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IN SILICO DOCKING, ADMET PREDICTION AND BIOLOGICAL ACTIVITY EVALUATION OF TRIAZOLOAZETIDIN-2-ONES

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1. ABSTRACT

A series of thirteen novel azetidinones T1-13 were synthesized by cyclocondensation of various Schiff bases of aldehydes with 1,2,4-triazole. Schiff's bases preparing from 1,2,4 triazole moiety by reacting the hydrazide of the parent compound with different aromatic or heterocyclic aldehydes under acidic conditions in ethanol and cyclocondensation of Schiff's bases with chloracetyl chloride in the presence of triethylamine and dioxane resulted in the formation of corresponding spectra. The synthesized compounds were evaluated for antibacterial and antifungal activities by disk diffusion method and also evaluated for anti-tubercular activity,

2. KEYWORDS: Molecular docking studies, ADMET prediction, Antitubercular activity, Azetidinones, Antimicrobial activity.

3. INTRODUCTION

It is widely recognized that antimicrobials play a crucial role in human medicine for effectively managing severe infectious diseases. However, the effectiveness of these antimicrobials is being compromised due to the emergence of antimicrobial resistance, which poses an escalating threat to morbidity and mortality in acute diseases.[5] Consequently, selecting optimal empirical and definitive antimicrobial treatment for serious infections with increasing levels of resistance is becoming increasingly complex.[6] The significance of discovering new heterocyclic compounds for the treatment of infectious diseases cannot be overstated. Among these, azetidinone-containing compounds have demonstrated a wide range of biological activities, Therefore, this study aims to investigate the potential of potent triazole-azetidinone derivatives in terms of their antitubercular and antimicrobial activity.[7]

Azetidinone is a saturated form of nitrogen containing cyclobutane having carbonyl group. The present review article explains the synthesis and various biological activities of azetidinones.[1]

All the compounds A1-A13 at a concentration of 1000, 500, 250, 125 and 62.5µg/ml and compounds were screened for their antibacterial activity against Staphylococcus aureus, (Gram positive bacteria) Escherichia coli, (Gram-negative bacteria) by disk diffusion method. A large number of 3-chloro monocyclic ß-lactam possesses powerful antibacterial, antimicrobial, anti inflammatory, anticonvulsant & antitubercular activities. They also function as enzyme inhibitors & are effective on the central nervous system.[2]

More than 1000 different β-lactamase proteins from several bacterial species were documented and are having various chemical structures and catalytic proficiencies. Apart from antibacterial property they have variety of significant activities like anti-tubercular, anti-convulsant, anti-hyperglycaemic, anti-cancer, analgesic, anti-inflammatory and enzyme inhibitory activities.[3]

synthesizing azetidinone derivatives, typically involving two steps. First, a Schiff base is formed by condensing aromatic or aliphatic aldehydes with primary amino compounds. Then, the Schiff base undergoes a cycloaddition reaction with ketene, resulting in the formation of 1,4-disubstituted azetidinones. Acid chloride reacts with a base like triethylamine to produce ketene.[4]

4. MATERIALS AND METHODS

4.1. Virtual Screening: The proposed structures of Schiff bases and triazoloazetidinones docking studies were analysed by Schrodinger Glide software version 2020_1. These compounds were docked against DNA Gyrase subunit B of Escherichia coli, Staphylococcus aureus and lanosterol 14α-demethylase of human and InhA inhibitor of Mycobacterium tuberculosis to establish their biological efficacy towards bacteria and fungi.

4.2. ADMET prediction: A online web tool of ADMETlab 2.0 used for the prediction of ADMET properties of the compounds. The SMILES form of the all compounds were loaded in the software in ADMET screening process and obtained the properties of physicochemical, medicinal chemistry, absorption, distribution, metabolism, excretion and toxicity.

4.3. Synthetic procedure

Step 1: Synthesis of Schiff bases

4-amino-1,2,4-triazole (1.68gm,0.02mol) taken in a tube of parallel synthesizer, dissolved entirely in 15ml methanol. To this 0.02mol of aliphatic/aromatic aldehyde (shown in scheme) added and refluxed for 4h with a catalytic amount (2-4 drops) of glacial acetic acid maintaining the temperature at 80°C. Allow cooling the solution after completion of reaction on ice, the product obtained was recrystallized with hot ethanol and dried. The reaction completion monitored by TLC using toluene: methanol as the mobile phase in the ratio of 7:3.[12]

Step 2: Synthesis of substituted azetidinones

To the 0.01mol of Schiff base (from the above step), add triethylamine (0.349ml, 0.025mol) and 10ml of dioxane. To this mixture, add chloroacetyl chloride (0.4ml, 0.025mol) dropwise by maintaining the temperature at 0 - 5°C, stirred for 5-6h, and then kept for two days at room temperature. The reaction mixture poured into crushed ice to develop simpler analogues of 2-azetidinones, it was filtered, dried, and recrystallized from ethanol. The completion of reaction monitored by TLC using Chloroform: methanol as the mobile phase in the ratio of 8:2.[10,11]

Aromatic aldehydes used in the Schiff bases synthesis

4-Nitro Benzaldehyde

Cinnamaldehyde

Vanillin

Salicylaldehyde

3-Nitro Benzaldehyde

2,4-dichloro benzaldehyde

4-Hydroxy-3,5-dimethyl benzaldehyde

Indole-3-carboxaldehyde

P-dimethyl amino benzaldehyde

Anisaldehyde

Ethylvanillin

4-Chloro benzaldehyde

4-Hydroxy benzaldehyde

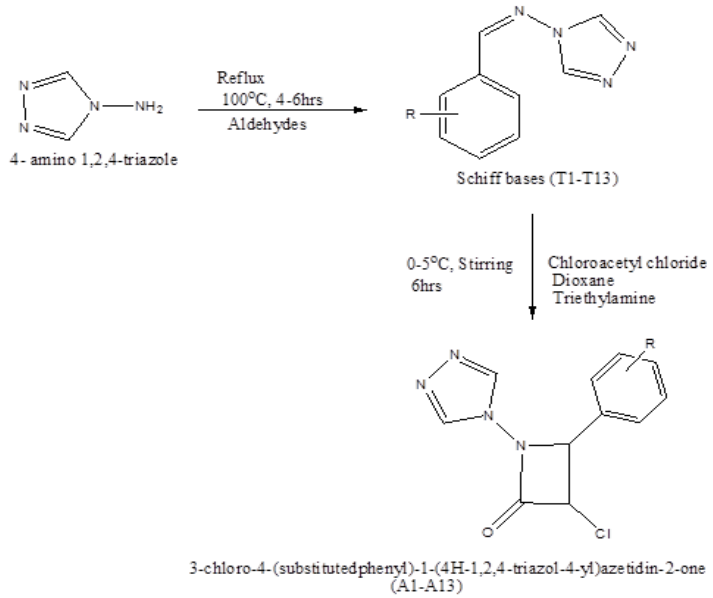

Fig.1. Synthesis of the final products.

