RESEARCH ARTICLE

In-silico ADMET Study and Molecular Docking of Triazole-3-thione Derivatives targeting Lipoteichoic Acid Synthase in Gram-positive Bacteria

Krishan Kumar Verma*, Manisha, Rahul Kaushik,Praveen Kumar Gaur, Kanak Lata
Metro College of Health Sciences & Research, Greater Noida, Uttar Pradesh, India.
Corresponding Author Email ID: krrishver@gmail.com

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Abstract

Introduction: Rapid global emergence of antimicrobial resistance led to the failure of many of the antibiotics available in the market. Among multi drug resistant Gram +ve bacteria, methicillin and vancomycin resistant, Staphylococcus aureus strains are leading causes of nosocomial infections involving skin, soft tissues and urinary tract. Therefore, search for new drug targets that are unique to gram positive bacteria is a promising strategy to design novel antibacterial agents. Recently, lipoteichoic acid, a bacterial cell wall glycopolymer, has emerged as a promising and novel target for antimicrobial action. The enzyme, lipoteichoic acid synthase (LtaS) has been recognized as a key enzyme in LTA biosynthesis.

Materials & Methods: In the present work, we designed triazole-3-thione based compounds and performed in- silico studies to predict and analyse some commonly used computational tools. The study involved assessment of drug likeliness, prediction and analysis of ADMET parameters by pkCSM and swissADME on the synthesized compounds. The molecular docking study was performed using autodock 4.2 targeting lipoteichoic acid synthase (LtaS) on PDB: 2W5S and interaction of the compounds and inhibition constant were studied.

Result & Discussion: All test compounds passed drug likeliness and majority of the compounds had potential to act as enzyme inhibitor or as ligand for G-protein coupled receptors in bioactivity prediction study. The ADMET analysis suggested the compounds suitable for oral administration due to better permeability and intestinal absorption, high tissue distribution, substrate for CYP3A4, CYP2C19 inhibitor, less mutagenic, low MRTD, less hepatotoxicity and none of the compound had potential for fatal ventricular arrythmia. In Docking study, 11 compounds were found to have better affinity with the active site and were able to make stable complexes based upon binding energy data. The compound K24 having thiophene at 5-position and 3-chloro phenyl at 4-position of triazole-3-thione ring was considered as most active compound based on binding energy, inhibition constant value and H-bond interaction with active residue.

Keywords: Lipoteichoic acid, Antimicrobial resistance, Troazole-3-thione, ADMET.

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Introduction

Antimicrobial resistance arises as a complication in the present scenario and current treatment of a constantly increasing variety of infections produced by fungi, viruses, bacteria and parasites and resistance against existing medicines available in the market. Antimicrobial Resistance (AMR) led to the failure of many of the antibiotics existing in the market. According to recent reports, approximately 1 million deaths are estimated by 2050 due to AMR with an associated 1 trillion-dollar global healthcare cost.[1,2] Multidrug-resistant and superbug bacteria are widespread in several parts of the world. Widespread use, misuse and overuse of antimicrobials through the last 80 years have been accompanied with a burst of antimicrobial resistance. The excessive use of antibiotics induces an adaptive evolutionary pressure that increases resistance.[3-5] Antimicrobial resistance (AMR) is a crucial threat to public health, and continues to rise. Microbiological research is the support for knowledge gaps for the development of diagnostics, new antibiotics and preventives and to inform plans to mitigate the spread of drug-resistant microorganisms. Nowadays, pharmaceutical companies have developed initiatives to boost the AMR research and development. Antibiotic pipelines remain insufficient to keep up with the increasing rates of resistance worldwide. AMR creates a lot of problems there is a requirement of a general platform and direction to ensure sufficient resources to fight against critical challenges arise by AMR, hence the creation of the worldwide AMR research and development hub to involve on this role.[6] AMR has been developing at an alarming rate with diseases caused by bacteria showing resistance to multiple antibiotics.[7-9] In healthcare and livestock farming, extreme usage of antibiotics cause antimicrobial resistance and has become a global threat in recent years.[10] The number of antibiotic-resistant bacteria found in water bodies has significantly increased as a result of the increased usage of antimicrobial agents in agriculture and the practice of dumping raw sewage into receiving waters.[11] The development of novel medications momentarily offers an advantage against

bacterial pathogens, but quick evolution of resistance restricts the longevity of a given drug's efficacy. The growth of infections with resistance can be curbed through surveillance and the creation of pharmacological regulations that promote the prudent use of antibiotics.[12] Antimicrobial medication resistance and bacterial evolution continue to make it difficult for medical practitioners working in both the community and hospitals to successfully treat illnesses. Even the most powerful and recent antimicrobial medicines are becoming susceptible to resistance. Although published susceptibility patterns can be used to make generalisations regarding the appropriateness of antimicrobial drugs, important regional, demographic, and interinstitutional characteristics necessitate each hospital creating its own antibiotic data bank and antibiogram.[13] The existing body of research provides evidence supporting the feasibility of addressing antimicrobial resistance through bacteriophages. There have only been a few numbers of completed and implemented clinical trials on bacteriophage therapy. However, more research and rigorous testing are required to determine the effectiveness of particular bacteriophage strains and the proper dosage.[14,15] The last antibiotic class introduced to the market was about 35 years back in 1987. There has been a lack of innovation in the antimicrobial discovery field, and today we have very few antibiotic classes in the drug discovery pipeline. As a consequence, more and more bacterial infections have become difficult to treat. The discovery void (Figure 1) refers to the period from 1987 until today, as the last antibiotic class introduced as treatment was discovered in 1987.[16,17]

