International Journal of Pharmaceutics and Drug Analysis

Available at www.ijpda.com

ISSN: 2348:8948

Antifungal docking studies of *Anacyclus pyrethrum* (L.) and *Senna Obtusifolia* (L.) against candida albicans

Vinayak Surawar*¹, Sanket Kore².

School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded-431606, Maharashtra State, India

Received: 12 March 2021 Revised: 14 May 2021 Accepted: 18 May 2021

Abstract

Today there are many antifungal drugs and their formulations like a tablet, capsule, cream, ointment, gel, soap etc are present in the market many of them are effective in treatment in minor or severe cases of fungal infection caused due to different fungal or yeast species. But there are many side effects or adverse effects that occur which makes them unpleasant. This challenges to drug developers to make drug or formulations which are effective in the treatment and have no or minimum side effects. This challenge makes our eyes focus on natural chemical constituents, which are known to be having fewer side effects with effective treatment of disease. Here we have taken two receptors from the RCSB PDB database which is a structural component of *Candida Albicans* (1CZ1 and 1A19) proven to be the target of many antifungal drugs, both are responsible for different mode of action. The *Anacyclus pyrethrum* (L.) and *Senna Obtusifolia* (L.) have many chemical constituents having different pharmacological activity like anti-inflammatory, anti-cancer, etc. from these two plant species some chemical constituents are selected depending on their structural characteristics. After selection ligand-receptor molecular docking (by using Autodock Vina) was carried out between them. For its verification of antifungal activity, it is then compared with selected Standard drugs which have already clinically proved as an antifungal drug. Sesamine from *Anacyclus pyrethrum* (L.) and Obtusifoline and Physcion from *Senna Obtusifolia* (L.) are potent lead compounds which on future structural modification gives us desired antifungal activity.

Keywords: Antifungal, Autodock Vina, Candida Albicans, Anidualfungin, dihydrofolate reductase etc..

This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License. Copyright © 2021 Author(s) retain the copyright of this article.

*Corresponding Author

Vinayak Surawar

Email: vinayaksurawar@gmail.comDOI: <https://doi.org/10.47957/ijpda.v9i2.457>

Produced and Published by

South Asian Academic Publications

Introduction:

The folate biosynthetic pathway is an important and effective mechanism that serves as source for novel Antifungal. Historically Dihydrofolate reductase (DHFR), a well-characterized and important drug target for many antineoplastic, antibacterial drugs but has not been mostly validated as an antifungal target [1]. Here, we carried out some in-silico experiments with help of some software (Autodock Vina) of DHFR inhibits

growth of *Candida albicans* which is a major human fungal pathogen. Inhibition of the enzyme dihydrofolate reductase (DHFR) is a well known mechanism of action in currently available antifungal compounds. The aim of this study was to find out effective lead molecule against dihydrofolate reductase as a valid antifungal target. The exo-b-(1,3)-glucanase (Exg) (preproenzyme of 438 amino acids) from *Candida albicans* [2] and The metabolism of cell wall glucan by systemically removal of a glucose residue at the b-1,3-glucan end and b-1,6-glucan end (at smaller extent) causes disruption of cell wall of *Candida albicans* [3]. These two polysaccharides are the major and the important structural pillars of the cell wall of *C. albicans*. So it helps in the development of antifungal drugs by acting on these sites. Molecular docking plays an important role in structure-based drug designing, functional sites prediction on protein molecular surfaces, protein ligand docking etc.

Molecular docking attempts to predict the binding mode by evaluating the energy scores of different bound conformations with a scoring function. Ligand-binding sites are among the most promising targets for drug candidates, whose actions depend upon the inhibition or regulation of the target protein functions. Ligand based methods use the shape similarity concept while the structure based methods rely on scoring functions i.e., reverse/inverse docking against a panel of targets. From the ancient time in east side of the country, *Anacyclus pyrethrum* (L.) is commonly used plant species in the traditional Ayurvedic and Unani system of medication for the treatment of various disorders [4]. The present study further elaborate study of chemical constituent's information about its antifungal activity. *Senna obtusifolia* (L.) is old Chinese medicinal herb used for many health disorders from the ancient time which contains many chemical constituents which have different pharmacological activity ranging from antimicrobial, anti-inflammatory, antioxidants and many more [5].

