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Synthesis and antimycobacterial activities of some new thiazolylhydrazone derivatives

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ABSTRACT

This Letter reports the synthesis and evaluation of some thiazolylhydrazone derivatives for their in vitro antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv. The cytotoxic activities of all compounds were also evaluated. The compounds exhibited promising antimycobacterial activity with MICs of 1.03–72.46 μM and weak cytotoxicity (8.9–36.8% at 50 μg/mL). Among them, 1-(4-(1*H*-1,2,4-triazol-1-yl)benzylidene)-2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazine **10** was found to be the most active compound (MIC of 1.03 μM) with a good safety profile (16.4% at 50 μg/mL). Molecular modeling studies were done to have an idea for the mechanism of the action of the target compounds. According the docking results it can be claimed that these compounds may bind most likely to TMPK than InhA or CYP121.

Mycobacterium tuberculosis is the causative agent of human tuberculosis. Tuberculosis is highly contagious and spreads through the air from coughing. If not treated, a person with TB infects an average of 10 to 15 new people each year. For HIV positive people the risk is much higher. According to the WHO report in 2012, there were almost 9 million new cases of tuberculosis and 1.4 million deaths.[1] The treatment regimen consists of an initial 2-month phase of treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol followed by a continuation phase of treatment lasting 4 months with isoniazid and rifampicin.[2] But a combination of isoniazid with other antibiotics is becoming ineffective against multi drug-resistant and extensively drug-resistant strains of *M. tuberculosis*. Hence, faster acting and effective new drugs to treat tuberculosis are needed. Hydrazones constitute an important class of compounds for new antitubercular drug development because several studies have been reported that hydrazone compounds could be prodrugs for antitubercular treatment.[3–5]

During recent years, some thiazolylhydrazone derivatives have been reported to exhibit remarkable growth inhibitory activity towards *M. tuberculosis* (Fig. 1).[6–8] On the other hand, many studies showed that azole heterocycles such as imidazole and triazole are useful pharmacophores for antimycobacterial activity.[9–13] These findings prompted us to synthesize new hybrid molecules by combining azole and thiazolylhydrazone structures and evaluate their antimycobacterial activity.

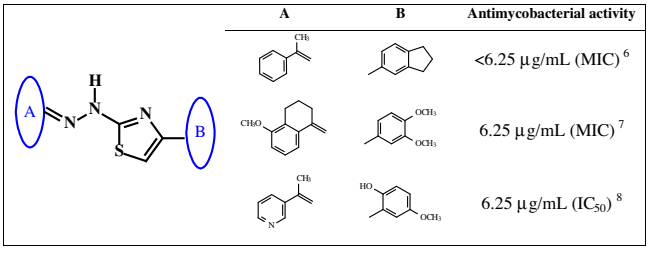

Figure 1. Some thiazolylhydrazones with antimycobacterial activity.

Although the number of three dimensional structures of *M. tuberculosis* proteins has been increasing rapidly in recent years, determination of biochemical pathways of the antimycobacterial drugs is still a challenge.[14,15] In order to find a clue for the mechanism of the action of the target compounds, molecular docking studies have been done with compound **10** and some crucial biochemical targets which are essential for the *M. tuberculosis* but different from human host. For this reason, cytochrome P450 monooxygenase (CYP121) which is inhibited by azole drugs,[16] the enoyl acyl carrier protein reductase (InhA) which is one of the key enzymes involved in the type II fatty acid biosynthesis pathway[17] and the thymidine kinase (TMPK) which catalyzes the reversible conversion of thymidine monophosphate to thymidine

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diphosphate, as important intermediate for DNA-building blocks[18] of *M. tuberculosis* were selected for the docking studies.

The starting compounds, 4-azolylbenzaldehydes **1a–b** were synthesized in accordance with the method described in the literature.[19] **1a–b** and thiosemicarbazide were refluxed in isopropanol in the presence of acetic acid to obtain thiosemicarbazones **2a–b**. The target compounds **3–18** were prepared by cyclization of **2a–b** with substituted phenacyl bromides (Scheme 1).

