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IN SILICO STUDIES OF *JUSTICIA ADHATODA, OCIMUM SANCTUM* PLANT COMPOUNDS AS *MYCOBACTERIUM TUBERCULOSIS* FTSZ INHIBITORS

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Abstract: Protein-ligand docking analysis was carried out using AutoDock Vina on 61 compounds from two different plants, *Justicia adhatoda* and *Ocimum sanctum* 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 61 compounds from two different plants, a final set of compounds were selected by filtering compounds that showed dock scores greater than 7.0kcal/mol. Following this criteria, 10 compounds each from *Justicia adhatoda* and *Ocimum sanctum* were finalized. In the next step, consensus scoring was employed to study the importance of various scoring functions available in other docking software's such as Molegro, GOLD, Patch dock and MEDock respectively. From the scoring generated based on rank-sum technique, Anisotine, Betasitosterol Beta-D glucoside, Lyoniside from *Justicia adhatoda*, Rosmarinic acid, Stigmasterol, Ursolic acid from *Ocimum sanctum* were found to be the best inhibitors of FtsZ protein.

Keywords: Virtual Screening, FtsZ protein, Molecular Docking, ME Dock, *Justicia adhatoda*, *Ocimum sanctum*,

INTRODUCTION

Tuberculosis is a common infectious disease caused by mycobacterium, in humans, mainly *Mycobacterium tuberculosis* [1]. According to WHO, Tuberculosis (TB) remains the leading cause of death worldwide from a single infectious disease agent. Indeed up to 1/2 of the world's population is infected with TB [2]. Multidrug-resistant TB (MDR-TB) is a form of TB that does not respond to the standard treatments using first line drugs. Development of drugs which display lasting anti mycobacterium activity in vivo is desirable. Since they can be administered with long intervals and consequently facilitate directly observed therapy and enhance patient compliance. There are a number of constraints that have deterred companies from investing in new anti-TB drugs [3]. Development of novel antituberculosis compounds to combat TB is needed.

Taking into consideration of protein in this current study FtsZ-Filamentation temperature sensitive protein Z is an essential bacterial cell division protein bacterial tubulin homologue [4]. It involves in the formation of cytokinetic ring and follows recruitment of other cell division proteins result the division of cell into two. Since inactivation of FtsZ or alteration of FtsZ assembly results in the inhibition of cell division. It is a very promising target for new antimicrobial drug development. FtsZ contains four main protein domains, as determined by the crystal structure of FtsZ from the thermophilic bacterium Thermotoga maritima and by Phylogenetic analysis [5].

Advances in computational techniques have enabled virtual screening to have a positive impact on the drug discovery process. In ligand-based virtual screening, the strategy is to use information provided by a compound or set of compounds that are known to bind to the desired target and to use this to identify other compounds in the corporate database or external databases with similar properties. When the structure of the target protein known, receptor-based computational methods can be employed. The majority of biological processes are well-known through protein-ligand interactions [6]. The three dimensional structure of the protein-ligand composite could be serve as a considerable source understanding the way of proteins interact with another and perform biological functions. Virtual Screening based studies on molecular level have become an integral part of many modern structure-based discovery efforts [7]. An advantage of this technique is that based on the predicted binding affinity data. Therefore activities can be quantified in a biochemical assay thereby reducing the time and expenditure in identifying new leads [8][9].

MATERIALS AND METHODS

Virtual screening: It is an Insilco tool for drug designing and a new approach attracting increasing levels of interest in the pharmaceutical industry as a productive and cost-effective technology in the search for novel lead compounds and widely used for lead identification in drug discovery programs [10][11].

Protein-ligand docking: Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design [12]. The goal of ligand and protein docking is mainly to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure.

Molegro Virtual Docker: It is an integrated platform for predicting protein -ligand interactions and it handles all aspects of the process, from preparing the molecules to determining the potential binding site of the target protein and predicting the binding mode of the ligand [13] It offers high-quality docking based on a novel optimization technique combined with a user interface experience.

