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Comparative, anti-infective screening and molecular docking studies of 4-amino-1, 2, 4-triazole derivatives

M P Toraskar*, Vinayak Pande, Sambodhan Dhawane, Rizwan Khan

Bharati Vidyapeeth's College of Pharmacy,Sector 8, C.B.D Belapur, Navi Mumbai, Maharashtra, India

Corresponding author: M P Toraskar, ✉ mrunmayee.toraskar@bvcop.in, **Orcid Id**: https://orcid.org/0000-0002-6855-0522

Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D Belapur, Navi Mumbai, Maharashtra, India

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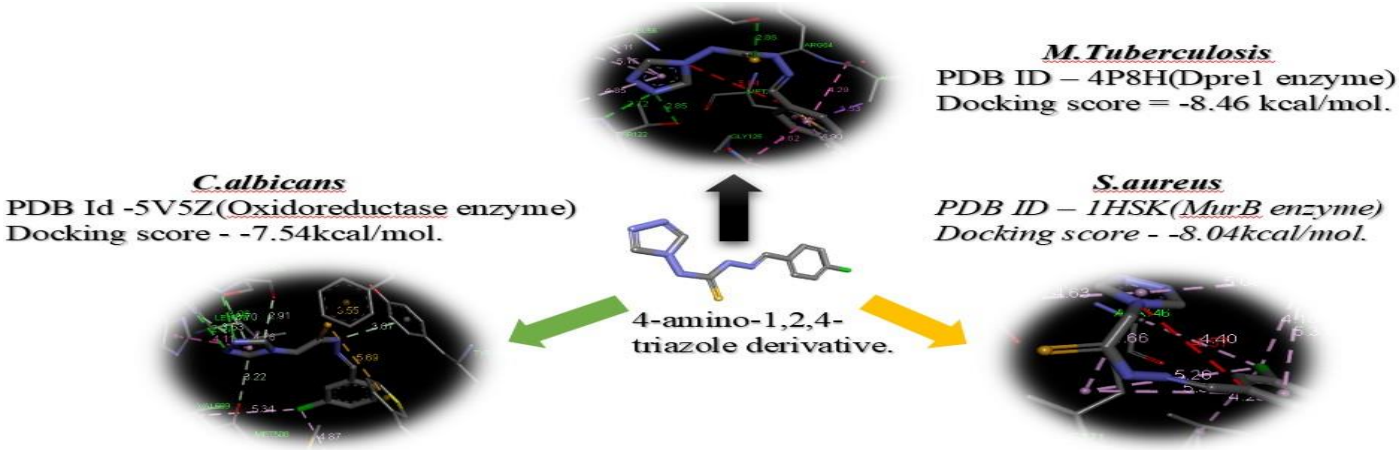
ABSTRACT

The rise of drug-resistant bacteria is a problem in medicine. This highlights the need to find new molecules that have anti-infective properties. In this study, thiosemicarbazone derivatives were made using a 4-amino-1,2,4-triazole structure. We have evaluated at the anti-infective activity against *E. coli, S. aureus, C. albicans, & M. tuberculosis*. Among the compounds tested, 4g showed activity at concentrations of 1 µg/ml & 2 µg/ml against *E. coli* and *S. aureus*. The molecule 4c had anti-infective activity at a concentration of 6.25 µg/ml against *M. tuberculosis*. Four compounds had nitro, anthracene, & chlorine (both mono & disubstituted) groups and were effective against all the bacteria we tested. Analysis of molecular binding was also done to see how these compounds bond with *E. coli* (PDB ID: 1HSK), *S. aureus* (PDB ID: 1HSK), *C. albicans* (PDB ID: 5V5Z), and *M. tuberculosis* (PDB ID: 4P8H).



Keywords: 4-Amino-1, 2, 4-Triazole, Anti-infective activity, Maba, Molecular docking, Pharmacokinetic Properties.

INTRODUCTION

Heterocyclic compounds are crucial for creating new structures that could be used in research study of anti-infective. Triazole is a kind of organic heterocycle. It has a five-membered ring made up of two carbon atoms and three nitrogen atoms. A key set of chemical compounds derived from 4-amino-1, 2, 4-triazole show various activities, like antifungal, antibacterial, and anti-tubercular effects [1]. Reports say that the main reason behind the biological activity comes from the features of 4-amino-1, 2, 4-triazoles. These include their mild dipole nature, the ability to form hydrogen bonds, stiffness & stability in metabolism [2]. Triazole has a slower metabolic rate, better oral bioavailability, less toxicity, more stability, and no