4.4. Characterization: The synthesized compounds characterized by m.p, IR spectral data, 1H NMR and 13C NMR data.

4.5. Pharmacological Screening

Antitubercular activity screening: MIC of the given compounds determined at the concentration ranging from 1000-7.81 µg/ml against Mtb H37Rv. The dilution made by using the 7H9 medium without altering the medium composition. The culture suspension was adjusted to 1 McFarland standard and diluted to a 1:10 ratio so that each well contained 1×105 cells. Rifampicin (Sigma-Aldrich) at the critical concentration of 1 µg/mL taken as a reference compound. Culture control and solvent control (DMSO) included in the assay. The plates observed under the microscope after five days incubating at 37°C for determining gradation based on the serpentine chord formation of Mtb culture growth.55 Screening performed in duplicate in a microtiter plate and the minimal concentration of the compounds that completely inhibited the growth of the Mtb cultures considered as MIC.[8,9]

Antimicrobial activity: All the synthesized compounds subjected to antimicrobial activity by using the micro-organisms like Staphylococcus aureus, Escherichia Coli, and Candida albicans. The drug concentrations used in range about 10µg/ml, 25µg/ml, 50µg/ml, and 100µg/ml. The micro-organisms are inoculated into test tube containing the peptone liquid media and incubated for 24 hours. The agar media is prepared and sterilized by using autoclave. The media is allowed to cool, poured in to the petri plates and micro-organisms

were streaked on the agar media and allow to grow by incubating at 35 37°C for 24 hours. After 24 hours the petri plates are collected and observed for the zone of inhibition. Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method on M. tuberculosis H37Rv.

5. RESULTS

The various results obtained in this research work are appended below.

5.1. Molecular docking studies

As per the standard procedure the designed molecules along with standard drugs were docked against 5L3J, 4P8O, 3LD6, and 4OHU by using Schrodinger Glide, results were showed in Table.

Table 1: Docking score and interactions of ligands on 5L3J Protein.

Compound code	Docking score	Glide energy	Interactions
6G9 Cocrystal	-6.57	-56.84	VAL 71, ASP73, ARG136
A3	-6.32	-36.43	VAL 43, GLY77
T11	-6.12	-32.30	No interactions
A11	-6.12	-34.20	No interactions
A8	-6.10	-38.08	ASP 73
A5	-5.92	-32.37	ASP 73
A9	-5.91	-35.04	No interactions
A6	-5.88	-33.42	No interactions
T8	-5.86	-33.69	ASP 73
T2	-5.85	-30.48	No interactions
A12	-5.78	-36.23	VAL 43, GLY 77
T4	-5.78	-32.44	ASP 73
A4	-5.76	-35.80	ASP 73
A13	-5.70	-33.45	No interactions
T5	-5.63	-33.05	ASP 73
A1	-5.61	-33.09	ASP 73
T13	-5.51	-32.03	ASP 73
T1	-5.32	-34.38	No interactions
T7	-5.30	-33.16	ASP 73
T3	-5.29	-32.18	ASP 73
A2	-5.11	-33.0	No interactions
A10	-5.03	-35.49	ARG 136
T6	-5.02	-32.06	No interactions
T9	-5.02	-28.29	No interactions
T12	-4.85	-32.64	ASP 73
A7	-4.69	-33.66	ASP 73
T10	-4.60	-29.34	ARG 136

Table 2: Docking score and interactions of ligands on 4P8O Protein.

Compound code	Docking Score	Glide Energy	Interactions
883 –Cocrystal	-7.12	-53.85	ASN 54, ASP 81, ARG 84, ARG 144
A8	-5.56	-39.08	ASN 54, ASP 57, ASP 81, GLY 85
A13	-5.56	-37.17	ASN 54, ASP 57, ASP 81, GLY 85
A3	-5.18	-35.39	ASP 81, GLY 85
T2	-5.15	-29.82	No interactions
T7	-5.07	-32.90	ASP 81, GLY 85
A1	-5.06	-33.95	GLY 85, ARG 144
T4	-5.04	-28.73	ASN 54, ASP 81, GLY 85
A4	-4.99	-32.22	ASP 57, GLY 85
A7	-4.85	-34.43	GLY 85
A10	-4.82	-32.35	ARG 84
T5	-4.81	-32.78	ASP 57
T1	-4.80	-32.20	ASN 54, ARG 84, ARG 144
T10	-4.80	-30.09	ARG 84, GLY 85, ARG 144
T9	-4.71	-28.25	No interactions
A5	-4.70	-33.67	GLU 58, ARG 84, ARG 144
A12	-4.67	-35.82	ASP 81, GLY 85
T3	-4.65	-30.13	ASP 81, GLY 85
T11	-4.64	-26.68	ASP 81, GLY 85
T13	-4.61	-25.79	ASP 81, GLY 85, ARG 144
A11	-4.58	-31.08	ARG 84, ARG 144
T6	-4.50	-30.80	ASN 54, ASP 81, GLY 85
T8	-4.49	-31.14	ASN 54, ASP 81, GLY 85
A6	-4.46	-31.17	ARG 84
A9	-4.44	-30.82	No interactions
A2	-4.30	-28.27	ARG 84, ARG 144
T12	-4.13	-32.50	ASP 81, GLY 85

Table 3: Docking score and interaction of ligands on 3LD6 protein.

