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Research Article

IDENTIFICATION OF NOVEL HIGH-AFFINITY CYTOPLASMIC ASPARAGINYL-TRNA SYNTHETASE INHIBITORS USING DOCKING AND MOLECULAR SIMULATION

RAHUL SINGH[1], TAZMEEN SHIRAZI[2]*

[1]Novo Informatics Pvt. Ltd., Gulmohar Community Center, New Delhi - 110 049, India. [2]Research Analyst, Email: Tazmeen.shirazi@novoinformatics.com *Received: 04 May 2015, Revised and Accepted: 18 May 2015*

ABSTRACT

Objective: To identify potential molecule, which can act as inhibitor for target asparaginyl tRNA synthetase (AsnRS) to treat Lymphatic filariasis.

Methods: Computational tools used in identifying potential molecule using ZINC pharmer. Consensus docking approach followed to validate the poses and to remove any false positives from the pool of the molecule screened. Molecules docked in high throughput manner using Vina and top 10% of highest scoring molecules selected which shows less than 2Å RMSD difference in the top pose predicted by both Autodock4 and Vina. Molecular dynamics simulations performed on molecules showing best interaction based on their binding energy and hydrogen bond formation.

Results: Eleven molecules identified which act as potential hit for the AsnRS to treat Lymphatic filariasis.

Conclusion: We were able to identify potential hit molecules for Lymphatic filariasis. These molecules seem suitable candidate to undergo in-vitro testing.

Keywords: Docking, Filariasis, Virtual screening, asparaginyl tRNA synthetase.

INTRODUCTION

At present, nearly one billion people harbor at least on worm infection (nematodes and platy-helminths) [1] and many individuals are simultaneously infected with multiple parasites from distantly related eukaryotic phyla [2]. Most of the tropical diseases were neglected by most of the pharma giants. A large number of people are suffering from lymphatic filariasis (LF) and there is an immediate need to suppress this disease. As per recent estimates, the LF infection is endemic in 83 countries with more than 1.33 billion people at risk and 120 million already infected. India accounts for approximately 67% of the 700 million people at risk from LF in the Southeast Asian region. Out of 60 million persons harboring microfilaremia (mf) or suffering from clinical manifestations of the disease, approximately 82% are from India. A total of 37 million people are in countries across Africa and Latin America, and more than 99% of the estimated population of onchocerciasis occurs in Sub-Saharan Africa. 500,000 of those infected with onchocerciasis are severely visually impaired, and another 270,000 have been rendered permanently blind.

No human-licensed vaccine exists for any eukaryotic disease; therefore drugs are a major component of intervention against most parasitic diseases [3]. Drug-based strategies include treatment of known or verified infection, mass drug administration to presumptive infected communities or individuals at risk of infection.

Most antibiotics that target protein translation interact with microbial ribosomes themselves - binding directly to the rRNA or ribosomal subunit proteins. Aminoacyl-tRNAsynthetase (aaRs) was a well-validated target antimicrobial therapeutics. The enzyme of this family catalyzes the binding or attachment of their cognate tRNA to produce the aminoacyl tRNA that are substrates for translation [4].

There are several reasons to support protein translation mechanism as an anti-filarial drug target and specifically aaRSs. First is the dependence of many parasites on abundant protein translation in fast growing cells. Because many parasites constitutively undergo active and constant proliferation and they are heavily reliant on efficient protein translation and might be sensitive to disruption of translation machinery. The second most important aspect of parasite protein translation which makes it a distinct plausible drug target is the immense evolutionary distance between the process in some parasites and human host. Several parasites have bacterial-like protein translation pathways that are not shared by humans [5,6].

We are targeting the *Brugia malayi* asparaginyl tRNA synthetase (AsnRS) for drug development against filariasis. It was an essential enzyme in protein synthesis expressed in both sexes of the nematode and in several stages of life cycle [7]. AsnRS is also specifically associated with chemokine activity toward human cells that may play a role in the massive inflammatory response associated with LF [8]. Here, in this study we had applied Pharmacophore based screening and structure-based drug design to identify a novel compound which can be potential drug candidate for LF.

