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New bithiazolyl hydrazones: novel synthesis, characterization and antitubercular evaluation

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Abstract

New bithiazolyl hydrazones (**6a-l**) have been first time synthesized by carrying novel one pot cyclocondensation of 5-acyl thiazoles (**1a-b**), thiosemicarbazide (**2**) and substituted phenacyl chlorides (**4a-f**) in freshly prepared ionic liquid, diisopropyl ethyl ammonium acetate (DIPEAc) at room temperature. The newly synthesized compounds have been evaluated for their antitubercular activity and the compounds **3b**, **6a**, **6b**, **6d**, **6e**, **6f**, **6g**, and **6l** have displayed noticeable antitubercular activity compared to Rifampicin with tolerable cytotoxicity. All these compounds were also screened for their antibacterial activity and found that, compounds **6j** and **6k** have exhibited a very good antibacterial activity. Molecular docking study has shown better harmony with the evaluation trend shown by these compounds under *in vitro* antitubercular screening.

Keywords: Thiosemicarbazones, Bithiazole, Ionic liquid, Antitubercular Activity, Antibacterial Activity, Cytotoxicity, Molecular Docking.

Tuberculosis (TB), a contagious disease responsible for high mortality worldwide, is now coexisting with human immunodeficiency virus (HIV) and causing serious threat to mankind.[1,2] Nowadays, the tuberculosis strains are found to become resistant to existing first line as well as second line multi drugs and therefore treating multi drug resistant (MDR) and extremely drug resistant (XDR) strains are becoming challenge for medicinal chemists.[3] Recently, new drug diarylquinoline (bedaquiline) has been introduced for treating the MDR-TB strains. However, these efforts are not completely satisfying the present threat of TB treatments. Therefore, in search of safer and potentially active new antitubercular agents attempts are found to generate library of new entities having various biodynamic heteryl scaffolds and active pharmacophores.[4]

Literature also reveals that, thiazole scaffold is well explored and it is a key constituent of various medicaments.[5-11] It is also reported that, thiazoles having other heteryl scaffolds and

pharmacophores viz. pyridyl,[12] aryl,[13,14] thiazolyl,[2] amino,[15] hydrazolyl,[16] triazolyl,[17] piperidinyl[18,19] and thiosemicarbazonyl[20,21] have displayed noticeable antitubercular activity.

Thiosemicarbazones and hydrazones/hydrazides have long attracted attention, due to their biological and pharmacological properties. Thiosemicarbazides are a key component of drugs like, triapine (anticancer), metisazone (small pox), MAIQ (anticancer), isonicotinaldehyde thiosemicarbazone (antitubercular), thiacetazone (antitubercular), and ambazone (antiseptic) (**Fig.** 1).[20] Hydrazones are well explored as prodrugs for antitubercular treatment.[22-24]

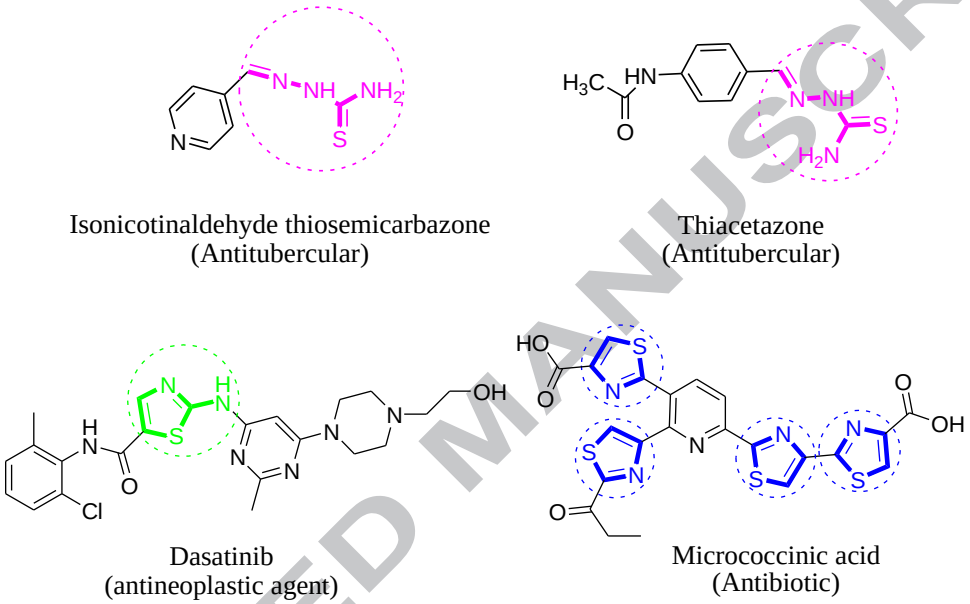


Figure 1. Thiazoles, amino thiazoles and thiosemicarbazones based drug candidates.

It is reviewed that, there is scanty information on the bithiazolyl scaffolds bridged with hydrazolyl pharmacophore in single molecular architectural frame. Therefore, it was thought to design and synthesize such molecular frameworks to achieve synergistic effect to get the entities with potential antitubercular activity. Hence, in the present work, it was decided to synthesize new bithiazolyl hydrazones (**6a-l**) and evaluate them for their antitubercular, cytotoxic, and antibacterial activities.

The synthesis of title bithiazolyl hydrazones (**6**) was initially attempted in two steps, path first and path second (**Scheme 1**).[25] To overcome the drawbacks of these paths and to obtain better yields of the title products, here then efforts were directed to carry one pot cyclocondensation of freshly prepared and readily available, 5-acyl thiazoles (**1a**, **1b**)

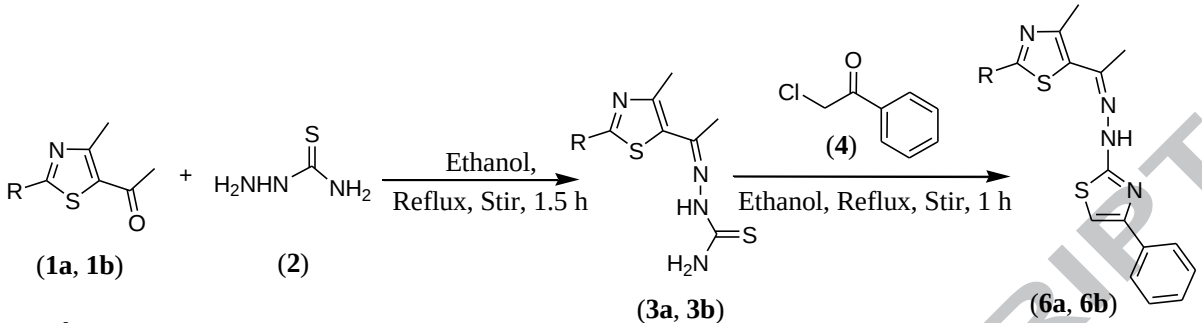
thiosemicarbazide (**2**) and substituted phenacyl chlorides (**4a-f**) in reflux ethanol (**Scheme 2, Route A**).[26] It was observed that though there was a little enhancement in the yields but time required for completion of cyclocondensation was pretty long i.e. more than 4 h (**Table S2**). To overcome these drawbacks and to practice green principles, here we then attempted this one pot cyclocondensation in various ionic liquids[27] at room temperature (**Table S1**). After screening the ionic liquids, it was found that diisopropyl ethyl ammonium acetate (DIPEAc)[28] was a suitable and novel medium for carrying the cyclocondensation leading to title products with excellent yields (**Table S1**, entry 7). The advantage of DIPEAc is that, it is stable, easily synthesized, cost effective, and recyclable.[28]

Hence, a mixture of three components, 5-acyl thiazoles (**1a-b**), thiosemicarbazide (**2**), and phenacyl chlorides (**4a-f**) (**Scheme 2, Route B**)[29] was dissolved in freshly prepared non-volatile organic solvent, DIPEAc and the solution was stirred at room temperature for about 30 min. Then, the products (**6a-l**) were isolated with enhanced yields, 82-96 % (**Table S2**).

