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Original Article

VIRTUAL SCREENING OF STILBENE ANALOGUE AND INSILICO, *IN VITRO* ANTIPROTOZOAL EVALUATION OF LEAD MOLECULES

G. LALASA*, S. VIJAYARAJ, K. THAMIZHVANAN, K. CHAITHANYAVEENA

Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Sri Sainath Nagar, Chandragiri (Mandal), Tirupati, Chittoor (Dist), A. P.
Email: lalasa.sudha@gmail.com

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ABSTRACT

Objective: The objective of present study is the virtual screening of stilbene analogues followed by the *in silico* and *in vitro* evaluation for its anti protozoal activity.

Methods: The method of virtual screening selected is the structure-based virtual screening using ChEMBL database. The *in silico* analysis was performed using auto dock tools 4.2. The docking was performed using 1T5F (Arginase I-OH complex) as the binding proteins which are drawn from the protein data bank.

Results: The stilbene analogues from virtual screening are allowed to dock with the proteins the binding energies and docking positions were determined using auto dock tools 4.2. The *in vitro* evaluation of anti protozoal activity was performed.

Conclusion: The stilbene analogues are capable of producing the antiprotozoal activity.

Keywords: Stilbene analogues, Virtual screening, Protein data bank, Docking

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INTRODUCTION

Stilbene analogues are generally used in the treatment of cancer. Combretastatin chemically known as 5-[(2s)-2-hydroxy-2-(3,4,5-tri methoxyphenyl)ethyl]-2-methoxyphenolfig. 1. Combretastatin shows their activity by binding to tubulin and also induce vascular shutdown and necrosis in tumours [1]. Clinical trials have revealed its positive effects, either as a single agent or in combination with chemotherapy, in patients with ovarian, lung or anaplastic thyroid cancer.

Tubulin represents a potent target in cancer chemotherapy, given its role in cell division. Combretastatin is a naturally occurring well-known tubulin polymerization inhibitor. Biochemical analyses revealed that CA4P rapidly diminished [2]. The articles have been reported that the repositioning of anti-cancer may also exhibit the anti protozoal activity by zone of inhibition method. Current research work is devoted to performing the virtual screening, *in-silico* analysis and *in-vitro* evaluation of stilbene analogues for its anti protozoal activity [3-5].

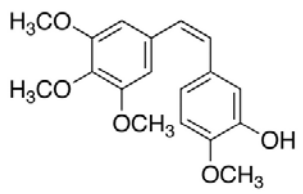

Fig. 1: Structure of combretastatin

MATERIALS AND METHODS

Softwares and applications used

ChEMBL is selected as the database to perform the virtual screening. Chembl or chembldb is a manually curated chemical database of bioactive molecules with drug-like properties. The chembio3ddraw is used to generate the pdb forms of the ligands which are visually screened ChEMBL, Chemdraw is a molecule editor used in the generation of molecules for *in silico* analysis. Autodock 4.2a software which performs the automated docking of flexible ligands to flexible receptors, introduced by Garret m. Morris *et al.*, popularly known as auto dock with version 4.2 were used in the present study to study the molecular docking [6].

Laboratory equipment's used

Electronic weighing balance (Shimadzu), autoclave, BOD incubator (biotechincs), and laminar air flow chamber. Combretastatin and quercetin were purchased from Sigma Aldrich, Dimethyl sulfoxide is used as a solvent.

Methodology

Virtual screening

Chembldata base was selected as screening software for the present study. In this study structure based mode of virtual screening was performed. Basic moiety of stilbene analogues was drawn in the screening software by using JSME drawer and the similarity was set to ≥ 70%. After completion of screening of stilbene analogues, 20 hit molecules were observed. Among them, combretastatin was selected.

In silico analysis

The auto dock 4.2 program was used to locate the appropriate binding orientations and conformations of combretastatin on arginase receptor (PDB Id: 1T5F). Autodockis an extensively used automated procedure for predicting the interaction of small molecules, such as peptides, enzyme inhibitors, and drugs, to macromolecules, such as proteins, enzymes, antibodies, DNA and RNA. The structure of the arginase receptor (PDB Id: 1T5F) were obtained from protein data bank.

Molecular structures of combretastatin were built using the chem-bio draw Ultra 11.0 version. Geometry optimisations of all derivatives were carried out using the Tripos force field with a distance-dependent dielectric and the Powell conjugate gradient algorithm. Gasteiger-huckel charges were used.

Docking procedure

All the water molecules, TMC 125 (ligand) and magnesium ions were removed from the original protein data bank file. Polar hydrogen atoms were added. Gasteiger charges, atomic solvation parameters and fragmental volumes were assigned to the protein. All the torisions in combretastatin were treated as flexible by allowing them to rotate freely. Autogrid 4.0+was used to calculate the grid maps with 40 x 40 x 40 points, a grid-point spacing of 0.375 å and the maps were centred on the ligand. The Lamarckian genetic algorithm (lga) in auto dock 4.0 was used to explore the energy landscape [7].