Mechanism of action

Amino-acyl-tRNA-synthetase catalyze a two-step reaction where an ATP and amino acid molecule (AA) enter the active site, forming an aminoacyl-adenylate (Equations 1 and 2) intermediate, followed by the esterification of the AA to the 30 end of the tRNA, forming the final "charged" amino-acyl-tRNA [9,10].

$$AA + ATP + AARS \Leftrightarrow AARS.AA\text{-}AMP + PPi \quad (1)$$

$$AARS.AA\text{-}AMP + tRNA \Leftrightarrow AARS + AA\text{-}tRNA + AMP \quad (2)$$

Based on the information obtained from Equations 1 and 2 several sites will be present on the aaRSenzymes, which can be used for drugging purposes; a binding pocket for ATP, AAs or aminoacyl-adenylate intermediates.

In this study, we had done a pharmacophore based computational ligand screening using Zinc Pharmer [11]. The X-ray crystal structure used for screening was PDB-ID 2XTI in complex with a non-hydrolysable analog of asparginyl adenyltae [12].

MATERIALS AND METHODS

Structure selection

The structure of the asparginyl t-rna synthetase (PDB-ID: 2XTI) of *B. malayi* was selected for virtual screening studies (Fig. 1).

Pharmacophore based virtual screening

Zinc Pharmer was used for screening of molecules from zinc database [13]. Zinc Pharmer a Pharmacophore based search tool, which identify Pharmacophore feature directly from the crystal structure. Pharmacophore features were selected in such a way so that entire scaffold of the molecule should be covered with the default parameters selected by Zinc Pharmer. The PDBID used for screening was 2XTI and the bound ligand selected was NB8 (5'-O-[(R)-{[(2S)-2-amino-4-(hydroxyamino)-4oxobutanoyl] oxy}(hydroxy) phosphoryl] adenosine). Root-mean-square deviation (RMSD) cut-off of less than 0.5 Å was given in the Zinc Pharmer tool, a total of 4133 molecules were screened out as first stage potential hits or molecules by Zinc Pharmer which can show good binding affinity with the receptor. These selected molecules were docked in a high throughput manner with Autodock4 [14] and VINA [15].

Docking analysis

Before starting the docking simulation for any receptor-ligand complex, we need to validate the parameters used in the docking protocol, which can reproduce the native pose under 2 Å RMSD. Autodock4 was used to find the grid centers and x-y-z spacing on which the given search function genetic algorithm was applied and only those set of parameters will be selected which can reproduce the native pose under 2 Å RMSD as compared to docked pose (Fig. 2).

The process of docking protocol fixation was also done for VINA using the same procedure as mentioned for Autodock4. Once the docking protocol was fixed for both Autodock4 and VINA, screened molecules were docked in a high throughput mode using VINA to select top 10% molecules based on their binding energy of the ligands as VINA is comparatively faster than Autodock4. These top 10% molecules highest negative binding energy pose was selected as seed structure for docking simulations in Autodock4.

All the 4133 compounds were docked using VINA to the A chain of PDB-ID 2XTI, these molecules were re-docked using Autodock4 and checked for the difference in the binding energy. Only those molecules were selected for interaction and pose analysis whose difference in the binding energy was under ±1 kcal/mol and the RMSD calculated between the top pose of VINA and Autodock was less than 2 Å [16]. After applying this RMSD cut-off value, we have 11 compounds.

Several comparative studies [17-21] had shown that no one scoring function for predicting ligand binding affinity performs consistently well across diverse protein families. Hence, to use a scoring protocol that can distinguish ligands from non-ligands and which can reliably identify the correct pose and binding mode. We had tested the predicted binding pose and predicted binding energy with two different scoring function one of Autodock VINA and Autodock4 Lamarckian genetic algorithm. The purpose of testing the binding pose on different tools is to remove the false positive from the potential hit pools after docking.