In this context, we have made an attempt to design and synthesize 4, 5-disubstituted triazole-3-thiones (Figure 2) and evaluated the compounds for antimicrobial activity. Triazole-3-thiones are five-membered heterocycles having 1,2,4 triazole ring with three nitrogen, two carbons and one sulfur atom at 3-position. Based on earlier reported work, triazol-3-thione based compounds have shown to possess good antimicrobial activity against many harmful bacterial and fungal pathogens.[6] Among multi-drug resistant Gram +ve bacteria, methicillin and vancomycin-resistant, *Staphylococcus aureus* strains are leading causes of nosocomial infections involving skin, soft tissues and urinary tract. Therefore, in the present work, we have synthesized 25 compounds bearing triazole-3-thiones nucleus and performed ADMET study along with a molecular docking study on lipoteichoic acid synthase (LtaS), a key enzyme for the cell wall biosynthesis of gram-+ve bacteria.[18]

Materials and Methods

Synthesis Of Triazole-3-Thiones

In the previous work, we reported the synthesis of novel triazole-3-thione derivatives as shown in Figure 3. The synthesis started with converting aromatic substituted carboxylic acids to corresponding ester, which were further converted to corresponding substituted acid hydrazides.[19,20]

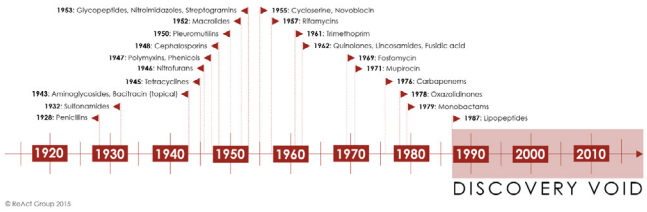


Figure 1: Antibiotic Discovery Timeline

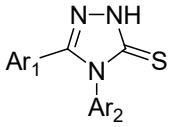


Figure 2: 4, 5-Disubstituted triazole-3-thione

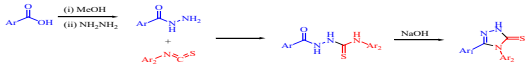


Figure 3: Synthetic scheme of 4, 5-disubstituted triazole-3-thiones

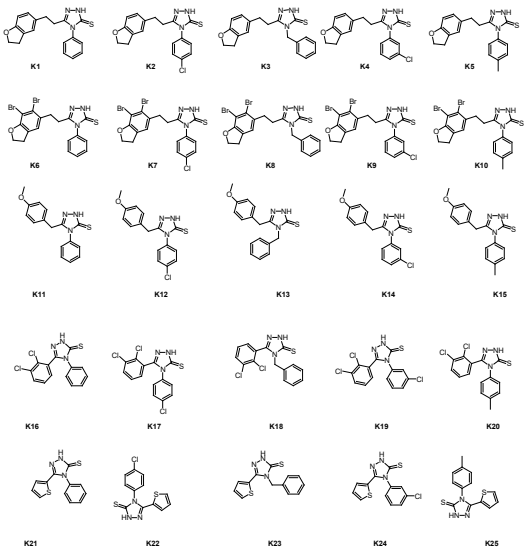


Figure 4: Structures of designed 4, 5-disubstituted triazole-3-thiones

The acid hydrazides were then converted to thiosemicarbazides, then triazole-3-thiones were obtained after cyclization.[21] The structures of the designed compounds are shown in Figure 4 and their smiles notations are given in Table 1.

In-silico Studies

Drug Likeliness study (As per Lipinski rule):

Drug likeliness study mainly involves comparison of molecular properties of the test compounds with structural features of known drugs. The classical method to determine the drug likeliness is to check compliance of Lipinski rule of five.[22] In general, as per the rule, any orally active drug should have not more than one violation of the following criteria: not more than 5 hydrogen bond donors; not more than 10 hydrogen bond acceptors; molecular mass should be less than 500 D and logP should be less than 5. The study

