



Synthesis, Characterization and Anti Mycobacterial Activity of Novel Hydrazones

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ABSTRACT

The hydrazone Schiff base analogues namely benzoic acid (4-allyloxy-benzylidene)-hydrazide and its series were designed and synthesized. The structures of newly synthesized compounds were characterized by analytical methods and spectral analysis and subjected to antibacterial and docking studies. The synthesized molecules were subjected to molecular docking studies using enoyl-acyl-carrier protein reductase (NADH) from *Mycobacterium tuberculosis* as the receptor. The docking results confirm the binding affinity of the synthesized compounds with the selected receptor. Preliminary in-vitro anti bacterial studies were carried out with Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Benzoic acid (4-allyloxy-benzylidene)-hydrazide was found to be most potent anti tuberculosis agent at 200 and 100µg per ml, the percentage of inhibition was 98.7% and 97.9%. The designed synthesized molecules were considered for evaluation for the molecular docking studies of their binding pattern with enoyl-acyl-carrier protein reductase from *Mycobacterium tuberculosis*. The antimycobacterial screening was performed against MTB H37Rv an isoniazid-resistant clinical isolate of MTB was used for the study.

Keywords: hydrazones, spectral studies, docking and anti mycobacterial activity.

INTRODUCTION

Tuberculosis (TB) is a worldwide pandemic caused by different species of mycobacteria. *Mycobacterium tuberculosis* is a successful pathogen, which causes tuberculosis and is the greatest single infectious cause of death worldwide, killing approximately two million people annually¹. Hydrazides are highly reactive bases with reducing

properties. They have been used as a synthetic intermediate to produce different type of drugs in the treatment of different chronic illnesses. Hydrazides used in medicine include the anti-tuberculosis drug isoniazid and the antihypertensive and peripheral vasodilator drug hydralazine, whose hydrochloride is an effective drug for emergency reduction of blood pressure in hypertensive crisis. In the same way the hydrazine derivatives have been widely used in the

treatment of tuberculosis and mental disorder. They can also be used as antimicrobial, antihypertensive, antimalarial and antitumoral agents²⁻¹¹.

Schiff bases are considered to be among the most important group of compounds in medicinal chemistry due to their preparative accessibility, structural variety and wide biological profile¹². The synthesized benzohydrazides were potent antimicrobial and anticancer agents¹³. Numbers of hydrazide-hydrazone derivatives possess antituberculosis activity¹⁴⁻²¹. The present study is aimed to carry out design, synthesis, *in silico* and *in-vitro* antimicrobial studies for the series of benzoic acid (4-allyloxy-benzylidene)-hydrazides.

EXPERIMENTAL

All the chemicals and solvents used are of analytical grade and were purchased from Royal scientific company and used without further purification. TLC was run on the silica coated aluminium sheets (TLC silica gel 60 F₂₅₄; Analytical chromatography) and visualized in low UV light. IR spectra in KBr pellets were recorded on the FT-IR Perkin Elmer Spectrum spectrophotometer. ¹H NMR and ¹³C NMR spectra were recorded on a 300 M Hz spectrometer with DMSO-d₆ as solvent and TMS as internal reference. Chemical shifts are quoted as δ ppm and the coupling constants J in Hz in signals are described as s (singlet), d (doublet), t (triplet), m (multiplet) and b (broad). Melting points were determined on a Sigma melting point apparatus without corrections. GC MS mass spectrum was taken in JEOL GCMATE II GC-MS with Data system in a high resolution, double focusing instrument.

General procedure for the synthesis of benzohydrazides (3a-e)

The substituted benzoic acid (2.46 m mol) was refluxed with methanol in sulphuric acid for 4h. The ester formed reacts with hydrazine and gets converted to benzohydrazides by hydrozoinolysis²².

Synthesis of Benzoic acid (4-allyloxy-benzylidene)-hydrazide (5a-e)

Benzaldehyde undergoes nucleophilic addition reaction with aromatic hydrazides resulting in the formation of hydrazones. The completion of reaction and the homogeneity of the synthesized

compounds were checked by TLC. Further the product formed was washed with petroleum ether, dried, recrystallized and characterized by spectral analysis.

