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Novel 1,3,4-(thiadiazol-2-ylamino) methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-thiones: synthesis, docking and antimycobacterial testing

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ABSTRACT

In the present study, a novel series of Mannich bases of 3-substituted 5-(pyridin-4-yl)-1,3, 4-oxadiazol-2-thione derivatives were synthesized. Docking study was performed to rationalize the possible interactions between the synthesized compounds and active site of 14DM. The test compounds were screened for antimycobacterial activity using Middlebrook 7H9 medium against *M. tuberculosis* H37Rv (ATCC 27294) as well as Isoniazid (INH) resistant clinical strain. Among the series 5c and 5a are found to be most potent (susceptible) while the compound 5f did not show activity against *M. tuberculosis* H37Rv (resistant). The SAR study reveals the importance of substitutions at *para* position for good activity.

Keywords: Mannich Base; Oxadiazole; Thiadiazole; Antimycobacterial; Docking

1. INTRODUCTION

Tuberculosis (TB) is a pandemic disease and its causative agent *Mycobacterium tuberculosis* is one of the most prolific infectious agents affecting humans. The 196 countries reporting to WHO in 2008 notified 5.6 million new and relapse cases in 2007, of which 2.6 million (46%) were new smear-positive cases [1]. Furthermore, treatment of tuberculosis with human immuno deficiency virus infected patients (HIV) is difficult and results as the leading cause of death among HIV positive patients worldwide. Another factor which contributes to more number of deaths is the emergence of multiple drug resistance (MDR) [2-5].

1,3,4-Oxadiazoles and thiadiazoles have been reported as potent heterocyclic ring system with wide spectrum of biological activities [6-10]. Recently, 1,3,4-oxadiazole derivatives has been reported as antimycobacterial agents also [11]. The conversion of Isoniazid (INH) to Oxadiazoles produces the corresponding 5- substituted 3H-1,3,4-oxadiazol-2-thione, 3H-1,3,4,-oxadiazol-2-one and their 3-alkyl or aralkyl derivatives and is responsible for potent activity against *M. tuberculosis* strain H37Rv [12,13]. Foroumadi *et al* have reported a series of alkyl (5-(nitroaryl)-1,3,4-thiadiazole-2-ylthio) propionates as antimycobacterial agents [14]. The biologically active derivatives synthesized as mannich bases are reported to be physiologically important because of the altered solubility in aqueous solvents and has been used as antitubercular, antimalarial, vasorelaxing, anticancer and analgesic drugs [15-18].

Till now, no new drug has been introduced since the discovery of Rifampin in spite of major advances that have been made in the drug discovery process. Hence, there is an overwhelming need to develop novel antimycobacterial agents. Literature survey revealed that genomic DNA from *the M. tuberculosis* (MT) H37 Rvstrain, CYP 51-like gene encodes a bacterial sterol 14α-demethylase (MT P450 14DM), which acts on 14 α -methyl sterols. Recently we have reported a series of novel 4-(morpholin-4-yl)-N'-(arylidene) benzohy-drazides as antimycobacterial agent [19]. In continuation of our project in development of new antimycobacterial agents, we thought of hybridizing the two potential pharmacophores *i.e.*, 1,3,4-oxadiazole and thiadiazole which as such has been reported as antimycobacterial agents. Furthermore, solubility plays an important role for the development of drug in tuberculosis. Thus our aim was further refined to synthesize mannich base of these two pharmacophores and evaluate them for anti antimycobacterial activity. Herein we report the synthesis, docking and *in vitro* antimycobacterial activity of a

series of mannich bases belonging to 5-(pyridine-4-yl)-1,3,4-oxadiazo l-2-thiones. The docking study was performed to rationalize the possible interactions between the synthesized compounds and the active site of 14DM.

