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Original Research Article

Virtual Screening of *Urtica dioica* Plant Compounds as *Mycobacterium tuberculosis* Cell Division Protein Inhibitors

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Abstract: Tuberculosis is a common infectious disease caused by mycobacterium; it remains the leading cause of death worldwide from a single infectious disease agent. Multi drug resistant TB is a form of TB that does not respond to the standard treatments using first line of drugs. By targeting TB related bacterial cell division protein FtsZ, due to inactivation of FtsZ results in the inhibition of cell division. It is a very potential target for new antimicrobial drug development**.** Protein-ligand docking analysis was carried out using Auto Dock Vina on 79 compounds from the plant *Urtica dioica*, with FtsZ protein of *Mycobacterium tuberculosis*. Various experimentally tested FtsZ inhibitors from literature were also studied before screening plant based compounds. The average dock score of the inhibitors taken from the literature was 7.2kcal/mol. After docking these compounds, a final set of compounds were selected by filtering compounds that showed dock scores greater than 7.0kcal/mol. From the scoring generated based on rank-sum technique, 5 compounds were found to be the best inhibitors of FtsZ protein.

Keywords: *Urtica dioica*, Virtual Screening, FtsZ protein, Molecular Docking

Introduction

Tuberculosis is one of the widely spread infectious disease which is caused by the bacterium, in humans, mainly Mycobacterium tuberculosis [1]. According to the World Health Organization (WHO) reports, Tuberculosis (TB) is the most frequent and important infectious disease causing morbidity and death. It estimates that about eight to ten million new TB cases occur annually worldwide and the incidence of TB is currently increasing. In this context, TB is in the top three, with malaria and HIV being the leading causes of death from a single infectious agent, and approximately two million deaths are attributable to TB annually [2]. In addition, during the past decade, multidrug-resistant TB (MDR-TB) is a form of TB and has been increasing in incidence in many areas, not only in developing countries but industrialized countries as well [3]. These situations, predominantly the global resurgence of TB and the rapid emergence of MDR-TB, underscore the importance of the development of novel anti tuberculosis compounds to combat TB is needed and new protocols for efficacious clinical control of TB patients using ordinary antimycobacterial drugs [4]. Concerning the development of new antituberculous drugs, the following work has been of particular importance.

From literature, it was observed that several attempts were made to build models for TB related proteins with its inhibitors. In this paper, we reported virtual screening studies to screen inhibitors for the selected protein FtsZ - Filamentation temperature sensitive protein Z to investigate the influence of molecular structure and biological activity with its receptor.

FtsZ is an essential bacterial cell division protein, bacterial tubulin homologue which contains four main protein domains [5] and mainly involves in the formation of cytokinetic ring and follows conscription of other cell division proteins result in the division of cell into two. While inactivation of FtsZ or variation of FtsZ assembly results in the inhibition of cell division. It is a very promising target for new antimicrobial drug development [6]. Several validations were reported which state the robustness and domain applicability of the screened compounds.

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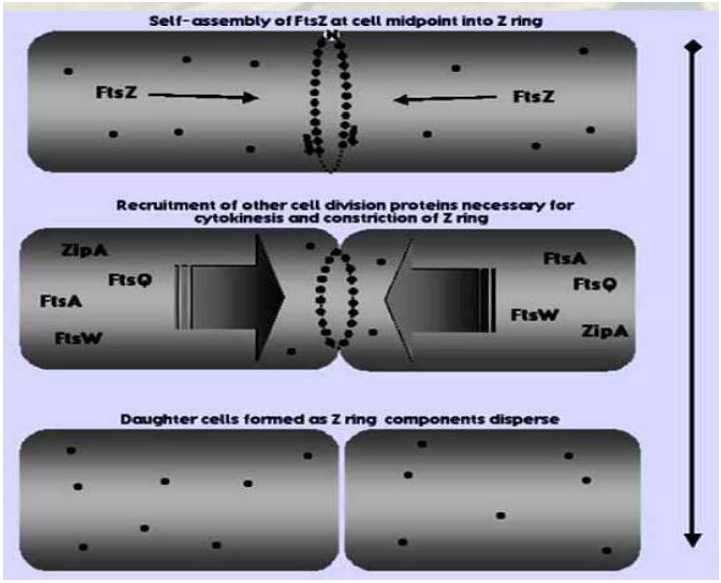


Figure.1: Diagram of prokaryotic cell division and Z ring in FtsZ

Molecular docking approaches are commonly used in modern drug design process to understand the three dimensional structure of the protein-ligand composite [7]. It could be serve as a considerable source of understanding the way of proteins interact with another and perform biological functions. Thus, knowing the detailed structure of protein-ligand and its complexes in atomic level is one of the considerable issues in biological sciences. Conversely, in the databank of proteins where in most of the docking studies, conformational changes occur on ligand binding. This may occupy small side chain rotations to increase interactions with the ligand. Prediction, Molecular Docking and Virtual Screening based studies on molecular level have become an integral part of many modern structure-based drug discovery efforts [8][9]. Hence, knowledge of the protein and ligand interactions with the specific drugs may provide a significant insight into the binding interactions and relativeness of the drug.