Molecular dynamics simulations

In this molecular simulation study, we used amber ff12SB [22] and gaff [23] force field to create topology file and am1bcc [24] method was used to calculate the charge. We run two stage energy minimization of our receptor-ligand complex. In the first stage, we minimized all the water positions keeping proteins positions fixed and in the second stage of minimization, the whole system was minimized. The method of minimization was switched from steepest descent to conjugate gradient. Once the minimization step was completed, we allowed the system to heat up from 0 K to 300 K running 50 ps molecular dynamics

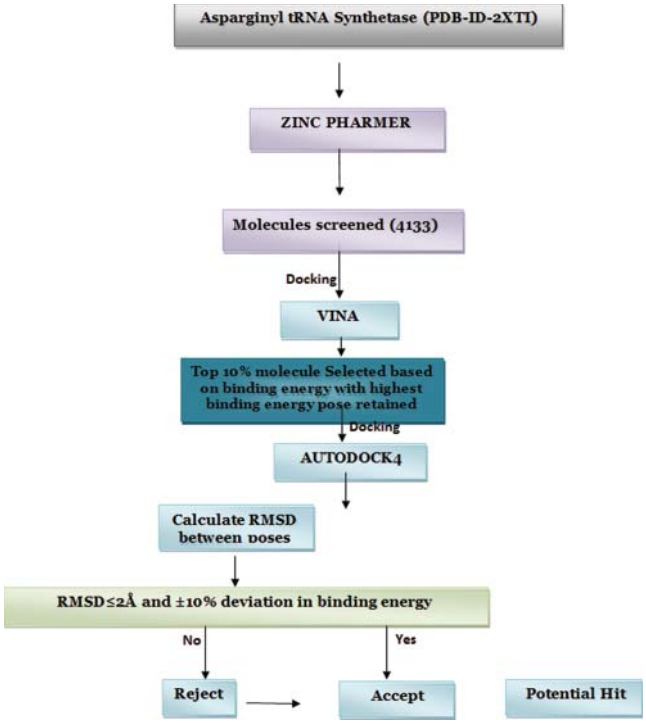

Fig. 1: Overall methodology flow chart

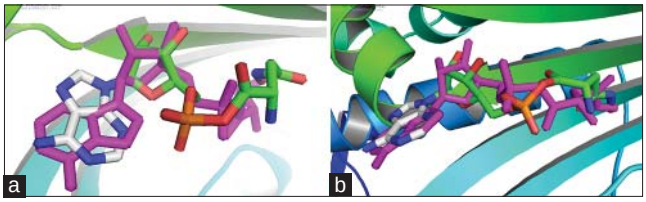

Fig. 2: (a) Docked pose of native ligand (NB8) Autodock VINA superimposed with X-ray crystal pose (root-mean-square deviation [RMSD] 1.3 Å) (b) Docked pose of native ligand (NB8) Autodock4.0 pose with the X-ray crystal pose (RMSD 0.67 Å)

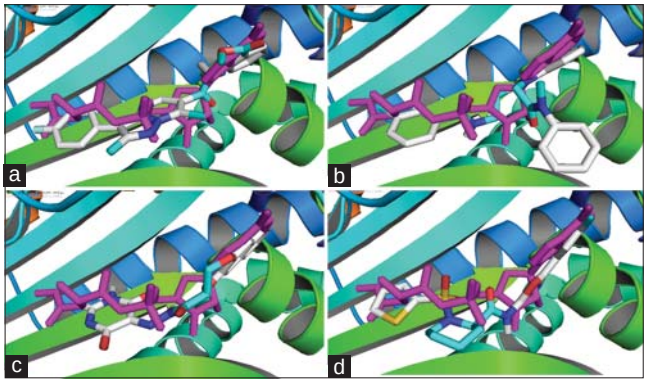

Fig. 3: Native ligand NB8 depicted in magenta color and the screened compound depicted in color by atom types. (a) Compound-1 (Zinc05650329) (b) Compound-2 (ZINC33039921), (c) Compound-3 (ZINC36682482) (d) Compound-4 (ZINC40201159)

with positioned restrained at constant volume. Before running the production stage, we equilibrated the system by running a 500ps molecular dynamics with positioned restrained at constant pressure. Once the equilibration step was completed, we checked Density, Total Energy and Pressure parameters so that they are converged and