The rate acceleration and enhancement of the yields of bithiazolyl hydrazones (**6a-l**) would be due to in situ successive condensations of 5-acyl thiazoles (**1a-b**), thiosemicarbazide (**2**), and phenacyl chlorides (**4a-f**). Initially, in situ phenacyl chlorides might be rapidly cyclocondensed with thiosemicarbazide yielding the intermediates, 2-hydrazinyl thiazoles (**5a-f**) which then subsequently be condensed in situ with available 5-acyl thiazoles, giving the titled, 1-(1-(2-phenyl/ methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6a-l**).

Here in this protocol DIPEAc might be displaying dual role. Its significant role could be a safer and non-volatile medium having ability to dissolve all the reactants at room temperature, generating their saturated solutions forming homogeneous mass. This initial high concentration of the reactants in the reaction solution might be helping for the rate acceleration of initial cyclocondensation and subsequent condensation. The other role of DIPEAc might be catalytical and the plausible mechanism, responsible for rate acceleration is depicted in the **Fig. 2**.

Path first:

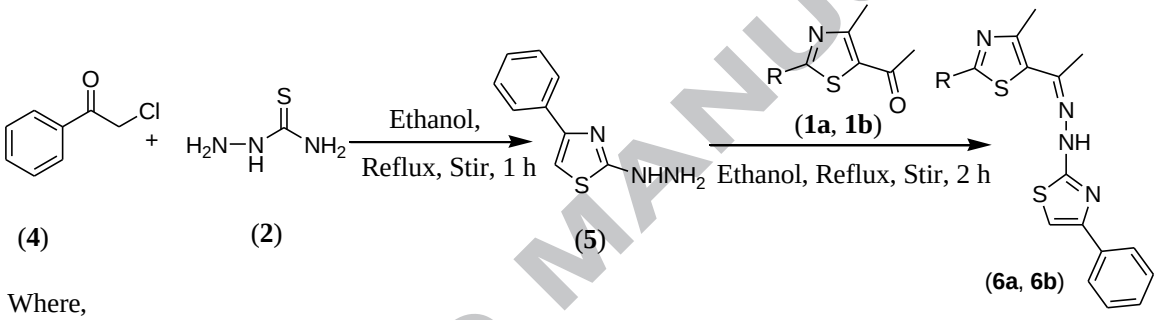


(**1a**, **1b**) (**2**) (**3a**, **3b**) (**6a**, **6b**)

Where,

R = -CH_3, -Ph

Path second:

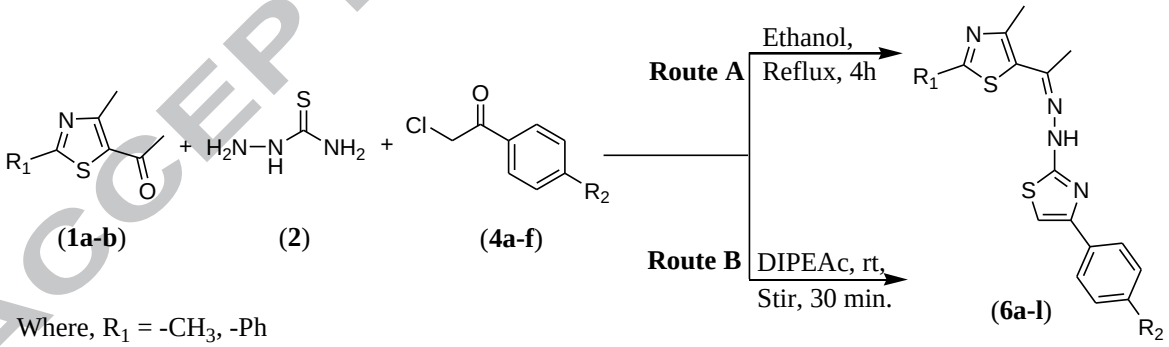


(**4**) (**2**) (**5**) (**6a**, **6b**)

Where,

R = -CH_3, -Ph

Scheme 1. Synthesis of 1-(1-(2,4-dimethylthiazol-5-yl) ethylidene)-2-(4-phenylthiazol-2-yl) hydrazine (**6**).



(**1a-b**) (**2**) (**4a-f**) (**6a-l**)

Where, R_1 = -CH_3, -Ph

R_2 = -H, -F, -Cl, -Br, -CH_3, -OCH_3

Scheme 2. One pot synthesis of 1-(1-(2-Phenyl/methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6a-l**) using ethanol and DIPEAc.