impact on human sterol production [3]. Because of metabolic breakdown, the majority of azole antimycotic have significant lipid solubility and are ineffective when taken orally [4]. Both triazole and imidazole nuclei function by the same mode of action. So triazole was substituted for imidazole [5]. The growth of microorganisms gets suppressed as a free nitrogen atom in the azole group links with iron present in the heme's prosthetic group within an enzyme's active site [6]. The triazole part interacts at a receptor's active site by acting as both a hydrogen bond acceptor & donor [3]. Lately, there's been growing resistance to common antibiotics; this raises the need for developing new broad-spectrum antibacterial drugs. One of the mechanisms for the inhibition of cell walls in bacteria is the inhibition of bacterial enzyme UDP-N-acetylenolpyruvylglucosamine reductase (encode MurB gene) [7]. In cases of tuberculosis, it interferes with an enzyme called decaprenylphosphoryl-beta-D-ribose oxidase (Dpre1) [8]. Fungi face inhibition in their cell membranes from Lanosterol 14 alpha-Demethylases [9]. Therefore we have selected this enzyme for our research studies. Antimicrobial activity was tested on four types of bacteria, covering both gram-positive & gram-negative strains. This study provides a summary of the synthesis of new triazole derivatives derived from triazole-hydrazine carbothioamide hybrids using 4-amino-1, 2, 4-triazole, along with their spectral characterization, molecular docking analysis, and biological evaluations.

MATERIALS AND METHODS

Chemicals

Compounds were synthesized using chemicals and purified solvents and obtained from standard commercial suppliers. VEEGO melting point apparatus is used, to determine the melting point of compounds. Thin-layer chromatography (TLC) was performed on 3x8 cm silica gel G plates (Sigma-Aldrich) and visualized under UV light or in an iodine chamber. Column chromatography, when required, was conducted using natural silica (mesh 120) in a 2.5 x 45 cm column with an appropriate eluent. IR spectra were obtained using a SHIMADZU FTIR 8400S infrared spectrophotometer. ^{1}H NMR spectra were obtained at 400 MHz frequency using deuterated DMSO-d6 solvent, with TetraMethylSilane as the standard reference, at the Sophisticated Analytical Instrument Facility (SAIF) at IIT Powai, Mumbai.

General procedure for the synthesis of N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (3)

In a 250ml round bottom flask, a mixture of 4-amino-1, 2, 4-triazole (0.05mol), distilled ethanol (50mL) and potassium hydroxide (0.08mol) was taken. Carbon disulfide (0.08mol) was added drop by drop to the mixture. The mixture kept for stirring for 1 hour at 0-5°C temperature to obtain the potassium salt. Then hydrazine monohydrate (0.08mol) was reacted using reflux. After 2hrs of reflux, the solution was kept under the solvent evaporator. The solid mixture was washed with water, separated out and recrystallized from ethanol to get pale white crystals [10].

N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (3)

Yield: 12.55%; M.P: 195-198°C; IR (cm-1): 3265 (N-H str.), 3211 (N-H str.), 2921 (Ar C-H str.), 1595 (N=C str.), 1087 (C=S str.); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 9.14 (s, 2H, CH triazole), 5.62 (s, 1H, NH), 5.27 (t, 1H, NH), 4.67 (d, 2H, NH2).

General procedure for the synthesis of (benzylidene/substituted benzylidene)-N (4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4a-4h)

In a 250mL round bottom flask, a same molar concentration of compound 3 and aromatic aldehyde (0.05mol) was mixed in 50mL ethanol by heating. A catalytic amount of glacial acetic acid was added and refluxed for 7-8 hrs. After reflux of 7-8, the solid mixture was kept under a solvent evaporator and poured over crushed ice. The crude product was washed with water, separated, and recrystallized from distilled ethanol [11].

(4-hydroxybenzylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4a)

Yield: 73.90%; m.p: 215-217°C; IR (cm-1): 3402 (O-H str.), 3323 (N-H str.), 2924 (Ar C-H str.), 1668 (N=C str.), 1049 (C=S str.); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 10.6 (s, 1H, OH), 9.78 (s, 2H, CH triazole), 9.14 (s, 1H, N=CH), 7.77 (d, 2H, Ar C-H), 6.94 (d, 2H, Ar CH), 5.27 (s, 1H, NH), 4.68 (s, 1H, NH).

[4-(dimethyl amino) benzylidene]-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4b)

Yield: 67%; M.P. 213-215°C; IR (cm-1): 3346 (N-H str.), 2929 (Ar C-H str.), 2852 (C-H str.), 1668 (N=CH str.), 1087 (C=S str.); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 9.66 (s, 2H, C-H triazole), 9.15 (s, 1H, N=CH), 7.69 (d, 2H, Ar C-H), 6.79 (d, 2H, Ar C-H), 5.28 (s, 1H, NH), 4.68 (s, 1H, NH), 3.1 (s, 6H, C-H dimethyl).

(2, 4-dichlorobenzylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4c)

Yield: 72.77%; M.P. 204-206°C; IR (cm-1): 3155 (N-H str.), 2968 (Ar C-H str.), 1600 (N=CH str.), 1099 (C=S str.), 619 (C-Cl); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 8.41 (s, 2H, C-H triazole), 7.89 (s, 1H, N=CH), 7.61 (s, 1H, Ar C-H), 7.52 (d, 2H, Ar C-H), 6.24 (s, 1H, NH), 5.66 (s, 1H, NH)

(4-bromobenzylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4d)

Yield: 62.97%; M.P. 205-207°C; IR (cm-1): 3273 (N-H str.), 2962 (Ar C-H str.), 1639 (N=CH str.), 1141 (C=S str.), 594 (C-Br str.); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 8.41 (s, 2H, CH triazole), 7.78 (s, 1H, N=CH), 7.57 (d, 2H, Ar C-H), 7.42 (d, 2H, Ar C-H), 6.16 (s, 1H, NH), 5.51 (s, 1H, NH).