Material & Methods

Protein preparation

The crystal structure of the candida albicans dihydrofolate reductase Fig. 1(A) (PDB ID: 1A19) and exo-beta-(1,3)-glucanase from candida albicans Fig. 1(B) (PDB ID: 1CZ1) were retrieved from the protein data bank with a resolution of 2.16 Å & 2.68 Å. the 3cl hydrolase enzyme has one chain with a total of 306 amino acids while structure of sars-cov-2 chimeric receptor-binding domain has two chain with total 597 amino acid [6]. Protein preparation was done by removal of ligand & adding the polar hydrogen. The polar hydrogen atoms & Kollman charges were added to the protein in order to correct the ionization and tautomeric states of the amino acid residues. Furthermore, the water molecules and complexes bound to receptor molecule were removed and converting it to PDBQT extension file before the docking.

Fig 01: (A) Crystal structure of the exo-beta-(1,3)-glucanase from candida albicans (pdb id: 1cz1), (B) Crystal Structure of candida albicans dihydrofolate reductase (pdb id: 1a19)

Ligand preparation

The structure of compounds was downloaded from drug bank database in pdb format [7]. These PDB formats of compound were then prone to energy minimization which avoids high energy conformations that leads to false result of binding to receptor. These natural chemical compounds and the standard drug used for the comparison were minimized by means of the steepest descent algorithm. The geometry optimization of these is done by Merck Molecular Force Field (MMFF 94) [8].

Ligand (Natural chemical compounds) selected from two plant species are of-[9]

1. *Anacyclus pyrethrum*:

1. Anacycline,
2. 2-Phenylethylamine
3. Pellitorine
4. Sesamine

2. *Senna obtusifolia*:

1. Obtusifoline
2. Physcion

Molecular Docking Process [10]

1. The molecular docking studies were performed using open source software (AutoDock Vina). AutoDock combines energy evaluation through pre-calculated grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein. Kollman united atom charges and polar hydrogens were added to the receptor PDB using AutoDock tools. The grid files of each target structure were generated to compute interactions between the binding pocket and ligands. Configuration file was created in text format where the entire grid dimensions, center points were added.
2. Binding site for the ligand was unknown hence blind docking is performed. Grid box included all amino acid residues of the receptor. The maximum grid box space is chosen for docking in order to maximize the active site hence possibility of finding lead molecule is more. Grid size are given in table no.01.
3. "Command prompt" was required for the

execution of the docking where each ligand molecule with each receptor were docked which gives energy values i.e. binding affinity which is used to calculate its effectiveness.

- The AutoDock Vina uses general functional form of the conformation-dependent part of the scoring function as below [11]:-

$$\sum_{i < j} \int t_i t_j (r_{ij})$$

Where, the summation is over all of the pairs of atoms that can move relative to each other, normally excluding 1-4 interactions, (i.e. atoms separated by 3 consecutive covalent bonds). Here, each atom i is assigned a type t_i ,

f_{ij} = a symmetric set of interaction functions

r_{ij} = interatomic distance.

Table 01: Grid Box co-ordinates & size parameters used in Autodock vina.

Receptor PDB ID	Number of points in			Centre Grid Box		
	X-dimension	Y-dimension	Z-dimension	X-centre	Y-centre	Z-centre
1CZ1	58	58	60	34.025	33.169	57.594
1A19	64	76	64	18.451	15.908	11.270

The standard docking protocol was applied which is based on the population size of 150 randomly placed individuals, a maximum number of 250000 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Twenty five independent docking runs were carried out for each inhibitor and cluster tolerance was kept at 1.0 Å [12]. After completion of docking, separate analysis of protein-ligand were done & docked pose with the least binding energy (kcal/mol) is selected. The least binding energy of final docked ligand structure is converted into pdb & study of their interaction were performed on pymol software [13] [14].