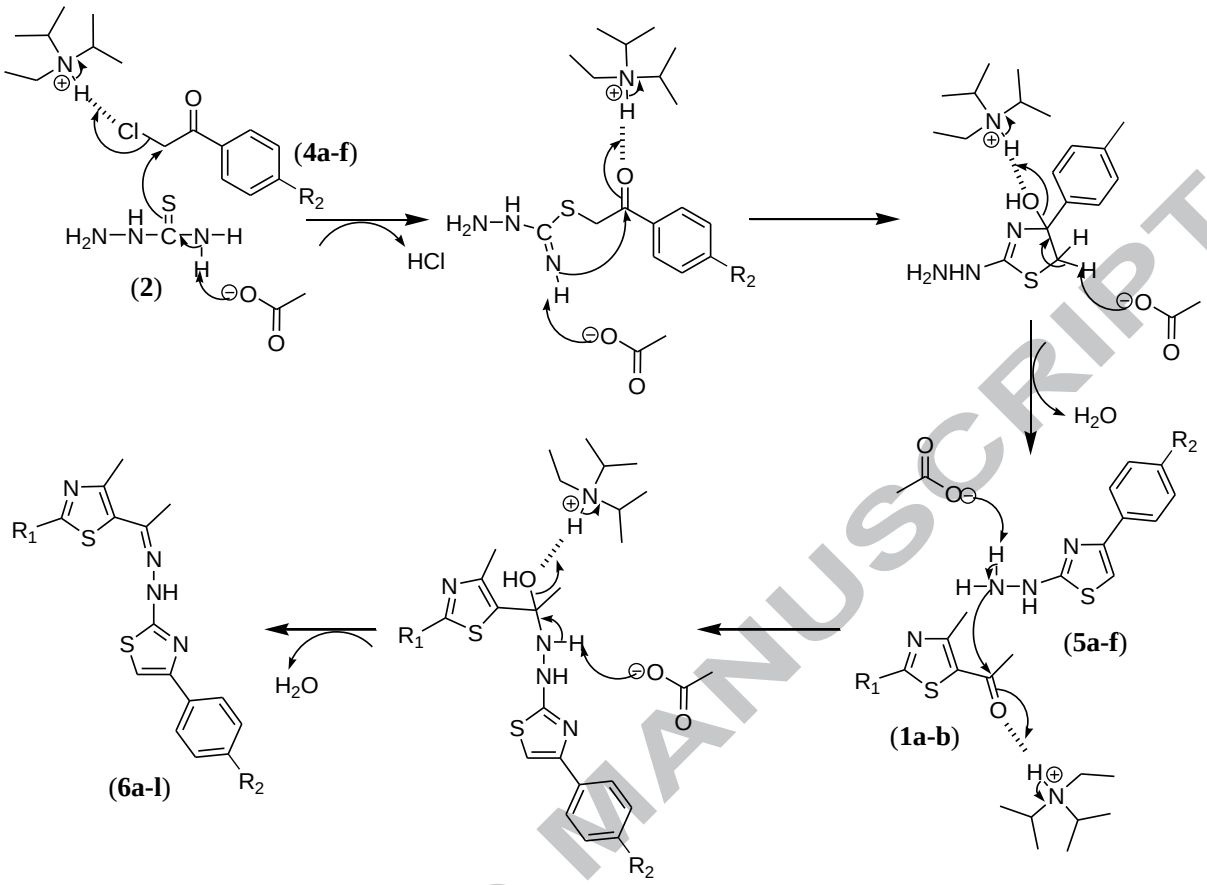


Figure 2. Plausible mechanism of successive one pot condensations leading to 1-(1-(2-Phenyl/methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6a-l**), carried in DIPEAc ionic liquid.

All the newly synthesized compounds were evaluated for their *in vitro antitubercular* activity against *Mycobacterium tuberculosis H37Ra (ATCC 25177) and Mycobacterium bovis Bacillus Calmette-Guerin (BCG) (ATCC 35743)* at dormant (12 days incubation) and active (8 days incubation) stages at concentrations of 0.7, 1.5, 3.1, 6.2, 12.5, 25, 50 and 100 μg/mL. Activity against *M. bovis* was estimated through nitrate reductase (NR) assay, reading absorbance at 540 nm.[30] XTT reduction menadione assay (XRMA) was performed to determine the inhibition of *M. tuberculosis*.[31] The absorbance of XRMA was measured at 470 nm.

In vitro studies against *M. tuberculosis H37Ra* and *M. bovis BCG* revealed notable antitubercular activity of the entities **3b**, **6a**, **6b**, **6d**, **6e**, **6f**, **6g**, and **6l** (**Table 1**). Among these

eight active compounds, 1-(1-(4-methyl-2-phenylthiazol-5-yl) ethylidene) thiosemicarbazide (**3b**) and 2-(4-(4-methoxyphenyl) thiazol-2-yl)-1-(1-(4-methyl-2-phenylthiazol-5-yl) ethylidene) hydrazine (**6l**) were found to be highly effective to inhibit both *M. tuberculosis* $H_{37}Ra$ and *M. bovis BCG* in active and dormant states, with MICs ranging from 1.78 to 14 μg/mL. MIC (<15 μg/mL) of thiazoles corroborate their well-known antimicrobial nature.[32] However, the activity is not profound as that of rifampicin (MIC 0.8 μg/mL).

Amongst the tested compounds, compound **3b** has thiosemicarbazonyl pharmacophore and phenyl moiety on thiazole ring came out as the most active compound against *M. tuberculosis* $H_{37}Ra$ (MIC in active stage, 1.78 μg/mL and in dormant stage, 2.05 μg/mL) and *M. bovis BCG* (MIC in active stage, 3.04 μg/mL and in dormant stage, 3.46 μg/mL). The compound **6l** has phenyl moiety in one of the thiazoles and 4-methoxy phenyl in other thiazole ring system has displayed better inhibition against *M. tuberculosis* $H_{37}Ra$ with MIC of 6.87 μg/mL in the active stage and 7.15 μg/mL in dormant stage.

The compounds **6b**, **6d**, and **6l** exhibited prominent antitubercular activity against *M. bovis BCG* with MICs in the range of 3.04-13.51 μg/mL and 3.46-14 μg/mL in the active and dormant stage respectively. From the standpoint of structure activity relationship, it was observed that the electronic environment of substituents on phenyl ring at R_2 position had no significant effect on biological activity as the active compounds had both electron withdrawing as well as donating substituents on them.

The pattern was similar in *ex vivo* THP-1 infection model assay with thiazolyl thiosemicarbazones (**3a**, **3b**) and bithiazolyl hydrazones (**6a-l**), having highest efficiency to inhibit *M. tuberculosis* (**Table 1**). MIC of dormant stage mycobacteria was observed to be greater than its active stage.

In view of this, the overall antitubercular activity exhibited by these thiazolyl thiosemicarbazones (**3a**, **3b**) and bithiazolyl hydrazones (**6a-l**) is significant, although they possess lower potencies as compared to that of standard drug, Rifampicin. Further experiments were carried out with the most effective compounds (**3b**, **6g** and **6l**).

Generally hydrazone derivatives are considered to be nontoxic.[33] Therefore, additionally all thiazolyl thiosemicarbazones (**3a**, **3b**) and bithiazolyl hydrazones (**6a-l**) were evaluated for their *in vitro* cytotoxic effect against three human cancer cell lines, MCF-7, HCT116 and THP-1 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with paclitaxel

Table 1

Antitubercular activities (*Ex vivo, in vitro*) and cytotoxicity profile (*In vitro*) against THP-1(Leukemia) human cancer cell line of thiazolyl thiosemicarbazones (**3a**, **3b**) and bithiazolyl hydrazones (**6a-l**)

Compound	*Ex vivo antitubercular activity* (*MIC_{90}, µg/mL*)		*In vitro antitubercular activity (*IC_{50}*, µg/mL)*				*In vitro antitubercular activity* (*MIC_{90}, µg/mL*)				Cytotoxicity profile (*GI_{50}, µg/mL*)
	MTB $H_{37}Ra$		*MTB $H_{37}Ra$*		*M. bovis BCG*		*MTB $H_{37}Ra$*		*M. bovis BCG*		THP-1
	(Active)	(Dormant)	(Active)	(Dormant)	(Active)	(Dormant)	(Active)	(Dormant)	(Active)	(Dormant)	(Leukemia)
3a	>100	>100	0.21	0.19	4.42	5.09	>100	30.54	>100	>100	>100
3b	1.94	3.67	0.12	0.78	0.94	0.97	1.78	2.05	3.04	3.46	81.51
6a	32.54	23.41	0.82	2.00	11.63	3.81	25.34	16.47	40.36	17.21	>100
6b	57.02	44.27	2.67	2.04	6.00	6.04	25.27	53.75	9.29	10.42	>100
6c	>100	>100	11.91	9.02	1.95	3.06	>100	>100	16.05	16.96	>100
6d	47.88	48.84	0.15	0.66	1.6	2.51	44.15	43.73	11.42	10.6	>100
6e	74.32	82.82	11.07	8.35	17.93	9.67	>100	65.76	54.76	83.17	>100
6f	48.23	46.17	0.88	1.24	7.37	8.41	74.07	20.49	67.33	24.04	>100
6g	15.58	11.97	0.17	0.98	8.75	7.95	9.46	12.76	21.2	18.71	>100
6h	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6i	>100	>100	0.12	0.24	16.98	26.58	>100	>100	77.85	83.68	>100
6j	>100	>100	0.18	0.14	>100	>100	>100	57.74	>100	>100	48.86
6k	>100	>100	0.16	0.075	5.21	5.29	>100	>100	19.66	57.49	>100
6l	12.31	9.38	0.078	0.075	2.43	2.82	6.87	7.15	13.51	14	>100
Rifampicin	0.5	0.7	0.51	0.75	0.45	0.81	0.51	0.75	0.45	0.81	>100
Paclitaxel	-	-	-	-	-	-	-	-	-	-	0.1374