AutoDock Vina: It is a new program for virtual screening and docking in drug discovery [14]. It offers multi-core capability, high performance, enhanced accuracy and ease of use. For its input and output, Vina uses the same PDBQT molecular structure file format used by AutoDock. PDBQT files can be generated and viewed using MGL Tools.

Patch Dock: Patch Dock is an algorithm for molecular docking. The input is two molecules of any type: proteins, DNA, peptides, drugs. The output is a list of potential complexes sorted by shape complementarity criteria [15]. It is inspired by object recognition and image segmentation techniques.

ME Dock: Maximum-Entropy based Docking web server is aimed at providing an efficient utility for prediction of ligand binding site [16]. A major distinction in the design of ME Dock is that its global search mechanism is based on a novel optimization algorithm that exploits the maximum entropy property of the Gaussian distribution.

GOLD: Genetic Optimization for Ligand Docking is a genetic algorithm for protein-ligand docking to investigate the rotational flexibility of receptor hydrogen's and ligand conformational flexibility including protein side chain with ability to dock into multiple models of the same or different proteins [17].

Consensus scoring: In general, docking programs calculate the protein-ligand complex structures with accuracy and speed. On the other hand, combinations of different scoring functions would reduce the error in single scoring schemes and then advance the probability of identifying true hits [18]. Therefore, it has been demonstrated that consensus scoring is generally effective way in getting improved hit rates in various virtual database screening studies than single scoring for molecular docking.

METHODOLOGY

In this study, the structures were drawn by using ISIS/Draw, a chemical structure drawing program for Windows [19]. By using Tsar's easy-to-use chemical spreadsheet interface (www.accelrys.com) the limits for ligands was observed and converted 2D structures to 3D with physicochemical properties to analyze and promote activity. When FtsZ was searched in the Protein Data Bank, 42 structural hits were obtained. Out of which 5 FtsZ entries are from *Mycobacterium tuberculosis*. The Root Mean Square Deviation [20] value was checked for the remaining proteins in AutoDock vina software. The protein 2Q1Y got the RMSD value 1.15 A°. Hence it is considered for the further studies.

Two plants were selected based on the studies reported in literature such as *Justicia adhatoda*, *Ocimum sanctum* followed by their compounds extracted from Duke's ethno botany database [21]. It has been well-documented in various literature sources that *Justicia adhatoda* plant was studied towards identifying potential anti-tubercular agents. However, little information was found on *Ocimum sanctum* plant. In this study, two plants were considered to study the effect of representative compounds against *Mycobacterium tuberculosis* FtsZ inhibition computationally. These two plants were known to inhibit bacterial proliferation. Hence, computational techniques were employed to evaluate the compounds present in each plant towards *Mycobacterium tuberculosis* FtsZ inhibition using AutoDock vina software. Before docking, the protein 3D structure from Protein Data Bank was validated and the runs were performed thrice for reproducibility.

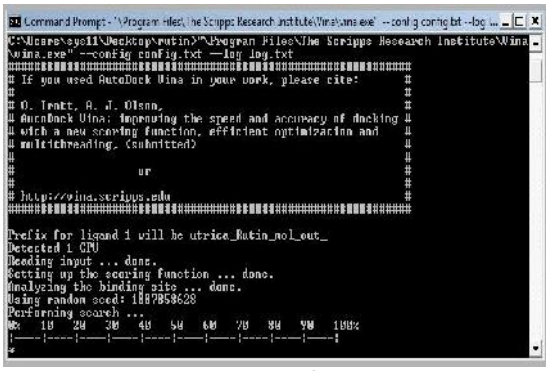

Figure 1: Image showing the running of docking

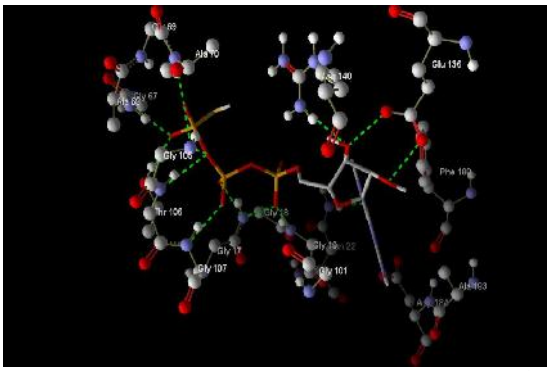

Figure 2: H- bonding interactions between original ligand (GTP-Gamma-S) and FtsZ amino acid residues. Green colored lines indicate hydrogen bonds.