Materials and Methods

In this study, the structures were drawn by using ISIS/Draw [10]. It was a chemical structure drawing program for Windows, published by MDL Information Systems for drawing chemical structures. By using Tsar's easy-to-use chemical spreadsheet interface (www.accelrys.com) the limits for ligands were observed and converted 2D structures to 3D with physicochemical properties to analyze and promote activity and the hydrogen bond interactions were studied using Molegro Virtual Docker [11]- It is an integrated platform for predicting protein -ligand interactions.

Virtual screening:

It is an Insilco tool for drug designing and widely used for lead identification in drug discovery programs [12]. 42 structure hits of FtsZ were found from PDB [13]. Among the 42 entries, only 5 FtsZ entries are from Mycobacterium tuberculosis. From the above 5 FtsZ entries, 1RQ7, 1RQ2 and 2Q1X are not taken because the co-crystallized GDP and citrate molecules are not considered as ligands. The RMSD (Root Mean Square Deviation) value was checked for the remaining two proteins in auto dock vina software [14]. The protein 2Q1Y got the RMSD value 1.15 A°. Hence it is considered for the further studies.

Auto Dock Vina:

It is a novel program for screening and docking compounds computationally in drug discovery. It offers multi capability, enhanced accuracy, high performance and ease of use. For its input and output, Vina uses the same PDBQT molecular structure file format used by Auto Dock. PDBQT files can be generated and viewed using MGL Tools.

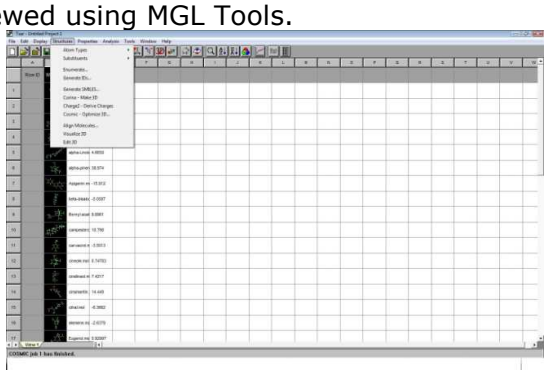


Figure.2: Image showing the conversion of 2D to 3D using Tsar Interface

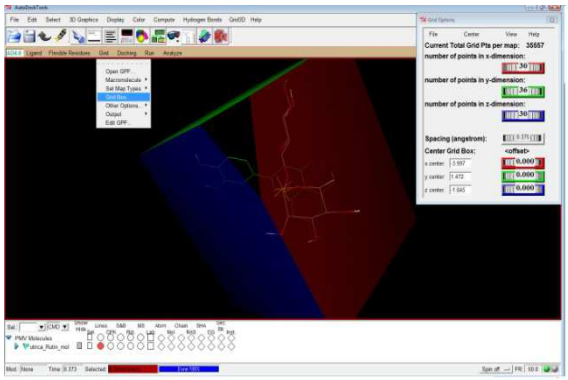


Figure.3: Image showing ligand placed in grid box by AutoDock tools

The plant *Urtica dioica* followed by their compounds extracted from Duke's ethno botany database [15] was selected and based on the studies reported in various literature sources that studied extensively towards identifying promising anti-tubercular agents. Using computational techniques, *Mycobacterium tuberculosis*, FtsZ inhibition was employed to evaluate the compounds present in the plant. This is used to reduce the time spent in synthesizing compounds by experimental analysis to identify the drug.

Results and Discussion

Virtual screening has become a crucial part of modern drug research. A range of computational tools are being developed and sophisticated to effectively employ fast screening methods to yield effective hits. The inhibitors from the literature [16, 17] were taken and docked with the FtsZ protein.

