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In-Silico Inhibition Studies of Phenothiazine Based Compounds on Chorismate Synthase (2 A86) Enzyme as A Potent Anti-Tuberculosis Agent.

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Abstract:

Mycobacterium tuberculosis is the single most deadly human pathogen and is responsible for nearly three million deaths every year[1]. Recent elucidation of the mode of action of frontline antimycobacterial drug, suggests that NAD metabolism is extremely critical for this micro-organism[2]. In the present study, the enzymes selected for docking which plays an important role in metabolism of tuberculosis. The enzymes is, chorismate synthase. The enzymes of the shikimate pathway are potential targets for the development of new therapies because they are essential for bacteria but absent from mammals. The substituted Phenothiazine molecules are found to be biological active[5] against mycobacterium tuberculosis, whereas their molecular level interactions are not studied with chorismate synthase (2A86). The knowledge of exact interactions between ligand and enzyme helps in enhancing the biological activities by designing the new and potent anti-tuberculosis agents.

Keywords: antimycobacterial drug,

Introduction

Mycobacterium tuberculosis is the single most deadly human pathogen and is responsible for nearly three million deaths every year[1]. Recent elucidation of the mode of action of frontline antimycobacterial drug, suggests that NAD metabolism is extremely critical for this micro-organism[2]. In the present study, the enzymes selected for docking which plays an important role in metabolism of tuberculosis. The enzymes is, chorismate synthase. The enzymes of the shikimate pathway are potential targets for the development of new therapies because they are essential for bacteria but absent from mammals. The last step in this pathway is performed by chorismate synthase (CS), which catalyzes the conversion of 5-enolpyruvylshikimate-3-phosphate to chorismate. Optimization of crystallization trials allowed the crystallization of homogeneous recombinant CS from Mycobacterium tuberculosis (MtCS)[3, 4].The substituted Phenothiazine molecules are found to be biological active[5] against mycobacterium tuberculosis, whereas their molecular level interactions are not studied with chorismate synthase(2A86). The knowledge of exact interactions between ligand and enzyme helps in enhancing the biological activities by designing the new and potent molecules.

Using computer simulation techniques, it is now possible to study the interaction of the ligand and enzyme for elucidating the binding energy. This process provides in-depth understanding about the inhibition strength of various ligands. This is generally achieved by designing series of derivatives of lead compound by varying the functional groups at various locations in the lead compound5.

Now days, various computational tools like autodock, amber, CHARMM etc are used to study the inhibition activities. These tools use ab initio or semi-empirical

techniques such as QM/MM. They help in understanding three major types of interactions; viz electrostatic interaction, hydrophobic interaction and steric interactions[6-11]. The combination of these interaction helps in understanding the strength of binding ligand with enzyme[12]. The computational tools provide the value of ΔG Kcal/mole as a binding energy between ligand and enzyme. The value of ΔG represents the inhibition strength of ligand with enzyme[6, 12].The aim of the present study is to understand the inhibition of chorismate synthase enzyme with the derivatives of substituted Pyridine. For the same computational tools were used to design the series of substituted Phenothiazine molecules. These molecules were docked with the chorismate synthase (2A86) enzyme for insilico studies.

Experimental

Chorismate synthasehaving PDB code 2A86 was selected as the target enzyme. Its 3D electronic structure having natural inhibitor was procured from protein repository databank. The position of natural inhibitor was selected as the center of active site and it was removed before docking the ligand.

The series of compounds (also called as small molecules), which are the derivatives of substituted Phenothiazine, were designed using computer based design tools ChemOffice11 and their 3D geometries were finalized by minimizing the total energy content using molecular mechanics techniques. While finalizing the geometry of small molecules, global minima were achieved and confirmed. Phenothiazine which is selected as lead compound provides four substitution sites viz. R1, R2, R3 & R4 as shown in **figure 1**.

This compound provides total 4 substitution sites from R1 to R4. The various pharmacophore used to substitutes these places were:

-H, -F, -CI, -Br, -OCH3, -OH, -NH2, -CH2CH3, -C3H7, -OCH2CH3, -NH2, -CONH2, -COCH3, -C6H4CI, -C6H4 (OH),-C6H5, -C6H4OC2H5, -OC6H5, -OC6H4OH, -CH3, -C2H5.