Benzylidene-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4e)

Yield: 65.2%; M.P. 203-205°C; IR (cm-1): 3250 (N-H str.), 3157 (Ar C-H str.), 1614 (N=CH str.), 1141 (C=S STR.). ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 8.41 (s, 2H, CH triazole), 7.78 (s, 1H, N=CH), 7.47 (d, 2H, Ar C-H), 7.42 (d, 3H, Ar C-H), 6.16 (s, 1H, NH), 5.51 (s, 1H, NH).

(3-nitrobenzylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4f)

Yield: 62.97%; M.P. 205-207°C; IR (cm-1): 3151 (N-H str.), 2941 (Ar C-H str.), 1639 (N=CH str.), 1516 (NO str.), 1112 (C=S str.). ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 9.88(s, 2H, C-H triazole), 9.15 (s, 1H, N=CH), 7.71 (d, 2H, Ar C-H), 6.74 (d, 2H, Ar C-H), 5.48 (s, 1H, NH), 4.68 (s, 1H, NH).

(9, 10-dihydroanthracen-9-ylmethylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4g)

Yield: 56.36%; M.P. 205-207°C; IR (cm-1): 3275 (N-H str.), 2960 (Ar C-H str.), 1668 (N=CH str.), 1141 (C=S str.); ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 9.73 (s, 2H, C-H triazole), 8.86 (s, 1H, N=CH), 8.46-7.80 (m, 9H, Ar C-H), 5.57 (s, 1H, NH), 4.3 (s, 1H, NH).

(4-chlorobenzylidene)-N-(4H-1,2,4-triazole-4-yl) hydrazine carbothioamide (4h)

Yield: 59%; M.P. 206-208°C; IR (cm-1): 3273 (N-H str.), 2960 (Ar C-H str.), 1647 (N=CH str.), 1080 (C=S str.), 754 (C-Cl str.). ^{1}H NMR (400MHz, DMSO-d6) δ ppm: 8.41 (s, 2H, C-H triazole), 7.85 (s, 1H, N=CH), 7.61 (s, 2H, Ar C-H), 7.52 (d, 3H, Ar C-H), 6.24 (s, 1H, NH), 5.56 (s, 1H, NH).

Computational Analysis

SwissADME is a dependable and free online tool that forecasts pharmacokinetic and physicochemical properties. These attributes are essential for advancing a lead molecule through preclinical and clinical stages. The 2D structures of compounds 4a-4h were created with ChemDraw Pro 8.0 and then imported into SwissADME (http://www.swissadme.ch/), where compounds SMILES were generated. The SwissADME tool provided data on various physicochemical and pharmacokinetic parameters, which were saved in CSV format for further analysis. The tool's bioavailability radar plots were utilized directly for interpretation [12].

Molecular Docking studies

Molecular docking was conducted using AutoDock-Cygwin software. The crystal structures of *S. aureus* (encode murb gene) (PDB ID: 1HSK) [13], CYP51 from *Candida albicans* (PDB ID: 5V5Z) [14], and *M. tuberculosis* DprE1 in complex with nitro-benzothiazole 6a (PDB ID: 4P8H) [15] were used. Water molecules and heteroatoms were removed from these structures, which were then saved as PDB files. Ligand molecules were analyzed, improved, and stored in PDB format using the Discovery Studio application. Torsions of the ligand molecules were set and stored in PDBQT format using AutoDockTools 1.5.6. Docking simulations were performed using the Lamarckian genetic algorithm. The resulting structural files were analyzed using the Discovery Studio visualization application.

Biological Activity

The synthesized compounds (4a-4h) were evaluated for their anti-infective properties. The biological activity studies was conducted at the Department of Microbiology, NGH Institute of Dental Sciences, and Karnataka.

Antibacterial Activity and Antifungal Activity

Using the tube dilution method, Minimal Inhibitory Concentrations (MIC) were calculated for the new compound (4a-4h). MIC was calculated *in vitro* for *E. coli*, *S. aureus* (anti-bacterial), and *C. albicans* (anti-fungal). Standard culture of bacteria & fungi on an agar plate medium. Nine dilutions of each compound were prepared using Brain Heart Infusion (BHI) broth to determine MIC. Each compound of 20 microlitres was mixed with 380 microlitres of BHI broth in each tube. Then, nine empty tubes were kept ready with labels. Each one had serial numbers on it & was filled with 200 microlitres of BHI broth. After that, we transferred 200 microlitres from the stock tube into the ninth tube containing 200 microlitres of BHI broth. This created a 10^{-1} dilution. This was repeated, transferring 200 microlitres from each tube to the next to make dilutions up to 10^{-9} for every single compound. Stock cultures of the test organisms were prepared by adding 5 microlitres of each bacteria into 2 milliliters of broth. In serially diluted tubes, 200 microlitres of each microbial suspension was added. After cultivating for 24 hours, we checked for turbidity in the tubes to determine our MIC values [16].