Table.1: List of compounds present in *Urtica dioica* plant

1	(-)-Pinoresinol	41	Isorhamnetin
2	(-)-Secoisolariciresinol	42	isorhamnetin-3-O-neohesperidoside
3	(+)-Isolariciresinol	43	Isorhamnetin-3-O-rutinoside
4	5-Hydroxytryptamine	44	Kaempferol
5	Acetic Acid	45	Kaempferol 3-O-glucoside;
6	Acetophenone	46	Lutein
7	Acetylcholine	47	Lycopene
8	Aesculetin	48	Lysophosphatidylcholines
9	Allantoic acid	49	Malic acid
10	Alpha-Tocopherol	50	Maltose
11	Arabinose	51	Mannose
12	Astragalin	52	Myo-inositol
13	Butyric Acid	53	Neoolivil
14	Caffeic acid	54	Olivil
15	Ceramide	55	Pantothenic Acid
16	Chlorogenic Acid	56	phosphotidyl choline
17	Choline	57	Phosphatidylethanolamines
18	Cinnamic acid	58	Phosphatidylinositols
19	Citric Acid	59	Phosphoric acid
20	Coumarin	60	Protoporphyrin
21	Flavonol 3-O-glycoside	61	Quercetin
22	Folate (Folacin, Folic Acid)	62	Quinic Acid
23	Formic acid	63	Raffinose
24	Fructose	64	Rhamnose
25	Fucose	65	Rutin
26	Fumaric acid	66	Sanglifehrin A (SFA)
27	Galactinol	67	Scopoletin
28	Gamma-Aminobutyric Acid(gaba)	68	Secoisolariciresinol
29	Glucosamine	69	Serotonin
30	Glucose	70	Sinapic Acid
31	Glucuronic Acid	71	stachyose
32	Glyceric acid	72	Stigmast-4-en-3-one
33	Glycerol	73	Succinic Acid
34	Glycolic acid	74	Tartaric acid
35	Histamine	75	Threonic acid
36	Homovanillin	76	Vanillin
37	Homovanillyl alcohol	77	Violaxanthin
38	Isocitric acid	78	Zeatin
39	Isopentenyladenosine	79	Zeatin-O-glucoside
40	Isoquercitrin		

Table.2: Docking score of FtsZ inhibitors collected from literature

S.No	Inhibitors	Affinity (K cal/mol)
1	Albendazole	-5.8
2	Bis-Ans	-7.9
3	Sanguinarine	-8.1
4	Thiabendazole	-5.6
5	Zantrin1	-8.2
6	Zantrin2	-8
7	Zantrin3	-7.2
8	Zantrin4	-7.2
9	Zantrin5	-6.8

The average docking score of the above inhibitors is 7.2kcal/mol, respectively. Therefore, keeping in view the average score of experimentally tested compounds, criteria has been adopted to filter those compounds from the plant, which exhibit a dock score greater than 7.0kcal/mol.