2. EXPERIMENTAL

2.1. Chemistry

The synthetic route used for the title compounds is outlined in **Scheme 1**. The thiosemicarbazones **2** were prepared by refluxing thiosemicarbazide and appropriate aromatic aldehydes **1** at 80˚C for 4 hr. 2-amino – 5-substituted 1,3,4-thiadiazole **3** were obtained by refluxing thiosemicarbazones **2** with ammonium ferric sulphate in water for 5 hrs. Oxadiazole-2-thione **4** was prepared according to the method reported in the literature using Isoniazid as starting material [13]. Mannich bases **5a-f** were obtained by reacting 2-amino-5-substituted 1,3,4-thiadiazole and oxadiazole-2-thione in DMF with formamide for 2-3 hr [20-22].

2.2. Modeling Studies

2.2.1. Molecular Docking Protocol

All computations were carried out on a Wipro Intel Pentium processor with Windows XP Operating System. All the compounds were constructed using standard fragment library of Maestro 8.0 and geometry optimization was done by Macromodel program Schrödinger, LLC) using Optimized Potentials for Liquid Simulations-all atom (OPLS-AA) force field [23]. The molecular docking tool, GLIDE (Schrodinger Inc., USA) was used for ligand docking studies into the X-ray crystal structure of cytochrome P450 14α-sterol Demethylase (14DM) from *Mycobacterium* in complex with 4-phenylimidazole (PDB entry code 1E9X) [24] were downloaded from the RCSB Protein Data Bank (PDB). The protein structure was prepared for docking using 'protein preparation wizard' in Maestro wizard 8.0. The protein preparation uses the OPLS force field 23 for this purpose. Grids were defined by centering them on the ligand in the crystal structure using the 10 Å box size. Ligprep 2.2 module utilized to produce the low energy conformer of ligands using MMFF94 force field [25]. The lower energy conformations of the ligands were selected and were docked into the grid generated from protein structures using standard precision (SP) docking mode [26].

2.2.2. Docking and Scoring Functions

The docking studies were performed for the designed compounds with 14DM enzyme and the results were compared with the natural ligand phenyl imidazole present within the receptor. The docked complexes of the designed compounds along with the ligand receptor poses have been shown in the **Figure 1**. The final evaluation is done with glide score (docking score) and single best pose is generated as the output for particular ligand.

$$\text{Gscore}=a^{*}\text{vdw}+b^{*}\text{cow}+\text{Lipo}+\text{H bond}+\text{Metal}+\text{BuryP}+\text{Rot B}+\text{Site}$$

where, vdW: Van der Waal energy; Coul: Coulomb energy; Lipo: lipophilic contact term; HBond: hydrogen-bonding term; Metal: metal-binding term; BuryP: penalty for buried polar groups; RotB: penalty for freezing rotatable bonds; Site: polar interactions at the active site; and the Coefficients of vdW and Coul are: a = 0.065, b = 0.130. If GScore was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to be reported to the user. Emodel combines GlideScore, the nonbonded interaction energy, and, for flexible docking, the excess internal energy of the generated ligand conformation.