Ethanol solution of 4-allyloxy benzaldehyde (10 m mol) was added to a 10 m mol of ethanolic solution of benzohydrazides and the reaction mixture was stirred for 30 minutes at room temperature in the presence of glacial acetic acid as catalyst. After refluxing for 3-5 h the completion of the reaction was monitored by TLC. The reaction mixture was cooled and placed in refrigerator overnight. The resulting solid was filtered and washed with petroleum ether and recrystallised with ethanol.

In silico Characterisation

The synthesized compounds were assessed *in silico* for their biological activity using PASS server²³. The three dimensional structure of the protein / enzymes was retrieved from Protein Data Bank²⁴. The compound under study was docked with the identified receptor using Autodock²⁵ software and further analyzed for interactions using Pymol.

Antimicrobial activity

Antibacterial screening of the synthesized compounds was evaluated against *Staphylococcus aureus* and *Escherichia coli*, and the zone of inhibition was measured in mm. The broth media was inoculated and grown at 37°C for 18 h to revive the bacteria from the stock cultures. The agar plates with wells were inoculated by spreading evenly with 18h old bacterial cultures (100 μ l, 10-4 cfu). After 20 min, various concentrations of the compound and the antibiotic were filled in the wells. Finally, the plates were incubated at 37°C for 24 h and the inhibition zone was observed²⁶.

Luciferase reporter phage (LRP) assay (27)

The MTB H37Rv and clinical isolate of MTB were grown in Middlebrook 7H9 complete medium 12 with and without test sample for 3 days at 37°C. LRP assay was done using concentrations of 200, 100 and 50 μ g/mL of test samples. 50 μ L bacterial suspension equivalent to MacFarlands. No.2 standard was added to 400 μ L of 7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72

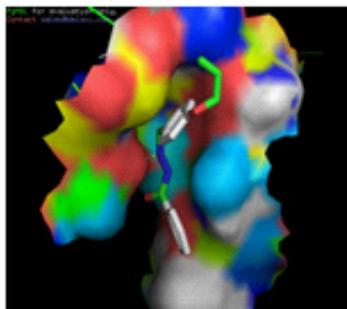


Fig 1: Binding interactions between Benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

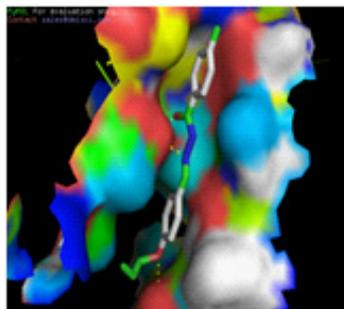


Fig 2: Binding interactions between 4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

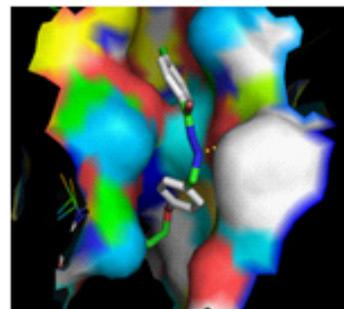


Fig 3: Binding interactions between 4-methyl-benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

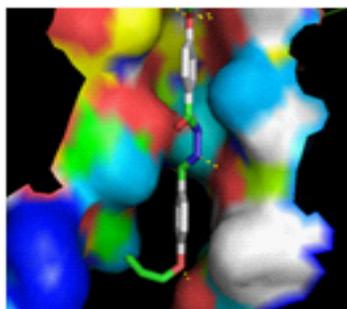


Fig 4: Binding interactions between 4-methoxy-benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

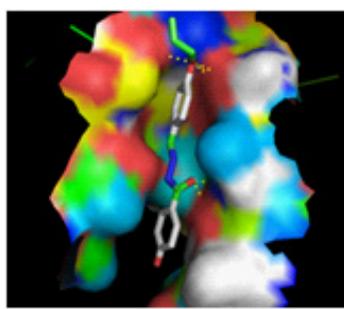


Fig 5: Binding interactions between 4-hydroxy-benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

