IN SILICO MOLECULAR DOCKING ANALYSIS OF [1,3,4]THIADIAZOLO[2',3':2,3]IMIDAZO[4,5-B]QUINOXALINE AS ANTITUBERCULAR AGENTS

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Abstract

[1,3,4]thiadiazolo[2',3':2,3]imidazo[4,5-b]quinoxaline are an important class of heterocyclic compounds that possess interesting biological activities like antimicrobial, antitubercular, antitumor, etc. Docking studies are proved to be an essential tool that facilitates the structural diversity to be harnessed in an organized manner. The objective of this study is to evaluate *in silico* antimycobacterial activity of some [1,3,4]thiadiazolo[2',3':2,3]imidazo[4,5-b]quinoxaline derivatives by using CLC Drug Discovery Workbench Software. For all ligands, docking studies were carried out and their scores were compared with the standard drug, Rifampicin. For the prediction of the most possible type of interaction, the binding affinities, and the orientations of the docked ligands at the active site of the target protein, the docking studies have been carried out. The results obtained are preliminary and experimental evaluation has to be carried out in near future.

Various online tools, databases, and software were used for this *in silico* studies. The proposed approaches were PDB, Molinspiration, Chemsketch, PyRx software, and many more. The binding scores were retrieved by PyRx software and no tumorigenicity, mutagenicity was there, and all parameters were in the desired range. The compounds used as ligands have shown energy minimization and can be further used as optimization, simulation, and *in vitro* and *in vivo* experimental validation.

Keywords: Imidazo[4,5-B]Quinoxaline, Antitubercular, Docking, PASS, Protein Data Bank

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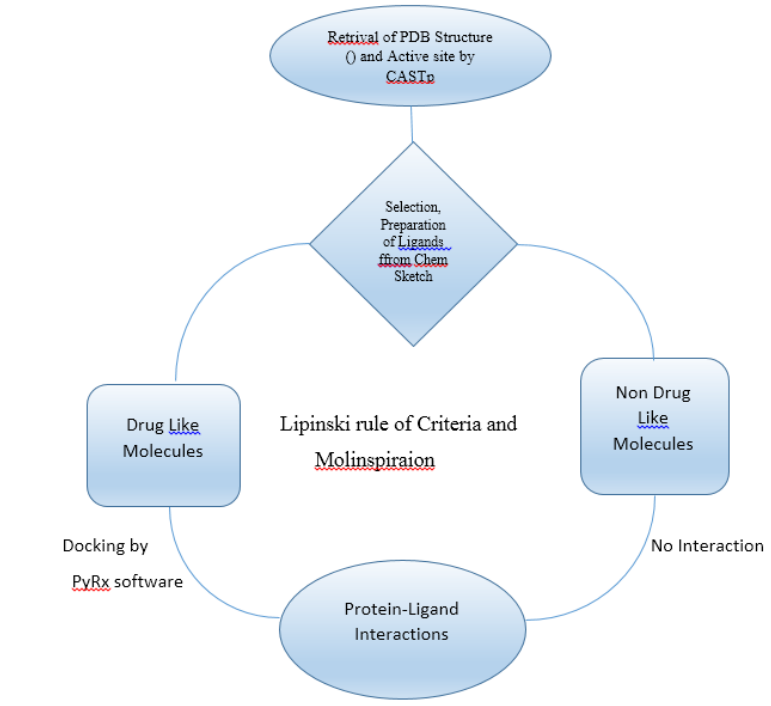
1. INTRODUCTION

Tuberculosis (TB) is a dangerous communicable infectious disease and a major cause of illness, especially in low-income countries. It is caused by various strains of mycobacteria bacillus, usually *Mycobacterium tuberculosis* (MTB) which primarily attacks the lungs (pulmonary) but later may affect other parts (extra-pulmonary) of the body (WHO, 2020). According to the reports given by World Health Organisation (WHO), TB is found to be one of the top 10 causes of death worldwide, and death rate is increased from a single infectious agent (WHO, 2020). In 2019, from reports it was found that TB resulted in nearly 1.4 million deaths, including 208,000 deaths among human immunodeficiency virus (HIV) positive patients (WHO, 2019). It is also observed that HIV-infected patients are 19 times more likely to develop TB than HIV-negative subjects (WHO, 2011; WHO, 2017). Majorly, TB infected people are cured with early diagnosis and with proper treatment. The mainstay of TB control strategies is by antibiotic therapy. In addition to the existing drugs, increase in resistance of Mycobacterium species has heightened alarm about TB in the international health community (Arul K *et al*., 2014).