RIF: Rifampicin; MIC_{90}: minimum inhibitory concentration for 90% (or greater) inhibition (µg/mL). GI_{50} indicates concentration to inhibit 50% growth of cells

as a positive control.[34,35] The corresponding GI_{50} and GI_{90} values of cell lines (MCF-7, HCT 116 and THP-1) have been calculated using OriginPro Software and are listed in **Table S3**. GI_{50} (>100 μg/mL) values of all compounds (except compounds **3b** and **6j**) against cell lines indicate that they are potent and specific inhibitors against MTB. The compounds, **3b** and **6j** have shown antiproliferative activity with GI_{50} values in between 25.99 to 81.51μg/mL against MCF-7, HCT 116 and THP-1 cell lines.

Selectivity index describes the selectivity of thiazolyl thiosemicarbazones (**3a**, **3b**) and bithiazolyl hydrazones (**6a-l**) toward human cell lines against *M. tuberculosis* (**Table 2** and **S4**). It reflects the amount of agent/drug is effective against mycobacteria without harming the host cells. GI_{50} of these compounds against the tested cell lines was >100 μg/mL, except **3b** and **6j**. Among these derivatives, **3b** showed the highest selectivity index (9–49) in dormant and active stage. Thus, a higher value of SI indicates that the compound can be used as a therapeutic agent. Moreover, the antimycobacterial activity of an agent is considered to be specific when selectivity index >10.[36] In our report **3b**, **6g** and **6l** exhibited high selectivity index, indicating their potential as antitubercular agent and these should be investigated further.

Table 2
Selectivity index (SI) of thiazolyl thiosemicarbazone (**3b**) and bithiazolyl hydrazones (**6g** and **6l**) on human cell lines against active as well as dormant stage of *M.tb. $H_{37}Ra$* and *M. bovis BCG*

Compound	SI on MCF-7		SI on HCT 116		SI on THP-1	
	Active Stage					
	Against *$H_{37}Ra$*	Against *BCG*	Against *$H_{37}Ra$*	Against *BCG*	Against *$H_{37}Ra$*	Against *BCG*
3b	15	9	27	16	46	27
6g	11	5	11	5	11	5
6l	15	7	15	7	15	7
RIF	196	222	196	222	196	222
	Dormant Stage					
3b	49	29	49	29	40	24
6g	8	5	8	5	8	5
6l	14	7	14	7	14	7
RIF	133	123	133	123	133	123

For investigating the specificity of (**3a**, **3b**) and (**6a-l**) compounds, all the compounds were screened for their anti-bacterial activity against four bacteria strains (Gram-negative strains: *Escherichia coli, Pseudomonas aeruginosa;* Gram-positive strains: *Staphylococcus aureus*, *Bacillus subtilis*) and it was observed that, two compounds (**6j**, **6k**) have displayed more than 90% inhibition in a compound concentration of 3 µg/ml (**Table 3** and **S5**). The compounds (**3a, 3b** and **6a-l**) exhibited MIC values >30 µg/ml. The compounds having good anti-bacterial activities were further analyzed for determining the Minimal Inhibitory Concentration (MIC). The calculation of MIC values for active anti-bacterial compounds was carried using Dose response curve. The compounds **6j** and **6k** showed a very good anti-bacterial activity against *E. coli, S. aureus, P. aeruginosa and B. subtilis,* with MIC values varying from 1.05 to 2.94 (**Table S6**).

Table 3

Antibacterial activities of bithiazolyl hydrazones (**6j-l**) against *E. coli, P. aeruginosa, S. aureus, B. subtilis*

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
	Gram-negative		Gram-positive	
	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)
6j	1.14	1.05	1.84	1.95
6k	1.43	1.53	2.46	2.94
6l	>30	>30	>30	>30
Ampicillin	1.46	4.36	1	10.32
Kanamycin	1.62	0.49	>30	1.35

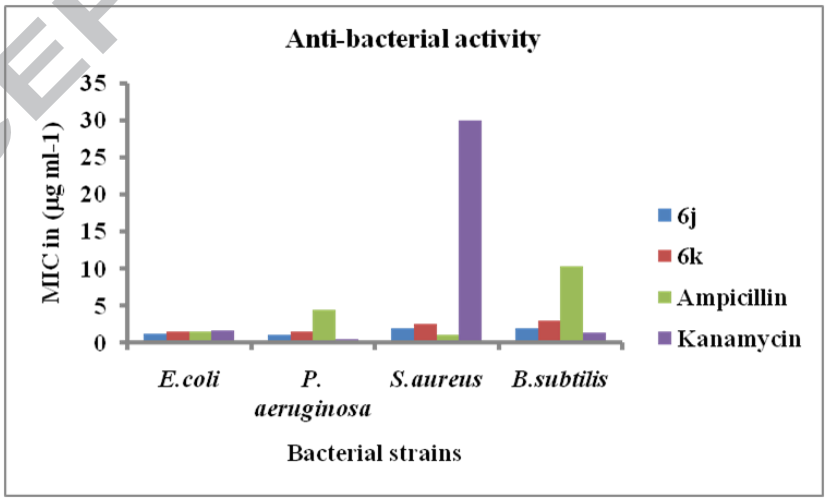


Figure 3. Comparison of MIC in (µg/ml) of compounds with some antibiotics.

The MICs of **6j** and **6k** compounds were found to be least as compared to others, not showing selectivity towards three human cancer cell lines, nil activity towards *M. tb.* and *BCG* shows that **6j** and **6k** can further be considered as potential anti-bacterial agent.

Molecular docking is a powerful tool in understanding different type of binding interactions of a molecule to the biological receptor. Therefore to rationalize the biological data and to investigate the possible interactions of the synthesized compounds (**3a-b** and **6a–l**), molecular docking study was performed with the target enzyme, decaprenylphosphoryl-β-D-ribose-2-epimerase (DprE1) of *M. tuberculosis*. The crystal structure of *M. tuberculosis* DprE1 enzyme, complexed with its inhibitor (pdb code: 4FDO) was used for the docking study.[37,38]

The objective of this work was to introduce bithiazolyl hydrazones as a potential scaffold against *M. tuberculosis* targeting DprE1. In the absence of available resources for the experimental enzyme-based assays, molecular docking has emerged as a very important tool to identify the targets for different ligands and the associated thermodynamic interactions with the target enzyme.[39-41] DprE1 is a highly conspicuous target being inhibited by multiple scaffold which motivated us to evaluate the binding affinity of the title class of molecules (bithiazolyl hydrazones) against this enzyme.[42-44] The significant correlation obtained between the docking scores and the *in vitro antitubercular* activity provides us with an opportunity to adopt the structure-based drug design approach i.e. molecular docking to optimize the title scaffold for this target DprE1. In order to carry out enzyme-based assay, we are utilizing the outcomes of current docking study to optimize this scaffold for designing more specific and tighter binding compounds. Once we arrive at compounds with very potent activity in the *in vitro* assay (which is one requirement to perform enzyme-based assay) we will perform assay against DprE1. In the future communication we will present the outcome of our designed bithiazolyl hydrazones based on molecular docking approach with enzyme-based activity against DprE1.