Antitubercular Activity

The compound (4a-4h) was synthesized for examining anti-tubercular activity against *M.Tb*. Using Middlebrook 7H-9 broth and the standard *M.tb H37Rv* strain. The Microplate Alamar Blue Assay (MABA) was employed to assess the anti-mycobacterial efficacy. To prevent the medium from evaporating it was kept in the incubator, put 200 µl of sterile deionized water in the wells on the outer edge of a sterile 96-well plate. Then, we added 100µL of Middlebrook 7H9 broth to that plate. Further stepwise dilutions of the compounds that were made ready to put onto the plate; the final amount of the compound ranged from 100 to 0.2 µg/mL. After that, the plate was wrapped in parafilm & kept for 5 days at 37°C. After 5 days, we mixed a 1:1 solution of Alamar Blue reagent with 10% tween 80 and poured it into each well for another 24 hours of incubation. A blue color shows bacterial growth is stopped, while a pink color means bacteria are growing. The lowest concentration of the compound that keeps the color from changing from blue to pink determines the MIC. Standard antibiotics used for comparison included Streptomycin at 7.5 µg/mL and Pyrazinamide at 7.5 µg/mL [17].

RESULTS AND DISCUSSION

The target compounds (4a–4h) were synthesized following the procedure and substitution pattern outlined in Scheme 1. To prepare the thiosemicarbazone derivative of 4-amino-1, 2, 4-triazole (3), 4-amino-1, 2, 4-triazole was reacted to hydrazine monohydrate in the presence of carbon disulphide and potassium hydroxide. The compounds (substituted benzylidene)-N-(4H1,2,4-triazole-4-yl) hydrazine carbothioamide (4a-4h) were then synthesized by reacting compound (3) with an aromatic aldehyde using glacial acetic acid in

catalytic amount and ethanol as a solvent.

Scheme 1: General scheme for the synthesis of (benzylidene/substituted benzylidene)-N-(4H-1, 2, 4-triazole-4-yl) hydrazine carbothioamide (4a-4h).

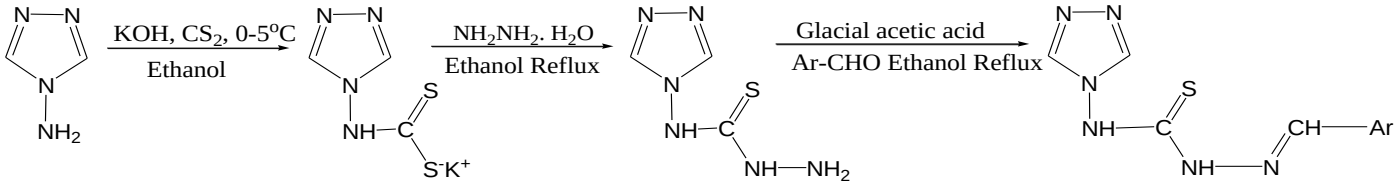
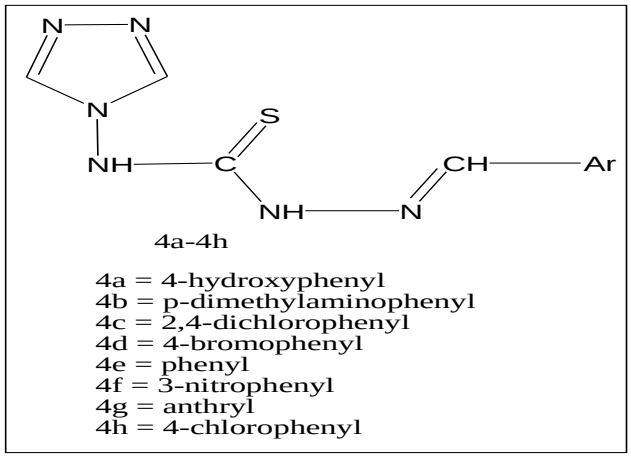


Table 1: Swiss ADME prediction.

Code	MW[a]	HBAb	HBD[c]	TPSA[d]	logP[e]	ESOL Class[f]	GIA[g]	Lipinski #violation	Bioavailability	P-gp[h]	BBB[i] permeability
4c	315.18	3	2	99.22	2.14	soluble	High	0	0.55	No	No
4f	291.29	5	2	145.0	0.48	soluble	Low	0	0.55	No	No
4h	280.74	3	2	99.22	1.6	soluble	High	0	0.55	No	No
4g	346.41	3	2	99.22	2.87	Moderately soluble	High	0	0.55	No	No

Note: a: Molecular weight; b: Hydrogen bond acceptors; c: Hydrogen bond donors; d: Topological surface area; e: octanol-water partition coefficient; f: Solubility class; g: Gastro-intestinal absorption; h: P-glycoprotein; i: Blood-Brain Barrier.
Note – Moreover synthesized compounds do not violate Lipinski's rule of 5.