Table 1: Smiles notations of designed 4, 5-disubstituted triazole-3-thiones

Compd.	*Smiles*
K1	S=C1N(C4=CC=CC=C4)C(CCC2=CC(CCO3)=C3C=C2)=NN1
K2	S=C1N(C4=CC=C(Cl)C=C4)C(CCC2=CC(CCO3)=C3C=C2)=NN1
K3	S=C1N(CC4=CC=CC=C4)C(CCC2=CC(CCO3)=C3C=C2)=NN1
K4	S=C1N(C4=CC=CC(Cl)=C4)C(CCC2=CC(CCO3)=C3C=C2)=NN1
K5	S=C1N(C4=CC=C(C)C=C4)C(CCC2=CC(CCO3)=C3C=C2)=NN1
K6	S=C1N(C4=CC=CC=C4)C(CCC2=CC(CCO3)=C3C(Br)=C2Br)=NN1
K7	S=C1N(C4=CC=C(Cl)C=C4)C(CCC2=CC(CCO3)=C3C(Br)=C2Br)=NN1
K8	S=C1N(CC4=CC=CC=C4)C(CCC2=CC(CCO3)=C3C(Br)=C2Br)=NN1
K9	S=C1N(C4=CC=CC(Cl)=C4)C(CCC2=CC(CCO3)=C3C(Br)=C2Br)=NN1
K10	S=C1N(C4=CC=C(C)C=C4)C(CCC2=CC(CCO3)=C3C(Br)=C2Br)=NN1
K11	S=C1N(C2=CC=CC=C2)C(CC3=CC=C(OC)C=C3)=NN1
K12	S=C1N(C2=CC=C(Cl)C=C2)C(CC3=CC=C(OC)C=C3)=NN1
K13	S=C1N(CC2=CC=CC=C2)C(CC3=CC=C(OC)C=C3)=NN1
K14	S=C1N(C2=CC=CC(Cl)=C2)C(CC3=CC=C(OC)C=C3)=NN1
K15	S=C1N(C2=CC=C(C)C=C2)C(CC3=CC=C(OC)C=C3)=NN1
K16	S=C1N(C3=CC=CC=C3)C(C2=C(Cl)C(Cl)=CC=C2)=NN1
K17	S=C1N(C3=CC=C(Cl)C=C3)C(C2=C(Cl)C(Cl)=CC=C2)=NN1
K18	S=C1N(CC3=CC=CC=C3)C(C2=C(Cl)C(Cl)=CC=C2)=NN1
K19	S=C1N(C3=CC=CC(Cl)=C3)C(C2=C(Cl)C(Cl)=CC=C2)=NN1
K20	S=C1N(C3=CC=C(C)C=C3)C(C2=C(Cl)C(Cl)=CC=C2)=NN1
K21	S=C1N(C3=CC=CC=C3)C(C2=CC=CS2)=NN1
K22	S=C1N(C3=CC=C(Cl)C=C3)C(C2=CC=CS2)=NN1
K23	S=C1N(CC3=CC=CC=C3)C(C2=CC=CS2)=NN1
K24	S=C1N(C3=CC(Cl)=CC=C3)C(C2=CC=CS2)=NN1
K25	S=C1N(C3=CC=C(C)C=C3)C(C2=CC=CS2)=NN1

was performed on DruLiTo software developed by National Institute of Pharmaceutical Education and Research, India.

Bioactivity scores and Biological Targets:

The "molinspiration" software was used for prediction of bioactivity scores for different drug targets. Another tool used for prediction of protein targets of small molecules was "SwissTargetPrediction". The website allows the prediction of the most probable target for bioactive molecules.

In-silico Pharmacokinetic studies

During the drug discovery process, knowledge of pharmacokinetics is of utmost importance. Pharmacokinetics tells about the fate of a drug compound in the living system by the knowledge of absorption, distribution, metabolism, excretion and toxicity parameters. The role of estimation and/or prediction of several ADMET parameters is increasing in drug discovery process, at a stage when measured compounds are frequent but access to limited physical samples. All the structure were constructed on chemdraw software and saved in mol file. The structures were converted to SMILES form to perform several *in-silico* studies on different web server tools. The pharmacokinetic studies involved the determination of individual parameters known as absorption, distribution, metabolism, excretion and toxicity (ADMET). The parameters were determined using computer models using SwissADME software and pkCSM. Additionally, biological targets were predicted using another web server-based tool, Swiss TargetPrediction and bioactivity scores of different targets were obtained by using molinspiration.[23,24]

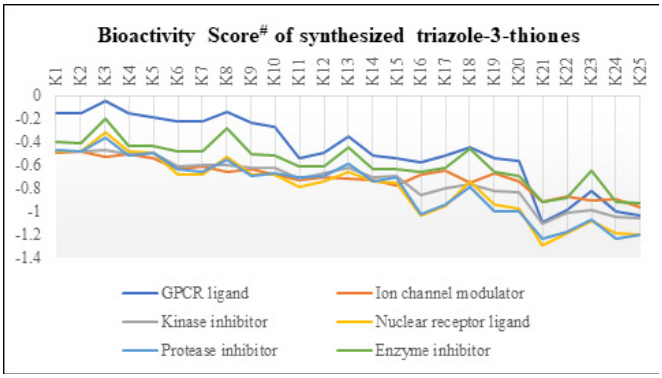
Molecular Docking studies:

All the synthesized compounds were investigated for binding properties with antibacterial target protein. In this study, we designed triazole-3-thione derivatives targeting bacterial cell wall glycopolymer, lipoteichoic acid (LTA) and performed molecular docking on lipoteichoic acid synthase (LtaS) (PDB ID: 2W5S) having resolution of 2.1 A^0. The docking study was performed by autodock. The RCSB Protein Data Bank was used to collect the crystal structures of LtaS, which were then cleaned by eliminating extra chains and cofactors from the protein structure. Hydrogen atoms were added after the water molecules were removed, and co crystal reference ligand was removed from the enzyme structures. The docking investigations were carried out using Autodock 4.2 and MGL Tools version 1.5.2. After making the necessary

Table 2: Drug Likeliness properties of triazole-3-thiones (As per Lipinski Rule).

