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Research Article

DESIGNING AND SCREENING OF POTENT INHIBITOR AGAINST INHA REDUCTASE OF *MYCOBACTERIUM TUBERCULOSIS*: A COMPUTATIONAL APPROACH

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ABSTRACT

Objectives: In this study, we attempt to design potent inhibitor specifically targeting the enoyl-acyl carrier protein reductase of *Mycobacterium tuberculosis*.

Methods: *In silico* docking studies were performed using FlexX and Autodock Vina with ligand1 (library compound) and known inhibitors against enoyl acyl carrier protein reductase of *Mycobacterium tuberculosis* i.e., drug target. Ten proven inhibitors of InhA were selected from literature with their IC_{50} value and were correlated using EasyQSAR to generate QSAR model.

Results and Discussions: By a two-step screening method, we identified a library compound expected to have high binding affinity to the enoyl acyl carrier protein reductase. Molecular docking with library compound showed good docking score better than known inhibitors. Drug like properties of these ligand1 were calculated by ADME/Tox calculations. The QSAR analysis of all standard compounds showed significant correlation with R square is 99.29 %.

Conclusion: We therefore, propose that (2-((3,5-dimethoxyphenyl)(hydroxy)carbamoyl)-5-methylphenyl)(7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-3-yl)azinic acid is presenting better bioactivity against InhA target. Thus, this library compound as a potent InhA inhibitor and may be used in designing new anti-tubercular therapy.

Keywords: Enoyl-acyl carrier protein reductase (InhA), ADME/Tox, *Mycobacterium tuberculosis,* Docking.

INTRODUCTION

Mycobacterium tuberculosis is a pathogenetic bacteria species in the genus Mycobacterium and the causative agent of most cases of tuberculosis [1].Tuberculosis currently remains the most common and important infectious disease involving both morbidity and mortality [2].It is the second leading cause of death worldwide [3].The World Health Organization (WHO) reports showed that there were an estimated 9.3 million incident cases and 13.7 million prevalent cases of TB in 2007 [4].TB kills more than 2-3 million people a year worldwide [5].One-third of the world's population is infected with Mtb, the etiological agent of TB [6]. *Mycobacterium tuberculosis* has two features that render it the deadliest infectious disease to date, its high virulence and its ability to enter latency for subsequent reactivation and that leads to a deadly synergy with AIDS [7].

InhA, the enoyl-ACP reductase in *Mycobacterium tuberculosis* is an attractive target for the development of new drugs against tuberculosis. InhA has type I and type II fatty acid synthesis which together function in the synthesis of mycolic acid. Mycolic acid is an essential component of the MTB cell wall [6].An active area for the search of new anti tuberculosis therapies is concerned with the use of computational approaches toward the discovery of new and potent anti TB agents [7].The increasing prevalence of tuberculosis in many area of the World, which is associated with the rise of drug resistant MTB strains, there is an urgent need for the designing and virtual screening of potent and versatile anti TB agents.

MATERIAL AND METHODS

Data collection

Enoyl acyl carrier protein reductase (InhA) from *Mycobacterium tuberculosis* (PDB ID-2NSD) was downloaded from protein data bank (PDB) and saved in pdb text format. From the binding database the existing inhibitors were downloaded in pdb format and N-(3, 5 - Dimethoxyphenyl) - 4 - methyl - 2- nitrobenzamide **(** ChemBL-558660) were selected as a main model for generation of library compounds. Descriptors i.e., LogP, Mass, Volume, Polarizability and Refractivity of each of the compound were calculated using hyperchem (www.hyper.com) software.

Generation of combinatorial library

For the generation of library compounds using the ilib diverse tool, the mol format was converted from pdb format of the main InhA inhibitor as the software only permits the mol format to be run and output library compounds were generated in smi format. A combinatorial library of about 300 derivatives were generated by adding the pharm group and hydroxyl group to the main inhibitor and the filtering properties were set as high likeliness (Ghose). The generated library compounds were automatically saved to the desktop.

Virtual screening of library compound

The virtual screening of library compounds for ADME/Tox were performed using mobyle@rpbs online portal and screened the drug likeness compounds and other toxic compounds were discarded and all the drug likeness compounds were subjected for docking with the target InhA protein.

Molecular docking

After screening the ADME/Tox, the molecular docking was performed using FlexX with the drug likeness library compounds in sdf format and Autodock Vina with the library compound in pdb format separately against target in pdb format. After docking, the highest docking score were recorded.

QSAR analysis and bioactivity prediction

The QSAR analysis was performed by taking the ten known InhA inhibitors. The activities have been calculated by taking the inverse logarithm of IC_{50} values. The descriptors were tabulated in an MS excel sheet against their bioactivities. The descriptors and activities were loaded in the EasyQsar software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activities for each molecule were predicted.