Table 1: List of compounds present in *Justicia adhatoda* plant.

1	Adhatodine	15	Peganidine
2	Anisotine	16	Peganine
3	Arachidic acid	17	Scopolamine
4	Ascorbic Acid	18	Scopoline
5	BehenicAcid	19	taraxerol
6	Beta Carotene	20	3-Alpha-Hydroxy-D-Friedoolean
7	Beta Sitosterol	21	Vasicine
8	Betaine	22	Vasicinol
9	Beta-Sitosterol-beta-D-glucoside	23	Vasicinolone
10	Cerotic acid	24	Vasicinone
11	Deoxyvasicinone	25	Vasicol
12	Lignoceric acid	26	Vasicoline
13	Linoleic Acid	27	Vasicolinone
14	Lyoniside (daucosterol)		

Table 2: List of compounds present in *Ocimum sanctum* plant

1	(E)-beta-ocimene	18	Linalol
2	Alpha-Bisabolene	19	Linalool
3	Alpha-Humulene	20	Linoleic Acid
4	Alpha-Linolenic Acid	21	Luteolin
5	Alpha-pinene	22	Methyleugenol
6	Apigenin	23	Ocimarin
7	Beta-bisabolene	24	Oleanolic Acid
8	Bornyl acetate	25	Palmitic Acid
9	Campesterol	26	Rosmarinic acid
10	Carvacrol	27	ß-sitosterol
11	Cineole	28	ß-carotene
12	Cirsilineol	29	Stearic acid
13	Cirsimaritin	30	Stigmasterol
14	Citral	31	Thymol
15	Elemene	32	Ursolic acid
16	Eugenol	33	vanillic acid
17	Germacrene D	34	Xylose

RESULTS AND DISCUSSION

Docking analysis was done by using AutoDock Vina. At the end of each run, docked orientations were saved and the resultant molecules are checked for geometry.

Table 3: Results of original protein ligands interactions on vina with various scores

S.NO	PDB ID	RUN1 (kcal/mol)	RUN2 (kcal/mol)	RUN3 (kcal/mol)
1.	2Q1Y	1.151	1.452	1.434

Table 4: 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 all the two plants, which exhibit a dock score greater than 7.0kcal/mol.

Table 5: Docking score of the *Justicia adhatoda* ligands with FtsZ

S.No	Compound name	Affinity (Kcal/mol)
1	Adhatodine	-7.8
2	Anisotine	-8.2
3	Arachidic acid	-4.5
4	Ascorbic Acid	-6
5	BehenicAcid	-4.9
6	Beta Carotene	-6.2
7	Beta Sitosterol	-6.7
8	Betaine	-4
9	Beta-Sitosterol-beta-D-glucoside	-7.8
10	Cerotic acid	-4.2
11	Deoxyvasicinone	-6.7
12	Lignoceric acid	-4.7
13	Linoleic Acid	-5.5
14	Lyoniside (daucosterol)	-7.1
15	Peganidine	-7.1
16	Peganine	-6.6
17	Scopolamine	-7.2
18	Scopoline	-4.7
19	taraxerol	-6.9
20	3-Alpha-Hydroxy-D-Friedoolean	-7.6
21	Vasicine	-5.8
22	Vasicinol	-5.8
23	Vasicinolone	-6.7
24	Vasicinone	-7
25	Vasicol	-6.6
26	Vasicoline	-7.5
27	Vasicolinone	-7.7