Compd.	*Mol. Wt.*	*HBA*	*HBD*	*Log P*
K1	325.43	2	1	3.27
K2	339.45	2	1	3.59
K3	339.45	2	1	3.28
K4	339.45	2	1	3.57
K5	339.45	2	1	3.59
K6	353.10	2	1	2.2
K7	367.12	2	1	2.49
K8	367.12	2	1	2.23
K9	367.12	2	1	2.49
K10	367.12	2	1	2.49
K11	299.39	2	1	2.83
K12	313.42	2	1	3.15
K13	313.42	2	1	2.86
K14	313.42	2	1	3.15
K15	311.40	2	1	3.62
K16	285.39	1	1	3.33
K17	297.42	1	1	3.66
K18	297.42	1	1	3.83
K19	297.42	1	1	3.65
K20	297.42	1	1	3.66
K21	261.37	1	1	2.76
K22	275.39	1	1	3.12
K23	275.39	1	1	2.79
K24	275.39	1	1	3.1
K25	275.39	1	1	3.12

alterations, all of the ligand and receptor structures were saved in the working directory in pdbqt format. After running the Autogrid and Autodock modules, the docking data were examined, and hydrogen bond interactions, docking score, and estimated KI values were recorded.



#predicted value

Figure 5: Bioactivity Score Chart

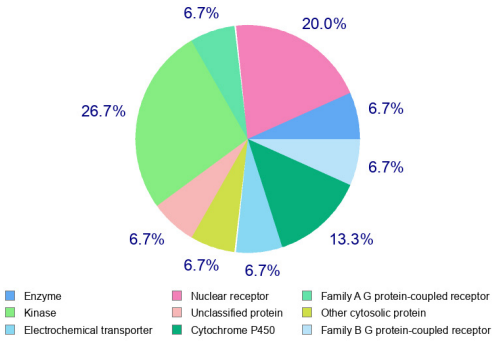


Figure 6: Predicted Biological targets for compound K3

Results and Discussion

Drug Likeliness study (As per Lipinski rule)

Lipinski rule states that for any compound to be a drug candidate, it should not violate more than one criterion. After performing the drug likeliness study of 25 compounds using the DruLiTo software, all the test compounds were found to satisfy all the criteria without any single violation to Lipinski rule. Therefore, all the compounds qualify all the requirements of Lipinski rule and are suitable as drug candidates. The results are summarized in Table 2.

Bioactivity scores and Biological Targets

The bioactivity scores of all the test compounds are shown in Table 3, and they were moderately active against given targets. The results indicated that all test compounds K1-K5 and K8 had the highest bioactivity score towards all biological targets. The best bioactivity score of -0.04 as GPCR ligand and -0.19 as enzyme inhibitor was shown by compound K3.

After looking into the visual trend of bioactivity score as shown in Figure 5, the conclusion can be made that majority of the compounds shown bioactivity as GPCR ligand and then as enzyme inhibitor. The data revealed that triazole-3-thiones can be considered as GPCR ligand or enzyme inhibitor for further research.

The biological target prediction study performed by Swiss target prediction program revealed *Kinase* as the target for most active compound K3 as shown in Figure 6.

In-silico Pharmacokinetic studies

A pharmacokinetic study involving the determination of ADMET parameters includes assessment of absorption, distribution, metabolism, excretion and toxicity and predicts the fate of any drug. The study is carried out to get insight into drug disposition within a living system. Therefore, ADMET study is considered one of the most crucial parts of computational drug design.

Absorption

The bioavailability of any drug greatly depends on absorption but certain factors that may be responsible for reducing

Table 3: Bioactivity Scores for triazole-3-thione derivatives

Compd.	*GPCR ligand*	*Ion channel modulator*	*Kinase inhibitor*	*Nuclear receptor ligand*	*Protease inhibitor*	*Enzyme inhibitor*
K1	-0.14	-0.49	-0.47	-0.48	-0.46	-0.39
K2	-0.14	-0.47	-0.47	-0.48	-0.47	-0.41
K3	-0.04	-0.52	-0.46	-0.31	-0.36	-0.19
K4	-0.15	-0.5	-0.5	-0.48	-0.51	-0.43
K5	-0.18	-0.54	-0.49	-0.49	-0.49	-0.43
K6	-0.22	-0.63	-0.6	-0.68	-0.63	-0.47
K7	-0.22	-0.61	-0.59	-0.68	-0.65	-0.48
K8	-0.13	-0.65	-0.59	-0.52	-0.55	-0.28
K9	-0.23	-0.63	-0.62	-0.68	-0.69	-0.5
K10	-0.26	-0.68	-0.62	-0.68	-0.67	-0.51
K11	-0.53	-0.72	-0.71	-0.78	-0.7	-0.6
K12	-0.49	-0.7	-0.67	-0.74	-0.69	-0.61
K13	-0.35	-0.71	-0.62	-0.65	-0.58	-0.44
K14	-0.51	-0.72	-0.7	-0.74	-0.73	-0.63
K15	-0.53	-0.77	-0.69	-0.75	-0.7	-0.63
K16	-0.57	-0.68	-0.85	-1.03	-1.02	-0.65
K17	-0.51	-0.64	-0.79	-0.95	-0.94	-0.62
K18	-0.44	-0.75	-0.76	-0.73	-0.78	-0.45
K19	-0.53	-0.67	-0.82	-0.94	-0.99	-0.65
K20	-0.56	-0.74	-0.83	-0.97	-1	-0.69
K21	-1.09	-0.91	-1.1	-1.29	-1.23	-0.91
K22	-0.98	-0.86	-1.01	-1.18	-1.17	-0.88
K23	-0.82	-0.9	-0.98	-1.08	-1.06	-0.64
K24	-1	-0.89	-1.04	-1.18	-1.23	-0.91
K25	-1.03	-0.96	-1.05	-1.19	-1.19	-0.92

Table 4: Absorption parameters of triazole-3-thiones.