The list of designed small molecules along with substituted pharmacophores is listed in table1. The table 1 shows only those molecules, which were successfully achieved global minima. The database of these molecules prepared and stored as an electronic library for docking process. To ascertain the validity of the 3D design, various thermodynamic properties was calculated in-Silico using ChemDraw. These molecules then subjected to docking studies with the binding site of Chorismate synthase enzymes using computer based tools Argus and Autodock.

The details of 2-d and 3-d designed molecules and their properties are listed in **table 1** and **table 2** respectively.

Result

The selected mode of docking was performed using genetical algorithm, which provides the most intelligent docking positions. The process gives the binding energy as the major of strength of interactions between small molecules and enzyme.

Table 3 shows the results of binding energy of various docked molecules, the number and distance of hydrogen bonding involved in the complex formation between enzyme and small molecules.

In case of 2A86, it is 3-D structure of complex having inhibitor. Using computer based tools this inhibitor was removed and hence the binding site identified. After determination of active site, the newly designed (in-silico) chemical compounds (ligand) were introduced having better chemical binding energies value than the existing ligand.

After evaluating the 2-D and 3-D properties of ligand molecules and also validating the 3-D structure of enzymes it is decided to go for calculating the binding energy values for the interaction between ligand and binding site of an enzyme. To study the interactions, docking procedure is used which is common practice in computational chemistry. There are various tools available for docking and for present study rigid-rigid docking procedure was selected. The docking results of various systems are depicted in **table 3**,

Those molecules show the better docking results are selected from each set of experiment and their in-detail interactions are reported in figure 2,3,4,5.

The various interactions studied are steric interactions, hydrophobic and electrostatic interactions.

The binding energy values (ΔG) vary from -9 to - 6.5 kcal/mol. approximately. This depends upon the steric, electrostatic and hydrophobic interaction properties of the molecules with binding site of enzymes. This proves that the designed molecules definitely have activity for selected enzymes.

The results shows that the binding energy values are favourable enough and could be better substitution alternative to the original ligands. It is possible to further get better values for activity of these molecules by using various mathematical and statistical calculations, but it is out of scope of this project.

Thus, the molecules which are designed in-silico can be potent anti-tuberculosis agents in the future. Also the synthesis path of these molecules is also validated since the parent base compounds are natural products or their derivatives; hence they can be either isolated from nature or can be synthesized in laboratory.

Nevertheless, many of the compounds described herein merit further investigation from both a chemical and biological perspective.

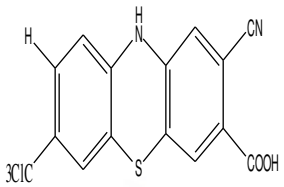
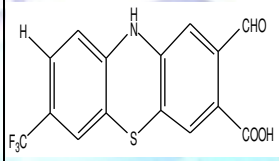
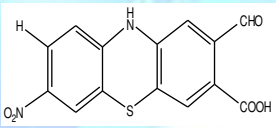
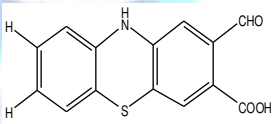
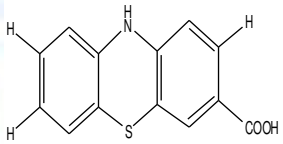
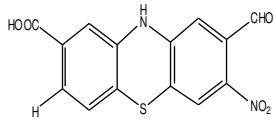
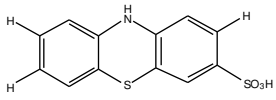
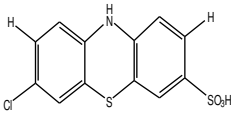
Table 1: Substituted Phenothiazine.

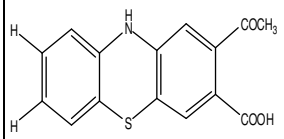
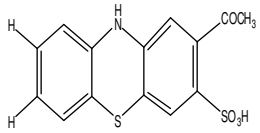
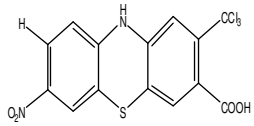
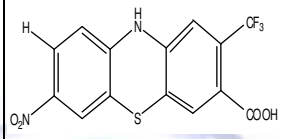
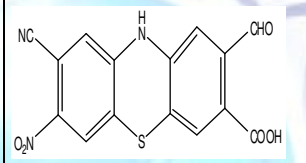
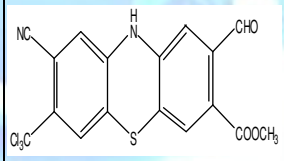
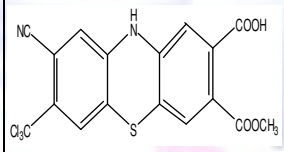
Molecule	R1	R2	R3	R4
1	CN	COOH	CCl3	H
2	CHO	COOH	CF3	H
3	CHO	COOH	NO2	H
4	CHO	COOH	H	H
5	H	COOH	H	H
6	CHO	NO2	H	COOH
7	H	SO3H	H	H
8	H	SO3H	Cl	H
9	COCH3	COOH	H	H
10	COCH3	SO3H	H	H
11	CCl3	COOH	NO2	H
12	CF3	COOH	NO2	H
13	CHO	COOH	NO2	CN
14	CHO	COOCH3	CCl3	CN
15	COOH	COOCH3	CCl3	CN