RESULT AND DISCUSSION

According to the World Health Organisation, the largest number of new TB cases was India and accounting for 40% of all TB cases globally [8]. The stable drug target is an important requirement for treating *Mycobacterium tuberculosis* infection [9]. In recent years, the pandemic of AIDS has had a major impact on the worldwide TB problem [10]. In our study, the ADME/Tox properties of the ligand1 (library compound) and known inhibitors proved their non-toxicity as they follow Lipinski's rule and were given in **(Table 1).**

Table 1: ADME/Tox properties of ligand and all standard inhibitors

Compound	MW	tpSA	Rotatable	Rigid Bond	Donor Bond	Accepter	Hydrogen	Ring Bond	Carbon
Ligand1	460.48	138.75	6	25	1	10	11	3	21
Ligand2	316.31	96.21	5	16	1	7	8	2	16
Comp1	314.40	23.55	2	20	0	3	3	3	18
Comp2	325.36	72.20	3	22	0	6	3	2	18
Comp3	318.77	23.55	2	20	0	3	3	3	17
Comp4	318.77	23.55	2	20	0	3	3	3	17
Comp5	335.23	23.55	2	20	0	3	3	3	17
Comp6	369.67	23.55	2	20	0	2	2	3	20
Comp7	293.40	20.31	3	20	0	2	2	3	20
Comp8	431.48	39.34	4	26	0	3	3	4	26
Comp9	406.47	23.55	4	26	0	3	3	4	25
Comp10	392.44	23.55	4	26	0	3	3	4	24

The active site residues ILE 21, MET 103,MET 147,ASP 148,PHE 149,MET 155,PRO 156,ALA 157,TYR 158,LYS 165,VAL 189,ALA 191,GLY 192,PRO 193,ILE 194,THR 196,MET 199,ILE 202,VAL 203,LEU 207,ILE 215,LEU 218,GLU 219,TRP 222,MET 232 etc were found out using Q-site finder portal. The generated library compound was showed high docking score result compared to all the existing inhibitors. The highest docking score is -32.4070 and the main standard inhibitor (ligand 2), from which the library compounds were generated, is -17.4574 (CID-45272830). In order to have more convincing result, ligand and all standard inhibitors were again docked in the same active site of the same target using Autodock Vina and again found that library compound is the best docking score **(Table 2)**.

Table 2: The list of ligands and all standard inhibitors and its database ID Molecular structure with their docking scores:

Sl. No	Standard Inhibitors	Database ID	Molecular Structure	FlexX	Autodock vina
1	**Ligand1**	Library compound	(2-((3,5-dimethoxyphenyl)(hydroxy)carbamoyl)-5-methylphenyl)(7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-3-yl)azinic acid	**-32.4070**	**-6.2**
2	**Ligand2**	CID- 45272830 (main compound)	N-(3,5-dimethoxyphenyl)-4-methyl-2-nitrobenzamide	**-17.4574**	**-4.8**
3	**Comp1**	CID-4006554	[4-(3-chlorophenyl) piperazin-1-yl]-(3-methylphenyl)methanone	**-18.9042**	**-5.4**
4	**Comp2**	CID-2838469	(3-methylphenyl)-[4-(4-nitrophenyl)piperazin-1-yl]methanone	**-19.6814**	**-5.4**
5	**Comp3**	CID-739897	[4-(3-chlorophenyl)piperazin-1-yl]-(2-fluorophenyl)methanone	**-22.0966**	**-5.5**
6	**Comp4**	CID-681767	-[4-(3-chlorophenyl)piperazin-1-yl]-(4-fluorophenyl)methanone	**-21.4822**	**-5.9**
7	**Comp5**	CID-702506	(3-chlorophenyl)-[4-(3-chlorophenyl)piperazin-1-yl]methanone	**-21.3402**	**-5.8**
8	**Comp6**	CID-1070143	[4-(3-chlorophenyl) piperazin-1-yl]-(3,4-dichlorophenyl) methanone	**-20.1587**	**-5.7**
9	**Comp7**	CID-1378917	(4-benzylpiperidin-1-yl)-(3-methylphenyl)methanone	**-20.855**	**-5.5**
10	**Comp8**	CID-25093354	(4-(Bis(4-fluorophenyl) methyl)piperazin-1-yl)(1H-indol-5-yl)methanone	**-24.453**	**-4.8**
11	**Comp9**	CID-17312855	(4-(Bis(4-fluorophenyl) methyl)piperazin-1-yl)(p-tolyl)methanone	**-18.1086**	**-5.3**
12	**Comp10**	CID-9378917	(4-(Bis(4-fluorophenyl) methyl)piperazin-1-yl)(phenyl)methanone	**-21.2095**	**-4.7**