Compd.	*Water solubility*	*Caco2 permeability*	*Intestinal absorption*	*Skin permeability*	*P-gp substrate*	*P-gp I inhibitor*	*P-gp II inhibitor*
K1	-4.284	1.44	93.06	-2.961	No	No	Yes
K2	-5.316	1.257	91.804	-2.97	No	Yes	No
K3	-3.867	1.205	91.064	-2.842	Yes	Yes	No
K4	-5.388	1.265	92.031	-2.968	No	yes	No
K5	-4.95	1.259	93.263	-2.975	No	yes	No
K6	-6.117	1.259	89.886	-2.966	Yes	Yes	Yes
K7	-6.712	1.245	88.631	-2.952	Yes	Yes	Yes
K8	-5.206	1.205	86.528	-2.824	Yes	Yes	Yes
K9	-6.822	1.253	88.858	-2.956	Yes	Yes	Yes
K10	-6.405	1.247	90.089	-2.974	Yes	Yes	Yes
K11	-4.395	1.399	93.027	-2.943	No	No	No
K12	-5.145	1.247	91.731	-2.952	No	Yes	No
K13	-3.72	1.374	91.087	-2.82	Yes	No	No
K14	-5.206	1.342	91.999	-2.941	No	Yes	No
K15	-3.989	1.047	91.449	-2.895	Yes	No	Yes
K16	-4.655	1.621	87.892	-2.79	No	No	No

K17	-5.277	1.469	87.055	-2.794	Yes	Yes	No
K18	-4.273	1.526	87.378	-2.788	Yes	No	No
K19	-5.329	1.473	87.102	-2.785	Yes	Yes	No
K20	-4.976	1.461	88.514	-2.796	Yes	No	No
K21	-3.672	1.669	89.092	-2.744	Yes	No	No
K22	-4.337	1.517	88.255	-2.757	Yes	No	No
K23	-3.776	1.572	88.532	-2.797	Yes	No	No
K24	-4.344	1.521	88.302	-2.741	Yes	No	No
K25	-3.999	1.51	89.714	-2.751	Yes	No	No

Table 5: Distribution properties of triazole-3-thiones

Compd.	*VD*	*Fraction unbound*	*BBB permeability*	*CNS permeability*
K1	-0.44	0.108	0.359	-1.783
K2	0.617	0.028	0.031	-1.609
K3	1.067	0.067	0.168	-2.242
K4	0.642	0.022	0.078	-1.682
K5	0.672	0.043	0.044	-1.609
K6	0.599	0	0.053	-1.626
K7	0.603	0	0.005	-1.61
K8	0.91	0	0.148	-2.259
K9	0.626	0	0.052	-1.683
K10	0.66	0	0.018	-1.61
K11	0.466	0.101	0.126	-1.602
K12	0.486	0.084	0.078	-1.584
K13	0.892	0.131	0.222	-2.168
K14	0.517	0.072	0.122	-1.662
K15	0.323	0.095	0.038	-1.531
K16	0.345	0.073	0.149	-1.162
K17	0.405	0.405	0.122	-1.153
K18	0.216	0.133	0.398	-1.093
K19	0.411	0.047	0.133	-1.222
K20	0.453	0.064	0.172	-1.153
K21	0.243	0.162	0.22	-1.313
K22	0.301	0.139	0.193	-1.308
K23	0.145	0.217	0.445	-1.306
K24	0.308	0.138	0.202	-1.367
K25	0.351	0.155	0.242	-1.308

absorption of orally administered drugs are poor solubility, intestinal transit time, gastric emptying time, inability to permeate the intestinal wall and gastric instability. The absorption properties computed in the current study are shown in Table 4.

In Table 4, water solubility indicates the solubility of the molecule as log mol/L in water at 25^0C. Caco2 permeability is used as in vitro model of human intestinal mucosa and predicts the apparent permeability coefficient. The Caco2