Table. 2-MM2 Parameters for Substituted Phenothiazine molecules**.**

Molecular Code	Heat of Formation Kcal/mole	Henry's Law Constant	LogP	Exact Mass Grams / Mole	Molecular Formula
1	-11.78	13.4082	5.0893	397.945	C16H9Cl3N2O2S
2	-211.7394	11.7837	3.5686	339.018	C15H8F3NO3S
3	-51.84991	0.6371	-	316.015	C14H8N2O5S
4	-55.85369	12.7227	2.6475	271.03	C14H9NO3S
5	-28.68739	10.1195	2.9005	243.035	C13H9NO2S
6	-49.95095	7.0154	-	316.015	C14H8N2O5S
7	-59.902181	6.2121	2.5019	279.002	C12H9NO3S
8	-63.28513	6.3423	3.0601-	312.963	C12H8ClNO3S2
9	-55.0274	12.8591	2.2126	285.046	C15H11NO3S
10	-76.98681	7.6051	1.814	321.013	C14H11NO4S2
11	-31.33672	0.6371	-	403.919	C14H7Cl3N2O4S
12	-172.154	0.6371	-	: 356.008	C14H7F3N2O4S
13	-13.46882	2.652	-	341.011	C15H7N3O5S
14	-21.083	13.5489	4.6123	425.94	C17H9Cl3N2O3S
15	-77.02184	15.6419	4.4223	441.935	C17H9Cl3N2O4S

Table 3: The selected substituted Phenothiazine molecules with their docked values.

Molecular No.	Molecular Formula & Name	Molecular structure	Bonding energy kcal/mol	No. of hydrogen bonds
1	**C16H9Cl3N2O2S** 7-(trichloromethyl)-2-cyano-10H-phenothiazine-3-carboxylic acid		-6.79715	9
2	**C15H8F3NO3S** 7-(trifluoromethyl)-2-formyl-10H-phenothiazine-3-carboxylic acid		-6.71829	11
3	**C14H8N2O5S** 2-formyl-7-nitro-10H-phenothiazine-3-carboxylic acid		-6.21275 kcal/mol	11
4	**C14H9NO3S** 2-formyl-10H-phenothiazine-3-carboxylic acid		-6.71626 kcal/mol	11
5	**C13H9NO2S** 10H-phenothiazine-3-carboxylic acid		-8.48696 kcal/mol	10
6	**C14H8N2O5S** 8-formyl-7-nitro-10H-phenothiazine-2-carboxylic acid		-6.54305 kcal/mol	11
7	**C12H9NO3S** 10H-phenothiazine-3-sulfonic acid		-7.3874 kcal/mol	11
8	**C12H8ClNO3S2** 7-chloro-10H-phenothiazine-3-sulfonic acid		-7.20094	12

9	**C15H11NO3S** 2-acetyl-10H-phenothiazine-3-carboxylic acid		-7.71655	11
10	**C14H11NO4S2** 2-acetyl-10H-phenothiazine-3-sulfonic acid		-7.88744	11
11	**C14H7Cl3N2O4S** 2-(trichloromethyl)-7-nitro-10H-phenothiazine-3-carboxylic acid		-6.80123	11
12	**C14H7F3N2O4S** 2-(trifluoromethyl)-7-nitro-10H-phenothiazine-3-carboxylic acid		-6.87749	12
13	**C15H7N3O5S** 8-cyano-2-formyl-7-nitro-10H-phenothiazine-3-carboxylic acid		-6.49281	11
14	**C17H9Cl3N2O3S** methyl 7-(trichloromethyl)-8-cyano-2-formyl-10H-phenothiazine-3-carboxylate		-6.94795	11
15	**C17H9Cl3N2O4S** 3-(methoxycarbonyl)-7-(trichloromethyl)-8-cyano-10H-phenothiazine-2-carboxylic acid		-8.26905	11

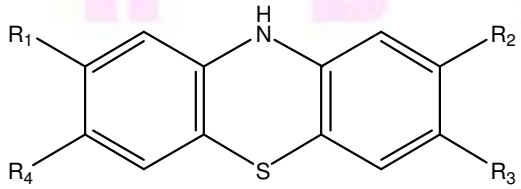


Figure.1-General Formula for Substituted Phenothiazine.