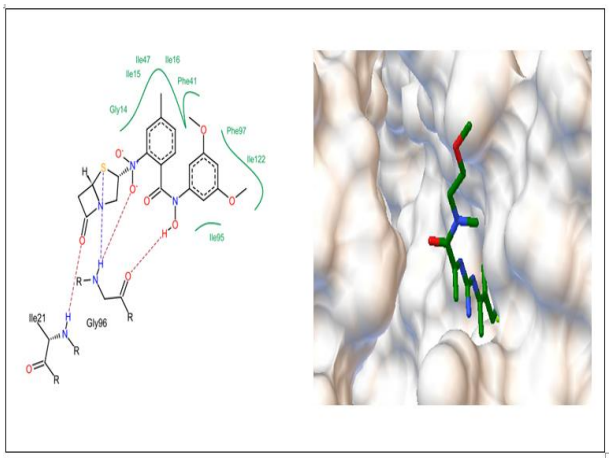

Fig 1: Docking poses of ligand1 (library compound) against InhA in FlexX and Autodock Vina

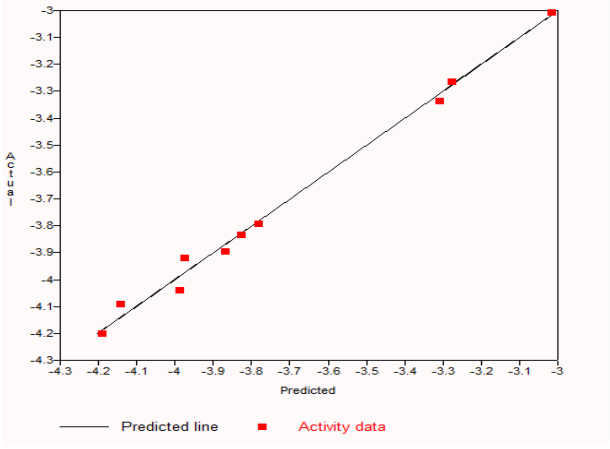

Fig 2: The multiple regression plots of ten known inhibitors

The QSAR descriptors viz. logP, refractivity, polarizability, mass and volume were generated each of the compounds **(Table 3)**.

Table 3: Descriptors of ligand and all standard inhibitors

Compounds	LogP	Refractivity	Polarizability	Mass	Volume	IC_{50}
Ligand1	-2.18	122.08	44.89	462.50	1183.49	0.31
Ligand2	-5.66	90.51	31.99	316.31	903.20	0.4
Comp1	0.83	98.21	35.05	314.81	919.90	1.16
Comp2	-3.62	98.70	34.96	325.37	931.41	5.16
Comp3	0.08	94.05	33.12	318.78	879.45	17.6
Comp4	0.08	94.05	33.12	318.78	873.69	15.4
Comp5	0.46	98.64	35.14	335.23	905.19	9.43
Comp6	0.24	103.36	37.07	369.68	947.08	7.39
Comp7	2.67	98.38	35.44	293.41	938.20	13.8
Comp8	-1.25	131.25	46.07	432.49	1168.34	31.5
Comp9	0.74	124.23	43.85	407.48	1131.51	16.6
Comp10	0.59	119.95	42.02	393.46	1088.50	9.74

The equation generated out of QSAR analysis is as follows

Activity= - 5.61 + 4.21×10^{-2} (logP) + 8.78×10^{-2}(Refractivity) + 5.49×10^{-3}(Polarizability) -3.22×10^{-3}(Mass) - 8.55×10^{-3}(volume)

Prediction of bioactivity (IC_{50}) of library compounds:

The IC_{50} value of unknown library compounds was calculated by using the generated QSAR equation. The library compounds are showing a low IC_{50} value, whereas by comparing the IC_{50} of the all known inhibitors. Multiple regression plot generated for QSAR model is shown in **(Figure 2)**.

Thus our library compound may act as a potential lead molecule for the inhibition of InhA. This indicates that the library compound bear character to be a orally active drug.

CONCLUSION

The World Health Organization estimates that about eight to ten million new TB cases occur annually worldwide and incidence of TB is currently increasing. Therefore new generation of drugs are needed for treating TB. In this study, we observed that InhA (Target) when docked with the (2-((3,5-dimethoxyphenyl) (hydroxy) carbamoyl)-5-methylphenyl)(7-oxo-4-thia-1-azabicyclo[3.2.0]hept-an -3-yl)azinic acid gave good result. So these can be potential drug for contributing in treatment of tuberculosis.

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