Figure 1: General structure of (benzylidene/substituted benzylidene)-N-(4H-1, 2, 4-triazole-4yl) hydrazine carbothioamide (4a-4h)



All the synthesized compounds (4a-4h) were characterized by spectral techniques such as IR, and ^{1}H NMR. The IR spectra showed the existence of (C=N) at 1600-1668 cm-1 conforming to Schiff's derivatives. In ^{1}H NMR spectra showed the existence of the singlet of methine proton at δ 7.78-9.15 ppm indicating aromatic aldehyde was reacted.

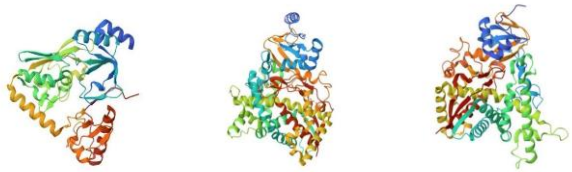
Computational Analysis

In silico studies for ADMET prediction have become very crucial in drug research innovation, offering a valuable method for evaluating various pharmacokinetic properties. Consequently, ADME (absorption, distribution, metabolism, and excretion) predictions were conducted for the most bioactive compounds, with the results detailed in Table 1.

Molecular Docking Studies

Molecular docking is a method used to demonstrate the binding and interaction modes of ligands within the binding pocket of macromolecules. It was employed to elucidate the enzyme action due to the most bioactive compounds using docking. The choice of receptor protein was determined solely through a review of the literature. It is recommended that docking poses be practiced to achieve optimal posture. Different types of receptor proteins with various 3D structures and interactions were examined to determine the effectiveness of ligands against infections. Target enzymes selected for docking were UDP-N-acetylenolpyruvylglucosamine reductase of *S.aureus* (PDB ID-1HSK), Lanosterol 14-α-demethylases of fungal pathogen *Candida albicans* (PDB ID-5V5Z), and anti-tubercular involving decaprenylphosphoryl-beta-D-ribose oxidase (DprE1)) of *Mycobacterium tuberculosis* (PDB ID-4P8H). Each of these enzymes plays a critical role in the execution of a vital cellular function. For example, the MurB gene aids in bacterial peptide synthesis in *S.aureus*, Lanosterol 14-α-demethylase aids in the synthesis of sterols in *Candida albicans*, and Dpre1 helps in cell wall synthesis of *M.tuberculosis bacteria*.

The crystal structures of the receptor proteins were obtained from the Protein Data Bank (https://www.rcsb.org) using the specific PDB IDs for each protein. To achieve a more favourable and stable configuration, unnecessary water molecules, heteroatoms, and other ligand coordinates were removed from the protein structures. For the docking process, polar hydrogens were added and Kollman charges were assigned to each target, which were then saved in PDBQT file type. The three dimensional structures of the receptors are shown below.



PDB ID: 1HSK RECEPTOR | PDB ID: 5V5Z RECEPTOR | PDB ID: 4P8H RECEPTOR

The four best optimal docked conformations with their respective

receptor found through molecular docking are illustrated in the fig with hydrogen and hydrophobic interaction. In the optimal docked conformations, hydrogen and hydrophobic interactions of compounds and their co-crystallized ligand to their PDB IDs are shown in Table 2, 3, 4.

Molecular docking studies for *Candida albicans*

The docking of molecules 4c and 4f inside the active site of *Candida albicans* oxidoreductase enzyme (PDB ID- 5V5Z) showed better binding energy than that of the co-crystallized ligand. The binding energy (-8.79kcal/mol) of compound 4f is superior to the binding energy (-6.22kcal/mol) of compound 4c. Molecule 4c exhibits hydrogen bonding with sulphur via ARG469, and CYS470 at distances 3.28Å, 3.64Å were as nitrogen of linker shows hydrogen bonding with GLY472 at distances 3.12Å. Molecule 4f shows hydrogen bond interaction through the nitrogen of the nitro group at distances 2.57Å and 2.85Å with TYR132, HIS468. In addition, residues formed hydrophobic pockets with ILE304, LEU150, GLY472, ALA146, and LYS143.

Figure 2: Interaction of compound 4f with PDB ID 5V5Z with their standard ligand 1YN.