Molecular docking study revealed that all the bithiazolyl hydrazones could perfectly fit into the active site of DprE1 with varying degree of affinities adopting a very similar orientation and at co-ordinates very close to that of the native ligand. Their complexation was stabilized by a network of steric and electrostatic interactions. The Glide docking score for each of these analogues ranged from -7.31 to -6.00 with a significant correlation between the docking score and the obtained biological activity. A plot of the antitubercular activity expressed as IC_{50} values versus the glide docking score of NAH derivatives is shown in **Figure 4**. The active compounds having higher scores while those with relatively low active are also predicted to have a lower

score (**Table S7**). The more negative value of docking score and binding energy signify a good binding affinity of the ligand towards the target and vice versa. The docking score for the native ligand was found to be -7.95.

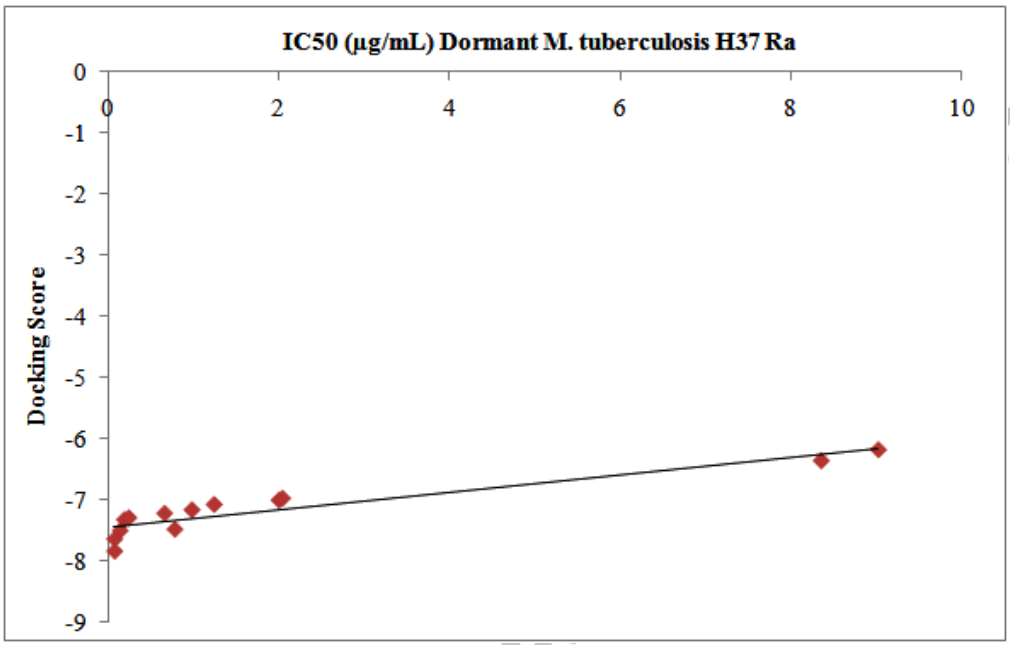


Figure 4. Correlation plot of the glide docking score vs activity against Dormant *M. tuberculosis* $H_{37}Ra$ (IC_{50}) for the bithiazolyl hydrazone derivatives.

A detailed per-residue interaction analysis between the docked bithiazolyl hydrazone derivatives and the residues lining the active site of the enzyme was carried out to identify the most significantly interacting the residues and the type of thermodynamic elements (bonded and non-bonded interactions) governing the binding of these molecules to the target. However in order to maintain the brevity of text, this analysis has been elucidated only for one of the most active analog (**6l)** while the results for the remaining compounds are summarized in (**Table S7**) and their binding modes are provided in the supplementary material as **Fig. 1S-13S**.

The lowest energy docked conformation of the most active bithiazolyl hydrazone (**6l**) into the active site of DprE1 enzyme (**Fig. 5**) showed, the inhibitor binds at the same site as the native ligand with a significantly higher binding affinity. The compound showed the highest binding affinity with a docking score of -7.84. This higher binding affinity can be explained in terms of the specific bonded and non-bonded per residue interactions with the residues lining the active site of DprE1.

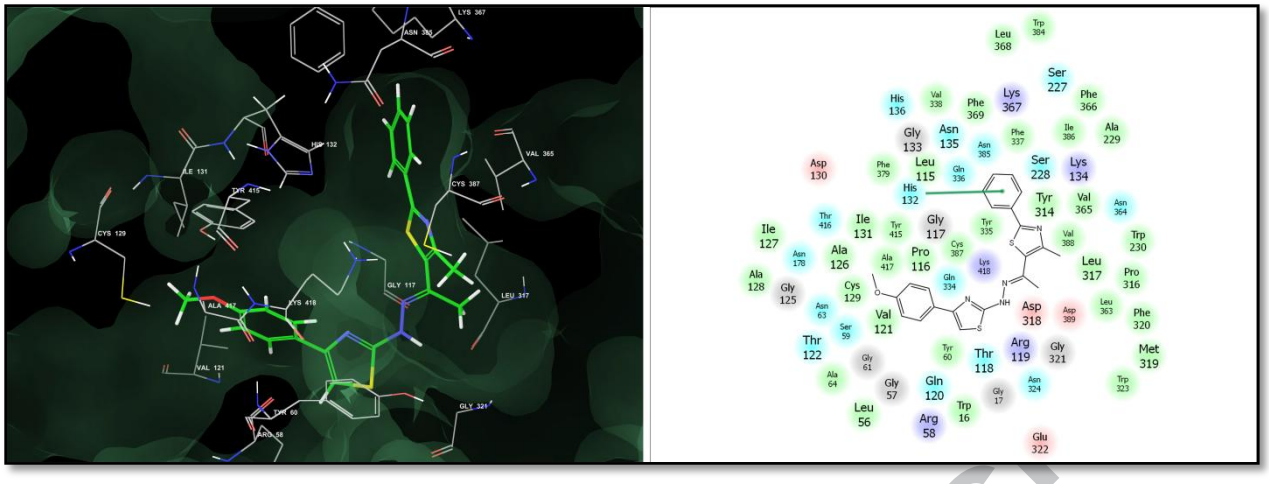


Figure 5. Binding mode of **6l** into the active site of DprE1 enzyme (the π-π stacking interaction has been represented using green lines).