Compound code	Docking score	Glide energy	Interactions
Ketoconazole KKK	-9.90	-57.52	TYR 131, LYS 156, HIE 236, TRP 239, MET 378
A8	-8.32	-41.17	No interactions
A9	-8.80	-37.64	HIE 236, MET 378
A10	-7.70	-37.59	No interactions
A5	-7.66	-40.91	HIE 236
A6	-7.58	-37.88	HIE 236, MET 378
A4	-7.58	-36.95	TYR 131, MET 378, ILE 379
A1	-7.31	-37.77	TYR 131, TRP 239, MET 378
A11	-7.25	-36.46	TYR 131
A13	-7.25	-37.67	TYR 131
T9	-7.20	-33.00	ILE 379
T8	-7.02	-35.14	LEU 310, MET 378
T1	-6.87	-34.42	TRP 239
A7	-6.81	-42.77	LEU 310, ILE 379
A12	-6.78	-43.84	LEU 310, ILE 379
T6	-6.70	-33.97	HIE 236, TRP 239

A2	-6.63	-37.34	MET 378, ILE 379
A3	-6.54	-40.56	LEU 310, ILE 379
T7	-6.48	-40.66	LEU 310, MET 378, ILE 379
T2	-6.38	-30.89	PHE 234, HIP 314, ILE 379
T10	-6.30	-32.33	ILE 379
T3	-6.10	-39.17	LEU 310, ILE 379, MET 378
T4	-6.01	-35.23	PHE 234, HIP 314, ILE 379
T12	-5.96	-37.56	ILE 379
T11	-5.84	-33.93	ILE 379
T5	-5.58	-34.84	PHE 234, MET 378, ILE 379
T13	-5.42	-31.78	MET 378, ILE 379

Table 4: Docking score and interactions of ligands on 4OHU protein

Compound code	Docking score	Glide energy	Interactions
Streptomycin	-8.12	-55.18	No interactions
A2	-7.95	-40.51	ILE 194
A9	-7.73	-38.91	LYS 165, ILE 194
T8	-7.59	-32.33	PHE 149, PRO 156
T2	-7.36	-35.78	ILE 21
A8	-7.29	-39.90	LYS 165
A13	-7.24	-40.34	LYS 165
A7	-7.15	-46.16	GLY 192, ILE 194
A11	-7.04	-35.89	LYS 165, ILE 194
A6	-6.99	-36.45	LYS 165, ILE 194
A1	-6.99	-38.81	ARG 43, GLY 96
T5	-6.92	-34.94	PHE 149
A4	-6.92	-39.01	LYS 165
A12	-6.85	-43.76	SER 20, ILE 21, GLY 192, ILE194
A3	-6.79	-42.03	ILE 21, GLY 192, ILE 194
A5	-6.79	-41.53	ARG 43, LEU 197
A10	-6.71	-39.35	ILE 21, SER 20
T3	-6.69	-34.51	THR 196
T13	-6.63	-32.79	GLY 192, ILE 194
T9	-6.52	-32.68	TYR 158
T6	-6.47	-32.90	SER 94
T4	-6.42	-30.77	ILE 194
T10	-6.14	-33.69	No interactions
T1	-6.11	-33.20	ILE 21, SER 94, PHE 149
T7	-5.93	-37.97	GLY 14
Isoniazid	-5.85	-24.93	GLY 14, SER 94, LYS 165
T11	-5.83	-30.96	PHE 149
T12	-5.81	-38.93	LYS 165, GLY 192, ILE 194,
Bedaquiline	-4.43	-34.85	ASP 150, ARG 153, ALA 154, PRO 156

5.2. ADMET Studies

Table 5: Physicochemical and Medicinal properties of Schiff bases.

Code	MW	Volume	Density	nHA	nHD	TPSA	nROT	Synthetic accessibility score	Lipinski	LogS	LogD	Logp
T1	217.06	201.216	1.079	7	0	86.21	3	2.512	Accepted	-3.345	1.544	1.662
T2	198.09	207.231	0.956	4	0	43.07	3	2.68	Accepted	-3.454	1.837	1.807
T3	218.08	210.152	1.038	6	1	72.53	3	2.53	Accepted	-1.555	0.343	-0.151
T4	188.07	184.066	1.022	5	1	63.3	2	2.621	Accepted	-1.376	0.723	0.466
T5	217.06	201.216	1.079	7	0	86.21	3	2.564	Accepted	-3.268	1.529	1.595
T6	240	205.698	1.167	4	0	43.07	2	2.594	Accepted	-3.788	2.587	2.934
T7	248.09	236.238	1.05	7	1	81.76	4	2.572	Accepted	-1.625	0.377	-0.141
T8	211.09	209.672	1.007	5	1	58.86	2	2.698	Accepted	-2.189	1.001	0.96
T9	215.12	220.864	0.974	5	0	46.31	3	2.514	Accepted	-2.383	1.654	1.574
T10	232.1	227.448	1.02	6	0	61.53	4	2.446	Accepted	-2.532	1.645	1.653
T11	202.09	201.362	1.004	5	0	52.3	3	2.326	Accepted	-2.404	1.414	1.515
T12	232.1	227.448	1.02	6	1	72.53	4	2.549	Accepted	-1.719	0.866	0.401
T13	188.07	184.066	1.022	5	1	63.3	2	2.531	Accepted	-1.537	0.32	-0.079
A1	293.03	251.253	1.166	8	0	94.16	3	3.682	Accepted	-3.175	2.094	1.14
A2	274.06	257.268	1.065	5	0	51.02	3	3.841	Accepted	-3.299	2.08	1.494
A3	294.05	260.189	1.13	7	1	80.48	3	3.718	Accepted	-2.41	1.29	0.281
A4	264.04	234.103	1.128	6	1	71.25	2	3.74	Accepted	-2.24	1.278	0.505
A5	293.03	251.253	1.166	8	0	94.16	3	3.734	Accepted	-3.038	2.101	1.149
A6	315.97	255.735	1.236	5	0	51.02	2	3.738	Accepted	-3.374	3.058	2.55
A7	324.06	286.275	1.132	8	1	89.71	4	3.743	Accepted	-2.795	1.272	0.168
A8	287.06	259.708	1.105	6	1	66.81	2	3.739	Accepted	-3.152	1.873	1.276
A9	291.09	270.901	1.075	6	0	54.26	3	3.684	Accepted	-2.504	1.887	1.217
A10	308.07	277.485	1.11	7	0	69.48	4	3.595	Accepted	-2.915	1.892	1.11
A11	278.06	251.399	1.106	6	0	60.25	3	3.539	Accepted	-2.792	1.799	1.141
A12	308.07	277.485	1.11	7	1	80.48	4	3.718	Accepted	-2.689	1.909	0.926
A13	264.04	234.103	1.128	6	1	71.25	2	3.723	Accepted	-2.354	1.344	0.426

Table 6: ADME properties of molecules.