Several factors is observed to the continuous health threat of TB globally, which includes the development of drug resistance such as multidrug-resistant tuberculosis (MDR-TB), extensively drug-resistant tuberculosis (XDR-TB) (Campbell PJ *et al*., 2011), and totally drug-resistant tuberculosis (TDR-TB) (Velayati AA *et al*., 2009), the co-morbidities with acquired immunodeficiency syndrome (AIDS) (Dheda K *et al*., 2010; Singh P *et al*., 2012) and the risks of developing diabetes mellitus among TB patients (Baker MA *et al*., 2012; Patpi SR et al., 2012). Several therapeutics have been approved by US-FDA to enhance the treatment of TB. Hence, the development of novel drugs with anti-tubercular activity is an urgent need to fulfill.

2. MATERIAL AND METHODS

The most important preliminary step in the rational drug designing of novel drugs was *in-silico* molecular modification. In the present study, different derivatives imidazo[4,5-*b*]quinoxaline were screened for different physico-chemical properties by using different software. *In silico* docking studies of derivatives were performed using Drug Discovery Studio. In this work, ACD Lab Chemsketch was used for 3-D drawing and also for calculating various molecular descriptors such as hydrophobicity, lipophilicity, steric and electronic parameters of the title molecules. For the calculation of log P values for proposed molecules, Lipinski'srule of five and drug likeness, Molinspiration software was used and then screened to find whether they obey the rule of five or not. Using PASS (Prediction of activity spectra for substances) software, the general biological activities of title molecules were predicted and similarly, using AdmetSAR programme, general pharmacokinetic properties of proposed molecules were predicted. The designed molecules having required physicochemical properties, drug-likeness and obeying Lipinski rule of five were selected for further docking studies. The flowchart study of methods used in this study was depicted in Figure 1.



Molecular docking studies

In the study of cell biology, the function of proteins is a studied by interacting (i.e., docking) with other proteins as well as other molecular components. When we understand how proteins interact (dock) with other molecules, the function of the protein can also be inferred. This docking results after calculation will help us to find the molecules that were effective against the particular disease. Search for docking with a molecule's (ligands) favoured orientation with receptors *i.e.*, a protein for the best binding affinity.

From the protein data bank (PDB), the 3 D structure of the protein was obtained using their specific PDB ID (5JFO). Ligands were preprocessed for optimizing and minimizing the structure before docking and generated conformers, respectively. For identifying the binding affinity with the targets using CHARM as force field, library docking was performed. Seven derivatives of [1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline were selected as ligands and by using ACD Lab Chemsketch their structures were drawn and converted to 3D form for the docking studies. The ligands are shown in table 1 and figure.1.

Table 1: Ligand molecules and their IUPAC names

Ligand code	Ligand IUPAC names
AB1	7,8-dintro-2-(4-fluoro-3-hydroxy)-[1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline
AB2	7,8-dintro-2-(2-fluoro-4,6-dihydroxy)-[1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline
AB3	7,8-dintro-2-(2,5-difluoro-3,4-dihydroxy)-[1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline
AB4	7,8-dintro-2-(2-fluoro-4-hydroxy)-[1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline
AB5	7,8-dintro-2-(2,4-dihydroxy)-[1,3,4]thiadiazolo[2',3':2,3]imidazo[4,5-*b*]quinoxaline
AB6	7,8-dintro-2-(2,3-dimethyl-4,5-dihydroxy)-[1,3,4]thiadiazolo [2',3':2,3]imidazo[4,5-*b*]quinoxaline
AB7	7,8-dintro-2-(2-methyl-4,5-dihydroxy)-[1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline