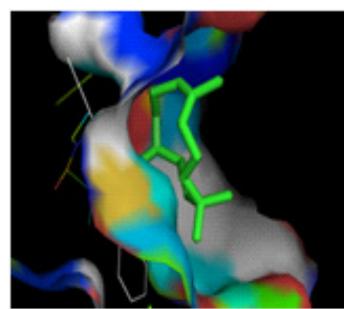


Fig 6: Binding interactions between isoniazid with the receptor

Table 2a: Docking results of Benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec.E	Total.IE	Tor.E	Unb.E	No. of H bonds	Hydrogen bond
1	-11.81	-0.56	2.22	-13.6	-13.59	-0.01	-0.92	1.79	-0.92	1	Met98:HN: :Lig1:O
2	-10.79	-0.51	12.37	-12.58	-12.58	0.0	-0.81	1.79	-0.81	0	-
3	-10.73	-0.51	13.61	-12.52	-12.49	-0.03	-0.97	1.79	-0.97	0	-
4	-10.72	-0.51	13.94	-12.51	-12.52	0.01	-1.03	1.79	-1.03	0	-
5	-10.68	-0.51	14.91	-12.47	-12.42	-0.05	-1.14	1.79	-1.14	1	Ile194:HN: :Lig1:N
6	-10.44	-0.5	22.27	-12.23	-12.2	-0.03	-1.07	1.79	-1.07	0	-
7	-10.33	-0.49	26.91	-12.12	-12.04	-0.07	-0.95	1.79	-0.95	1	Tyr158:HH: :Lig1:O
8	-9.84	-0.47	61.29	-11.63	-11.62	-0.01	-0.85	1.79	-0.85	2	Lig1:N: :Ala201: OLig1:N: :Ala201:O
9	-9.82	-0.47	63.87	-11.61	-11.58	-0.03	-1.23	1.79	-1.23	1	Ile194:HN: :Lig1:N
10	-9.56	-0.46	98.8	-11.35	-11.26	-0.09	-1.18	1.79	-1.18	1	Tyr158:HH: :Lig1:N

4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide (5b) was prepared by the above mentioned method. 4-chlorobenzohydrazide (0.171g 0.001mol 1 equivalent) 4-allyloxybenzaldehyde (0.15ml 0.001mol 1 equivalent), pale yellow amorphous solid, yield: 92%. m.p. 158-160°C. FT-IR (ν_{\max} KBr pellets, cm^{-1}) 3434 or 3283 (N-H), 2930Ar (C-H) str, 2849 Ali (CH) str, 1652 (C=O) str, 1506 (C=N) str, DMSO, 300 MHz) δ H: 5.2-5.3 (d, d, 2H, 1.5Hz), 5.9-6.1 (CH 1H m), 4.63 (d 2H, 5.1 Hz), 7.0(d, 2H, 8.7 Hz), 7.61 (d, 2H, 8.7Hz), 5.3-5.4 (d, d 1.5-1.8Hz, 1H), 8.38 (1H, s), 7.9 (d, 2H, 8.7Hz), 7.6 (d, 2H, 9 Hz). ^{13}C NMR (DMSO 300 MHz) 161, 159, 147 136, 133, 132, 129, 128, 128, 117, 115 and 68. m/z = 315 (M+1). Molecular Formula $\text{C}_{17}\text{H}_{15}\text{ClN}_2\text{O}_2$.

4-methyl-benzoic acid (4-allyloxy-benzylidene)-hydrazide (5c) was prepared by the above mentioned method. 4-methyl benzohydrazide (0.15g 0.001mol 1 equivalent) 4-allyloxybenzaldehyde (0.15ml 0.001mol 1 equivalent), yellow amorphous solid, yield: 88%. m.p. 168°C. FT-IR (ν_{\max} KBr pellets, cm^{-1}) 937 (N=N), 1654 (C=O), 1526 (C=N). ^1H NMR (DMSO, 300 MHz) δ H: 5.23- 5.24 (d, 2H), 5.89(s, 1H), 4.61 (2H), 6.8(d, 2H), 7.5(d, 2H), 8.1(s, 1H), 8(s, 1H), 7.83(d, 2H), 7.24(d, 2H), 2.35(S, 3H). ^{13}C NMR (DMSO 300 MHz) 115, 137, 75, 164, 114, 130, 123, 154, 127, 130.5, 129, 141 and 20. m/z = 292 (M+1). Molecular Formula $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2$.