The target compounds **3–18** were evaluated for their antimycobacterial activity in vitro against *M. tuberculosis* $H_{37}R_v$ using the microplate alamar blue assay method[20] in duplicate. The results of the antimycobacterial activity (MIC values) were reported in Table 1. It was observed that, 1-(4-(1*H*-1,2,4-triazol-1-yl)benzylidene)-2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazine **10** with MIC of 1.03 μM, was more active than standard compounds, ethambutol and ciprofloxacin (MIC = 7.65 and 4.71 μM respectively). Additionally, **4**, **9**, **12**, **15**, **16** and **18** were found as active as ethambutol and ciprofloxacin. Non substituted derivatives exhibited weakest activity in this series (**3** and **11** with MIC of 72.46 and 72.25 μM respectively). These findings show that the presence of substituent on the phenyl ring (R group) is important for the activity. Introduction of NO_2 which could be probably converted to active amino group by reduction enhanced the antimycobacterial activity (**10** and **18** with MIC of 1.03 and 3.99 μM respectively). The gap of activity between nitro substituted and other compounds may be explained by this reduction. It was also observed that the presence of Cl and F atoms on phenyl ring increased antimycobacterial activity remarkably (**4**, **5**, **12** and **13**; MIC = 4.29–8.22 μM). However the substitution of Br to the phenyl ring did not significantly affect the activity (**6** and **14**; MIC = 59.10 and 29.48 μM respectively). However, no relationship can be established between other substituents (2,4-diCl, CH_3 and OCH_3) and the antimycobacterial activity in the series. When the activities of **3** and **11** are compared, it can be assumed that the type of azole structure is not a decisive factor for the activity.

All compounds were also tested for in vitro cytotoxicity against THP1cell line at 50 μg/mL by MTT assay method. Percentage growth of cells was reported in Table 1. None of the compounds showed significant cytotoxicity (8.9–36.8%).

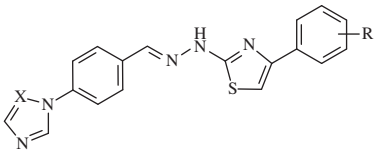
In order to further rationalize the biological results, molecular docking studies were performed on compound **10**, the most active derivative, with selected enzymes, CYP121, InhA and TMPK of *M. tuberculosis*. To check the docking procedure, the ligands which were taken from crystal structure of the enzyme-ligand complexes, were rebuilt and redocked. RMSD values of the docking pose from the original orientation of the ligands were found between 0.33–0.51 Å. The crucial residues of enzymes that interact with ligands were given in Table 2.

Firstly, CYP121, which is known to bind azole drugs (ex. fluconazole),[21] were selected for docking studies. Unfortunately, docking results (Fig. 2A) showed that **10** did not make any interaction with CYP121 (Table 2) and the orientation of **10** was completely different from that of fluconazole (Fig. 3A).

Then, docking studies were carried out with InhA and TMPK. According to docking results with InhA, it was observed that **10** interacts with Pro156 and Tyr158 (Fig. 2B). For InhA, it is known that Tyr158 is a key residue for activity[22] and it forms a direct hydrogen bond with inhibitors by its hydroxyl group.[23] Differently from mentioned interaction, π–H interaction was observed between phenyl ring of **10** and methylene of Tyr158. Docking studies with TMPK revealed that **10** interacted with Tyr103, Asp163 and Glu166 (Fig. 2C). It was reported that Tyr103 is a residue which is responsible for the affinity of TMPK,[24] whereas Asp163 and Glu166 are the residues of lid region[25] of TMPK. By regarding the interactions (Table 2) and orientations (Fig. 3B and C respectively), it can be proposed that the docking results of InhA and TMPK are more reasonable than that of CYP121.

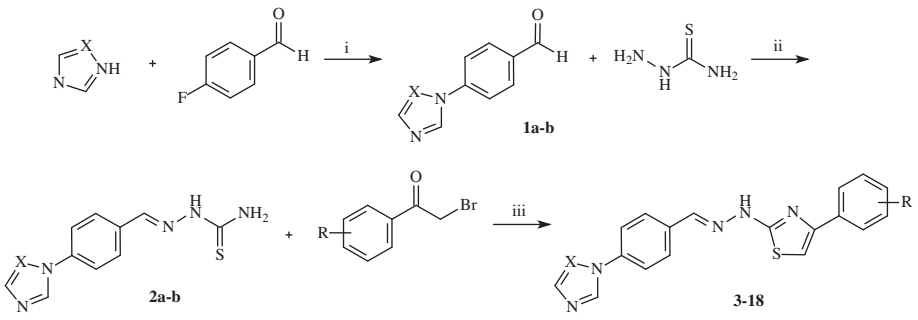
On the other hand, the order of the energies of the conformers of **10** in enzymes was found as $10_{InhA} > 10_{Cyp121} > 10_{TMPK}$. Having

Table 1
Antimycobacterial activity and cytotoxicity of the compounds



Compound	X	R	MIC[a] (μM)	Cytotoxicity[b] (% inhibition)
3	CH	4-H	72.46	21.8
4	CH	4-F	4.30	18.7
5	CH	4-Cl	8.22	32.1
6	CH	4-Br	59.10	36.8
7	CH	2,4-DiCl	15.10	28.8
8	CH	4-CH_3	32.98	36.0
9	CH	4-OCH_3	4.16	26.8
10	CH	4-NO_2	1.03	16.4
11	N	4-H	72.25	28.4
12	N	4-F	4.29	35.9
13	N	4-Cl	8.20	28.4
14	N	4-Br	29.48	32.6
15	N	2,4-DiCl	3.76	8.9
16	N	4-CH_3	4.33	13.6
17	N	4-OCH_3	16.62	23.0
18	N	4-NO_2	3.99	12.9
INH			0.36	—
Rifampin			0.12	—
Ethambutol			7.65	—
Ciprofloxacin			4.71	—

[a] MIC = minimum inhibitory concentration.
[b] Cytotoxicity against THP1 cell line at 50 μg/mL.