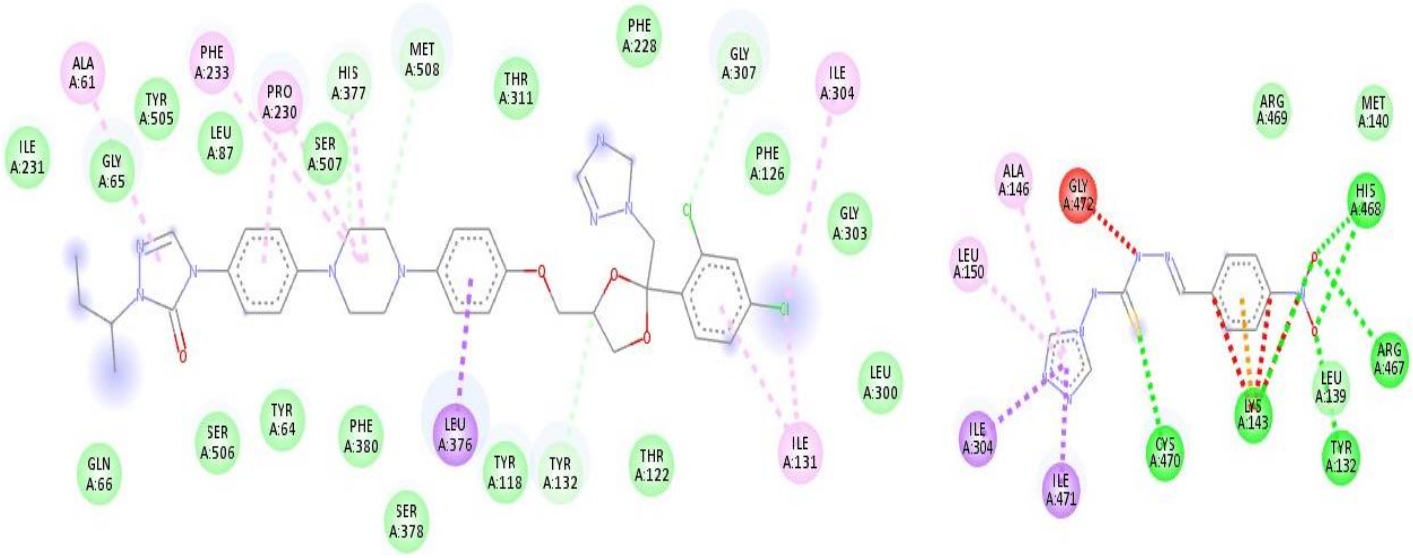


Table 2: For PDB ID -5V5Z

Cpd. Code	MOLECULAR DOCKING ON PDB ID – 5V5Z			
	Estimated Free Binding Energy.	Estimated Inhibition Constant(Ki)	Interaction	
			Hydrogen Bonding	Hydrophobic Bonding
4c.	-6.22	27.44µM	GLY472, ARG469	CYS470, LYS143
4f.	-8.79	265.79nM	CYS470, HIS468, TYR132, LYS143	GLY472, ILE304, LEU150, ALA146, LYS143
4g.	-9.09	66nM		ILE471, CYS470, GLY307, ILE304, LEU300, LYS143, LEU139, ILE131
4h.	-7.54	2.67µM	SER378, HIS377, LEU376	PHE380, HIS377, LEU376
Co-crystallized ligand(1YN)	-6.62	14.12µM	THR311, GLN142, TYR132, TYR118	ILE471, CYS470, PRO462, ARG381, LEU376, ILE304, LEU300, ALA146, LYS143. ILE131

Molecular docking studies for *Mycobacterium tuberculosis*

Docking simulation of the most potent anti-tubercular inhibitor 4c with decaprenylphosphoryl-beta-D-ribose oxidase (PDB ID- 4P8H) indicated that the docking score, as well as the binding energy, is better than that of co-crystallized ligand FAD (FLAVIN-ADENINE DINUCLEOTIDE). From the interaction study, it is identified that in compound 4c dichloro moiety forms intermolecular hydrophobic interaction with TYR60, PRO116, VAL121,GLY125, CYS129, ILE131, ALA417 and LYS418 at,5.32 Å,5.11 Å,5.49 Å,3.63 Å,5.01 Å,5.47 Å,4.03 Å,4.52 Å also pi electron between carbon and the nitrogen atom of linker have shown hydrophobic interaction with GLY125, at distance 4.08 Å to 3.98 Å respectively, while 1,2,4-triazole displayed exhibit intermolecular unfavourable interaction with ARG54,GLY55,ALA64, at distance 4.88 Å,4.0 Å,3.91 Å, and sulphur of linker chain shown hydrogen bonding interaction with LEU56,THR122 at distance 3.13Å and 2.91Å respectively. For Compound 4f, the Nitrogen of triazole and linker showed hydrogen bonding interaction with ILE184 and pi electron of triazole have shown hydrophobic interaction with ALA53,GLY124,ALA128,ILE183, and ILE184 at distance 2.53 Å,3.58 Å,4.64 Å,5.02 Å,2.99 Å. Nitro substituent showed carbon-hydrogen bond interaction with LEU56 and THR122. Benzene ring showed hydrophobic interaction with ALA64 at distance4.93 Å

Figure 3: Interaction between compound 4C with PDB ID – 4P8H with their standard ligand FAD.