Table.3: Docking score of *Urtica dioica* ligands with FtsZ protein

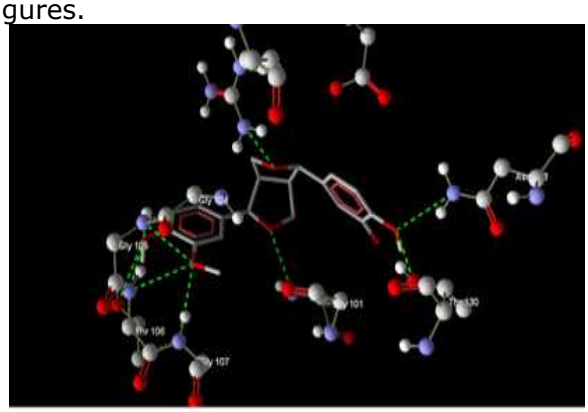
S.No	Compound Name	Affinity (K cal/mol)	S.No	Compound Name	Affinity(K cal/mol)
1	(-)-Pinoresinol	-8.5	41	Isorhamnetin	-7.8
2	(-)-Secoisolariciresinol	-6.9	42	Isorhamnetin-3-O-neohesperidoside	-8.8
3	(+)-Isolariciresinol	-7.2	43	Isorhamnetin-3-O-rutinoside	-8.4
4	5-Hydroxytryptamine	-6	44	Kaempferol	-7.7
5	Acetic Acid	-3.3	45	Kaempferol 3-O-glucoside	-8.3
6	Acetophenone	-5.1	46	Lutein	-7.6
7	Acetylcholine	-4.5	47	Lycopene	-7
8	Aesculetin	-6.8	48	Lysophosphatidylcholines	-6.2
9	Allantoic acid	-5.8	49	Malic acid	-4.8
10	Alpha-Tocopherol	-6.9	50	Maltose	-6.5
11	Arabinose	-4.8	51	Mannose	-5.7
12	Astragalin	-8.7	52	Myo-inositol	-5.4
13	Butyric Acid	-3.7	53	Neoolivil	-7.2
14	Caffeic acid	-6.6	54	Olivil	-8
15	Ceramide	-5.8	55	Pantothenic Acid	-6.2
16	Chlorogenic Acid	-8.4	56	Phosphotidyl choline	-6
17	Choline	-3.5	57	Phosphatidylethanolamines	-5.6
18	Cinnamic acid	-5.7	58	Phosphatidylinositols	-7.3
19	Citric Acid	-5.9	59	Phosphoric acid	-3.4
20	Coumarin	-6.3	60	Protoporphyrin	-8.4
21	Flavonol 3-O-glycoside	-7.7	61	Quercetin	-8
22	Folate (Folacin, Folic Acid)	-9.1	62	Quinic Acid	-6.1
23	Formic acid	-2.8	63	Raffinose	-8.3
24	Fructose	-5.2	64	Rhamnose	-5.2
25	Fucose	-5.3	65	Rutin	-9.7
26	Fumaric acid	-4.4	66	Sanglifehrin A (SFA)	-6.9
27	Galactinol	-7.3	67	Scopoletin	-6.5
28	Gamma-Aminobutyric Acid(gaba)	-4.1	68	Secoisolariciresinol	-7.6
29	Glucosamine	-5.3	69	Serotonin	-5.9
30	Glucose	-5.6	70	Sinapic Acid	-6.2
31	Glucuronic Acid	-5.6	71	Stachyose	-7.9
32	Glyceric acid	-4.3	72	Stigmast-4-en-3-one	-7.4
33	Glycerol	-3.8	73	Succinic Acid	-4.7
34	Glycolic acid	-3.6	74	Tartaric acid	-5.2
35	Histamine	-4.2	75	Threonic acid	-4.7
36	Homovanillin	-5.5	76	Vanillin	-5.2
37	Homovanillyl alcohol	-5.4	77	Violaxanthin	-7.5
38	Isocitric acid	-6	78	Zeatin	-6.5
39	Isopentenyladenosine	-8	79	Zeatin-O-glucoside	-8.1
40	Isoquercitrin	-8			

The average affinity of the inhibitors taken from the literature was 7.2kcal/mol. From the above plant compounds, the compounds showing affinity more than 7.0kcal/mol were taken as the best compounds such as –(-)pinoresinol, isorhamnetin-3-o-neohesperidoside, Lutein, Olivil, Rutin respectively.

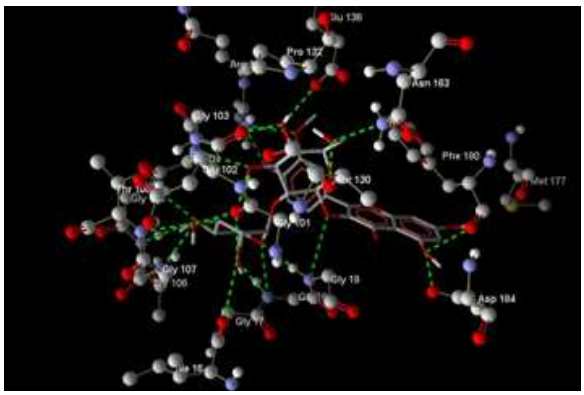
Table.4: The hydrogen bonding interactions of *Urtica dioica* ligands

S.NO	LIGAND	SCORE	NO. OF INTERACTING RESIDUES	INTERACTING RESIDUES
1	(-)-PINORESINOL	-8.5	10	ND2-ASN 163, OG1-THR 130,N-GLY 19,N(2)-GLY105,N (2)-THR 106,OG1-THR106,N-GLY 107, NH1-ARG 140
2	ISORHAMNETIN-3-O-NEOHESPERIDOSIDE	-8.8	21	OG1-THR130,N-THR106,N-THR 108,ND2-ASN163,NH1-ARG140,NH2-ARG140,OD1-ASP184,O-GLU102, OE2-GLU136,N(2)-GLY 18,N (2)-GLY 19,O(3)-GLY 101,N-GLY 104,O-GLY 105,N-GLY 107,O-LLE 16,O-PHE 180.
3	LUTEIN	-7.6	1	O-ASN 25
4	OLIVIL	-8	9	N-ALA 68, NH1-ARG 140, NH2-ARG 140, ND2-ASN 163, O-GLY 101, N-GLY 104, N-THR 106, OG1-THR 106, OG1-THR 130.
5	RUTIN	-9.7	18	N (2)-ALA 68,N (2)-ALA 70,NH1(2)-ARG 140,NH2-ARG 140,ND2-ASN 163,N-GLY 18,N-GLY 69,O(2)-GLY 101,N-GLY 104,N-GLY 105,OG1-THR 42,OG1-THR 106,OG1(2)-THR 130.