Code	Caco-2	BBB	HIA	PPB	VDss	CYP1 A2-Inh	CYP1 A2-sub	CYP2 C19-inh	CYP2 C19-sub	CYP 2 C9-inh	CYP 2 C9-sub	CYP 2 D6-inh	CYP 2 D6-sub	CYP 3 A4-inh	CYP 3 A4-sub	CL	T1/2
T1	-4.625	0.147	0.007	64.60%	0.935	0.987	0.184	0.79	0.069	0.62	0.23	0.026	0.057	0.053	0.215	9.31	0.856
T2	-4.639	0.216	0.009	84.30%	1.565	0.996	0.391	0.941	0.088	0.806	0.554	0.028	0.067	0.085	0.264	11.26	0.909
T3	-4.848	0.385	0.008	73.96%	1.07	0.996	0.852	0.918	0.066	0.863	0.759	0.487	0.102	0.341	0.346	8.425	0.955
T4	-4.853	0.339	0.015	57.77%	0.996	0.996	0.517	0.946	0.066	0.884	0.759	0.476	0.043	0.244	0.322	8.794	0.961
T5	-4.646	0.186	0.007	65.83%	0.844	0.991	0.275	0.874	0.076	0.721	0.123	0.034	0.047	0.102	0.222	9.36	0.886
T6	-4.494	0.033	0.004	94.50%	1.786	0.994	0.48	0.966	0.192	0.874	0.151	0.055	0.031	0.107	0.43	10.45	0.598
T7	-4.873	0.297	0.013	84.21%	1.052	0.993	0.947	0.89	0.198	0.817	0.656	0.189	0.134	0.299	0.545	7.632	0.951
T8	-4.786	0.54	0.011	77.69%	1.233	0.997	0.508	0.969	0.072	0.902	0.621	0.537	0.052	0.524	0.322	7.376	0.956
T9	-4.724	0.772	0.008	75.47%	1.226	0.994	0.603	0.868	0.186	0.77	0.053	0.03	0.021	0.076	0.303	10.31	0.921
T10	-4.67	0.579	0.007	74.65%	1.383	0.992	0.921	0.912	0.646	0.63	0.181	0.042	0.034	0.167	0.488	10.06	0.902
T11	-4.697	0.518	0.006	58.82%	1.555	0.993	0.71	0.896	0.194	0.707	0.096	0.064	0.017	0.11	0.396	9.512	0.904
T12	-4.866	0.224	0.006	77.26%	0.986	0.995	0.638	0.946	0.068	0.907	0.755	0.439	0.073	0.305	0.375	7.621	0.942
T13	-4.904	0.332	0.012	48.70%	1.061	0.996	0.486	0.934	0.06	0.849	0.721	0.361	0.052	0.131	0.25	8.595	0.959
A1	-4.797	0.113	0.005	54.74%	0.804	0.635	0.889	0.243	0.161	0.138	0.095	0.032	0.051	0.124	0.639	9.505	0.867
A2	-4.708	0.531	0.006	53.46%	0.965	0.96	0.78	0.192	0.159	0.183	0.075	0.024	0.046	0.115	0.601	8.398	0.928
A3	-5.039	0.239	0.005	62.27%	0.756	0.646	0.961	0.109	0.25	0.124	0.253	0.018	0.063	0.207	0.716	13.25	0.945
A4	-5.161	0.193	0.005	53.93%	0.674	0.726	0.881	0.127	0.168	0.139	0.361	0.026	0.049	0.14	0.621	12.46	0.955
A5	-4.783	0.139	0.005	55.56%	0.828	0.735	0.892	0.268	0.138	0.191	0.072	0.045	0.045	0.261	0.636	9.871	0.906
A6	-4.59	0.031	0.004	91.93%	1.073	0.94	0.868	0.644	0.569	0.352	0.082	0.041	0.034	0.507	0.844	8.981	0.743
A7	-4.927	0.129	0.008	74.89%	0.713	0.472	0.978	0.062	0.59	0.079	0.277	0.013	0.071	0.162	0.846	12.57	0.933
A8	-4.635	0.22	0.004	68.33%	0.871	0.938	0.884	0.357	0.271	0.288	0.168	0.054	0.046	0.489	0.651	10.51	0.941
A9	-4.745	0.775	0.006	72.74%	1.235	0.846	0.928	0.286	0.674	0.263	0.057	0.018	0.065	0.146	0.733	11.42	0.921
A10	-4.759	0.432	0.005	73.70%	0.905	0.878	0.967	0.342	0.8	0.165	0.15	0.019	0.085	0.258	0.851	11.27	0.921
A11	-4.762	0.547	0.004	56.10%	1.027	0.776	0.933	0.388	0.692	0.182	0.102	0.029	0.062	0.245	0.749	11.57	0.909
A12	-5.076	0.07	0.004	66.54%	0.669	0.871	0.903	0.214	0.261	0.311	0.292	0.024	0.051	0.288	0.717	11.99	0.92
A13	-5.171	0.235	0.005	50.49%	0.769	0.586	0.882	0.126	0.09	0.088	0.168	0.017	0.046	0.071	0.543	13.26	0.952

Table 7: Toxicity parameters of compounds.