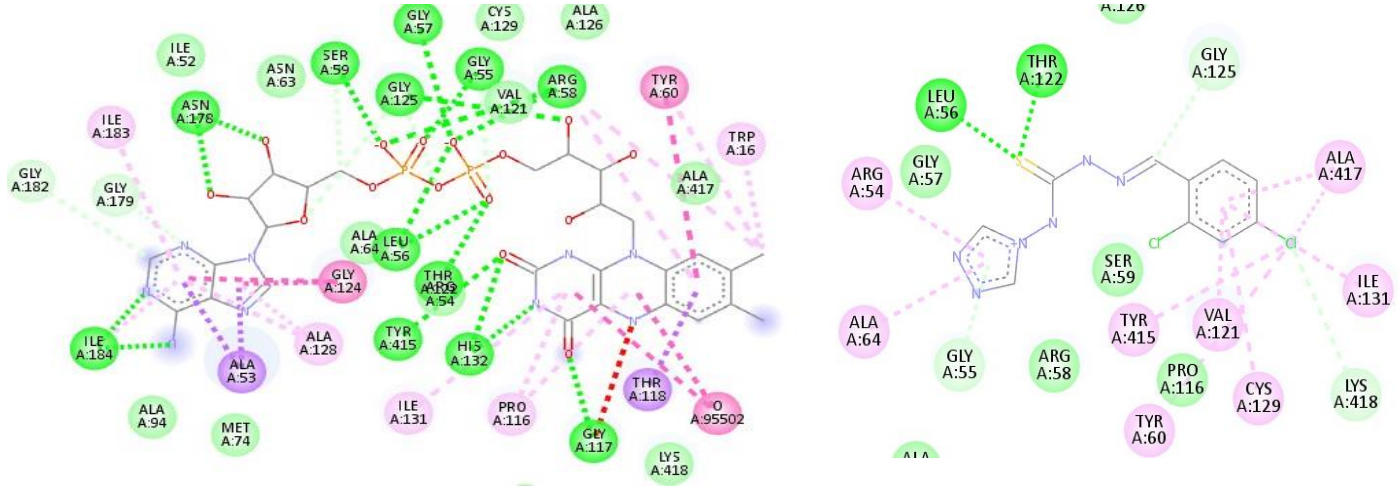


Table 3: for PDB ID – 4P8H.

		MOLECULAR DOCKING ON PDB ID – 4P8H		
Cpd. Code	**Estimated Free Binding Energy.**	**Estimated Inhibition Constant (Ki)**	**Interaction**	
			Hydrogen Bonding	**Hydrophobic Bonding**
4c.	-8.53	2.71 µM	LEU56,THR122	ARG54,GLY55, ALA64, TYR60,VAL121,GLY125,CYS129,ILE131,ALA417,LYS418
4f.	-8.67	217.68nM	ALA53,LEU56,THR122,ILE184	ALA53,ALA64,GLY124,ALA128,ILE183,ILE184
4g.	-9.62	482.68 nM	THR122	TRP16,LEU56,ARG58,SER59,TYR60,PRO116,VAL121,ILE131,HIS132,TYR415,ALA417
4h.	-8.47	10.03 µM	SER59,THR122,ILE184	ALA53,LEU56,ARG58,MET74,VAL121,GLY124,ALA128
Co-crystallized ligand(FAD)	-6.26	55.98µM	ARG54,ARG58,LEU56,GLY57,SER59,THR122,ILLE184	TRP16,ALA53,TYR60,PRO116,GLY124,ALA128,ILE131,GLY179,GLY182

Molecular docking studies for *S.aureus*

A simulation of the most promising chemical compound 4g and 4h with the active site of UDP-N-acetylenopyruvylglucosamine reductase (pdb id-1HSK) of *S.aureus.* Compound 4g (-9.86kcal/Mol) has shown good binding energy than 4h (-8.04kcal/Mol). Nitrogen of 1,2,4-triazole ring of compound 4h has shown hydrogen bonding with GLN 193 at distance 2.75 Å whereas compound 4g has shown hydrogen bonding with SER 143 at distance 3.22 Å. Residue TYR 77 in compound 4g, ARG 310 in compound 4h also shown interaction with nitrogen, carbon of triazole ring as well as of linker. Residue TYR77 and ARG310 are showing both hydrogen and hydrophobic interaction in compound 4g and 4h. Sulphur of thiosemicarbazide has shown hydrophobic interaction in compound 4g with ARG 310 at distance 3.94Å whereas in compound 4h sulphur have shown hydrogen bonding interaction with TYR77 at distance 2.90Å. Chlorine substitution on compound 4h shows hydrogen bond interaction with two amino residue SER115 at distance 3.33 Å. Compared with complex, compound 4h has shown additional interaction stacking around aromatic ring are LEU98, SER143, and VAL199 at distance 3.28Å, 3.33Å, 3.2.

Figure 4: Interaction between compound 4h with PDB ID - 1HSK with their standard ligand FAD