4-methoxy-benzoic acid (4-allyloxy-benzylidene)hydrazide (5d) was prepared by the above mentioned method. 4-methoxy

Table 2b: Docking results of 4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec. E	Total. IE	Tor.E	Unb. E	No. of H bonds	Hydrogen bond
1	-11.87	-0.54	1.99	-13.66	-13.66	-0.01	-0.85	1.79	-0.85	3	Lig1:O::4oim: Ala201:OLig1:N: :4oim:Ala201: OLig1:N::4oim: Ala201:O
2	-11.31	-0.51	5.09	-13.1	-13.11	0.01	-0.81	1.79	-0.81	3	Lig1:O::Gly205: OLig1:N::Ala201 :OLig1:N: :Ala201:O
3	-11.07	-0.5	7.67	-12.86	-12.89	0.03	-0.83	1.79	-0.83	1	Lig1:O::Gly96:O
4	-11.04	-0.5	8.12	-12.83	-12.86	0.04	-0.79	1.79	-0.79	3	Lig1:O::Ala201: OLig1:O::Gly96: OLig1:N: :Met98:O
5	-11.01	-0.5	8.44	-12.8	-12.8	0.0	-0.81	1.79	-0.81	0	-
6	-10.54	-0.48	18.78	-12.33	-12.34	0.01	-0.63	1.79	-0.63	1	Met98:HN: :Lig1:O
7	-10.51	-0.48	19.66	-12.3	-12.36	0.05	-0.54	1.79	-0.54	0	-
8	-10.42	-0.47	22.98	-12.21	-12.18	-0.03	-0.73	1.79	-0.73	1	Lig1:O::Ile194:O
9	-9.86	-0.45	58.91	-11.65	-11.63	-0.02	-0.8	1.79	-0.8	1	Lig1:N::Ala201:O
10	-8.71	-0.4	414.7	-10.5	-10.47	-0.02	-0.78	1.79	-0.78	3	Ala157:HN::Lig1 :N,NAasn 159: HD21::Lig1: OLig1:N: :Asn159:OD1

benzohydrazide (0.166g 0.001mol 1 equivalent)
4-allyloxybenzaldehyde (0.15ml 0.001mol 1 equivalent), light yellow amorphous solid, yield: 88%. m.p.162°C. FT-IR (ν_{\max} KBr pellets, cm^{-1}) 1640 (C=N), 937 (N-N), 1410 (C-O), 2918 Ar (C-H). ^1H NMR (DMSO, 300 MHz) δH : 5.23- 5.24 (d, 2H), 5.89(s, 1H), 4.61 (2H), 6.8(d, 2H), 7.5(d, 2H), 8.1(s, 1H), 8(s, 1H), 7.84(d, 2H), 6.95(d, 2H), 3.73(s, 3H). ^{13}C NMR (DMSO 300 MHz) 115, 137, 75, 164, 114, 130, 123, 154, 125, 128, 114, 165 and 56. $m/z = 292$ (M+1). Molecular Formula $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2$.

4- hydroxy -benzoic acid (4-allyloxy-benzylidene)- hydrazide (5e) was prepared by the above mentioned method. 4-hydroxy benzohydrazide (0.152g 0.001mol 1 equivalent) 4-allyloxybenzaldehyde (0.15ml 0.001mol 1 equivalent), yellow amorphous solid, yield: 84%. m.p. 178°C. FT-IR (ν_{\max} KBr pellets, cm^{-1}) 1535 (C=C), 1625 (C=N), 3250 (N-H), 1658 (C=O). ^1H NMR (DMSO, 300 MHz) δH : 5.23- 5.24 (d, 2H), 5.89(s, 1H), 4.61 (2H), 6.8(d, 2H), 7.5(d, 2H), 8.1(s, 1H), 8(s, 1H), 7.78 (d, 2H), 6.91 (d, 2H), 5 (S, 1H). ^{13}C NMR (DMSO 300 MHz) 115, 137, 75, 164, 114, 130, 123, 154, 126, 128, 115 and 160. $m/z = 310$ (M+1). Molecular Formula $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_3$.

Synthesized compounds (Benzoic acid (4-allyloxy-benzylidene)-hydrazide), (4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide), were subjected to a preliminary anti bacterial screening against the strain gram positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria to assess the antibacterial susceptibility of the test compounds. The compounds showed good inhibition at lower concentration.

It is evident from table-1 that the compound 5a exhibits maximum anti-tuberculosis activity among all the study compounds as its probability of being active (Pa) value is 0.831 and Probability of being inactive (Pi) is only 0.003. Also all the other compounds demonstrate anti-tuberculosic activity with Pa between 0.827 and 0.781. Hence, all the study compounds were docked with the target Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis*. The interaction of the synthesized compound with the target was evaluated using Docking studies. The docking scores along with the other parameters for the different poses of the docked molecules are presented in Tables 2(a) – 2(e).