Figure. 2- Interaction of molecule 5 with 2A88 protein binding site of tuberculosis along with hydrogen bonds.	**Figure. 3-** Interaction of molecule 7 with 2A88 protein binding site of tuberculosis along with hydrogen bonds.

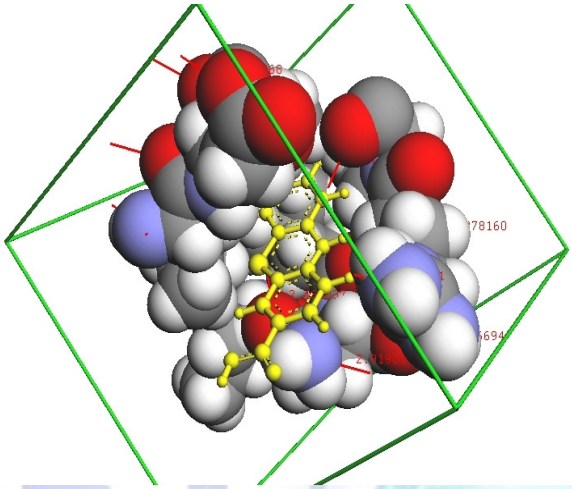
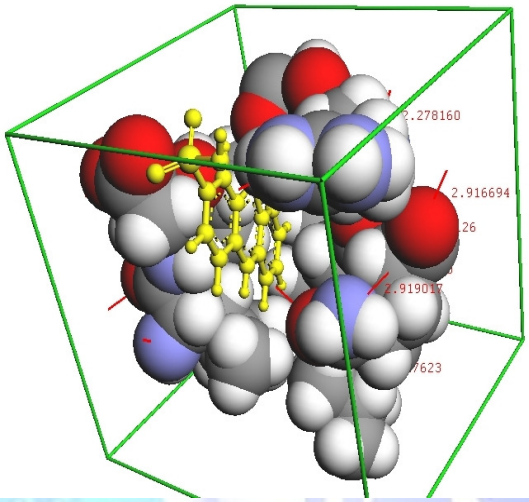
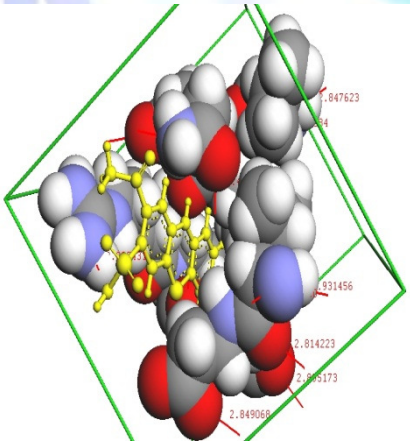
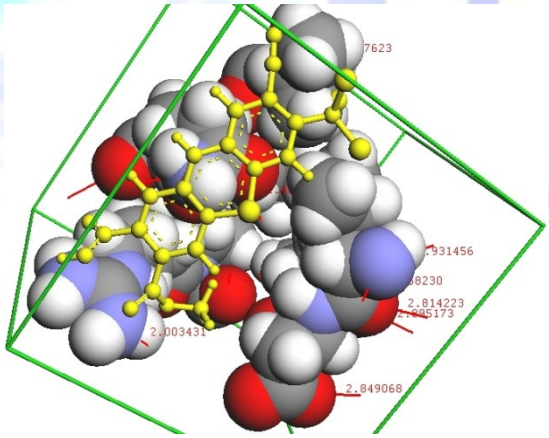

	

Figure. 4- Interaction of molecule 10 with 2A88 protein binding site of tuberculosis along with hydrogen bonds.	**Figure. 5-** Interaction of molecule 15 with 2A88 protein binding site of tuberculosis along with hydrogen bonds.
	

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