Result

Docking studies of 3D receptor of exo-beta-(1,3)-glucanase from *Candida albicans* (PDB ID: 1CZ1) and crystal structure of the *Candida albicans* dihydrofolate reductase (PDB ID: 1A19) with Six phytochemicals

revealed most of them have good binding affinity to the receptor as shown in the Fig. 2A, 2a to 2f and 3A, 3a to 3f. Sesamine exhibiting highest affinity {Binding energy of -11.4 kcal/mole against exo-beta-(1,3)-glucanase (1CZ1) and -9.1 kcal/mole against dihydrofolate reductase (PDB ID: 1A19)}. (As shown in the Table no. 02) Besides sesamine (from *Anacyclus pyrethrum*), Obtusifoline and Physcion (from *Senna obtusifolia*) shows good binding affinity against both receptor, exo-beta -(1,3)-glucanase from *Candida albicans* (PDB ID: 1CZ1) (-9.8 kcal/mol and -10.1 kcal/mol respectively) and dihydrofolate reductase of *Candida albicans* (PDB ID: 1A19) (-8.5 kcal/mol and -8.2 kcal/mol respectively) Although they show good binding affinity against receptor but not strong enough as standard drug taken for the comparison which have already proved effectiveness for antifungal activity, they are anidulfungin against exo-beta-(1,3)-glucanase from *Candida albicans* (PDB ID: 1CZ1) (-13.9 kcal/mol) which is far more than natural compounds taken for the experiments. Methotrexate which is taken as standard for activity against dihydrofolate reductase of *Candida albicans* (PDB ID: 1A19) shows binding affinity (-9.0 kcal/mol) whereas, sesamine shows -9.1 kcal/mol binding affinity which is approximately same as methotrexate and obtusifoline and Physcion (from *Senna obtusifolia*) shows (-8.5 kcal/mol and -8.2 kcal/mol respectively) which is also close to the standard drug.

Table 02: PDB ID of receptor, plant species of chemical constituents, chemical constituents (compound), binding energy carried out by autodock vina on chemical constituents of *Anacyclus pyrethrum* and *Senna obtusifolia* on *Candida albicans*.

Pdb ID	Plant species/category	Compound	Binding energy (kcal/mol)
1CZ1	<i>Anacyclus pyrethrum</i>	Anacycline	-7.0
		2-Phenylethylamine	-5.2
		Pellitorine	-6.3
		Sesamine	-11.4
	<i>Senna obtusifolia</i>	Obtusifoline	-9.8
		Physcion	-10.1
	Standard Drug	Anidulfungin	-13.9
	<i>Anacyclus</i>	Anacycline	-5.7

1AI9	<i>pyrethrum</i>	2-Phenylethylamine	-4.6
		Pellitorine	-5.3
		Sesamine	-9.1
	<i>Senna obtusifolia</i>	Obtusifoline	-8.5
		Phycion	-8.2
	Standard Drug	Methotrexate	-9.0

Graph 01: Binding affinity on exo-beta-(1,3)-glucanase (1CZ1) by standard drug aniduaifu and natural chemical constituents obtained from *Anacyclus pyrethrum* and *senna obtusifolia* on *candida albicans*.

Graph no. 02: Binding affinity on dihydrofolate reductase (1AI9) by standard drug methotrexate and natural chemical constituents obtained from *Anacyclus pyrethrum* and *senna obtusifolia* on *candida albicans*.

2 (A)

Fig 2A: Complex of DHFRase receptor (1AI9) and Methotrexate showing attachment position.