Compound code	Herg blocker	DILI	ROA	Ame's Mutagenicity	Skin sensitisation	FDAMDD	EI	Carcino genecity	Hepato toxicity	Respiratory	BCF
T1	0.002	0.974	0.399	0.97	0.803	0.032	0.979	0.853	0.78	0.765	0.501
T2	0.003	0.978	0.782	0.781	0.921	0.061	0.976	0.257	0.328	0.952	0.713
T3	0.013	0.95	0.718	0.972	0.127	0.078	0.827	0.972	0.789	0.951	0.629
T4	0.005	0.969	0.78	0.978	0.627	0.029	0.943	0.964	0.705	0.951	0.592
T5	0.003	0.97	0.505	0.959	0.795	0.051	0.975	0.876	0.814	0.763	0.468
T6	0.001	0.976	0.151	0.762	0.709	0.093	0.922	0.803	0.233	0.693	1.334
T7	0.04	0.937	0.526	0.952	0.083	0.043	0.46	0.98	0.818	0.954	0.465
T8	0.011	0.978	0.977	0.986	0.587	0.763	0.768	0.903	0.844	0.972	0.454
T9	0.018	0.968	0.502	0.963	0.737	0.06	0.955	0.896	0.303	0.953	0.602
T10	0.012	0.945	0.566	0.953	0.268	0.125	0.857	0.954	0.7	0.933	0.752
T11	0.012	0.967	0.394	0.964	0.541	0.106	0.911	0.932	0.685	0.909	0.701
T12	0.01	0.953	0.436	0.975	0.13	0.042	0.813	0.97	0.651	0.876	0.724
T13	0.01	0.962	0.76	0.975	0.374	0.048	0.925	0.965	0.648	0.94	0.54
A1	0.029	0.986	0.304	0.99	0.513	0.414	0.051	0.976	0.856	0.75	0.388
A2	0.01	0.987	0.729	0.969	0.688	0.095	0.151	0.966	0.851	0.817	0.661
A3	0.009	0.98	0.697	0.907	0.099	0.234	0.028	0.98	0.806	0.769	0.472
A4	0.006	0.984	0.822	0.918	0.428	0.101	0.07	0.97	0.469	0.77	0.445
A5	0.038	0.983	0.342	0.989	0.464	0.647	0.046	0.975	0.87	0.796	0.392
A6	0.021	0.985	0.149	0.91	0.245	0.867	0.017	0.967	0.582	0.761	0.898
A7	0.012	0.974	0.709	0.775	0.042	0.319	0.018	0.984	0.872	0.779	0.478
A8	0.022	0.986	0.938	0.903	0.443	0.923	0.029	0.949	0.691	0.856	0.283
A9	0.019	0.985	0.406	0.931	0.327	0.231	0.038	0.979	0.77	0.779	0.382
A10	0.015	0.979	0.846	0.9	0.089	0.624	0.024	0.989	0.9	0.782	0.489
A11	0.015	0.985	0.726	0.924	0.186	0.272	0.032	0.986	0.856	0.638	0.472
A12	0.01	0.981	0.355	0.863	0.072	0.197	0.029	0.979	0.529	0.505	0.555
A13	0.01	0.984	0.681	0.915	0.238	0.081	0.052	0.975	0.479	0.655	0.382

5.3. Synthesis and Characterization

Table 8: IUPAC names of synthesized compounds.

code	Schiff bases	code	Triazoloazetidinones
T1	N-(4-nitrobenzylidene)-4H- 1,2,4-triazol-4-amine	**A1**	3-chloro-4-(4-nitrophenyl)-1-(4H- 1,2,4-triazol-4-yl) azetidine-2-one
T2	N-(3-phenylallylidene)-4H- 1,2,4-triazol-4-amine	**A2**	3-chloro-4-styryl-1-(4H-1,2,4- triazol-4-yl) azetidine-2-one
T3	N-(4-hydroxy-3-methoxybenzylidene)-4H- 1,2,4-triazol-4-amine	**A3**	3-chloro-4-(4-hydroxy-3- methoxyphenyl)-1-(4H-1,2,4- triazol-4-yl) azetidine-2-one
T4	N-(2-hydroxybenzylidene)-4H-1,2,4-triazol-4-amine	**A4**	3-chloro-4-(2-hydroxyphenyl)-1- (4H-1,2,4-triazol-4-yl) azetidine-2- one
T5	N-(3-nitrobenzylidene)-4H- 1,2,4-triazol-4-amine	**A5**	3-chloro-4-(3-nitrophenyl)-1-(4H- 1,2,4-triazol-4-yl) azetidine-2-one
T6	N-(2,4-dichlorobenzylidene)- 4H-1,2,4-triazol-4-amine	**A6**	3-chloro-4-(2,4-dichlorophenyl)-1- (4H-1,2,4-triazol-4-yl) azetidine-2-one
T7	N-(4-hydroxy-3,5- dimethoxy benzylidene)-4H- 1,2,4-triazol-4-amine	**A7**	3-chloro-4-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4H-1,2,4- triazol-4-yl) azetidine-2-one
T8	N-[(1H-indol-3-yl) methylene]-4H-1,2,4-triazol-4-amine	**A8**	3-chloro-4-(1H-indol-3-yl)-1-(4H- 1,2,4-triazol-4-yl) azetidine-2-one
T9	dimethyaminobenzylidene)- 4H-1,2,4-triazol-4-amine	**A9**	3-chloro-4-(4- dimethylamino) phenyl)-1-(4H- 1,2,4-triazol-4-yl) azetidine-2-one
T10	N-(2,4-dimethoxybenzylidene)-4H- 1,2,4-triazol-4-amine	**A10**	3-chloro-4-(2,4-dimethoxyphenyl)- 1-(4H-1,2,4-triazol-4-yl) azetidine- 2-one
T11	N-(4-methoxybenzylidene)- 4H-1,2,4-triazol-4-amine	**A11**	3-chloro-4-(4-methoxyphenyl)-1- (4H-1,2,4-triazol-4-yl) azetidine-2- one
T12	N-(3-ethoxy-4- hydroxy benzylidene)-4H- 1,2,4-triazol-4-ami	**A12**	3-chloro-4-(3-ethoxy-4- hydroxyphenyl)-1-(4H-1,2,4- triazol-4-yl) azetidine-2-one
T13	N-(4-hydroxybenzylidene)-4H-1,2,4-triazol-4-amine	**A13**	3-chloro-4-(4-hydroxyphenyl)-1- (4H-1,2,4-triazol-4-yl) azetidine-2- one

Characterization

Table 9: Physicochemical data of the compounds.