Table 2c: Docking results of 4-methyl-benzoic acid (4-allyloxy-benylidene)- hydrazide with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec. E	Total. IE	Tor.E	Unb.E	No. of H bonds	Hydrogen bond
1	-11.37	-0.52	4.64	-13.46	-13.48	0.03	-0.8	2.09	-0.8	2	LIG1:N: :MET98:OLIG1 :N::MET98:O
2	-11.17	-0.51	6.48	-13.26	-13.25	-0.01	-0.85	2.09	-0.85	0	-
3	-10.69	-0.49	14.59	-12.78	-12.78	0.0	-0.85	2.09	-0.85	0	-
4	-10.61	-0.48	16.73	-12.7	-12.68	-0.02	-0.91	2.09	-0.91	2	LIG1:O: :MET98: OMET98:HN: :LIG1:O
5	-10.4	-0.47	23.61	-12.49	-12.47	-0.03	-0.92	2.09	-0.92	0	-
6	-10.3	-0.47	28.01	-12.39	-12.34	-0.05	-0.83	2.09	-0.83	1	ILE194:HN: :LIG1:O
7	-10.02	-0.46	45.14	-12.11	-12.08	-0.02	-0.94	2.09	-0.94	0	-
8	-9.98	-0.45	48.37	-12.07	-12.01	-0.05	-1.09	2.09	-1.09	1	TYR158:HH: :LIG1:O
9	-9.74	-0.44	72.73	-11.83	-11.72	-0.11	-1.05	2.09	-1.05	0	-
10	-9.7	-0.44	77.03	-11.79	-11.76	-0.03	-0.94	2.09	-0.94	0	-

The docking results of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand Benzoic acid (4-allyloxy-benzylidene)-hydrazide is presented in table 2a. The binding energies of the different binding conformations range between -11.81 and -9.56 kcal/mol. Among the ten different docked conformations conformation 8 had a binding energy of -9.84 kcal/mol with 2 hydrogen bonds between the ligand Benzoic acid (4-allyloxy-benzylidene)-hydrazide and Ala 201. The conformation 1, Conformation 5, Conformation 7, Conformation 9 and Conformation 10 had only one hydrogen bond formed with binding energies -11.81, -10.68, -10.33, -9.82 and -9.56 kcal/mol respectively. Hence, conformation 8 with 2 hydrogen bonds and binding energy of -9.84 kcal/mol is considered the best docked conformation and represented in figure-1

The table 2b presents the ten different binding conformations of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand 4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide. The binding energy ranges from -11.87 kcal/mol to -8.71 kcal/mol. The conformations 1,2,4 and 10 had three hydrogen bonds between the receptor, Enoyl-[acyl-carrier-protein] reductase [NADH] and ligand 4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide. In the first binding conformation, all the three hydrogen bonds are formed between the ligand and Alanine 201 in Enoyl-[acyl-carrier-protein] reductase [NADH] protein. But in the second binding conformation two hydrogen bonds are formed between the ligand and Alanine 201 and the third hydrogen bond is formed between the ligand and Glycine 205 in the receptor. Alternatively in the binding conformation 4, Alanine 201, Glycine 96 and Methionine 98 in the receptor

Table 2d: Docking results of 4-methoxy-benzoic acid (4-allyloxy-benzylidene)- hydrazide with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec. E	Total. IE	Tor.E	Unb.E	No. of H bonds	Hydrogen bond
1	-11.89	-0.52	1.93	-13.98	-13.97	-0.01	-0.85	2.09	-0.85	1	LIG1:N: :ALA201:O
2	-10.96	-0.48	9.29	-13.05	-13.09	0.04	-0.82	2.09	-0.82	1	LIG1:O: :PRO156:O
3	-10.89	-0.47	10.41	-12.98	-12.98	0.0	-0.85	2.09	-0.85	2	LIG:N::ALA201: OLIG1:N: :ALA201:O
4	-10.8	-0.47	12.02	-12.89	-12.89	0.0	-0.91	2.09	-0.91	4	LIG1:N::ALA201: :OLIG1:O: :ASN106: OD1LIG1:O: :GLY96:OLIG1: O::ALA201:O
5	-10.62	-0.46	16.4	-12.71	-12.67	-0.03	-0.87	2.09	-0.87	0	-
6	-10.41	-0.45	23.36	-12.5	-12.46	-0.04	-0.93	2.09	-0.93	1	LIG1:O: :MET98:O
7	-10.16	-0.44	35.45	-12.25	-12.27	0.02	-0.73	2.09	-0.73	1	LIG1:O: :PRO156:O
8	-9.7	-0.42	77.07	-11.79	-11.67	-0.12	-0.84	2.09	-0.84	1	MET98:HN: :LIG1:O
9	-9.41	-0.41	125.97	-11.5	-11.46	-0.04	-0.9	2.09	-0.9	1	LIG1:O: :ALA201:O
10	-8.98	-0.39	259.88	-11.07	-10.94	-0.13	-0.85	2.09	-0.85	0	-