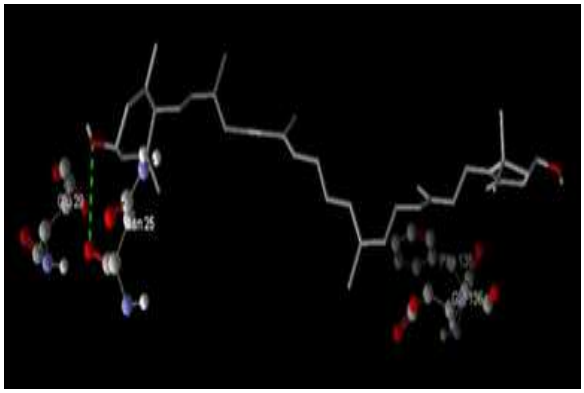
Among all the H bonding interacting residues Arg 140 and Gly 105 amino acid residues appeared more number of times in each case, hence, it can be suggested that these two residue interactions with any FtsZ inhibitor would possess high affinity and should be regarded as an important parameter that should be assessed during docking studies. The hydrogen bond interactions are shown in the following figures.



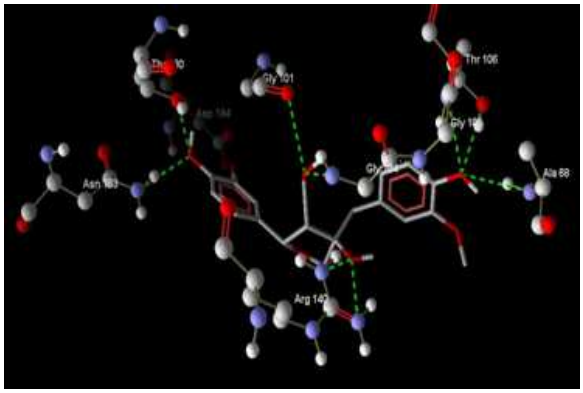
– (-) pinoresinol



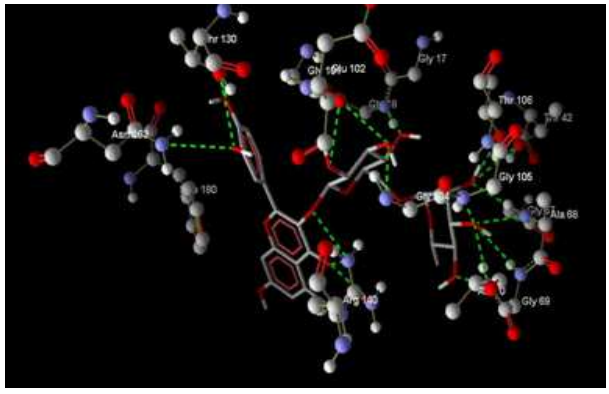
Isorhamnetin-3-O-Neohesperidoside



Lutein



Olivil



Rutin

Figure.4: H-bonding interactions (green colored) between best selected ligands and FtsZ amino acid residues.

Conclusion

Protein interactions with ligands can help X-ray crystallography and structural genomic projects to overcome their limitations and provide experimentalists with clues for the identification of the proteins potential function. Screening methods are routinely and extensively used to reduce cost and time of drug discovery. In this study of docking analysis, screening various compounds from the plant *Urtica dioica* is reported based on docking analysis against *Mycobacterium tuberculosis* FtsZ using Auto Dock Vina software. Based on rank-sum technique, revealed 5 best mycobacterial FtsZ inhibitors from the plant under study. Hydrogen bond interactions analyzed for top scoring compounds revealed Arg 140 and Gly 105 residue interactions and it has been hypothesized that any FtsZ inhibitor that possess these two H-bond interactions would provide high affinity and shall be regarded as an important parameter during docking studies. Finally, the work justifies that with minimal effort, computational techniques can be used to reduce the time spent in synthesizing compounds and further experimental procedures on these 5 identified novel compounds would lead to potent compounds and provides a way to screen various plant compounds towards identifying and achieving best inhibitory properties. The future scope of our work towards creating a novel treatment against the *Mycobacterium tuberculosis.*

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