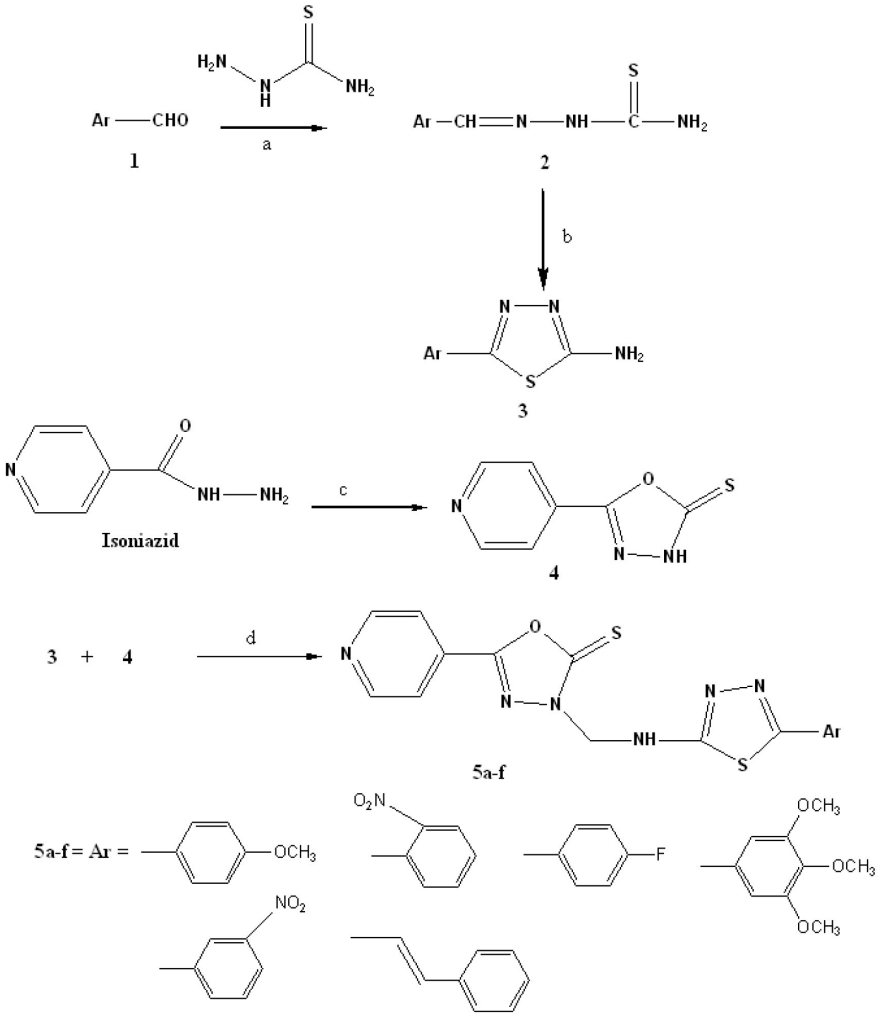
2.2.3. Antimycobacterial Activity

All the newly synthesized mannich bases were assayed *in vitro* for antitubercular activity against *M. tuberculosis* H37Rv (ATCC 27294) and Isoniazid resistant strain. The anti-TB screening was carried out by using Middlebrook 7H9 medium [27,28].

3. RESULTS

3.1. Modeling

The docked complex of the designed compounds is shown in **Figure 1**. The designed compounds were found to display good binding affinity to the receptor. *G-score, H-Bond Interaction and Contacts* The more negative value of G-score indicates that the compound is more potent and good binding affinity (**Table 1**). The Standard (CoCrystalisedLigand) and Isoniazid have shown G-Score of –4.73 and –5.02. The G-score of the designed compounds were found to be **5c** > **5a** > Isoni azid > **5b** > **5e** > standard co-crystallized ligand > **5d**. Besides the G-score, other parameters like energy and the E-model were also taken into consideration for the evaluation of the docking results; the values of the energy and E-model were found to be significantly more than that of the values of the standard co-crystallized ligand and Isoniazid. The designed compounds, **5a** and **5d** found to display 1 and 2 H- bonds with HIS 259 respectively. Standard (CoCrystalised Ligand) showed 1 H-Bond with HIS 259. It is well established and accepted fact that number of good van der Waals interactions decides the binding affinity for any ligand with receptor enzyme protein and bad, ugly contacts indicate steric clashes after docking which should be less for good activity. Therefore we have analyzed the binding



Scheme 1. Synthesis of 1,3,4-(thiadiazol-2-ylamino)methyl-5-(pyridine-4-yl)-1,3,4- oxadiazol-2-thione. Reagents and conditions (a) Ethanol, HCl, Reflux (b) Ammonium ferric sulphate, H_2O, Reflux, (c) CS_2, KOH, (d) CH_2O, 37%.