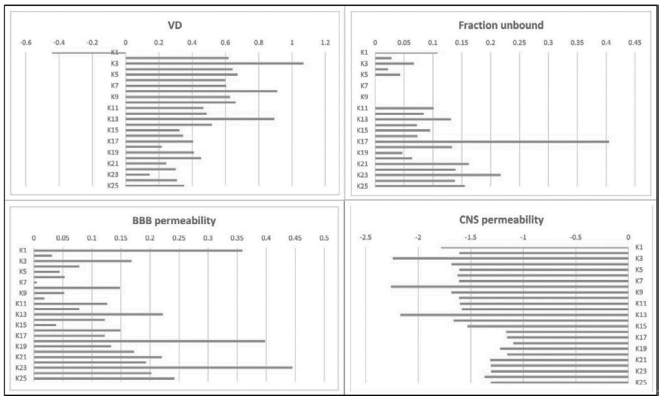


Figure 7: Computed distribution parameters of triazole-3-thiones

permeability values greater than 0.90 indicates higher permeation and absorption. The intestinal absorption value of less than 30% indicates poor absorption. The skin permeability is expressed as $\log K_p$ (cm/h). The skin permeability is considered low for any compound with a $\log K_p$ value greater than -2.5. The P-glycoprotein functions as a biological barrier, and it removes toxins and xenobiotics from the cells. This parameter is indicative of whether any compound will be a substrate for P-glycoprotein or not. Finally, P-glycoprotein I & II inhibition ability is useful to ascertain improved functioning of P-glycoprotein substrates. Based upon the results, we can conclude that all the test compounds were have better permeability and intestinal absorption, making them suitable candidates for oral administration. The majority of the compounds were predicted to be P-glycoprotein substrate and 5 compounds (K6-K10) were found to inhibit P-glycoprotein I and II both, whereas 10 compounds (K11, K13, K16, K18, K20-K25) were not inhibiting both P-glycoproteins. The Rest of the compounds could only inhibit any one P-glycoprotein type.[25]

Distribution

The computed values of distribution parameters are presented in Table 5 and visual comparison is shown in Figure 7. The VD represents the theoretical volume of any drug to be distributed in blood uniformly. The larger values of VD indicate greater distribution of drug in tissue than in plasma. The results indicate that 14 compounds had VD greater than 0.45. Another parameter, fraction unbound predicts the part

Table 6: computed metabolism parameters of triazole-3-thiones

	CYP2D6 substrate	*CYP3A4 substrate*	*CYP1A2 inhibitor*	*CYP2C19 inhibitor*	*CYP2C9 inhibitors*	*CYP2D6 inhibitors*	*CYP3A4 inhibitors*
K1	No	Yes	Yes	Yes	Yes	Yes	Yes
K2	No	Yes	Yes	Yes	Yes	No	Yes
K3	Yes	Yes	Yes	Yes	No	No	No
K4	No	Yes	Yes	Yes	yes	No	Yes
K5	No	Yes	Yes	Yes	yes	Yes	No
K6	No	Yes	Yes	Yes	Yes	Yes	No
K7	No	Yes	Yes	Yes	Yes	No	Yes
K8	Yes	Yes	Yes	Yes	Yes	Yes	No
K9	No	Yes	Yes	Yes	Yes	No	Yes
K10	No	Yes	Yes	Yes	Yes	No	Yes
K11	No	Yes	Yes	Yes	Yes	No	No
K12	No	Yes	Yes	Yes	Yes	No	No
K13	Yes	Yes	Yes	Yes	Yes	No	No
K14	No	Yes	Yes	Yes	Yes	No	No
K15	No	Yes	Yes	Yes	Yes	No	Yes
K16	No	Yes	Yes	Yes	Yes	Yes	No
K17	No	Yes	Yes	Yes	Yes	No	No
K18	Yes	Yes	Yes	Yes	Yes	Yes	No
K19	No	Yes	Yes	Yes	Yes	No	No
K20	No	Yes	Yes	Yes	Yes	No	No
K21	No	Yes	Yes	Yes	No	No	No
K22	No	Yes	Yes	yes	Yes	No	No
K23	Yes	Yes	Yes	Yes	No	No	No
K24	No	Yes	Yes	Yes	Yes	No	No
K25	No	Yes	Yes	Yes	Yes	No	No

of drug that remains unbound in plasma. The majority of compounds had lower values of fraction unbound as shown in Table 5.

The ability of any drug to cross blood-brain barrier (BBB) is a critical parameter and can be useful in reducing CNS side effects or improving the efficacy of CNS-acting drugs. The compounds with logBB value greater than 0.3 are considered to easily cross the BBB, on the other hand, compounds with logBB value less than –1 are considered poorly distributed in the brain. The results suggested that none of the compounds had poor brain penetration and, in fact, three compounds had greater BBB permeability. Finally, CNS Permeability, where compounds with logPS greater than -2 can enter the CNS and those with logPS smaller than -3 cannot penetrate the CNS. The results indicated that all compounds have ability to penetrate CNS efficiently.[25]

Metabolism

The important metabolism parameters computed for the all the test compounds are represented in Table 6. The cytochrome P450 enzyme system is considered the most important detoxification system in the body. The different isoforms of the enzyme are available and responsible for deactivation of many drugs or xenobiotics while some can be activated.

The computed values shown in Table 6 indicated 5 compounds to be the substrate for CYP2D6, whereas all compounds were shown to be a substrate for CYP3A4 isoform. Additionally, all compounds were shown to inhibit CYP1A2 and CYP2C19 isoforms. The results of other three isoforms, CYP2C9, CYP2D6 and CYP3A4 of cytochrome P450