Table 1: Ligand interaction table showing zinc IDs with lowest binding energy and residues involved in hydrogen-bond and pie-pie interaction

Zinc ID	Hydrogen-bond (residues)	Pie-pie interaction (residues)	Binding energy (Kcal/mol)
ZINC0138092	LYS-445-2, GLU-471, ARG-522, TYR-334, ARG-321	ARG-321, ARG-522, TYR-334	−10.92
Zinc05650329	HIS-336, GLY-474, GLU-471	TYR-334-2, ARG-522-2, ARG-321	−10.47
ZINC071794727	ARG-522, TYR-334, ARG-321	ARG-522, TYR-334, LYS-445	−11.1
ZINC09355732	ARG-321-2	ARG-522-2, TYR-334-2, ARG-321	−10.48
ZINC09668447	ARG-321-2	ARG-522-2, TYR-334-2, ARG-321	−10.77
ZINC31397455	NA	ARG-522-3, TYR-334-2	−9.3
ZINC33039921	ARG-478, TYR-334	ARG-321, ARG-522, TYR-334	−11.60
ZINC33144921	ARG-321, GLU-471	ARG-321, ARG-522-2, TYR-334	−9.88
ZINC36682482	GLU-471, ARG-321, GLY-519	ARG-522-2, TYR-334-2	−12.71
ZINC40201158	GLY-517, ARG-522	ARG-522-2, TYR-334-2, ARG-321	−10.70
ZINC40201159	TYR-334, ARG-321, GLY-517, ARG-522	TYR-334-2, ARG-522-2, ARG-321	−11.0
ZINC63447979	ARG-321, TYR-334-2	ARG-522, ARG-321	−10.81