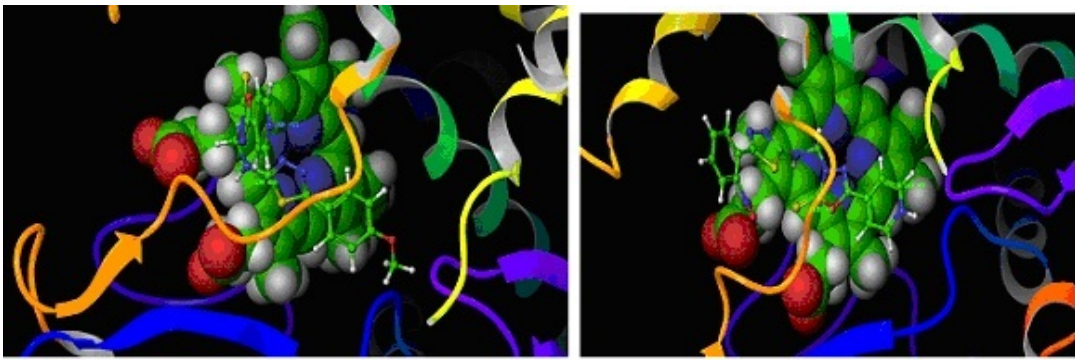


Figure 1. (a) Binding mode of 5c inside the pocket of crystal structure 14 demethylase; (b) Binding of 5a in 14 demethylase.

modes and abilities, considering the number of good, bad and ugly van der Waals (vdW) interactions of the standard and designed compounds with 14-DM active binding site.

3.2. ADME Properties

We have analyzed 44 physical descriptors and pharmaceutically relevant properties of mannich bases of

3-substituted 5-(pyridine-4-yl)-1,3,4-oxadiazol-2-thione derivatives using Qikprop, among which significant descriptors are reported (**Table 2**) and are important for predicting the drug-like properties of molecules. These properties were:

1) Molecular weight (Mol_MW) (130 - 460)

2) Octanol/water partition coefficient (Log Po/w) (–2 - 3.6)

3) Aqueous solubility (QPlogS) (–5.5 - 1)

4) Apparent MDCK cell permeability (QPPMDCK) (<25 poor, >500 great)

5) Brain/blood partition coefficient (QPlogBB) (<–1.7)

6) Percent human oral absorption (C80% is high, B25% is poor)

3.3. Antimycobacterial Activity

Amongst the compound tested **5c** and **5a** have shown good antimycobacterial activity (susceptible) against *M. tuberculosis* H37Rv (ATCC 27294) among the series. The effect of other test compounds on H37Rv and Isoniazid resistant strain is given in **Table 3**.

4. DISCUSSION

The results obtained reveals that the nature of substituents on the aromatic ring have a considerable impact on the antitubercular activities of the test compounds. Literature survey indicates that electrons-withdrawing groups amend lipophilicity of the test compounds, which in turn alters permeability across the mycobacterial cell membrane. Furthermore, the presence of electronegative atom such as fluoro in **5c** has shown better activity than **5e** and **5b** which have nitro at ortho and meta position respectively, clearly indicating the importance of para positions for substitutions. Also **5a** bearing a methoxy group at para position have shown good activity. Nitro group in spite of being electron withdrawing does not show significant biological response, revealing the importance of para substitution than ortho and meta. Complete lose of activity in **5d** and **5f** observed may be due to bulky less and steric factors. This is well supported by the docking studies performed, as more the G score of the test compounds better the activity and binding ability of molecule into the active site. Our docking indicates good van der Waals interaction than the standard with 14DM. Unlike antifungal azoles, it had shown just a stacking effect with Fe of 14DM and not binding with Fe.

5. CONCLUSIONS

In conclusion a series of mannich bases containing two pharmacophores were synthesized and characterized. Molecular docking studies were performed in 14 DM protein docked with ligand to identify the possible interaction. However two test compounds have shown better G score than Isoniazid. The test compounds were subjected to antimycobacterial study against H37Rv and INH Resistant Clinical Strain. Larger G score better the binding affinity of test molecules and is reflected in antimycobacterial activity indicating a direct correlation between observed activity and energy score. This indicates that the designed series of mannich bases possessing electron withdrawing group at para position have shown antimycobacterial activity. So, these factors collectively indicate the importance, simplicity and wide applicability of designed series as antimycobacterial activity.