form one hydrogen bond each with the ligand. Finally, the conformation 10 had 3 hydrogen bonds formed each between Alanine 157, Asparagine 159, Asparagine 159 and the ligand with a binding energy of -8.71 kcal/mol. Among the ten different binding conformations, the conformation 1 with the least

binding energy and three hydrogen bonds is chosen as the best possible orientation which is displayed in Figure-2

The table 2c depicts the different binding orientations of the ligand K1b with the receptor

Table 2e: Docking results of 4- hydroxy -benzoic acid (4-allyloxy-benylidene)- hydrazide with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec. E	Total. IE	Tor.E	Unb.E	No. of H bonds	Hydrogen bond
1	-11.35	-0.52	4.83	-13.14	-13.12	-0.02	-0.61	1.79	-0.61	1	LIG:N: :ALA201:O
2	-10.26	-0.47	30.34	-12.05	-12.03	-0.02	-0.81	1.79	-0.81	0	-
3	-10.25	-0.47	30.41	-12.04	-12.08	0.03	-0.81	1.79	-0.81	0	-
4	9.97	-0.45	49.19	-11.76	-11.74	-0.02	-0.58	1.79	-0.58	0	-
5	-9.95	-0.45	50.58	-11.74	-11.74	-0.01	-0.64	1.79	-0.64	2	LIG1:N::ALA201 :OLIG1:O: :ASN106:OD1
6	-9.78	-0.44	67.28	-11.57	-11.59	0.02	-0.73	1.79	-0.73	0	-
7	-9.76	-0.44	70.34	-11.55	-11.62	0.07	-0.79	1.79	-0.79	1	LIG:O::MET98:O
8	-9.74	-0.44	72.95	-11.53	-11.49	-0.04	-0.77	1.79	-0.77	2	LIG1:O::MET98: OMET98:HN: :LIG1:O
9	-9.73	-0.44	73.19	-11.52	-11.53	0.01	-0.84	1.79	-0.84	1	LIG1:N: :ALA201:O
10	-7.1	-0.32	6.29	-8.89	-8.84	-0.05	-0.77	1.79	-0.77	0	-

Table 2f: Docking results of isoniazid with the receptor

Pose	B.E	L.E	IC (nM)	Int.E	Vdw	Elec. E	Total. IE	Tor.E	Unb.E	No. of H bonds	Hydrogen bond
1	-6.73	-0.67	11.61	-7.03	-7.0	-0.04	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O
2	-6.73	-0.67	11.69	-7.03	-6.99	-0.04	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O
3	-6.71	-0.67	12.11	-7.01	-6.97	-0.03	-0.09	0.3	-0.09	1	Iso:O::Ile 202:O
4	-6.67	-0.67	12.94	-6.97	-6.98	0.01	-0.09	0.3	-0.09	1	Iso:O::Ala 201:O
5	-6.67	-0.67	13.01	-6.96	-6.98	0.02	-0.09	0.3	-0.09	1	Iso:O::Ala 201:O
6	-6.54	-0.65	16.15	-6.84	-6.73	-0.11	-0.09	0.3	-0.09	0	-
7	-6.04	-0.60	37.35	-6.34	-6.26	-0.08	-0.09	0.3	-0.09	2	Tyr158:HH::Iso:O Ile194:HN::Iso:N
8	-6.04	-0.60	37.62	-6.33	-6.25	-0.08	-0.09	0.3	-0.09	2	Tyr158:HH::Iso: Olle194:HN: :Iso:N
9	-6.00	-0.60	39.78	-6.30	-6.21	-0.09	-0.09	0.3	-0.09	1	Ile194:HN::Iso:N
10	-5.83	-0.58	53.14	-6.13	-6.06	-0.07	-0.09	0.3	-0.09	1	Ile194:HN::Iso:O

