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Original Article

IN SILICO STUDY FOR IDENTIFICATION OF DRUG LIKE INHIBITOR FROM NATURAL COMPOUNDS AGAINST INHA REDUCTASE OF *MYCOBACTERIUM TUBERCULOSIS*

URIKHIMBAM JOYLAXMI, M D CHOUDHURY

Department of Life Science and Bioinformatics, Assam University Silchar, Assam 788011
Email: joyalaxmi.u4@gmail.com

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ABSTRACT

Objective: Natural products have played an important role for developing new drugs and becoming popular due to toxicity and side effects of allopathic medicine. The main objective of this research work is to find drug-like inhibitor from natural compounds that can help to treat tuberculosis.

Methods: *In silico* docking studies were performed with four different compounds (isopimpinellin, pimpinellin, malic acid, and psoralen) from *Angelica archangelica* against enoyl acyl carrier protein reductase of *Mycobacterium tuberculosis* i.e., drug target. Flex X and Autodock Vina were used to dock the compound onto an active site of InhA to determine the probable binding of these inhibitors.

Results: Among various natural compounds that were screened as inhibitors, psoralen was found to bind in closest proximity to the InhA binding site. This is compared to the commonly recommended anti-tubercular drugs. Drug like properties of these compounds were calculated by ADME/Tox calculations.

Conclusion: According to molecular docking studies and ADME values the compound (psoralen) from *Angelica archangelica* was conformed as a promising lead compound and also will be the good starting point for natural plant based pharmaceutical chemistry.

Keywords: *A. archangelica,* InhA, Docking, ADME, Mycobacterium *Tuberculosis.*

INTRODUCTION

Tuberculosis (TB) is regarded as one of the most deadly infectious diseases caused by *Mycobacterium tuberculosis.* This bacterium is responsible for more human deaths than other throughout the centuries of human history [1, 2]. TB kills more than 2-3 million people a year worldwide [3-5]. One-third population of the worlds is infected with Mtb, the etiological agent of TB [6, 7]. The two features of *M. tuberculosis* that renders it the deadliest infectious disease to date, its high virulence and its ability to enter latency for subsequent reactivation [8].

M. tuberculosis has an extremely rigid cell wall containing mycolic acid. Such characteristics in the cell envelope are important in the virulence and persistence of MTB. Since a strong cell wall confers high resistance of the bacteria [9]. Mycolic acid is an essential component for the formation of *M. tuberculosis* cell wall [10, 11]. InhA is an NADH dependent [12] trans enoyl-acyl ACP carrier protein that is part of the fatty acid biosynthesis system and member of the short chain dehydrogenase/reductase family [13-15].

Fatty acid biosynthesis in *Mycobacterium tuberculosis* is mediated by fatty acid syntheses I and II. While FAS-II is a collection of individual enzymes, FAS-I is a polypeptide with multiple active site that performs catalytic reactions in the pathway. FAS-II are absent in humans thus they are considered as an important target for new drug development [16-18].

Angelica archangelica Linn. belong to the family *Apiaceae*is native to Europe including Austria, Belgium, Germany, UK and Poland. Many of these species have long been used in ancient traditional medicine systems, especially in the far-east. It is described as "Gandrayan Bhaid" in Traditional System of Medicine (Ayurveda) and "Rickhchoru" (means pseudoangelica) in Garhwal, North-West Himalaya. *A. archangelica* revealed the presence of various types of secondary metabolites, predominantly 2-4 furanocoumarins [19, 20].

Angelica archangelica has been used widely and is one of the most respected medicinal herbs in Nordic countries [21]. It is commonly used in folk medicine as a remedy for nervousness, insomnia, stomach and intestinal disturbances and arthritis. The plant is generally cultivated for its roots, which are richer in oils than the other organs and whose oil is esteemed for its use in flavouring and in making perfumes [22].

In the current study, we sought to design a drug like inhibitor for InhA reductase of *Mycobacterium tuberculosis* through *In silico* studies. These compounds from *Angelica archangelica* inhibit the activity of InhA thereby preventing the initial step of fatty acid biosynthesis and can be effective against *M. tuberculosis.*

MATERIALS AND METHODS

Drugs target

The protein InhA of the fatty acid biosynthesis pathway is indispensable for the organism and hence could be a promising drug target against *M. tuberculosis* [10]. 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.

Phyto compound preparation

Active compound against InhA target was collected from the Dr. Duke Photochemical and ethanobotanical database. These databases contain information on the activity of chemical in plants, and ethanobotanical uses for plants [23]. Databases are searchable with the activity of antitubercular from these plants were screened and searchable by plant (scientific or common name), chemical (e. g., ascorbic acid), or activity (e. g., antiviral) [24]. The 2D structure of these phyto compounds was searched against pubchem database and then with the help of open label, these 2D structures were converted to 3D structure for docking.