Analysis of the binding pose of (**6l)** revealed that it is stabilized within the active site of DprE1 through an extensive network of favorable van der Waals interactions with Lys418 (-2.792 Kcal/mol), Tyr415 (-1.883 Kcal/mol), Cys387 (-2.662 Kcal/mol), Val365 (-4.113 Kcal/mol), Gly321 (-1.974 Kcal/mol), Leu317 (-4.706 Kcal/mol), Thr118 (-3.046 Kcal/mol), Tyr60 (-2.426 Kcal/mol), Trp16 (-2.083 Kcal/mol) through the bithiazolyl hydrazone scaffold. While a similar network of interaction observed with the Ala417 (-1.664 Kcal/mol), Asn385 (-1.844 Kcal/mol), Lys367 (-1.984 Kcal/mol), Gln336 (-1.929 Kcal/mol), His132 (-2.590 Kcal/mol), Ile131 (-1.904 Kcal/mol), Gly117 (-2.820 Kcal/mol), Pro116 (-1.893 Kcal/mol), Ser59 (-1.951 Kcal/mol), Arg58 (-3.444 Kcal/mol) lining the active site via the aromatic substitution on both terminal of the scaffold contributed to the binding affinity. Similarly the compound was engaged in a set of relatively few but significant electrostatic interactions as well observed with Lys418 (-4.413 Kcal/mol), Asp389 (-1.935 Kcal/mol), Glu322 (-1.833 Kcal/mol), Leu317 (-1.307 Kcal/mol) and Gly117 (-1.518 Kcal/mol) residues. The enhanced binding affinity of **6l** can also be attributed to a close pi-pi **(π- π)** stacking interactions observed between the aromatic ring and His132 residue (2.871Å) which serve as an "anchor", intensely determining the 3D orientation of the ligand in the active site. These strong thermodynamic interactions of **6l** with DprE1 account for its good *in silico* binding and provide a clue for the significant *in vitro antitubercular* activity.

A similar binding mode and network of interactions was observed for the other bithiazolyl hydrazones (please refer supplementary material) as well but decreasing gradually with their observed antitubercular activity. The per residue ligand interaction energy distribution

for all these bithiazolyl hydrazones showed that the primary driving forces for mechanical interlocking was the steric complementarity between the ligand and the active site residues of DprE1 as is reflected in the relatively higher number of favorable van der Waals interaction over other components contributing to the overall binding affinity. Overall, it is evident from this docking simulation and more specifically from the per-residue interaction analysis that these molecules have promising affinity for the DprE1 enzyme making them pertinent starting points for structure-based drug design.

New thiazolyl thiosemicarbazones (**3a** and **3b**) and bithiazolyl hydrazones (**6a-l**) have been conveniently synthesized. A novel one pot multi component synthetic protocol, mediated and catalyzed by diisopropyl ethyl ammonium acetate (DIPEAc), operating at room temperature have been first time developed for obtaining the title products (**6a-l**) with better to excellent yields. Thiosemicarbazones (**3a** and **3b**) and the hydrazones (**6a-l**) having thiazolyl motif were screened for their *in vitro antitubercular* and cytotoxicity activities and observed that most of them have displayed prominent activities. The compounds **3b**, **6g**, **6j**, **6k**, and **6l** have shown excellent inhibitory activity against active as well as dormant *M. tuberculosis* $H_{37}Ra$ (ATCC 25177) and *M. bovis Bacillus Calmette-Guerin* (*BCG*) (ATCC 35743) and found to have lower toxicity. In the current report compounds **3b**, **6g**, and **6l** exhibited a moderate selectivity index (SI) of >10, indicating their potential as an antitubercular agent. The compounds **3b** and **6j** have displayed notable antiproliferative activity against MCF7, HCT 116 and THP-1 human cancer cell lines. Compounds **6j** and **6k** have shown excellent antibacterial activity against strains *E. coli, S. aureus, P. aeruginosa, and B. subtilis*. The theoretical predictions from the molecular docking study were found to be in harmony with the trend observed in the *in vitro antitubercular* activity. A detailed per-residue interaction analysis was carried to gain an insight into the various thermodynamic components involved in the binding of these molecules within the enzyme cavity. These findings suggest that systematic alterations around these leads through iterative synthesis and docking could gradually lead to identification of potent antitubercular candidates.

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25. General procedure for the preparation of 1-(1-(2-methyl/phenyl-4-methylthiazol-5-yl) ethylidene) thiosemicarbazides (**3a**, **3b**). A mixture of 5-acyl thiazole (4.6 mmol) and thiosemicarbazide (4.6 mmol) was refluxed in dry ethanol for 1.5 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, reaction mass was allowed to cool at room temperature and then the reaction

mass was poured on crushed ice. The obtained solid mass was filtered, washed with water and dried. The crude products were crystallized using dry ethanol.

1-(1-(2,4-Dimethylthiazol-5-yl) ethylidene) thiosemicarbazide (**3a**). Yield 60%; yellow powder; mp 156-158℃. FTIR (KBr pellet) cm^{-1}: 3442 (N-H str.), 1570 (C=N str. hydrazolyl), 1086 (C-S-C str., aromatic); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.30 (s, 2H, -CH_3), 2.58 (s, 3H, -CH_3), 2.67 (s, 3H, -CH_3), 8.66 (1H, br, NH) ppm; HRMS (ESI, m/z): calcd for $C_8H_{13}N_4S_2$ ($M + H^+$) 229.0503; found: 229.05761.

1-(1-(4-Methyl-2-phenylthiazol-5-yl) ethylidene) thiosemicarbazide (**3b**). Yield 58%; yellow powder; mp 160-162℃. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.39 (s, 3H, -CH_3), 2.60 (s, 3H, -CH_3), 7.43-7.93 (m, 5H, Ar-H), 8.39 (1H, br, NH), 10.48 (1H, br, NH) ppm; HRMS (ESI, m/z): calcd for $C_{13}H_{15}N_4S_2$ ($M + H^+$) 291.066; found: 291.07326.

General procedure for the synthesis of 1-(1-(2-phenyl/methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6**). A mixture of thiazolyl thiosemicarbazone (**3)** (4.4 mmol) and phenacyl chloride (**4**) (4.4 mmol) was allowed to reflux in dry ethanol for 1 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the reaction mixture was allowed to cool at room temperature and poured on crushed ice and then neutralized by aqueous ammonia. The obtained solid compound was filtered, washed with water and dried. The crude compound was crystallized using DMF-Ethanol.

26. General procedure for one pot synthesis of 1-(1-(2-Phenyl/Methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6**) using ethanol as a solvent. 5-Acyl thiazole (**1**) (4.6 mmol), thiosemicarbazide (**2**) (4.6 mmol) and phenacyl chloride (**4**) (4.6 mmol) were refluxed in ethanol (5 ml) for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was poured on crushed ice and neutralized by aqueous ammonia. The obtained solid was filtered, washed with water and dried. The crude compound was crystallized using DMF-Ethanol.
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29. General procedure for one pot synthesis of 1-(1-(2-Phenyl/Methyl-4-methylthiazol-5-yl) ethylidene)-2-(4-substitutedphenylthiazol-2-yl) hydrazines (**6**) using diisopropyl ethyl ammonium acetate (DIPEAc). 5-Acyl thiazole (**1**) (4.6 mmol), thiosemicarbazide (**2**) (4.6

mmol) and phenacyl chloride (**4**) (4.6 mmol) were dissolved in freshly prepared diisopropyl ethyl ammonium acetate (DIPEAc) (5 ml) and stirred at room temperature for 30 min. After stirring the reaction mixture for 30 min., the reaction mass was poured on crushed ice and neutralized by aqueous ammonia. The obtained solid was filtered, washed with water and dried. The crude compound was crystallized using DMF-Ethanol.