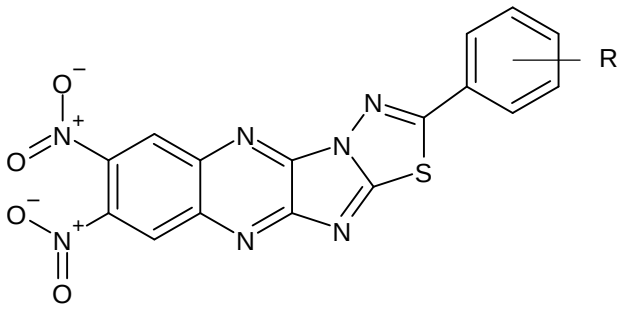


Fig. 1: Structure of novel [1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline derivatives

Table 2: Molecular descriptors of proposed molecules

Compound	Molar refractivity, cm^3	Molar volume, cm^3	Parachor, cm^3	Surface tension dynes/cm	Polarizability, cm^3
AB1	99.56 ± 0.5	202.4 ± 7.0	657.5 ± 8.0	111.3 ± 7.0	39.46 ± 0.5 10^{-24}
AB2	100.41 ± 0.5	199.6 ± 7.0	663.2 ± 8.0	121.6 ± 7.0	39.80 ± 0.5 10^{-24}
AB3	100.28 ± 0.5	202.5 ± 7.0	663.4 ± 8.0	115.0 ± 7.0	39.75 ± 0.5 10^{-24}
AB4	99.56 ± 0.5	202.4 ± 7.0	657.5 ± 8.0	111.3 ± 7.0	39.46 ± 0.5 10^{-24}
AB5	100.54 ± 0.5	196.8 ± 7.0	663.0 ± 8.0	128.7 ± 7.0	39.85 ± 0.5 10^{-24}
AB6	109.39 ± 0.5	227.1 ± 7.0	725.2 ± 8.0	103.8 ± 7.0	43.36 ± 0.5 10^{-24}
AB7	104.96 ± 0.5	212.0 ± 7.0	694.1 ± 8.0	114.9 ± 7.0	41.61 ± 0.5 10^{-24}

Table 3: Analysis of Lipinski's rule of 5

Compound	LogP	Mol. Wt	NHDon	nHAcc	Nrotb	Lipinski's rule alert index

AB1	3.91	427.33	1	12	3	1
AB2	3.35	443.33	2	13	3	1
AB3	3.71	461.32	2	13	3	1
AB4	3.65	427.33	1	12	3	1
AB5	3.26	425.34	2	13	3	1
AB6	4.05	453.40	2	13	3	1
AB7	3.44	439.37	2	13	3	1

Table 4: Drug-likeness analysis of ligands

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
AB1	-0.40	-0.46	-0.07	-0.61	-0.82	-0.34
AB2	-0.41	-0.50	-0.19	-0.68	-0.83	-0.42
AB3	-0.35	-0.54	-0.14	-0.73	-0.78	-0.42
AB4	-0.41	-0.51	-0.17	-0.72	-0.85	-0.40
AB5	-0.45	-0.60	-0.20	-0.71	-0.88	-0.47
AB6	-0.41	-0.52	-0.33	-0.69	-0.87	-0.38
AB7	-0.43	-0.52	-0.26	-0.72	-0.87	-0.42

TABLE 5: ADMET property/descriptors of ligands

Compound	ADME prediction				Toxicity prediction	
	BBB	Caco2 cell permeability	HIA	Cytochrome P450	Ames test	Carcinogenicity
AB1	0.5500	0.7862	0.9861	Inhibitory	0.8300	0.3963
AB2	0.5500	0.7333	0.9724	Inhibitory	0.8000	0.6544
AB3	0.5250	0.7908	0.9611	Inhibitory	0.7600	0.6644
AB4	0.5500	0.6967	0.9861	Inhibitory	0.8900	0.6644
AB5	0.5500	0.7629	0.9501	Inhibitory	0.8400	0.7325
AB6	0.6000	0.7823	0.9276	Inhibitory	0.7700	0.7100
AB7	0.6000	0.7820	0.9357	Inhibitory	0.8200	0.7025