Compound code	Mol. Formula	Molecular weight	Melting point °C	% Yield	Rf value
T1	$C_9H_7NO_2$	217.06	270-271	54.3	---
T2	$C_{11}H_{10}N_4$	198.09	195-197	20.3	---
T3	$C_{10}H_{10}N_4O_2$	218.08	250-251	32.11	---
T4	$C_9H_8N_4O$	188.07	207-208	59	---
T5	$C_9H_7N_5O_2$	217.06	250-252	41.9	---
T6	$C_9H_6Cl_2N_4$	240	170-172	25.7	---
T7	$C_{11}H_6N_4O_3$	248.09	295-297	20.66	---
T8	$C_{11}H_9N_5$	211.09	200-202	40.7	---
T9	$C_{11}H_{13}N_5$	215.12	100-101	40	---

T10	$C_{11}H_6N_4O_2$	232.1	110-112	11.06	---
T11	$C_{11}H_7N_4O$	202.09	97-99	62.3	---
T12	$C_{11}H_7N_4O_2$	232.1	195-197	37	---
T13	$C_9H_8N_4O$	188.07	255-256	66.4	---
A1	$C_{11}H_8ClN_5O_3$	293.03	195-197	54	0.54
A2	$C_{13}H_{11}ClN_4O$	274.06	75-77	30.3	0.47
A3	$C_{12}H_{11}ClN_4O_3$	294.05	170-171	44	0.62
A4	$C_{11}H_9ClN_4O_2$	264.04	180-181	16.1	0.55
A5	$C_{11}H_8ClN_5O_3$	293.03	200-202	43.4	0.73
A6	$C_{11}H_7Cl_3N_4O$	315.97	140-142	36	0.32
A7	$C_{13}H_{13}ClN_4O_4$	324.06	215-217	50	0.50
A8	$C_{13}H_{10}ClN_5O$	287.06	160-161	18.8	0.42
A9	$C_{13}H_{14}ClN_5O$	291.09	45-48	38	0.65
A10	$C_{13}H_{13}ClN_4O_3$	308.07	50-52	46	0.3
A11	$C_{12}H_{11}ClN_4O_2$	278.06	80-82	28.72	0.75
A12	$C_{13}H_{13}ClN_4O_3$	308.07	90-91	47	0.58
A13	$C_{11}H_9ClN_4O_2$	264.04	220-221	38	0.70

Table 10: IR (Cm^{-1}) spectral data of the synthesized compounds.

Compound code	IR spectral values in cm^{-1}
T1	1680.81 (N = C stretch), 1318.17 (C-N stretch), 3019.20 (C-H stretch), 1163.00 (C-H deformation), 1560.54 (C = C stretch), 1405.54, 1318.17 (N=O stretch), 812.67 (C-N stretch)
T2	1628.72 (C=N stretch), 1332.40 (C-N stretch), 3078.38 (C-H stretch), 1168.66 (C-H deformation), 1503.55 (C=C stretch)
T3	1629.61 (C=N stretch), 1342.23 (C-N stretch), 3085.36 (C-H stretch) 1114.23 (C-H deformation), 1514.27 (C=C stretch), 1269.52 (C-O stretch), 3646.02 (O-H stretch), 2936.86 (C-H stretch), 1422.52 (C-H deformation)
T4	1660.08 (C=N stretch), 1328.93 (C-N stretch), 3086.06 (C-H stretch), 1064.52 (C-H deformation), 1550.04 (C=C stretch), 3648.15 (O-H stretch) 1375.71 (C-O stretch)
T5	1640.95 (C=N stretch), 1357.43 (C-N stretch), 3076.06 (C-H stretch), 1050.46 (C-H deformation), 1507.29 (C=C stretch), 1507.29,1357.43 (N=O stretch), 805.07 (C-N stretch)
T6	1671.95 (C=N stretch), 1342.06 (C-N stretch), 3027.69 (C-H Stretch), 1047.71 (C-H deformation), 857.39 (C=C stretch), 735.17 (C-Cl stretch)
T7	1655.92 (C=N stretch), 1334.74 (C-N stretch), 3013 (C-H stretch), 1028.31 (C-H deformation), 1505.27 (C=C stretch), 3628.78 (O-H stretch), 1334.58 (C-O stretch), 2968.11 (C-H Stretch), 1444.85 (C-H deformation), 1212.65 (C-O stretch aryl -ether), 1028.31 (C-O stretch alkyl-ether)
T8	1629.32 (C=N stretch), 1336.99 (C-N stretch), 3055.20 (C-H stretch), 1134.18 (C-H deformation), 1518.85 (C=C stretch), 1572.40 (N-H stretch), 1689.32 (C=C stretch), 870.09 (C-H deformation), 6336.44 (C-N stretch)
T9	1656.78 (C=N stretch), 1345.01 (C-N stretch), 3044.08 (C-H stretch), 1156.809 (C-H deformation), 1507.47 (C=C stretch), 1345.01 (N-C stretch),