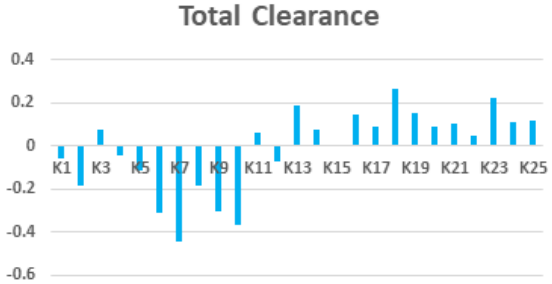


Figure 8: Total clearance values of triazole-3-thiones

Table 7: Computed excretion parameters of triazole-3-thiones

	Total Clearance	*Renal OCT2 substrate*
K1	-0.055	No
K2	-0.186	No
K3	0.074	No
K4	-0.046	No
K5	-0.112	No
K6	-0.312	No
K7	-0.443	No
K8	-0.183	No
K9	-0.303	No
K10	-0.369	No
K11	0.063	No
K12	-0.069	No
K13	0.189	No
K14	0.072	No
K15	0.006	No
K16	0.145	No
K17	0.089	No
K18	0.266	No
K19	0.152	No
K20	0.086	No
K21	0.1	No
K22	0.044	No
K23	0.219	No
K24	0.108	No
K25	0.118	No

system suggested the inhibition by few compounds only.[25]

Excretion

The excretion parameters computed are shown in Table 7. Total clearance involves both hepatic clearance and renal clearance. The predicted total clearance values are represented as log(ml/min/kg) shown in Figure 8. Organic cation transporter 2 (OCT2) is a renal uptake transporter and plays an important role in the disposition and renal clearance of drugs.

OCT2 substrates may have potential for interactions with OCT2 inhibitors, and provide insight into clearance and contraindications. In the current study, none of the investigated compounds is predicted to be OCT2 substrate.[25]

Toxicity

The toxicity computation results for all the derivatives are shown in Table 8 and represents AMES toxicity, MRTD, hERG I and II inhibitor, ORAT, ORCT, hepatotoxicity, skin sensitization and *T. pyriformis* toxicity.

AMES Toxicity provides insights into the mutagenic potential of any compound. The positive result indicates that the compound under investigation could be mutagenic. The computation results indicated sixteen compounds

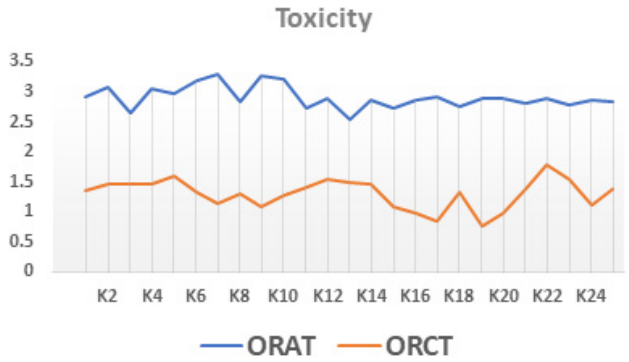


Figure 9: Predicted toxic doses of triazole-3-thiones

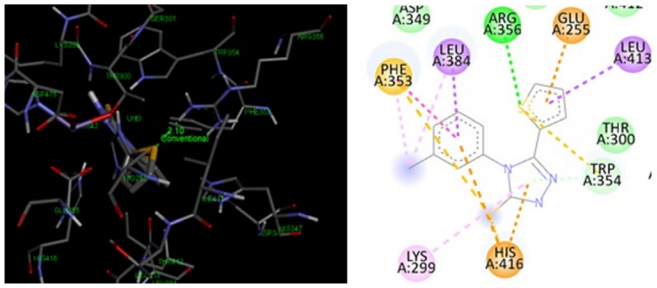


Figure 10: Docking Interactions of K24 with 2w5s

without mutagenic potential. MRTD indicates maximum recommended tolerated dose of any compound in humans. The MRTD value less than or equal to 0.477 is considered low, whereas, value greater than 0.477 is considered high. The results indicated that all compounds had low MRTD values except K1 and K8. The hERG channels inhibition has been key factor in the withdrawal of many drugs from the market because inhibition of K-channels associated with hERG leads to fatal ventricular arrythmia. None of the test compounds was predicted to be hERG I inhibitors but for hERG II All the test compounds were unlikely to be hERG I inhibitors, but in case of hERG II, only nine compounds were unlikely to be the inhibitors. Acute toxicity is generally measured by LD_{50} values that is defined as the amount of drug causing death in 50% animal population and are measured through ORAT and ORCT (Figure 9) indices given in Table 8.

Hepatoxicity is mainly associated with disrupted normal function of the liver. The predicted values for test compounds indicated only six compounds to be hepatotoxic, and only one compound was associated with skin sensitization. Lastly, *T. pyriformis* is a protozoa bacterium whose toxicity is generally used as a toxicity test. A value greater than -0.5 is considered to be toxic, and the predicted value of all the compounds was greater and marked as toxic.[25]

Molecular Docking studies

Docking studies were performed on all synthesized compounds to investigate their ability to occupy and interact with the active site of LtaS (PDB ID: 2w5s) by means of Autodock4.2 software. Based upon earlier reported work, the main residues essential for LtaS function are Thr300 and