TABLE 6: Summary of PASS values of ligands

Compound	Antitubercular activity	
	P_a	P_i
AB1	0.175	0.161
AB2	0.232	0.130
AB3	0.114	0.113
AB4	0.194	0.177
AB5	0.337	0.054
AB6	0.250	0.110
AB7	0.257	0.103

TABLE 7: Docking scores

Compd	Substituition (-R)	Docking score
AB1	3-OH, 4-F	74.8838
AB2	2,4-dihyroxy, 6-F	92.8493
AB3	3,4-dihyroxy, 2,5-difluoro	80.9771
AB4	4-OH, 2-F	74.9953
AB5	2,4-dihyroxy	78.3421
AB6	2,3-dimethyl,4,5-dihydroxy	72.3866
AB7	2-CH_3, 4,5-dihydroxy	70.5887

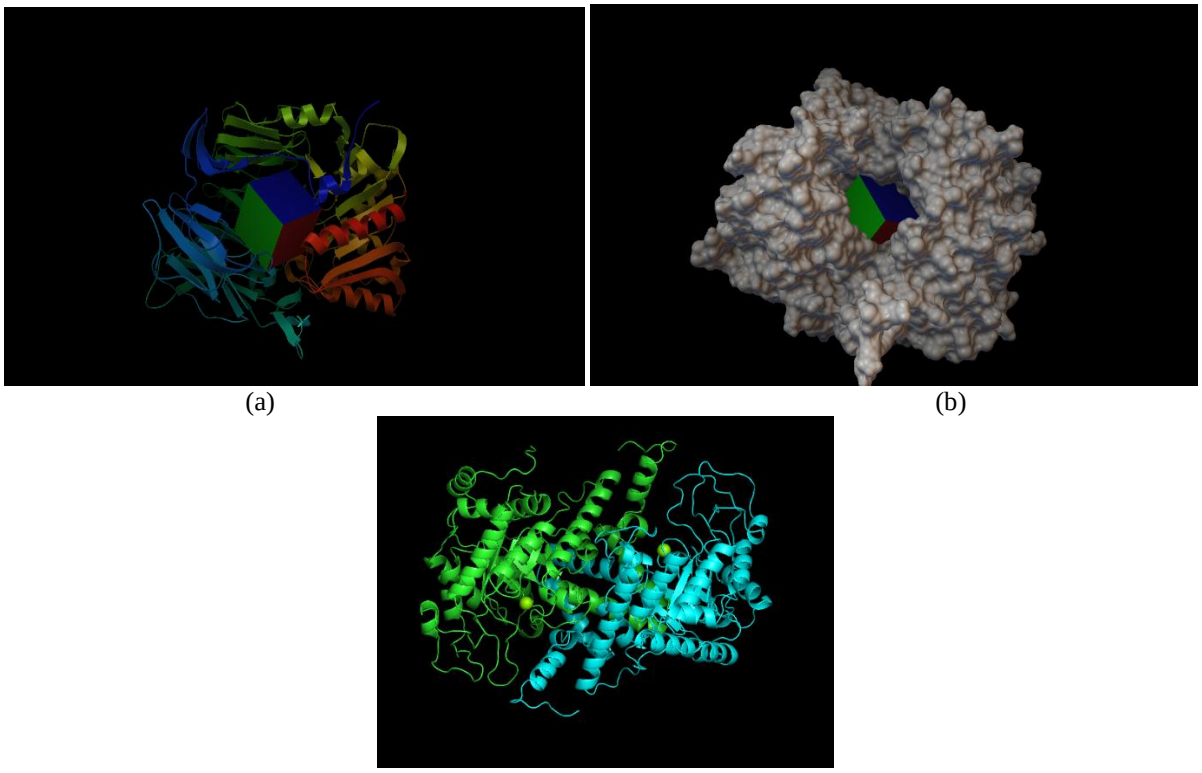


Figure 2: Docking Image of molecule with PDB.

