield: 83 %. FTIR (in cm^{-1}): 620.71, 1178.75, 1335.61, 1510.94, 1616.37, 3475.71; Elemental Analysis Found (Calc.) %: C, 44.68; H, 3.17; N, 20.04; O, 4.58; S, 27.53; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.53 (s, 1H, NH–CO), 7.90 (m, 2H, aromatic), 7.66 (s, 2H, NH_2), 7.56 (s, 1H, aromatic thiazole), 7.44 (m, 1H, aromatic), 7.37–7.29 (m, 2H, aromatic), 4.11 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.01, 166.39, 148.84, 148.68, 134.06, 128.64 (strong), 128.48, 127.74 (strong), 125.55, 108.25, 37.19, $C_{13}H_{11}N_5OS_3$, Exact Mass: 349.4450; Observed Mass: 349.7523.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-bromophenyl) thiazol-2-yl) acetamide (5 b)

Color: Off White; Yield: 93 %. FTIR (in cm^{-1}): 620.73, 1177.97, 1383.05, 1516.65, 1617.57, 3470.61; Elemental Analysis Found (Calc.) %: C, 44.68; H, 3.17; N, 20.04; O, 4.58; S, 27.53 Elemental Analysis Found (Calc.) %: C, 36.45; H, 2.35; Br, 18.65; N, 16.35; O, 3.74; S, 22.46; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.65 (s, 1H, NH–CO), 7.94 (d, J = 8.5 Hz, 2H, aromatic), 7.82 (s, 1H, aromatic thiazole), 7.72 (d, J = 8.5 Hz, 2H, aromatic), 7.42 (s, 2H, NH_2), 4.20 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.01, 166.48, 157.71, 148.66, 147.65, 133.27, 131.58 (strong), 127.58 (strong), 120.81, 109.08, 37.17. $C_{13}H_{10}BrN_5OS_3$, Exact Mass: 428.3410; Observed Mass: 428.00.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide (5 c)

Color: Off White; Yield: 90 %. FTIR (in cm^{-1}): 620.54, 744.74, 1181.57, 1385.57, 1502.23, 1617.46, 3476.72; Elemental Analysis Found (Calc.) %: C, 40.67; H, 2.63; Cl, 9.23; N, 18.24; O, 4.17; S, 25.06; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.55 (s, 1H, NH–CO), 7.91 (d, J = 6.0 Hz, 2H, aromatic), 7.72 (s, 1H, aromatic thiazole), 7.50 (d, J = 6.0 Hz, 2H, aromatic), 7.33 (s, 2H, NH_2), 4.11 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.00,166.47, 157.71, 148.56, 147.61, 132.93, 132.21, 128.67 (strong), 127.27 (strong), 109.00, 37.17. $C_{13}H_{10}ClN_5OS_3$, Exact Mass: 383.8870; Observed Mass: 384.00.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-methoxyphenyl) thiazol-2-yl) acetamide (5 d)

Color: Light Brown; Yield: 77 %. FTIR (in cm^{-1}): 619.89, 732.11, 814.13, 1041.71, 1384.58, 1502.29, 1616.32, 3476.55; Elemental Analysis Found (Calc.) %: C, 44.31; H, 3.45; N, 18.46; O, 8.43; S, 25.35; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.54 (s, 1H, NH–CO), 7.93 (d, 2H, aromatic), 7.63 (s, 1H, aromatic thiazole), 7.34 (d, J = 8.8 Hz, 2H, aromatic), 7.25 (s, 2H, NH_2), 4.12 (s, 2H, CH_2), 3.38 (s, 3H, CH_3). 13 C NMR (126 MHz, DMSO) δ 169.35, 167.61, 164.72, 150.79, 148.57, 136.41, 132.51, 128.88 (strong), 125.35 (strong), 106.34, 66.98, 21.14. $C_{14}H_{13}N_5O_2S_3$, Exact Mass: 379.4710; Observed Mass: 380.00.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-p–tolylthiazol-2-yl) acetamide (5 e)

Color: Brown; Yield: 80 %. FTIR (in cm^{-1}): 620.53, 736.47, 825.92, 1052.26, 1384.16, 1518.44, 1617.58, 3475.42; Elemental Analysis Found (Calc.) %: C, 46.26; H, 3.60; N, 19.27; O, 4.40; S, 26.46 ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.77 (d, J = 8.1 Hz, 2H, aromatic), 7.42 (s, 1H, NH–CO), 7.34 (s, 1H, aromatic thiazole), 7.28 (s, 2H, NH_2), 7.20 (d, J = 7.9 Hz, 2H, aromatic), 4.02 (s, 2H, CH_2), 2.32 (s, 3H, CH_3). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.06, 166.45, 160.74, 157.66, 147.86, 130.72, 127.63, 127.57, 115.60, 115.43, 108.04, 40.32, 37.22. $C_{14}H_{13}N_5OS_3$, Exact Mass: 363.4720; Observed Mass: 363.0960.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-nitrophenyl) thiazol-2-yl) acetamide (5 f)