1-(1-(2,4-Dimethylthiazol-5-yl) ethylidene)-2-(4-phenylthiazol-2-yl) hydrazine (**6a**). Yield 96%; brown powder; mp 154-156℃. FTIR (KBr pellet) cm^{-1}: 1550 (C=N str. hydrazolyl), 1100 (C-S-C str., aromatic) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H, -CH_3), 2.62 (s, 3H, -CH_3), 2.67 (s, 3H, -CH_3), 6.81 (s, 1H, thiazolyl), 7.41-7.73 (m, 5H, Ar-H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.55, 164.84, 159.13, 153.64, 148.31, 133.61, 129.31, 129.18, 125.76, 125.76, 114.27, 102.38, 19.19, 18.65, 18.50 ppm; HRMS (ESI, m/z): calcd for $C_{16}H_{17}N_4S_2$ ($M + H^+$) 329.0816; found: 329.0889.

2-(4-(4-Fluorophenyl) thiazol-2-yl)-1-(1-(2,4-dimethylthiazol-5-yl) ethylidene) hydrazine (**6b**). Yield 94%; brown powder; mp 172-174℃. FTIR (KBr pellet) cm^{-1}: 3448 (N-H str.), 1567 (C=N str. hydrazolyl), 1093 (C-S-C str., aromatic), 1131 (C-F str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.12 (s, 3H, -CH_3), 2.59 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 6.79 (s, 1H, thiazolyl), 7.04 (d, 2H, Ar-H, J = 8 Hz), and 7.69 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.75, 163.74, 163.46, 161.49, 150.23, 141.62, 130.96, 127.67, 127.61, 115.66, 115.49, 103.46, 19.12, 18.34, 16.92 ppm; HRMS (ESI, m/z): calcd for $C_{16}H_{16}FN_4S_2$ ($M + H^+$) 347.0722; found: 347.0790.

2-(4-(4-Chlorophenyl) thiazol-2-yl)-1-(1-(2,4-dimethylthiazol-5-yl) ethylidene) hydrazine (**6c**). Yield 85%; yellow powder; mp 164-166℃. FTIR (KBr pellet) cm^{-1}: 3321 (N-H str.), 1550 (C=N str. hydrazolyl), 1091 (C-S-C str.), 731 (C-Cl str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.16 (s, 3H, -CH_3), 2.61 (s, 3H, -CH_3), 2.65 (s, 3H, -CH_3), 6.87 (s, 1H, thiazolyl), 7.32 (d, 2H, Ar-H, J = 8 Hz), and 7.67 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.59, 163.80, 161.17, 150.03, 141.95, 133.62, 130.00, 128.86, 128.86, 127.18, 127.18, 115.64, 104.28, 19.17, 18.38, 16.96 ppm. HRMS (ESI, m/z): calcd for $C_{16}H_{16}ClN_4S_2$ ($M + H^+$) 363.0427; found: 363.0625.

2-(4-(4-Bromophenyl) thiazol-2-yl)-1-(1-(2,4-dimethylthiazol-5-yl) ethylidene) hydrazine (**6d**). Yield 90%; green powder; mp 176-178℃. FTIR (KBr pellet) cm^{-1}: 3380 (N-H str.), 1548 (C=N str. hydrazolyl), 1096 (C-S-C str.), 723 (C-Br str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.18 (s, 3H, -CH_3), 2.61 (s, 3H, -CH_3), 2.65 (s, 3H, -CH_3), 6.86 (s, 1H,

thiazolyl), 7.48-7.60 (m, 4H, Ar-H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.04, 169.65, 164.00, 153.30, 142.69, 131.86, 131.86, 130.08, 127.42, 127.42, 122.06, 114.25, 104.16, 19.14, 18.44, 17.21 ppm; HRMS (ESI, m/z): calcd for $C_{16}H_{16}BrN_4S_2$ ($M + H^+$) 406.9922; found: 406.9994.

1-(1-(2,4-Dimethylthiazol-5-yl) ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**6e**). Yield 82%; brown powder; mp 188-190℃. FTIR (KBr pellet) cm^{-1}: 3335 (N-H str.), 1553 (C=N str. hydrazolyl), 1103 (C-S-C str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.11 (s, 3H, -CH_3), 2.29 (s, 3H, -CH_3), 2.54 (s, 3H, -CH_3), 2.61 (s, 3H, -CH_3), 6.73 (s, 1H, thiazolyl), 7.13 (d, 2H, Ar-H, J = 8 Hz), and 7.57 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.85, 164.07, 161.17, 150.56, 144.12, 138.41, 130.11, 129.53, 129.53, 125.77, 125.77, 115.94, 102.29, 21.25, 19.14, 18.44, 17.74 ppm; HRMS (ESI, m/z): calcd for $C_{17}H_{19}N_4S_2$ ($M + H^+$) 343.0973; found: 343.1046.

2-(4-(4-Methoxyphenyl) thiazol-2-yl)-1-(1-(2,4-dimethylthiazol-5-yl) ethylidene) hydrazine (**6f**). Yield 93%; brown powder; mp 202-204℃. FTIR (KBr pellet) cm^{-1}: 3420 (N-H str.), 1550 (C=N str. hydrazolyl), 1098 (C-S-C str.), 1021 (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.09 (s, 3H, -CH_3), 2.58 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 3.76 (s, 3H, -OCH_3), 6.69 (s, 1H, thiazolyl), 6.87 (d, 2H, Ar-H, J = 8 Hz), and 7.64 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.41, 163.69, 159.43, 150.30, 150.02, 142.38, 133.40, 130.48, 127.27, 127.16, 114.66, 114.04, 114.04, 101.79, 55.24, 19.09, 18.34, 17.18 ppm; HRMS (ESI, m/z): calcd for $C_{17}H_{19}N_4OS_2$ ($M + H^+$) 359.0922; found: 359.0995.

1-(1-(4-Methyl-2-phenylthiazol-5-yl) ethylidene)-2-(4-phenylthiazol-2-yl) hydrazine (**6g**). Yield 92%; brown powder; mp 196-198℃. FTIR (KBr pellet) cm^{-1}: 3450 (N-H str.), 1549 (C=N str. hydrazolyl), 1095 (C-S-C str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H, -CH_3), 2.70 (s, 3H, -CH_3), 6.82 (s, 1H, thiazolyl), 7.36-7.94 (m, 10H, Ar-H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.39, 165.88, 161.46, 153.14, 146.35, 133.12, 130.41, 129.15, 129.15, 129.01, 129.01, 129.01, 129.01, 126.00, 126.00, 125.74, 125.74, 113.80, 102.45, 19.19, 18.49 ppm; HRMS (ESI, m/z): calcd for $C_{21}H_{19}N_4S_2$ ($M + H^+$) 391.0973; found: 391.1046.

2-(4-(4-Fluorophenyl) thiazol-2-yl)-1-(1-(4-methyl-2-phenylthiazol-5-yl) ethylidene) hydrazine (**6h**). Yield 89%; yellow powder; mp 192-194℃. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.22 (s, 3H, -CH_3), 2.68 (s, 3H, -CH_3), 6.77 (s, 1H, thiazolyl), 7.05 (d, 2H, Ar-H, J = 4

Hz), 7.40 (t, 3H, Ar-H, J = 4 Hz), 7.72 (d, 2H, Ar-H, J = 8 Hz), and 7.92 (d, 2H, Ar-H, J = 8 Hz) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.62, 165.16, 163.87, 161.41, 152.10, 148.80, 142.77, 133.27, 130.23, 130.23, 128.97, 128.97, 127.00, 127.00, 126.39, 126.39, 115.86, 115.64, 103.17, 18.95, 17.29 ppm. HRMS (ESI, m/z): calcd for $C_{21}H_{18}FN_4S_2$ (M + H^+) 409.0879; found: 409.09514.