	2982.40 (C-H stretch), 1433.83 (C-H deformation)
T10	1666.94 (C=N stretch), 1325.81 (C-N stretch), 3001.77 (C-H stretch), 1592.03 (C=C stretch), 2941.27 (C-H stretch, -CH3), 1414.09 (C-H deformation, -CH3), 1263.59 (C-O stretch aryl-ether), 1055.06 (C-O stretch alkyl-ether)
T11	1685.69 (C=N stretch), 1365.31 (C-N stretch), 3059.65 (C-H stretch), 1160.28 (C-H deformation), 1498.42 (C=C), 1254.90 (C-O stretch aryl ether), 1053.25 (C-O stretch alkyl ether), 2971.34 (C-H stretch, -CH3), 1444.30 (C-H deformation, -CH3)
T12	1687.44 (C=N stretch), 1380.00 (C-N stretch), 1562.07 (C-H stretch), 3035 (C-C stretch), 1014.49 (C-C deformation), 1053.91, 1248.69, 1380.00 (C-O stretch), 3608.42 (O-H stretch).
A1	1391.47 (C-N stretch, 3°amine), 1710.71 (C=O stretch), 1510.41, 1343.45 (NO2 stretch, aromatic), 847.30 (C-N stretch, NO2), 751.19 (C-Cl stretch).
A2	1351.73 (C-N stretch, 3°amine), 1710.50 (C=O stretch), 752.53 (C-Cl stretch), 3027.78 (=C-H stretch, -CH=CH- trans), 974.77 (CH out of plane, deformation), 1299.38 (CH in plane, deformation), 1623.16 (C=C stretch).
A4	1366.01 (C-N stretch, 3°amine), 1682.49 (C=O stretch), 754.71 (C-Cl stretch), 3539.98 (O-H stretch), 1248.45 (C-O stretch), 1449.82 (O-H deformation).
A5	1349.11 (C-N stretch, 3°amine), 1606.58 (C=O stretch), 1509.52, 1285.81 (NO2 stretch, aromatic), 846.86 (C-N stretch, NO2), 721.75 (C-Cl stretch).
A6	1386.98 (C-N stretch, 3°amine), 1612.71 (C=O stretch), 786.86, 723.45 (C-Cl stretch),
A7	1368.97 (C-N stretch, 3°amine), 1626.79 (C=O stretch), 728.72 (C-Cl stretch), 3740.85 (O-H stretch), 1212.46 (C-O stretch), 1328.40 (O-H deformation), 2809.84 (C-H stretch, CH3-O), 1464.60 (C-H deformation, CH3-O), 1251.33, 1052.36 (C-O stretch, Ar-O-CH3).
A8	1388.70 (C-N stretch, 3°amine), 1629.15 (C=O stretch), 752.40 (C-Cl stretch), 1331.09 (C-N stretch, 2°amine), 1570.73 (N-H deformation).
A10	1329.57 (C-N stretch, 3°amine), 1662.54 (C=O stretch), 734.10 (C-Cl stretch), 2851.85 (C-H stretch, CH_3-O), 1466.27 (C-H deformation, CH_3-O), 1261.61, 1011.95 (C-O stretch, Ar-O-CH_3).
A12	1301.97 (C-N stretch, 3°amine), 1730.82 (C=O stretch), 751.56 (C-Cl stretch), 3624.95 (O-H stretch), 1166.74 (C-O stretch), 2924.38 (-CH_2-stretch), 1469.12 (-CH_2-deformation), 1424.78 (-CH_3 deformation), 1246.10, 1051.77 (C-O stretch, Ar-O-CH_3).
A13	1340.22 (C-N stretch, 3°amine), 1705.78 (C=O stretch), 748.68 (C-Cl stretch), 3611.80 (O-H stretch), 1213.64 (C-O stretch), 1386.67 (O-H deformation).

Table 11: ^{1}H NMR and ^{13}C NMR spectral data of the synthesized compounds.

Compound Code	Chemical shift value in ppm	
	^{1}H NMR	^{13}C NMR
A1	1.2037 (1H, CH, azetidinones), 2.5161 (1H, CH, azetidinones), 8.0966-8.4124 (4H, aromatic ring), 9.2135, 9.2694 (2H, triazole ring)	51.05 (1C, C-Cl), 64.91 (1C, C-H, azetidinone), 126.90-132.51 (4C, aromatic), 142.35, 149.96 (2C, aromatic), 151.94, 152.65 (2C, triazole), 173.77 (1C, C=O)
A2	4.1385 (2H, CH, azetidinones), 6.7517 (1H, CH, ethylene bridge), 6.9948-7.0453 (1H, ethylene bridge), 7.2709-7.5659 (5H, aromatic ring), 8.6720 (2H, triazole ring)	48.34 (1C, C-Cl), 60.37 (1C, C-H, azetidinone), 126.86-130.99 (6C, aromatic), 132.99, 135.48 (2C, - CH=CH-), 146.88 (2C, triazole), 171.94 (1C, C=O)
A4	2.5178 (1H, CH, azetidinones), 3.4351 (1H, CH, azetidinones), 6.9455-7.8096 (4H, aromatic ring), 9.1748, 9.1802 (2H, triazole ring), 10.5057 (1H, -OH)	46.02 (1C, C-Cl), 62.63 (1C, C-H, azetidinone), 117.92 – 125.48 (5C, aromatic), 145.48, 146.88 (2C, triazole), 157.94 (1C, C-OH), 169.54 (1C, C=O)
A5	2.5162 (1H, CH, azetidinones), 3.4520 (1H, CH, azetidinones), 7.8045-8.6427 (4H, aromatic ring), 9.2152, 9.2905 (2H, triazole ring)	49.90 (1C, C-Cl), 63.81 (1C, C-H, azetidinone), 109.11-116.06 (4C, aromatic), 145.52, 148.59 (2C, aromatic), 147.25, 147.96 (2C, triazole), 168.37 (1C, C=O)
A6	1.2012 (1H, CH, azetidinones), 3.0635-3.0974 (1H, CH, azetidinones), 7.6060-8.0719 (3H, aromatic ring), 9.1880, 9.2678 (2H, triazole ring)	50.04 (1C, C-Cl), 60.41 (1C, C-H, azetidinone), 121.17-139.55 (6C, aromatic), 143.69, 144.53 (2C, triazole), 169.25 (1C, C=O)
A7	2.4998-2.5141 (1H, CH, azetidinones), 3.5692-3.6931 (1H, CH, azetidinones), 3.8406, 3.8477 (6H, -OCH3), 7.1563, 7.2120 (2H, aromatic), 9.3276 (2H, triazole ring), 9.7803 (1H, - OH)	48.52 (1C, C-Cl), 56.90 (2C, - OCH3), 62.37 (1C, C-H, azetidinone), 109.11, 110.60, 131.51, 132.06, 148.16, 148.59 (6C, aromatic), 145.52, 147.25 (2C, triazole), 174.23 (1C, C=O)
A8	2.5110-2.5255 (1H, CH, azetidinones), 3.4356 (1H, CH, azetidinones), 7.2201-8.2978 (5H, aromatic), 9.9621 (2H, triazole ring), 10.1577 (1H, -NH, pyrrole ring)	47.52 (1C, C-Cl), 54.30 (1C, C-H, azetidinone), 112.93-139.41 (8C, indole), 150.43, 151.18 (2C, triazole), 166.51 (1C, C=O)
A9	3.0892 (6H, -CH3), 5.1089, 5.3356 (2H, CH, azetidinones), 6.7221-7.7531 (4H, aromatic), 9.1490 (2H, triazole ring)	36.08 (2C, -CH3), 45.47 (1C, C-Cl), 55.72 (1C, C-H, azetidinone), 112.70-143.89 (6C, indole), 155.13, 155.96 (2C, triazole), 170.42 (1C, C=O)
A10	3.8739, 3.9002 (6H, -CH3), 5.1209, 5.3056 (2H, CH, azetidinones), 6.4437-7.8120 (3H, aromatic), 9.2863, 9.2875 (2H, triazole ring)	46.48 (1C, C-Cl), 55.28, 55.40 (2C, -OCH3), 57.09 (1C, C-H,azetidinone), 108.42, 110.32, 114.75, 132.48, 157.59, 162.27 (6C, aromatic), 153.88, 154.40 (2C, triazole), 168.91 (1C, C=O)
A12	3.8359-3.8725 (3H, -CH3), 4.1637, 4.1810 (2H, -CH2-),5.5345, 5.6526 (2H, CH, azetidinones),7.1258-7.8710 (3H, aromatic), 9.1345 (1H, -OH), 9.5233, 9.5336 (2H, triazole ring)	17.59 (1C, -CH3), 45.40 (1C, C-Cl), 55.88 (1C, C-H, azetidinone), 72.94, 73.32 (2H, -CH2-), 101.72, 115.14, 123.10, 133.24, 149.04, 155.77 (6C, aromatic), 150.57, 151.82 (2C, triazole), 169.83 (1C, C=O)