X: CH (a), N (b); **R**: H, 4-F, 4-Cl, 4-Br, 2,4-diCl, 4-CH_3, 4-OCH_3, 4-NO_2

Scheme 1. Synthesis of the compounds. Reagents and conditions: (i) K_2CO_3, DMSO, ultrasonic irradiation; (ii) CH_3COOH, isopropanol, reflux; (iii) THF, rt.

Table 2
The data obtained from docking studies

Enzyme (ligand)	PDB	RMSD	Residues that interact with bound ligand	Residues that interact with compound 10
CYP121 (fluconazole)	2ij7	0.33	Met62, Val78, Val82, Val83, Phe168	—
InhA ((3*S*)-1-cyclohexyl-5-oxo-N-phenylpyrrolidine-3-carboxamide)	2h7i	0.37	Gly96, Lys165, Tyr158, Met103, Ile215, Pro156, Ala157, Met199, Gln100	Pro156, Tyr158
TMPK (3′-azido-3′-deoxythymidine-5′-monophosphate)	1w2h	0.51	Asp9, Pro37, Phe70, Arg74, Arg95, Asn100, Tyr103, Asp163	Tyr103, Asp163, Glu166

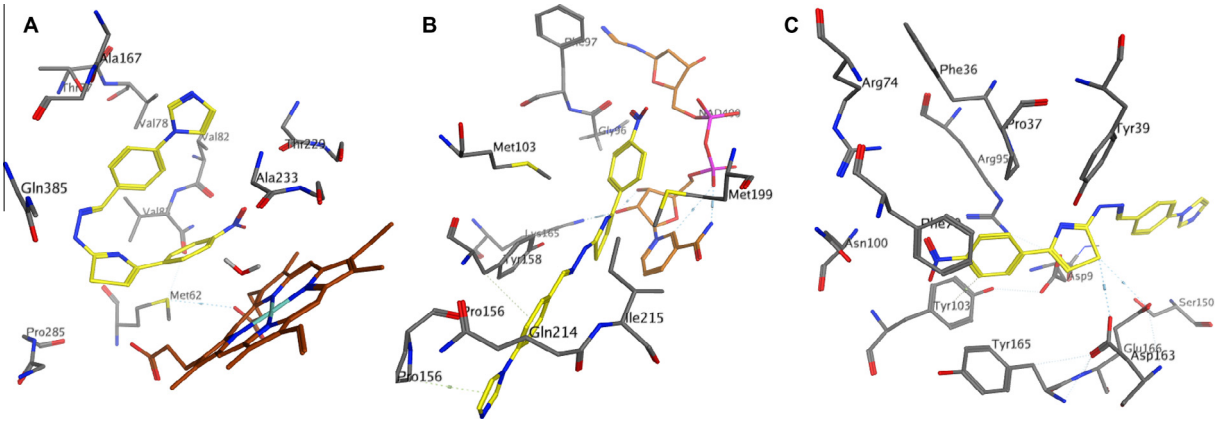


Figure 2. The best docking pose of **10** in **(A)** CYP121; **(B)** InhA; **(C)** TMPK.

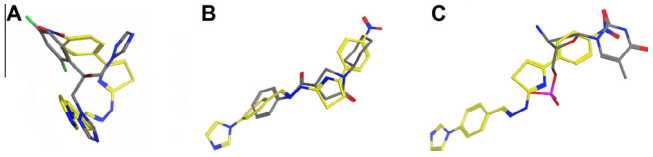


Figure 3. Superimposition of best pose of **10** with (A) fluconazole; (B) (3*S*)-1-cyclohexyl-5-oxo-N-phenylpyrrolidine-3-carboxamide; (C) 3′-azido-3′-deoxythymidine-5′-monophosphate.

taken the mentioned data into account, it can be assumed that **10** fits better into active site of TMPK than that of CYP121 and InhA.

In summary, a new series of thiazolylhydrazones as antimycobacterial agents was reported in the study. Among them, 1-(4-(1*H*-1,2,4-triazol-1-yl)benzylidene)-2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazine **10** was found to be the most active compound (MIC of 1.03 μM) with a good safety profile (16.4% at 50 μg/mL). It is interesting to note that substitutions on the phenyl ring make wide impact on antimycobacterial activity rather than types of azole ring. The docking results presented herein have shown that these compounds bind most likely to TMPK than InhA or CYP121.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.052.

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