Molecules highlighted green were top hits

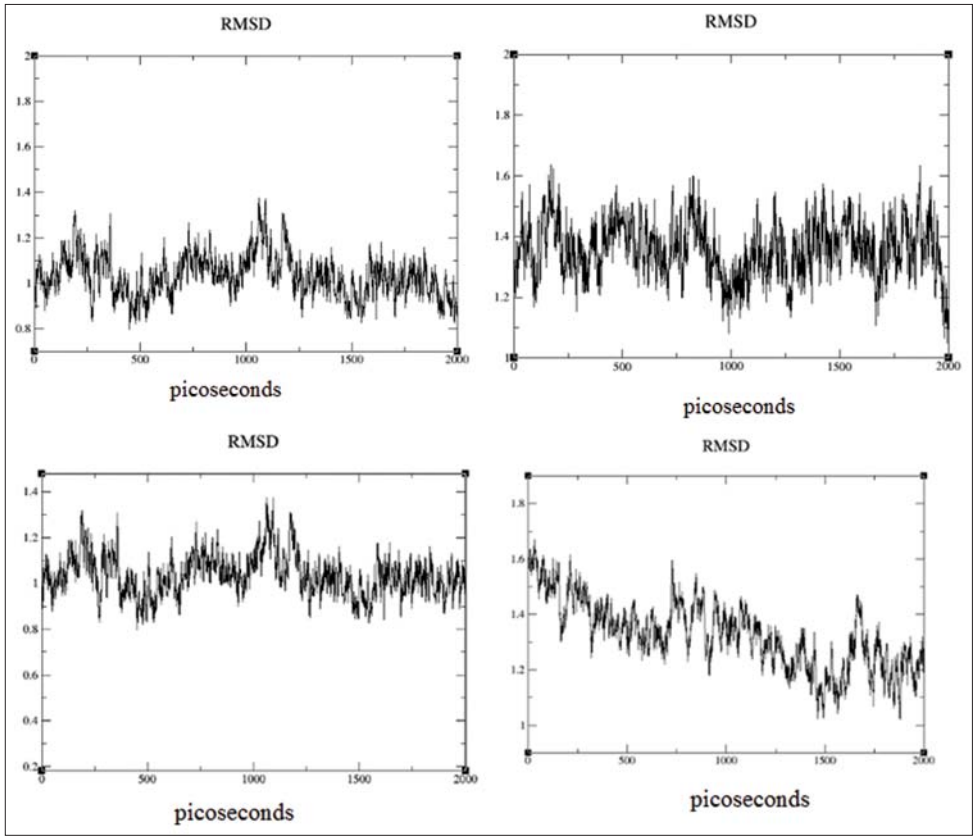

Fig. 4: Root-mean-square deviation plot of backbone atoms of top hits

reached near to a constant value. MD simulations for 4 ns were run using sander [25-27].

To ensure the dynamic stability of the MD trajectories and the difference instabilities of MD simulations we plotted the RMSD values of the protein backbone atoms relative to the initial minimized structure.

RESULTS AND DISCUSSION

Docking and pose analysis

Once docking studies were completed, we ranked compounds based on their binding energy and the receptor-ligand interaction. Apart from these considerations we gave more preference to those compounds which shows all the top 10 poses under 2 Å RMSD or all the top 10 poses fall in one cluster or the largest cluster of poses. The molecules which show less fluctuation in the top poses are considered to be most stable throughout the docking simulation.

The docked poses of the top four hits (green Table 1) were further analyzed and found that these molecules follow similar binding pattern as of the native ligand (2XTI). The superimposed pose (Fig. 3), which clearly indicates that docked pose is well occupied in the active site of Asparginyl t-RNA synthase and the binding pattern is similar to that of native ligand (2XTI).

Molecular dynamics simulations

To show the stability of the top hits in their receptor bound complex, MD simulation were performed for each complex. The dynamic nature of proteins is responsible for their numerous hidden biological functions

which can be revealed by studying their internal motions. Similarly, for accurate recognition of the drug binding pattern and to understand the interaction of protein receptor with drugs, it is important to consider.

To ensure the dynamic stability of the MD trajectories and the differences in stabilities of MD simulations, the RMSD values for the protein backbone atoms relative to the initial minimized structure through the phase of the simulation were calculated (Fig. 4).

Based on the RMSD plot of the top four compounds we can say that compound four shows minor fluctuations in the CA RMSD and all other compounds shows stable fluctuations in the RMSD. we conclude computationally that molecule one, two and three were most promising candidate, which can be tested in wet lab facility to further confirm the exact binding effect of the compound on the target AsnRS.

CONCLUSION

Various Insilico methods were employed to search for novel and selective inhibitors for AsnRS. The compound library was searched to select best virtual AsnRS inhibitors using different docking and virtual screening protocols. These protocols helped us to find four molecules as the best virtual dockers. These molecules shows pose similar to the native ligand by both the docking tools Autodock4 and vina, these molecules also shows a similar type of binding energy by both the tools. Molecular simulation study also reveals that these molecules were tightly bound in the active site. This molecule can go for *in vitro* testing for future studies.