6. SUPPLEMENTARY INFORMATION (SI)

Synthesis of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione 4.

The compound, 5-(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione were prepared as per the reported procedure [13].

Synthesis of Thiosemicarbazones **2**.

A mixture of appropriate aromatic aldehyde **1** (1 g, 8.06 mmol), thiosemicarbazide (0.73g, 8.06 mmol) and catalytic amount of HCl in 10 ml ethanol were refluxed at 80˚C. The progress of the reaction was monitored by TLC. On completion, solvent were removed under vacuum. The solid obtained was recrystallized from 50% aqueous ethanol.

Synthesis of 2-Amino thiadiazoles **3.**

A mixture of thiosemicarbazone **2** (1g, 5.07 mmol) and ammonium ferric sulfate dodecahydrate (0.1 g) in H_2O (5 ml) were refluxed for 1 h. Then 1 g ammonium ferric sulfate in H_2O (10 ml) were added and further continued for 5 hr. On completion of reaction, the mixture was chilled; the solid separated was filtered off, washed and crystallized from EtOH-H_2O.

Synthesis of Mannich bases **5a-f**

General procedure for the synthesis of 1,3,4-thiadiazol-2-ylamino)methyl)-5-(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione.

Oxadiazole-2-thione **4** (0.5 g, 2.79 mmol) and substituted 2-amino thiadiazoles **3** (0.54 g, 2.79 mmol) were mixed in 15 ml absolute ethanol and stirred. To the stirred suspension (2.79 mmol, 37%) formaldehyde was added drop wise and heated to reflux for 10 - 12 hrs. The progress of the reaction was monitored by TLC. On completion, reaction mixture was concentrated under reduced pressure and the residue obtained were recrysatllised from appropriate solvent.

Representative data from the series

3-((5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-ylamino) methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione

Table 1. Results of molecular docking studies using standard precision mode of Glide.

Sr. No	Title	G-score	E-Model	Energy	H-Bond	Good VDW	Bad VDW	Ugly VDW
1	**5c**	–5.70	–59.7	-46.3	0	167	5	0
2	**5a**	–5.66	–52.3	-40.7	1	193	5	1
3	**Isoniazid**	–5.02	–32.4	-21.8	2	82	1	1
4	**5b**	–4.91	–55.9	-43.3	0	168	1	0
5	**5e**	–4.73	–57.3	-44.3	0	157	0	0
6	**Standard (CoCrystalised Ligand)**	–4.73	–34.3	-21.6	1	119	1	0
7	**5d**	–4.60	–55.0	-42.7	2	170	0	0
8	**5f**	–4.57	–52.0	-41.3	0	160	0	0

Table 2. Prediction of ADME properties of designed derivatives using qikprop.

Compd. no.	Mol_MW	Log Po/w	Log S	Log BB	PMDCK	Human oral absorption (%)
5a	398.45	3.025	–5.076	–0.652	1191.76	96.55
5b	413.42	2.397	–4.856	–1.379	209.62	80.46
5d	458.50	2.226	–5.253	–0.802	1193.20	100
5e	413.42	3.663	–5.007	–1.671	120.54	75.37
5f	394.46	–1.592	–5.564	–0.822	1266.35	100
5c	386.42	3.195	–5.342	–0.477	2127.74	100
Isoniazid	137.14	–0.646	–0.052	–0.843	123.74	66.89

Higher the value of MDCK cell, higher the cell permeability. All designed compounds have shown the ADME properties in acceptable range.