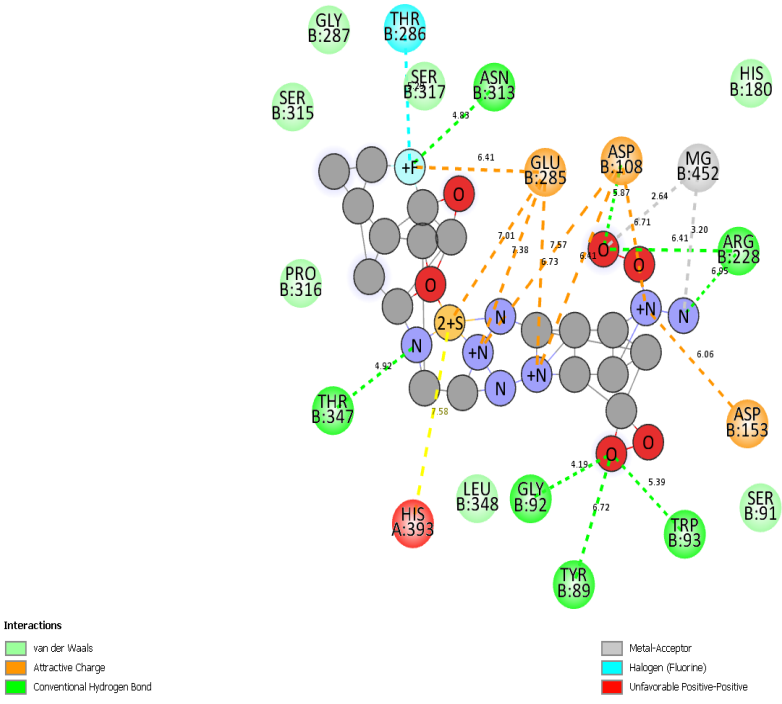


Figure 3: Ligand Interactions of molecule with PDB.

3. RESULTS AND DISCUSSION

In silico Design of [1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline

By using different software, *In silico* molecular modifications of title derivatives were done. 3-D drawing, optimizing and calculating various descriptors of [1,3,4]thiadiazolo[2',3':2,3] imidazo[4,5-*b*]quinoxaline derivatives were done by using ACD Lab Chemsketch 12.0. The results of the those study were shown in Table 2. Later, analysis of Lipinski's Rule of Five and Drug- likeness

Molinspiration software were used to study the logP values, violation of Lipinski's rule of five and drug likeness. The results of Lipinski's rule of five and drug-likeness were shown in Table 3 and Table 4.

ADMET Property/Descriptors of ligands

Prediction of ADMET (absorption, distribution, metabolism and excretion and toxicity) profiles were done by AdmetSAR programme. The results of the prediction confirmed that all the ligands tested exhibited positive result to cross blood-brain barrier, human intestinal absorption, Caco-2 permeability. Similarly, all the studied compounds scored negative result for carcinogenic character. The results were shown in Table 5.

Molecular docking studies

The crystal structure of the M.tuberculosis enoyl-reductase InhA were retrieved from PDB. In it, the protein consists of polypeptide, chain with sequence length of 269 amino acids. Docking scores of novel [1,3,4]thiadiazolo[2',3':2,3]imidazo[4,5-*b*]quinoxaline derivatives with enzyme M. tuberculosis enoyl-reductase InhA for anti-tubercular activity were given in Table 7. All the seven compounds showed better docking score. Among the 7 compounds **AB2** showed better interaction with the target active site amino acid by Hydrogen bond interaction with good docking score of 92.8493. Thus, this molecule may be considered as ideal lead molecule in drug discovery after scientific validation.(figure 2, figure 3).

4. CONCLUSION

TB which was caused by *M. tuberculosis*, has been a life threatening for the mankind since ages. This research work was mainly focused on the rational approach in designing and development of derivatives of well-known antitubercular drug INH not only as a mode to improve its antitubercular activity but also to minimize other problems that were associated with INH therapy. A series of [1,3,4]thiadiazolo[2',3':2,3]imidazo[4,5-*b*]quinoxaline derivatives were taken to preliminary *in silico* designing. Docking studies of the derivatives were performed using Bio via Discovery studio 2018. Among the 7 derivatives docked at the active site of enoyl-reductase InhA to study anti tubercular effect, the one containing hydroxyl and fluoro group showed better docking score and also found that all the compounds showed better docking scores.

5. REFERENCE

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