Table 6: Docking score of *Ocimum sanctum* ligands with FtsZ protein

S.No	Compound Name	Affinity (Kcal/mol)
1	(E)-beta-ocimene	-4.7
2	Alpha-Bisabolene	-5.9
3	Alpha-Humulene	-6.1
4	Alpha-Linolenic Acid	-5.6
5	Alpha-pinene	-4.8
6	Apigenin	-7.6
7	Beta-bisabolene	-6
8	Bornyl acetate	-5.1
9	Campesterol	-6.9
10	Carvacrol	-5.7
11	Cineole	-4.8
12	Cirsilineol	-7.3
13	Cirsimaritin	-7.3
14	Citral	-4.9
15	Elemene	-5.7
16	Eugenol	-5.4
17	Germacrene D	-5.9
18	Linalol	-4.5
19	Linalool	-4.6
20	Linoleic Acid	-5.7
21	Luteolin	-7.8
22	Methyleugenol	-5.3
23	Ocimarin	-7.3
24	Oleanolic Acid	-7.3
25	Palmitic Acid	-5.1
26	Rosmarinic acid	-7.9
27	ß-sitosterol	-6.7
28	ß-carotene	-7
29	Stearic acid	-5.2
30	Stigmasterol	-8.6
31	Thymol	-5.4
32	Ursolic acid	-7.8
33	vanillic acid	-5.4
34	Xylose	-5.1

From the above plant compounds, the compounds showing affinity more than 7.0kcal/mol were taken as the best compounds. The best compounds from each of the above plants are shown below.

Table 7: *Justicia adhatoda* ligand scores obtained from different docking softwares.

S. No.	Compound Name	Vina (kcal/mol) (kcal/mol)	Molegro (kcal/mol)	GOLD (kcal/mol)	Patchdock (kcal/mol)	Medock (kcal/mol)
1	Adhatodine	-7.8	-112.625	26.37	4590	-9.63
2	Anisotine	-8.2	-115.507	41.53	4522	-9.81
3	Beta sitosterol beta D glucoside	-7.8	-125.541	-186.88	6342	-8.3
4	Lyoniside	7.1	-143.186	-40.62	5820	-8.83
5	Scopolamine	-7.2	-98.364	32.53	4126	-9
6	3 alpha hydoxy D fridoolean	-7.6	-79.093	0	5256	-10.67
7	Peganidine	-7.1	-74.613	28.78	3626	-8.72
8	Vasicinone	-7	-88.219	31.57	3182	-22.76
9	Vasicoline	-7.5	-111.577	31.99	4282	-8.92
10	Vasicolinone	-7.7	-112.566	29.84	4382	-9.05

Table 8: *Ocimum sanctum* ligand scores obtained from different docking softwares

S. No.	Compound Name	Vina (kcal/mol)	Molegro (kcal/mol)	GOLD (kcal/mol)	Patchdock (kcal/mol)	Medock (kcal/mol)
1	Apigenin	-7.6	-104.125	17.81	3980	-9.97
2	Cirsilineol	-7.3	-109.986	34.79	4558	-11.46
3	Luteolin	-7.8	-102.017	39.65	3764	-10.39
4	Ocimarin	-7.3	-96.49	27.42	3388	-9.86
5	Cirsimaritin	-7.3	-87.472	33.78	4388	-9.98
6	Beta carotene	-7	-139.315	0	7334	-9.12
7	Oleanolic acid	-7.3	-64.976	0	5332	-49.3
8	Rosmarinic acid	-7.9	-113.779	-1.75	4150	-52.7
9	Stigmasterol	-8.6	-136.056	8.14	5338	-8.62
10	Ursolic acid	-7.8	-87.695	-55.75	5294	-49.61

From the above analysis, the following compounds are found as best active compounds derived based on rank sum technique [22]. From *Justicia adhatoda*, Anisotine, Beta-sitosterol Beta-D glucoside, Lyoniside and from *Ocimum sanctum*, Stigma sterol, Rosmarinic Acid, Ursolic Acid respectively. For the above 6 compounds, the hydrogen bond interactions were obtained using Molegro software. 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.