A13	2.5064-2.5201 (1H, azetidinone), 3.3757 (1H, azetidinone), 8.0946-8.4131 (4H, aromatic), 9.2544 (1H, -OH), 9.2067 (2H, triazole ring)	43.97 (1C, C-Cl), 62.32 (1C, C-H, azetidinone), 115.30, 115.35, 121.37, 121.87, 142.33, 158.75 (6C, aromatic), 156.04, 156.36 (2C, triazole), 164.61 (1C, C=O)

5.4. Biological screening

5.4.1. Antitubercular activity

Solvent used: DMSO (2%)

Concentration for the compounds used to validate the MIC:1000, 500,250,125, 62.5, 31.25, 15.62, 7.81µg/ml.

Control Drug: Rifampicin (conc. 1 µg/ml)

Table 12: MIC data of compounds on Mtb H37Rv.

Compound	MIC (µg/ml)	Compound	MIC (µg/ml)
A1	125	T1	>1000
A2	>1000	T 2	>1000
A3	>1000	T3	>1000
A4	>1000	T4	>1000
A5	1000	T5	>1000
A6	>1000	T6	---
A7	>1000	T7	>1000
A8	500	T8	>1000
A9	>1000	T9	>1000
A10	>1000	T10	>1000
A11	>1000	T11	>1000
A12	250	T12	>1000
A13	250	T13	>1000

TB screening completed as two replicas (n=2), a total of twelve compounds from schiff bases and thirteen azetidinone series screened for anti TB activity against *Mycobacterium tuberculosis* H37Rv. From the results **Table 12**, it is clear that in azetidinone series A1 showed inhibition at MIC 125µg/mL, compound A12, A13 exhibited MIC at 250µg/mL, and compound A8 at 500 µg/mL. None of the Schiff base showed activity against H37Rv.

Antimicrobial activity – Among the synthesized compounds, the compounds showed antiTB activity were screened for their antimicrobial activity by disc plate method against the microorganisms like *Staphylococcus aureus, Escherichia Coli, and Candida albicans* and allowed to grow by incubating at 35-37 °C for 24 hours in the concentration of 50µg/ml,100µg/ml,150µg/ml,200µg/ml and 250µg/ml. After 24 hours the petri plates are collected and observed that no compound showed promising zone of inhibition compared

with the standards.

CONCLUSION

The docking studies reveal that while standard drugs like the 6G9 cocrystal, 883 cocrystal, Ketoconazole, and Streptomycin show strong binding affinities and stable interactions with their respective proteins, several designed molecules also exhibit promising docking scores and interactions. Compounds such as A3, A8, A9, and A2 stand out across different proteins, demonstrating potential as effective antimicrobial agents. However, the varied interaction profiles suggest that further optimization and testing are necessary to fully assess their efficacy and stability as therapeutic agents. The ADMET profiles highlight the necessity for a balanced approach in drug development, considering both pharmacokinetics and safety. Compounds with favourable absorption, distribution, metabolism, and low toxicity profiles (e.g., T_1, T_2) present promising candidates for further development, while others with higher toxicity risks need more optimization or may be considered for alternative therapeutic uses where the risk can be managed. Further *in vivo* studies and clinical evaluations will be essential to confirm these findings.

The IR spectra of compounds A1 to A13 reveal C=O stretches (1606-1730 cm^{-1}), C-N stretches (1300-1400 cm^{-1}), C-Cl stretches (720-750 cm^{-1}), O-H stretches (3539-3740 cm^{-1}), NO2 stretches (1500-1600 cm^{-1}), and C-O stretches (1200-1300 cm^{-1}), indicating varied functional groups and substitutions.

The ^{1}H NMR spectra of compounds A1 to A13 reveal characteristic proton signals indicating different structural elements. Azetidinone protons are consistently seen around 1-5 ppm. Aromatic protons typically appear in the 6-8 ppm range, indicating aromatic rings in the compounds. Triazole ring protons resonate around 9-10 ppm, confirming the presence of triazole rings. Methoxy groups (OCH_3) are observed at 3.8-3.9 ppm in some compounds (e.g., A7, A10). Hydroxyl and amine protons are seen around 9-10 ppm in compounds with OH or NH groups (e.g., A4, A7, A8). These spectra collectively suggest the presence of azetidinone, aromatic, and triazole moieties in these compounds. The ^{13}C NMR spectra of A1- A13 reveal characteristic peaks: C=O signals (164-174 ppm), azetidinone carbons (54-65 ppm), C-Cl carbons (43-51 ppm), aromatic carbons (109-143 ppm), and triazole carbons (145-157 ppm). Methoxy groups show signals near 56-57 ppm, while methyl groups appear around 17-36 ppm, reflecting diverse structural motifs. TB screening of twelve Schiff bases

and thirteen azetidinone compounds against Mycobacterium tuberculosis H37Rv revealed A1 at MIC 125µg/mL, while A_{12}, A_{13}, and A_8 showed MIC at 250µg/mL and 500µg/mL, respectively. However, none of the Schiff bases displayed activity against H37Rv. Antimicrobial tests against various microorganisms showed no significant inhibition compared to standards across different concentrations 50µg/ml to 250µg/ml.

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