Active site prediction

Active site prediction of target protein InhA was performed using the Q-site finder portal to predict the location of the active site in which an inhibitor is bound [25]. In this prediction of an active site of target protein InhA, it was uploaded in active site server in pdb format and submitted.

Docking

Molecular docking were performed to obtain more insight into the binding mode and to predict the potential compounds. Flex X and Autodock vina were used to dock all the compounds onto the active site of InhA in order to identify the probable binding conformation of these inhibitors.

ADME/Tox (absorption, distribution, metabolism and excretion/ toxicity)

The drug like properties for the phyto compounds ADME calculation was performed by using mobyl@rpbs online portal [26]. To identify the new antituberculosis compounds from the database, we collected the best compound based on two distinct parameters: antituberculosis activity and Lipinski's rule of 5. This rule would have good absorption and permeation in the body when

a. No more than 5 H-bond donors

b. No more than 10 H-bond acceptors

c. Molecular weight no higher than 500

d. The log *P* is less than

RESULTS AND DISCUSSION

The chemical compounds with antitubercular activity from *Angelica archangelica* plant were screened from Dr. Duke's Photochemical and ethno botanical data. The structure of molecules that concerned with the Lipinski's rule of five was downloaded from the chemical database Pubchem. Commonly recommended antitubercular drug was taken as reference inhibitor. Ligands with their IUPAC name, Pubchem ID, and structure were found out and are depicted in (table 1)

Table 1: Inhibitors with their IUPAC names, cid numbers and structures

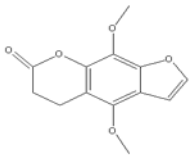
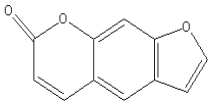
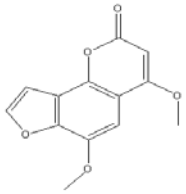
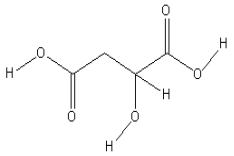
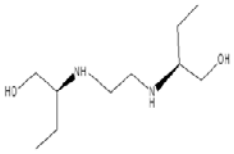
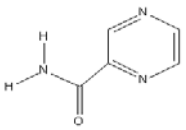
Inhibitors	IUPAC name	Structure	Pubchem (CID)
Isopimpinellin	4,9-dimethoxyfuro[3,2-g]chromen-7-one		CID-68079
Psoralen	furo[3,2-g]chromen-7-one		CID-6199
Pimpinellin	5,6-dimethoxyfuro[2,3-h]chromen-2-one		CID-4825
Mallic acid	2-hydroxybutanedioic acid		CID-525
Ethambutol	(2S)-2-[2-[[(2S)-1-hydroxybutan-2-yl]amino]ethylamino]butan-1-ol		CID-14052
Pyrazinamide	Pyrazine-2-carboxamide		CID-1046

Table 2: Comparison of various compounds derived from *A. Archentia* as inhibitor based on FlexX results

Pose name	Score	Match	Lipo	Ambig	Clash Rot
Isopimpinellin	9.9864	12.6169	3.6598	4.1010	2.1914
Pimpinellin	10.4024	9.4024	4.3268	6.9318	1.8237
Psoralen	20.9446	22.266	3.3247	4.0043	0.3111
Malic acid	7.3419	12.3251	1.5905	4.5093	0.0830

In order to identify suitable drug-like inhibitors for InhA of *Mycobacterium tuberculosis* docking study, was performed by using FlexX, The affinity for binding of the inhibitors to the InhA binding site, the binding free energies of inhibitor-receptor complexes are obtained by the FlexX runs.

The result showed that Psoralen is the most potent inhibitor as it has the highest free energy of binding which are the most desired characteristics as inhibitors. Inhibitors in search of most potent drug-like agent are compared and listed in table 2.

Validation of result is essential to optimise the uniformity and error. FlexX result is compared with the Autodock vina which shows the same pattern of results. Negative value of the results signified better conjugation of inhibitor to binding pocket of the receptor. Inhibitor-target interaction leads to overall changes in the conformation of the protein structure and hence arrests the activity of the enzyme. After comparing the FlexX with Autodock vina, psoralen is confirmed as the most potent anti tubercular with the favourable results like highest dock score values. The comparison of docking result and best inhibitor (psoralen) with commercial inhibitors are shown in table 3.