Color: Brown ; Yield: 91 %. FTIR (in cm^{-1}): 620.53, 736.47, 825.92, 1052.26, 1384.16, 1518.44, 1617.58, 3475.42; Elemental Analysis Found (Calc.) %: C, 39.58; H, 2.56; N, 21.31; O, 12.17; S, 24.39; Elemental Analysis Found (Calc.) %: C, 44.68; H, 3.17; N, 20.04; O, 4.58; S, 27.53; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.67 (bs, 3H, NH–CO, and NH_2), 8.23 (d, J = 9.0 Hz, 2H, aromatic), 8.08 (d, J = 9.0 Hz, 2H, aromatic), 7.97 (s, 1H, aromatic thiazole), 4.35 (s, 2H, CH_2). $C_{13}H_{10}N_6O_3S_3$, Exact Mass: 394.4420; Observed Mass: 395.00.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-fluorophenyl) thiazol-2-yl) acetamide (5 g)

Color: Light Brown; Yield: 79 %. FTIR (cm^{-1}): 621.12, 754.81, 1055.32, 1381.31, 1514.22, 1617.04, 3414.20; Elemental Analysis Found (Calc.) %: C, 42.49; H, 2.74; F, 5.17; N, 19.06; O, 4.35; S, 26.18; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 3H, NH–CO), 7.95 –7.19 (m, 4H, aromatic), 7.06 (s, 2H NH_2), 6.98 (s, 1H, aromatic thiazole), 4.57 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 168.17, 162.23, 160.29, 148.67, 131.42, 127.35 (strong), 127.29, 115.20 (strong), 115.03, 101.06, 42.20. $C_{13}H_{10}FN_5OS_3$, Exact Mass: 367.4354; Observed Mass: 367.0978.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(3-nitrophenyl) thiazol-2-yl) acetamide (5 h)

Color: Light Brown; Yield: 87 %. FTIR (cm^{-1}): 621.12, 754.81, 1055.32, 1381.31, 1514.22, 1617.04, 3414.20; Elemental Analysis Found (Calc.) %: C, 39.58; H, 2.56; N, 21.31; O, 12.17; S, 24.39; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.67 (s, 1H, NH–CO), 8.73 (dt, J = 2.3 Hz, J = 7.6 Hz, 1H, aromatic), 8.35 (dt, J = 2.3 Hz, J = 7.6 Hz, 1H, aromatic), 8.18 (t, J = 2.3 Hz, 1H, aromatic) 7.98 (s, 1H, aromatic thiazole), 7.74 (t, J = 7.6 Hz, 1H, aromatic), 7.35 (s, 2H, NH_2), 4.14 (s, 2H, CH_2). ^{13}C-NMR

(126 MHz, DMSO-d_6) δ 170.04, 166.83, 158.28, 148.66, 148.22, 146.59, 135.54, 131.86, 130.42, 122.23, 120.11, 110.72, 37.17. $C_{13}H_{10}N_6O_3S_3$, Exact Mass: 394.4420; Observed Mass: 394.7724.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(3-bromophenyl) thiazol-2-yl) acetamide (5 i)

Color: Light Brown; Yield: 75 %. FTIR (cm^{-1}): 480.77, 621.19, 802.61, 1074.34, 1384.89, 1514.22, 1617.48, 3472.96; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.63 (s, 1H, NH–CO), 8.10 (s, 1H, aromatic), 7.91 (d, J = 7.7 Hz, 1H, aromatic), 7.81 (s, 1H, aromatic thiazole), 7.53–7.37 (m, 4H, NH_2 and aromatic proton), 4.13 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.06, 166.55, 157.69, 148.78, 147.09, 136.29, 130.86, 130.34, 128.17, 124.46, 122.08, 109.70, 37.28. $C_{13}H_{10}BrN_5OS_3$, Exact Mass: 428.3410; Observed Mass: 428.3617.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(3-methoxyphenyl) thiazol-2-yl) acetamide (5 j)