2-(4-(4-Chlorophenyl) thiazol-2-yl)-1-(1-(4-methyl-2-phenylthiazol-5-yl) ethylidene) hydrazine (**6i**). Yield 90%; brown powder; mp 186-188℃. FTIR (KBr pellet) cm^{-1}: 3470 (N-H str.), 1564 (C=N str. hydrazolyl), 1089 (C-S-C str.), 725 (C-Cl str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.21 (s, 3H, $-CH_3$), 2.73 (s, 3H, $-CH_3$), 6.90 (s, 1H, thiazolyl), 7.36 (d, 2H, Ar-H, J = 4 Hz), 7.44 (t, 3H, Ar-H, J = 4 Hz), 7.73 (d, 2H, Ar-H, J = 8 Hz), 7.96 (d, 2H, Ar-H, J = 8 Hz), and 8.05 (s, 1H, =N-N-H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.52, 164.96, 162.61, 151.83, 150.18, 141.51, 133.61, 133.38, 130.15, 128.96, 128.96, 128.86, 128.86, 127.20, 127.20, 126.40, 126.40, 115.94, 104.39, 18.86, 16.95 ppm; HRMS (ESI, m/z): calcd for $C_{21}H_{18}ClN_4S_2$ (M + H^+) 425.0583; found: 425.0656.

2-(4-(4-Bromophenyl) thiazol-2-yl)-1-(1-(4-methyl-2-phenylthiazol-5-yl) ethylidene) hydrazine (**6j**). Yield 94%; tan powder; mp 182-184℃. FTIR (KBr pellet) cm^{-1}: 3430 (N-H str.), 1552 (C=N str. hydrazolyl), 1088 (C-S-C str.), 664 (C-Br str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H, $-CH_3$), 2.75 (s, 3H, $-CH_3$), 6.93 (s, 1H, thiazolyl), 7.45 (t, 3H, Ar-H, J = 4 Hz), 7.52 (d, 2H, Ar-H, J = 4 Hz), 7.66 (d, 2H, Ar-H, J = 8 Hz), and 7.96 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.41, 167.41, 165.01, 151.85, 148.35, 133.61, 133.36, 131.80, 131.80, 128.96, 128.96, 128.96, 128.96, 128.00, 126.41, 126.41, 121.79, 116.16, 104.55, 18.88, 16.88 ppm; HRMS (ESI, m/z): calcd for $C_{21}H_{18}BrN_4NaS_2$ (M + H^+) 490.9999; found: 490.9980.

1-(1-(4-Methyl-2-phenylthiazol-5-yl) ethylidene)-2-(4-p-tolylthiazol-2-yl) hydrazine (**6k**). Yield 85%; yellow powder; mp 196-198℃. FTIR (KBr pellet) cm^{-1}: 3350 (N-H str.), 1550 (C=N str. hydrazolyl), 1091 (C-S-C str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.16 (s, 3H, $-CH_3$), 2.36 (s, 3H, $-CH_3$), 2.73 (s, 3H, $-CH_3$), 6.86 (s, 1H, thiazolyl), 7.20 (d, 2H, Ar-H, J = 4 Hz), 7.44 (t, 3H, Ar-H, J = 4 Hz), 7.68 (d, 2H, Ar-H, J = 8 Hz), and 7.96 (d, 2H, Ar-H, J = 8 Hz) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.63, 167.06, 164.85, 151.65, 141.47, 137.74, 133.43, 130.10, 129.39, 129.39, 128.95, 128.95, 126.39, 126.39, 126.39, 125.92, 125.92, 118.72, 103.14, 21.24, 18.78, 16.95 ppm; HRMS (ESI, m/z): calcd for $C_{22}H_{21}N_4S_2$ (M + H^+) 405.1129; found: 405.1202.

2-(4-(4-Methoxyphenyl) thiazol-2-yl)-1-(1-(4-methyl-2-phenyl thiazol-5-yl) ethylidene) hydrazine (**6l**). Yield 93%; brown powder; mp 184-186℃. HRMS (ESI, m/z): calcd for $C_{22}H_{21}N_4OS_2$ ($M + H^+$) 421.1079; found: 421.1151.

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Graphical Abstract

New bithiazolyl hydrazones: novel synthesis, characterization and antitubercular evaluation

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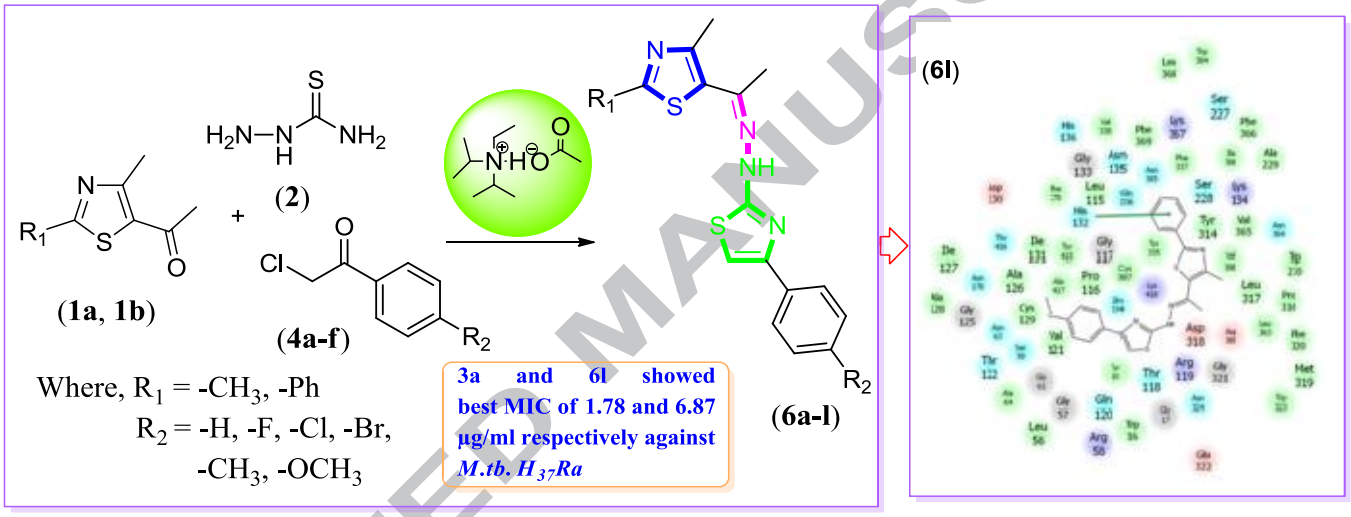

Highlights

- New bithiazolyl hydrazones have been synthesized by one pot novel synthetic route.
- Bithiazolyl hydrazones displayed noticeable antiTB activity compared to Rifampicin.
- Compounds, **3b**, **6g**, **6j**, **6k**, and **6l** have shown excellent antiTB activity.
- Molecular docking study has shown better harmony with the evaluation trend.