Table 3: Comparision of docking between commercial inhibitor and psoralen

Ligands	FlexX Score	Autodockvina
Psoralen	20.9446	7.5645
Ethambutol	11.7038	5.2984
Pyrazinamide	12.2757	5.6547

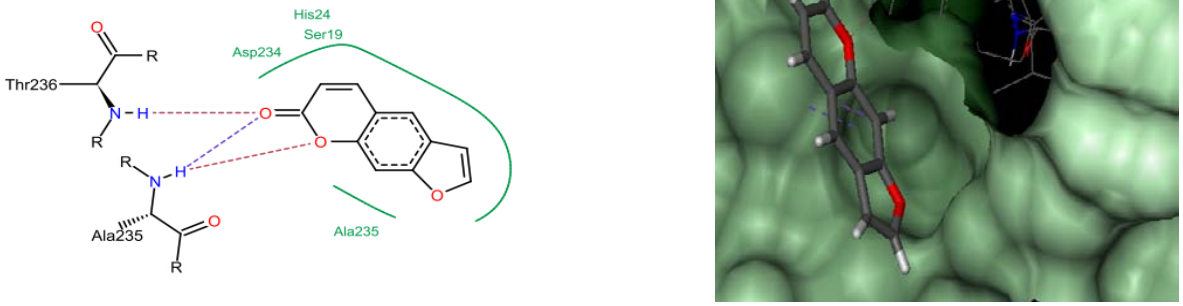


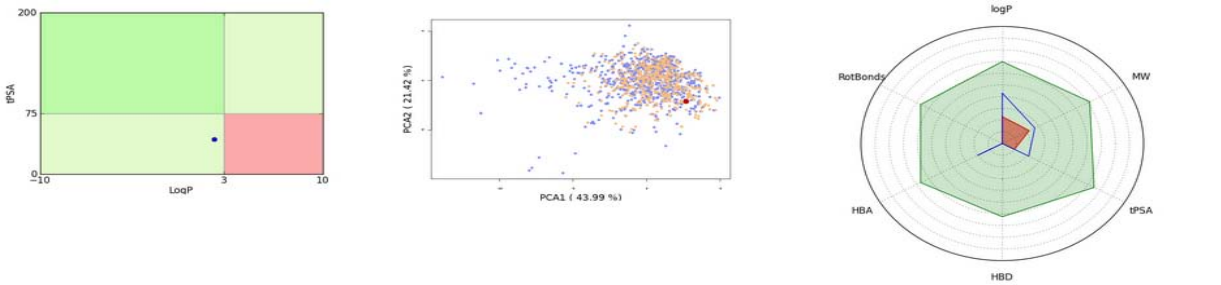
Fig. 1: Docked structure of InhA protein with psoralen (Left) and binding to target active site (right)

Table 4: Bonded residues, bond energy and bond length of psoralen

Inhibitor	Bond properties		
	Bond Residues	Bond Energy (Kcal/mol)	Bond Length (Å)
Psoralen	O10-GLU219A	-5.2	2.33
	O22-GLU219A	-4.4	1.85
	H20-THR 165A	-4.7	1.68
	H20-ASP 148A	-6.8	2.17

Table 5: Molecular properties and drug-likeness of psoralen and commercial drug

Molecular properties	Lipinski's values	Psoralen	Ethambutol	Pyrazinamide
Molecular weight	<500	186.16	204.31	123.04
Number of hydrogen bond acceptor	<10	3	4	2
Number of hydrogen bond donor	<5	3	4	4
Log P (octanol-water partition Coefficient value)	<5	2.31	-0.08	-1.01
Polar surface area, Å^2	<140	43.35	64.52	68.87



a. Pfizer 3/75 rule positional **b. Oral property space** **c. Oral absorption estimation**

Fig. 2: ADME/Tox profiling of psoralen

(a). Compounds located in the red square are likely to cause toxicity and experimental promiscuity (b). The chemical property space was obtained by applying a Principal Component Analysis (PCA) of the 15 principal physico-chemical descriptors of user's compound (red), compared to two oral sub-libraries extracted from Drugs and DrugBank (c). Compound values (blue line) should fall within RO5 and Veber rules area (light green).

Our study showed that the natural compound derived from *A. archangelica* can be used as the inhibitory compound using molecular docking method. Based on docking of inhibitor (psoralen) to InhA target, a docking protocol involving flexible ligand docking of the inhibitor best result are obtained. Psoralen satisfies all the criteria of the Lipinski's rule of five and its properties without violating any rule, So, it can be developed as a promising drug-like inhibitor of tuberculosis. As it is a natural compound it might have fewer side effects.

Based on the docking result and ADME values, this approach revealed different binding pattern of proposed inhibitors that can be attributed to structural differences of the inhibitors. We revealed that psoralen having the desired potential to block the active site of the receptor InhA. These studies conclusively revealed psoralen as a potent lead compound better than commercially available drug (ethambutol and pyrazinamide) based on best values of docking energy and hydrogen bond intereaction. Therefore the compound (psoralen) is confirmed as the most potent antitubercular agent. Further studies are needed to

isolate and characterise the structure of the bioactive compounds of this plant for industrial drug formulation.

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CONFLICT OF INTERESTS

Declared None

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