Table 3: Antimicrobial activity data of 5a and 5b by LPR assay

Comp. code	Conc. (mL)	CFU (col/mL)	% inhibition
5a	10	3.2x10 ⁶	19.1
	20		0
	50		0
	200		
5b	100	4.4x10 ⁶	97.9
	200	2.4x10 ⁶	98.7
	400		
Control-1	-	5.8x10 ⁶	-
Control-2	-	5.0x10 ⁶	-

Control-1 -H37Rv
control-2 -H37Rv

Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis*. Poses 1 and 4 have two hydrogen bonds formed by 'O' of Met98 and 'N' and 'O' atoms in the ligand. Poses 6 and 8 had one hydrogen bond each between 'H' atom of Ile 194 and Tyr 158 respectively with the 'O' atom of the Ligand. Among the ten different binding conformations, the conformation 1 with binding energy of -11.37 kcal/mol and two hydrogen bonds is chosen as the best possible orientation which is displayed in Figure 3.

The table 2d presents the ten different binding conformations of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand 5d. The binding energy ranges from -11.89 kcal/mol to -8.98 kcal/mol. The poses 1, 2, 6, 7, 8 and 9 had one hydrogen bond between the receptor, Enoyl-[acyl-carrier-protein]reductase [NADH] and ligand 5d. But pose 3 and 4 had 2 and four hydrogen bonds between the receptor and the ligand. Hence, among the ten different binding conformations, the conformation 4 with the binding energy of -10.8 and 4 hydrogen bonds is chosen as the best possible orientation which is displayed in Figure 4.

The table 2e presents the ten different binding conformations of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the ligand 5e. The binding energy ranges from -11.35 kcal/mol to -7.1 kcal/mol. The poses 1, 7 and

9 had one hydrogen bond between the receptor, Enoyl-[acyl-carrier-protein]reductase [NADH] and ligand 5e, whereas conformations 5 and 8 had two hydrogen bonds. Among the ten different binding conformations, the conformation 5 with binding energy of -9.95 kcal/mol and two hydrogen bonds is chosen as the best possible orientation which is displayed in Figure 5

The docking results of Enoyl-[acyl-carrier-protein] reductase [NADH] from *Mycobacterium tuberculosis* with the standard drug Isoniazid is presented in table 2f. The binding energies of the different binding conformations range between -6.73 and -5.83 kcal/mol. Among the ten different docked conformations conformation 7 and 8 show 2 hydrogen bonds between the drug and Tyr158 and Ile194. The conformation 1, Conformation 2, Conformation 3, Conformation 4 and Conformation 5 had only one hydrogen bond formed with binding energies -11.81, -10.68, -10.33, -9.82 and -9.56 kcal/mol respectively. Hence, conformation 8 with 2 hydrogen bonds and binding energy of -9.84 kcal/mol is considered the best docked conformation and represented in Figure-6

Synthesized compounds were evaluated for antimycobacterial activity using Luciferase Reporter Phase (LRP) assay on MTB H37Rv and one isoniazid-resistant (INH-resistant) clinical isolate at National Institute for Research in Tuberculosis (NIRT), Chennai (28).

The percentage growth inhibition was recorded at the concentrations of 200, 100 and 50 µg/mL (Table 3). Seeing the good inhibition of the synthesized compound further biological assays were conducted to determine their MIC values on different strains of *Mycobacterium*.

Results of these investigations indicate that some of the test compounds exhibited significant antimycobacterial activity along with antibacterial activity against all the tested strains.

CONCLUSION

A series of hydrazone Schiff base analogues were synthesized in good yield adopting standard procedures. The compounds were

characterized by spectral studies. Docking studies of the above mentioned compounds revealed scope for antibacterial activity especially anti-tuberculosis activity. Further, all the study compounds exhibit a binding better than the existing drug isoniazid for Tuberculosis. This was further confirmed using *in vivo* studies where compound 4-chloro-benzoic acid

(4-allyloxy-benzylidene)-hydrazide had maximum inhibition (97.9%) 100µmL. Hence, it can be concluded from the study that compound 4-chloro-benzoic acid (4-allyloxy-benzylidene)-hydrazide can be further evaluated for its efficiency in treating Tuberculosis.

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