2a: 1AI9 -2Phenylethylamine complex

2b:1AI9 -Anacycline complex

2c: 1AI9 –Obtusifolin complex

2d:1AI9 –Pellitorine complex

2e: 1AI9 -Phycion complex

2f: 1AI9 -Sesamine complex

3 (A)

Fig 3A: Complex of α -D-glucanase (1CZ1) receptor and anidulafungin showing attachment position.

3a: 1CZ1-2Phenylethyamine complex

3b: 1CZ1-Anacycline complex

3c: 1CZ1-obtusifoline complex

3d: 1CZ1- Pellitorine complex

3e: 1CZ1- Physcion complex

3f: 1CZ1- Sesamine complex

Conclusion

We conclude that the docking study carried out with the natural chemical constituents Anacycline from *Anacyclus pyrethrum* and obtusifoline from *senna obtusifolia* shows good binding affinity as compared with Standard drug as shown in the Graph No. 1 and Graph No. 2 with given receptor, These proved to be a promising lead compound which could be potentially used as an

efficient drug for the treatment of antifungal disorder against fungal infection caused due to *candida albicans*. As these chemical constituents from natural origin they have low side effects and better effectiveness in terms of long term use in case of chronic disorder. It may have advantage over chemical drugs in case of immune-compromised condition.

Future Scope

As Docking is the first step of new drug development, structural modification with help of in-silico techniques of this natural compounds may show new chemical entity that have strong antifungal activity.

Conflict of interest

No Potential conflict of interest was declared by authors.

References

- DeJarnette C, Luna-Tapia A, Estredge LR, Palmer GE. Dihydrofolate reductase is a valid target for antifungal development in the human pathogen *Candida albicans* 2020; mSphere5: e00374-20.
- Chambers, R. S., Broughton, M. J., Cannon, R. D., Carne, A., Emerson, G. W. & Sullivan, P. A. An exo-b-(1,3)-glucanase of *Candida albicans*: purification of the enzyme and molecular cloning of the gene. J. Gen. Microbiol. 1993b; 139, 325-334.
- Stubbs, H. J., Brasch, D. J., Emerson, G. W. & Sullivan, P. A. Hydrolase and transferase activities of the b-1,3-exoglucanase of *Candida albicans*. Eur. J. Biochem. 1999; 263, 889-895.
- Afreen Usmani, Mohd Khushtar, Muhammad Arif, Mohd. Aftab Siddiqui, Satya PrakashSing, Md Mujahid. Pharmacognostic and phytopharmacology study of *Anacyclus pyrethrum*: An insight, Journal of Applied Pharmaceutical Science Vol. 6 (03) 2016; pp. 144-150.
- Doughari, J. H, El-mahmood, A. M. and Tyoyina, I. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L) African Journal of Pharmacy and Pharmacology Vol. 2(1), 2008; pp. 007-013.
- Bank, R. (2020). RCSB PDB: Homepage. Retrieved 8 November 2020, available from: <https://www.rcsb.org/>.
- DrugBank Pharmaceutical Knowledge Base API Integrations. 2020. Retrieved 8 November 2020, available from <https://www.drugbank.com/>.
- "Avogadro - Free Cross-Platform Molecular Editor". *Avogadro*, 2020, available from: <http://avogadro.cc/>.
- Dr. Duke's Phytochemical and Ethnobotanical Databases, U.S. Department of Agriculture. Homepage. Retrieved 2021. Available from: <https://phytochem.nal.usda.gov/phytochem/search>.
- Autodock Vina Manual. Available from: <http://vina.scripps.edu/manual.html>
- O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31- 2010; 455-461.
- Parvathaneni, Vineela et al. "Drug Repurposing: A Promising Tool To Accelerate The Drug Discovery Process". *Drug Discovery Today*, vol 24, no. 10, 2019, pp. 2076-2085.
- PyMOL Molecular Graphics System (Version Version 2.0) [Windows]. San Carlos, CA, 700.
- Fukunishi, Y., & Nakamura, H. Prediction of ligand-binding sites of proteins by molecular docking calculation for a random ligand library. *Protein Science*, 20(1), 2011, pp 95-106.