(5a)

83%, mp 243-245. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.73 (s, 3H, OCH_3), 4.0 (s, 1H, NH), 4.81(s, 2H, CH_2), 7.21-7.65 (m, 4H, phenyl), 7.73-7.85 (d, 2H, pyridyl), 8.12-8.31(d, 2H, pyridyl). MS (m/z %) 398 (M^+), 399 (M+1). Anal. Calcd for $C_{17}H_{14}N_6O_2S_2$: C, 51.24; H, 3.54; N, 21.09; Found C, 51.41; H, 3.44; N, 21.11

3-((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-ylamino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione **(5c)**

79.2%, mp 257-259. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.32 (s, 1H, NH), 4.61 (s, 2H, CH_2), 7.17 - 7.44 (m, 4H, phenyl), 7.53 - 7.72 (d, 2H, pyridyn), 7.98 - 8.21(d, 2H, pyridyn). MS (m/z %) 386 (M^+), 387 (M+1). Anal. Calcd for $C_{16}H_{11}FN_6OS_2$: C, 49.73; H, 2.87; F,4.92; N, 21.75; O, 4.14; S, 16.60; Found C, 49.63; H, 2.76; N, 21.69

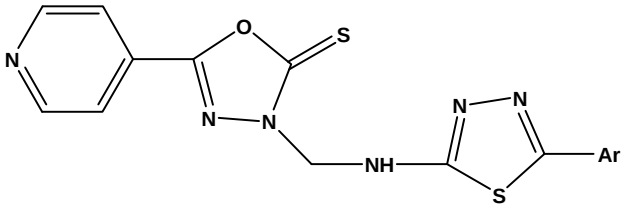
3-((5-(3-nitrophenyl)-1,3,4-thiadiazol-2-ylamino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione **(5e)**

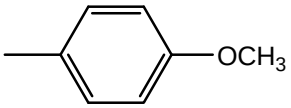
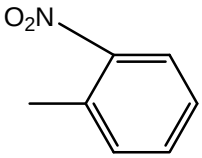
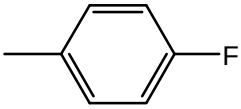
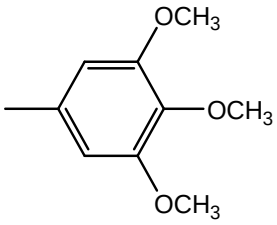
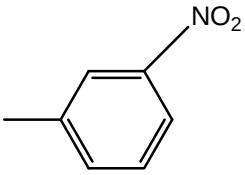
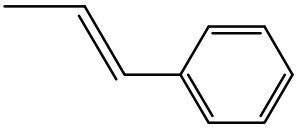
82.8%, mp 272-273˚C. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.35 (s, 1H, NH), 4.58 (s, 2H, CH_2), 7.61-7.91 (m, 4H, phenyl), 7.96-8.12 (d, 2H, pyridin), 8.23-8.48 (d, 2H, pyridyn). MS (m/z%) 413 (M^+), 414 (M+1). Anal. Calcd for $C_{16}H_{11}N_7O_3S_2$: C, 46.48; H, 2.68; N, 23.72; Found C, 46.53; H, 2.84; N, 23.79

Anti-TB sensitivity test by Middlebrook 7H-9 broth (Macro)

The anti-TB screening was carried out by using Middlebrook 7H9 medium [25,26] against M. tuberculosis H37Rv (ATCC 27294). The basal medium was prepared, sterilized and to 4.5 ml of this broth, 0.5 ml of ADC (al-bumin-dextrose-catalase) supplement was added. Then a stock solution (10 mg/ml) of the test compounds was prepared and the final concentrations of 10, 25 and 50 µg/ml were transferred to media bottles. Finally, 10 µg suspension of M. tuberculosis H37Rv strain (100,000 organisms/ml adjusted by McFarland's turbidity standard) was transferred to each of the tubes and incubated at 37˚C along with one growth control without compound and drug control was also set up. The bottles were observed for growth twice a week for a period of three weeks.The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a Zeil-Nelson stain at the end of 4 weeks.

Table 3. Results of H37Rv and INH Resistant clinical strain testing.



Compound Code	Ar	MIC in μg ml^{-1} (H37Rv)	MIC in μg ml^{-1} (INH Resistant Clinical Strain)
5a		**12.5**	**>25**
5b		**>50**	**>100**
5c		**6.25**	**25**
5d		**>100**	**nd**
5e		**>25**	**>100**
5f		**>100**	**nd**
Std	Isoniazid	**0.25**	**--**

nd = not determined

7. ACKNOWLEDGMENTS

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