Table 8: Computed toxicity parameters of triazole-3-thiones

Compd	*AMES toxicity*	*MRTD*	*hERG I Inhibitor*	*hERG II Inhibitor*	*ORAT*	*ORCT*	*Hepatotoxicity*	*Skin sensitisation*	*T. Pyriformis toxicity*
K1	Yes	0.483	No	Yes	2.914	1.351	No	No	0.397
K2	No	-0.102	No	Yes	3.074	1.469	No	No	1.746
K3	No	0.02	No	Yes	2.644	1.47	No	No	0.886
K4	Yes	-0.12	No	Yes	3.047	1.46	No	No	1.777
K5	No	-0.106	No	Yes	2.964	1.595	Yes	No	1.727
K6	No	-0.149	No	Yes	3.2	1.32	No	No	1.678
K7	No	-0.108	No	Yes	3.291	1.147	No	No	1.594
K8	No	0.483	No	Yes	2.832	1.302	No	No	0.984
K9	No	-0.142	No	Yes	3.275	1.094	No	No	1.602
K10	No	-0.113	No	No	3.213	1.273	No	No	1.651
K11	Yes	0.225	No	Yes	2.734	1.406	Yes	No	2.127
K12	No	0.251	No	Yes	2.879	1.54	Yes	No	2.281
K13	No	0.323	No	Yes	2.534	1.499	Yes	No	1.09
K14	No	0.228	No	Yes	2.857	1.475	Yes	No	2.302
K15	Yes	0.161	No	Yes	2.737	1.08	No	No	0.603
K16	No	0.069	No	No	2.868	0.976	No	No	2.052
K17	No	0.007	No	No	2.93	0.853	No	No	2.139
K18	No	0.179	No	Yes	2.763	1.323	No	No	1.129
K19	Yes	0.019	No	No	2.886	0.754	No	No	2.201
K20	Yes	0.009	No	No	2.895	0.979	No	No	2.124
K21	Yes	0.059	No	No	2.816	1.375	No	No	1.802
K22	Yes	-0.002	No	No	2.9	1.777	No	No	2.021
K23	No	0.129	No	No	2.772	1.556	Yes	Yes	1.038
K24	No	0.001	No	No	2.857	1.123	No	No	2.096
K25	Yes	0.005	No	No	2.84	1.388	No	No	0.973

Table 9: The docking results of triazole-3-thiones with lipoteichoic acid synthase (LtaS)

Compd.	*Binding Energy (kcal/mol)*	*Inhibition Constant (KI)*	*H Bond Residue*	*H-Bond length*
K11	-5.94	43.92 mM	HIS 347 ARG356	1.845 1.836
K12	-0.13	809.28 mM	ARG356	2.006
K13	-2.57	13.07 mM	ARG356	2.102
K14	-4.21	842.12 µM	ARG356 HIS 347	2.175, 2.09 2.1
K16	-3.39	3.3 mM	-	-
K18	-0.49	435.17 mM	-	-
K21	-5.78	58.02 µM	-	-
K22	-3.67	2.03 mM	ARG356	2.136
K23	-6.14	31.61 µM	-	-
K24	-5.94	44.624 µM	ARG356	2.095
K25	-5.8	56.44 µM	ARG356	2.197

Arg356. All the test compounds were evaluated for hydrogen bonding interaction with Arg356 and Thr300 and inhibition constant (KI) values were also estimated along with binding energy determination. Total 21 compounds exhibited interaction with active residues of LtaS. Among all interacting compounds, seventeen compounds participated in H-bond formation with ARG356 and/or Thr300. A total 11 compounds showed inhibition of LtaS with stable binding energy values and KI values as shown in Table 9.

None of the active compounds was able to make H-bond with Thr300 residue. Based upon binding energy data and estimated inhibition constant values, K24 was found to be most active compound. Compound K24 had a binding score of -5.94 kcal/mol with an estimated inhibition constant of 44.624 µM and H bond length of 2.095 with Arg356 shown in Figure 10.

Conclusion

In the present study, we studied twenty-five triazole-3-thione derivatives synthesized previously as per the reported

procedure. The designed compound smiles were obtained and utilized for several *in-silico* studies performed during current work. The present study involved use of some commonly used computational techniques employed in drug discovery and development process. All the designed compounds passed the Lipinski rule criteria for drug likeliness. The bioactivity prediction and bioactivity score computation suggested that the majority of the compounds could be used as enzyme inhibitors or as ligand for G-protein coupled receptors. Further investigation aimed to predict and analyze ADMET parameters that could be related to the bioavailability and pharmacokinetics of the designed compounds. The ADMET analysis suggested that the designed compounds are suitable candidates for oral administration due to better permeability and intestinal absorption, high tissue distribution, substrate for CYP3A4**,** CYP2C19 inhibitor, less mutagenic, low MRTD, less hepatotoxicity and none of the compounds had potential for fatal ventricular arrhythmia. Finally, after prediction and analysis of ADMET parameters, molecular docking was also performed to study the ability of the designed compounds to inhibit lipoteichoic acid synthase (LtaS) in gram-positive bacteria. Total 11 compounds were found to have better affinity with the active site and were able to make stable complexes based upon binding energy data. The compound K24 having thiophene at 5-position and 3-chloro phenyl at 4-position of triazole-3-thione ring was considered the most active compound based on binding energy, inhibition constant value and H-bond interaction with active residue. The designed compounds are potential drug candidate based on drug likeliness study and ADMET analysis. Further, molecular docking study indicated inhibition of lipoteichoic acid synthase by triazole-3-thione derivatives. The results showed triazole-3-thiones as a promising lead for drug discovery and development targeting LtaS, but further *in-vitro* activity data is needed to validate the results of *in-silico* studies.

Declarations

Ethical Approval
Not Applicable

Funding

None

Availability of data and materials

The supporting data will be made available by the authors, without undue reservation.

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