Color: Light Brown; Yield: 70 %. FTIR (cm^{-1}): 480.43, 621.77, 723.23, 849.01, 1059.38, 1382.59, 1506.59, 1617.01, 3473.89; Elemental Analysis Found (Calc.) %: C, 36.45; H, 2.35; Br, 18.65; N, 16.35; O, 3.74; S, 22.46; Elemental Analysis Found (Calc.) %: C, 44.31; H, 3.45; N, 18.46; O, 8.43; S, 25.35; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.81 (s, 1H, NH–CO), 7.77 (s, 1H, aromatic thiazole), 7.37 −7.32 (m, 4H, aromatic), 6.98 (broad, 2H, NH_2), 4.25 (s, 2H, CH_2), 3.81 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.74, 168.99, 166.45, 159.69, 157.56, 148.58, 135.36, 130.00, 118.13, 113.85, 110.60, 108.83, 55.47, 37.28. $C_{14}H_{13}N_5O_2S_3$, Exact Mass: 379.4710; Observed Mass: 378.5019.

2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl) acetamide (5 k)

Color: Brown; Yield: 76 %. FTIR (cm^{-1}): 1382.7, 1510.10, 1620.22,1759.20, 3415.20; Elemental Analysis Found (Calc.) %: C, 46.03; H, 2.66; N, 16.78; O, 11.50; S, 23.04; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.62 (s, 1H, NH–CO), 8.61 (s, 1H), 7.87 (d, J = 7.3 Hz, 1H, aromatic), 7.64 (m, 1H, aromatic), 7.47 (d, J = 8.2 Hz, 1H, aromatic), 7.41 (t, J = 7.4 Hz, 1H, aromatic), 7.36 (s, 1H, aromatic thiazole), 7.13 (s, 2H, NH_2), 4.44 (s, 2H, CH_2). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 171.86, 170.06, 166.84, 158.58, 157.55, 152.36, 142.08, 138.53, 131.86, 128.83, 124.68, 120.22, 118.10, 115.84, 114.42, 36.90. $C_{16}H_{11}N_5O_3S_3$, Exact Mass: 417.4760; Observed Mass: 417.6170.

Ehyl2-(2-(5-amino-1,3,4-thiadiazol-2-ylthio) acetamido)-4-methyl thiazole-5-carboxylate (5 l)

Color: Faint Yellow; Yield: 74 %. FTIR (cm^{-1}): Elemental Analysis Found (Calc.) %: C, 36.76; H, 3.65; N, 19.48; O, 13.35; S, 26.76; 1789.20; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.84 (s, 1H, NH–CO), 7.40 (s, 2H, NH_2), 4.24 (q, J = 6.9 Hz, 2H CH_2), 4.13 (s, 2H, CH_2), 3.36 (s, 3H, CH_3), 1.28 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.10, 167.18, 161.91, 159.12, 156.14, 148.39, 114.19, 60.45, 37.19, 16.88, 14.07. $C_{11}H_{13}N_5O_3S_3$, Exact Mass: 359.4370; Observed Mass: 359.3464.

Author Contributions

Mr. Samin A. Shaikh proposed the research idea. Samin A. Shaikh, Dr. Deepak Boraste and Dr. Satish Waghchaure wrote the manuscript. All authors have given their approval to the final version of the manuscript. Mr. Samin Shaikh, Dr Shivaji Labhade, Dr Raju Kale, Mr. Kamlesh Jain, and Rushikesh Labhade have completed the experimental process for synthesizing intermediate and compounds (**5 a**–**5 l**). Dr. Rahul More and Ms. Prajakta Y. Pachorkar have conducted MICs, DPPH radical scavengers, and anti-oxidant activity. Dr. Rohan Meshram and Ms. Debopriya Ballabh have carried out the molecular docking study. Dr. Santosh Chobe and Mr. Samin A. Shaikh contributed significantly to the spectral analysis and interpretations.

Conflict of Interests

The authors declare no conflict of interest.

Keywords: Antituberculosis thiazole · 1,3,4-dithiadiazole · molecular docking

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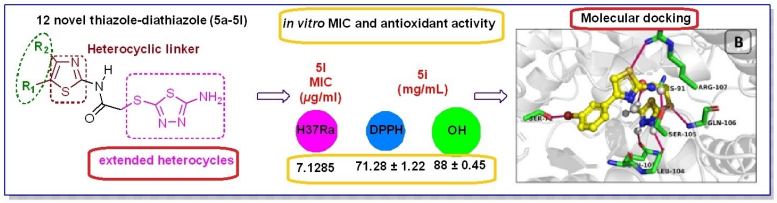

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1 – 9

Synthesis, Biological and Molecular Docking Studies of Thiazole-Thiadiazole derivatives as potential Anti-Tuberculosis Agents

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