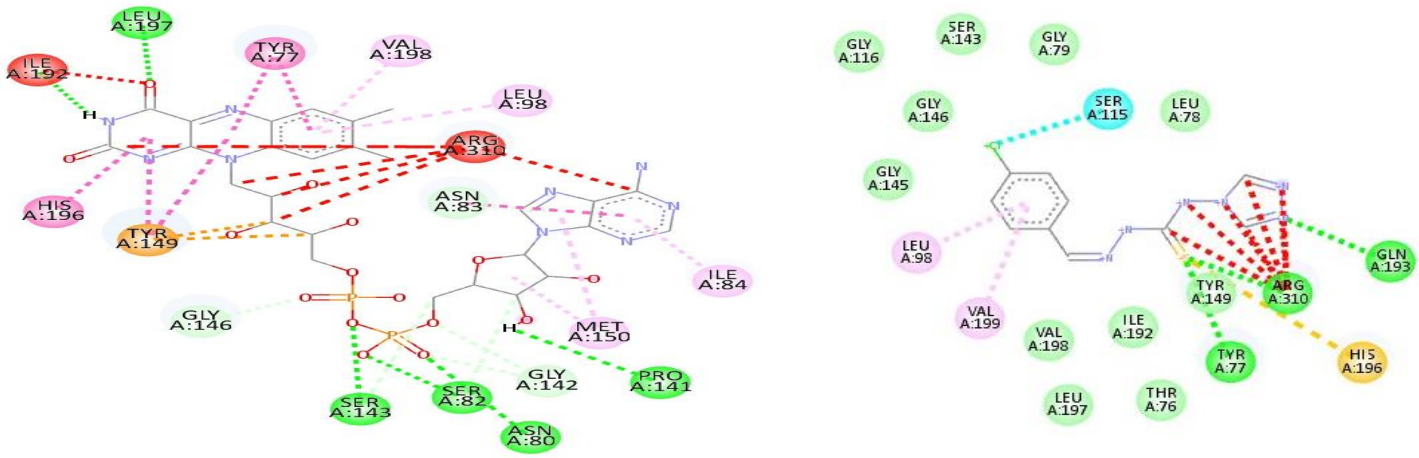


Table 4: for PDB ID – 1HSK.

	MOLECULAR DOCKING ON PDB ID – 1HSK			
Cpd. Code	**Estimated Free Binding Energy.**	**Estimated Inhibition Constant(Ki)**	**Interaction**	
			Hydrogen Bonding	**Hydrophobic Bonding**
4c.	-8.20	970.52nM	LEU197, SER143, TYR77	ARG310, VAL199, HIS196, LEU98
4f.	-9.48	112.98nM	LEU197, GLY145, SER143	ARG310, TYR149, SER143, SER82, GLY81
4g.	-9.86	1.31nM	TYR77, SER143,	VAL199,LEU98,ARG310,
4h.	-8. 04	1.29µM	ARG310, GLN193, TYR77	VAL199, HIS196, SER115, LEU98
Co- crystallized ligand(FAD)	-4.52	488.98µM	HIS271, ARG242, SER143, TYR77, GLY153, LEU197	LEU98, GLU308, GLN241, GLN229, ARG310, TYR149, GLU158

Biological Activity

The research demonstrated that triazole derivatives with various substituents exhibit antimicrobial activity. The results for minimal inhibitory concentration were displayed in Table no 3 for *E. coli, S. aureus, C. albicans, and M. tuberculosis (H37Rv).*

Table 5: The minimal concentration (µg/mL) of synthesized compounds needed to prevent the growth of the tested microbes.

Code	**Bacterial strains**		**Fungal strain**	**Mycobacterium strain**
	E. coli	***S. aureus***	***C. albicans***	***H37Rv***
4a	125	250	250	50
4b	250	250	250	50
4c	125	16	62.5	6.25
4d	125	31.25	125	50
4e	125	125	125	50
4f	125	16	62.5	25
4g	1	2	500	50
4h	1	8	500	50

All compounds tested showed strong efficacy in preventing the growth of bacteria and fungi. Based on structural substitution biological activity varies. In the case of antibacterial studies, 4g and 4h containing anthracene and chloro substituents appear to be beneficial. Compounds 4c and 4f containing dichloro and nitro substituents are moderately active against fungal and mycobacterium strains. Both hydrophobic and Hydrogen bonding are favourable for the activity. Generally, anthracene shows hydrophobic interaction with the target at the 9 and 10 positions because these positions are electron-rich. It was observed that sulphur and nitrogen of thiosemicarbazide are likely to show hydrogen bond interaction with the target molecule. The compounds 4g and 4h (1µg/mL) were observed to possess good antimicrobial activity against *E. coli*. Compound 4g (2µg/ml) exhibited superior antibacterial efficacy against *S. aureus* while compound 4h (8µg/ml) showed similar action.

From the results of the antifungal evaluation, it was observed that the compounds 4c and 4f were identified as moderately active against *C albicans*. The anti-tubercular results showed that in the di-substitution of chlorine at the second and fourth positions on the benzene ring, the compound 4c (6.25µg/mL) displayed good inhibitory activity against *M. tuberculosis*.

CONCLUSION

The anti-microbial activities of all the compounds were validated by *E. coli, S. aureus, C. albicans, and M. tuberculosis (H37Rv*). Significantly inhibitory activities were observed especially for *E. coli* and *S. aureus*. Bioactive Compound 4g exhibits strong inhibition for *E. coli* and *S. aureus.*

The results of the antimicrobial screening suggested the following assumptions regarding the structure-activity relationship (SAR).

The availability of an activating substituent on the ring, for instance, the chlorine group, and an electron-withdrawing group like the nitro group on the ring is dependable for the compound activity.

The results showed that the synthesized analogs have potential antimicrobial activity.

When all the data was combined, it was concluded that compounds 4c, 4f, 4g, and 4h would provide better lead molecules for the synthesis of effective antibacterial drugs.

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