CONCLUSION

Virtual Screening methods are consistently and extensively used to reduce cost and time of drug discovery. In this work, screening various compounds from two plants is reported based on docking analysis against *Mycobacterium tuberculosis* FtsZ protein using AutoDock vina software. Consensus scoring applied to retrieve best hits based on rank-sum technique revealed best compounds from two plants under study. The analysis has identified 3 novel compounds each from *Justicia adhatoda* and *Ocimum sanctum* with FtsZ inhibitors respectively. 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, based on our docking studies it can be stated that the identified novel compounds shall act as effective anti-tubercular agents, as they were not studied till date towards anti-tubercular activities experimentally. Therefore, the proposed study provides a way to screen various plant compounds towards identifying and achieving best inhibitory properties.

REFERNCES

1. Kumar Vinay, Abbas Abul K, Robbins Basic Pathology. 8th ed. Saunders. Elsevier. pp. 516-522. 2007.
2. World Health Organization (WHO). Tuberculosis Fact sheet N°104- Global and regional incidence. March 2006.
3. Tomioka H, Namba K, Development of anti-tuberculosis drugs: Current status and future prospects. 2006 Dec; 81(12):753-74.
4. Erickson, HP FtsZ, a prokaryotic homolog of tubulin? Cell 80, 367–70.
5. Vaughan S, Wickstead B, Gull K & Addinall SG, Molecular evolution of FtsZ protein sequences encoded within the genomes of archaea, bacteria, and eukaryote. J. Mol. Evol. 58, 19–29, 2004.
6. Jones G, Wilett P, Glen RC, Leach AR, Taylor R, Development and validation of a genetic algorithm for flexible docking. J Mol Biol. 267, 1997, 727-748.
7. Paul D. Lyne, Structure-based virtual screening: an overview. DDT Vol. 7, No. 20 October 2002 1047-1055.
8. Rarey M, Kramer B, Lengauer T, Klebe G, A fast flexible docking method using an incremental construction algorithm. J Mol Biol. 261, 1996, 470-489.
9. Morris, GM, Good sell DS, Halliday R, Huey R, Hart WE, Belew RK, Arthur,J. J Comput Chem. 19, 1998, 1639-1644.
10. Shen J, Xu X, Cheng F, Liu H, Luo X, Shen J, Chen K, Zhao W, Shen X, Jiang H, Virtual screening on natural products for discovering active compounds and target information. Curr Med Chem. 10, 2003, 2327-2342.
11. Schapira M, Abagyan R, Totrov M, Nuclear hormone receptor targeted virtual screening. J Med Chem. 2003; 46:3045–3059.
12. W Walters, Drug Discovery Today, 1998, 3:160.
13. R Thomsen, MH Christensen, J. Med. Chem. 2006, 49, 3315–3321.
14. O Trott, AJ Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry 2009.
15. Schneidman-Duhovny D, Inbar Y, Polak V, Shatsky M, Halperin I, Benyamini H, Barzilai A, Dror O, Haspel N, Nussinov R, Wolfson HJ. Taking geometry to its edge: fast unbound rigid and hing e-bent docking. Proteins, 2003, Jul 1; 52(1): 107-112.
16. DTH Chang, YJ Oyang, JH Lin, MEDock: a web server for efficient prediction of ligand binding sites based on a novel optimization algorithm. Nucleic acids research, 33, 2005, W233 -W238.
17. Jones G, Willet P, Glen RC, Leach AR, Taylor R, Development and Validation of a Genetic Algorithm for Flexible Docking. J. Mol. Biol. 1997, 267:727-748.
18. Kitchen DB, Decornez H, Furr JR, 2004, docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov 3: 935-949.
19. Li Z, Wan H, Shi Y, Ouyang P, 2004, Personal Experience with Four Kinds of Chemical Structure Drawing Software: Review on ChemDraw, Chem Window, ISIS/Draw, and ChemSketch. J. Chem. Inf. Comput. Sci. 44 (5): 1886–1890.
20. Coutsias EA, Seok C, Dill KA, 2004, using quaternions to calculate RMSD. J Comput Chem 25 (15): 1849–1857.
21. http://www.ars-grin.gov/duke/
22. Honglin Li, Hailei Zhang, Mingyue Zheng, An effective docking strategy for virtual screening based on multi-objective optimization algorithm. BMC Bioinformatics. 2009; 10: 58.

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