REFERENCES

1. Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, McCarthy JS, *et al.* A research agenda for helminth diseases of humans: The problem of helminthiases. PLoS Negl Trop Dis 2012;6(4):e1582.
2. Fèvre EM, Wissmann BV, Welburn SC, Lutumba P. The burden of human African trypanosomiasis. PLoS Negl Trop Dis 2008;2(12):e333.
3. Prichard RK, Basáñez MG, Boatin BA, McCarthy JS, García HH, Yang GJ, *et al.* A research agenda for helminth diseases of humans: Intervention for control and elimination. PLoS Negl Trop Dis 2012;6(4):e1549.
4. Ibba M, Soll D. Aminoacyl-tRNA synthesis. Annu Rev Biochem 2000;69:617-50.
5. Jackson KE, Habib S, Frugier M, Hoen R, Khan S, Pham JS, *et al.* Protein translation in plasmodium parasites. Trends Parasitol 2011;27(10):467-76.
6. Pham JS, Dawson KL, Jackson KE, Lim EE, Pasaje CF, Turner KE, *et al*. Aminoacyl-tRNA synthetases as drug targets in eukaryotic parasites. Int J Parasitol 2014;4(1):1-13.
7. Nilsen TW, Maroney PA, Goodwin RG, Perrine KG, Denker JA, Nanduri J, *et al.* Cloning and characterization of a potentially protective antigen in lymphatic filariasis. Proc Natl Acad Sci U S A 1988;85(10):3604-7.
8. Kron M, Petridis M, Milev Y, Leykam J, Härtlein M. Expression, localization and alternative function of cytoplasmic asparaginyl-tRNA synthetase in Brugia malayi. Mol Biochem Parasitol 2003;129(1):33-9.
9. Ahel I, Korencic D, Ibba M, Söll D. Trans-editing of mischarged tRNAs. Proc Natl Acad Sci U S A 2003;100(26):15422-7.
10. Ali V, Nozaki T. Current therapeutics, their problems, and sulfur-containing-amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. Clin Microbiol Rev 2007;20(1):164-87.
11. Koes DR, Camacho CJ. Zinc Pharmer: Pharmacophore search of the ZINC database. Nucleic Acids Res 2012;40(Web Server issue):W409-14.
12. Crepin T, Peterson F, Haertlein M, Jensen D, Wang C, Cusack S, *et al.* A hybrid structural model of the complete *Brugia malayi* cytoplasmic asparaginyl-tRNA synthetase. J Mol Biol 2011;405(4):1056-69.
13. Irwin JJ, Sterling T, Mysinger MM, Bolstad ES, Coleman RG. ZINC: A free tool to discover chemistry for biology. J Chem Inf Model 2012;52(7):1757-68.
14. Trott O, Olson AJ. Auto dock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010 30;31(2):455-61.
15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. Autodock4 and auto dock tools4: Automated docking with selective receptor flexiblity. J Comput Chem 2009;16(16):2785-91.
16. Houston DR, Walkinshaw MD. Consensus docking: Improving the reliability of docking in a virtual screening context. J Chem Inf Model 2013;53(2):384-90.
17. Ferrara P, Gohlke H, Price DJ, Klebe G, Brooks CL 3rd. Assessing scoring functions for protein-ligand interactions. J Med Chem 2004;47(12):3032-47.
18. Perola E, Walters WP, Charifson PS. A detailed comparison of current docking and scoring methods on systems of pharmaceutical relevance. Proteins 2004;56(2):235-49.
19. Halperin I, Ma B, Wolfson H, Nussinov R. Principles of docking: An overview of search algorithms and a guide to scoring functions. Proteins 2002;47(4):409-43.
20. Stahl M, Rarey M. Detailed analysis of scoring functions for virtual screening. J Med Chem 2001;44(7):1035-42.
21. Bissantz C, Folkers G, Rognan D. Protein-based virtual screening of chemical databases 1. Evaluation of different docking/scoring combinations. J Med Chem 2000;43(25):4759-67.
22. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins 2006;65(3):712-25.
23. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general amber force field. J Comput Chem 2004;25(9):1157-74.
24. Jakalian A, Jack DB, Bayly CI. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. J Comput Chem 2002;23(16):1623-41.
25. Tejero R, Bassolino-Klimas D, Bruccoleri RE, Montelione GT. Simulated annealing with restrained molecular dynamics using CONGEN: Energy refinement of the NMR solution structures of epidermal and type-alpha transforming growth factors. Protein Sci 1996;5(4):578-92.
26. Dror RO, Dirks RM, Grossman JP, Xu H, Shaw DE. Biomolecular simulation: A computational microscope for molecular biology. Annu Rev Biophys 2012;41:429-52.
27. Lee EH, Hsin J, Sotomayor M, Comellas G, Schulten K. Discovery through the computational microscope. Structure 2009;17(10):1295-306.