The hybrid search technique consists of a global optimizer modified from a genetic algorithm with 2-point crossover, random mutation, and a local optimizer with a solid and wets algorithm. A docking box of 40 x 40 x 40 points with a grid spacing of 0.375 å was used in the calculations. Random conditions were used in the settings of seed, initial quaternion, coordinates, and torsions. A 0.2 å step was used for translation and a 25-degree was used for quaternion and torsion [8]. The maximum number of energy evaluation was 250000, and the maximum number of generations was 27000.

The rate of gene mutation was 0.02, and the rate of crossover was 0.8. The number of cycles was set to 10. So a total of 10 docking configurations were determined in each docking calculation. A "preferable" docking configuration was chosen based on the lowest empirical binding free energy and the most frequent cluster [9, 10].

***In vitro* antiprotozoal activity**

In this study, rhizopodial-culture was selected as protozoal strain. The microorganism was allowed to grow overnight at 37 °C in 2% nutrient broth at pH 7.

Preparation of inoculums

The inoculum was prepared by inoculating a loop of each protozoal strain from 24 h old culture into a sterile nutrient broth aseptically in the laminar air flow unit. The culture growth was allowed for 24 h in an incubator at 37 °C.

Determination of antiprotozoal activity

By using agar well diffusion method determination of anti protozoal activity was performed. The agar plates were prepared by pouring 20 ml of sterile molten Muller-Hinton (MH) agar. The protozoal culture was prepared by adding the seed culture in the autoclaved agar medium followed by pouring into the Petri plates. The solid agar medium was gently punctured with the aid of 8 mm sterile cork borer to make a proper well. 1 ml of Combretastatin was added in the pre-labelled wells together with standard antiprotozoal drug Quercetin. The standard Quercetin drug is used in the concentration of 1000µg/ml. It was taken care that the sample should be placed at the level of the cavity. The diffusion of the sample was allowed for 1hr at room temperature on a sterile bench.

Then the Petri plates were incubated for 48 h at 37 °C. After 48 h the plates were observed for the presence of inhibition of protozoal growth and that was indicated by an aclear zone of inhibition of protozoal growth around wells. The size of the inhibitory zone was measured in millimetres.

RESULTS AND DISCUSSION

Virtual screening of stilbene analogues was performed using ChEMBL. The structure-based virtual screening was done by using 70% similarity. After screening, 20 hits were observed. Among 20hits combretastatin was selected for the present study based on the commercial availability. *In silico* studies were performed following the virtual screening.

In silico analysis of stilbene analogues ie., combretastatin and quercetin which was selected as standard were performed and docking positions and binding energies were determined. The docking studies of target compounds were performed using the binding pocket of Arginase I-OH complex (PDB Id: 1F5F).

***In silico* analysis of quercetin with arginase receptor (1t5f)**

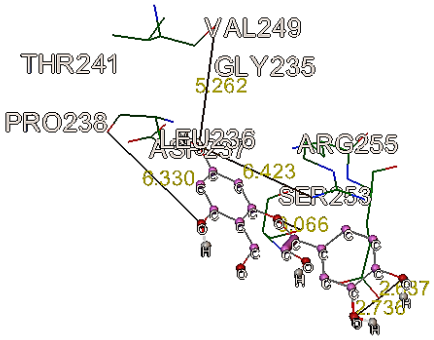

Fig. 2: Docking position of quercetin with arginase receptor (PDB Code: 1T5F)

The docking results disclosed that targeted molecules exhibited considerable and diverse bindingffinities of quercetin towards 1T5F (14.28 to-11.41) along with the formation of numerous hydrogen bonds, π–σ interactions with ARG 255, VAL 249, VAL 239, ASP 237, SER 253 and GLU 256 amino acid residues of 1T5F (Arginase I-OH complex). The bond length of the interactions was estimated and illustrated in table 1.

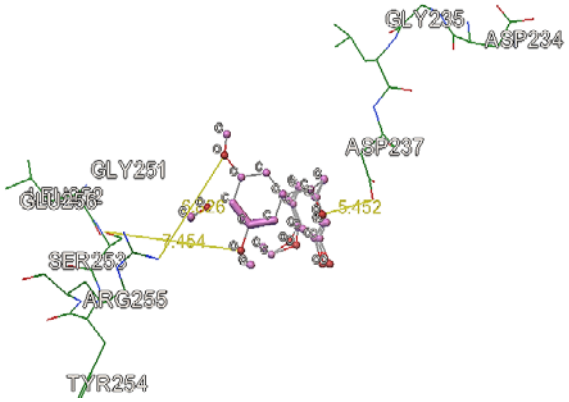

Fig. 3: Docking position of combretastatin on arginase receptor ((PDB Code: 1T5F)

The docking results disclosed that targeted molecules exhibited considerable and diverse binding affinities of combretastatin towards 1T5F (-12.27 to 10.11) along with the formation of numerous hydrogen bonds and π–σ interactions with ASP 237, ASP 255 and SER 253 amino acid residues of 1T5F (Arginase I-OH complex). The bond length of the interactions was estimated.

***In vitro* antiprotozoal studies**

Fig. 4: Zone of inhibition of quercetin against rhizopoda.

Table 1: Table representing various bond lengths, bond angles and amino acid residues

S. No.	Drug name	1T5F		Amino acid residues	Binding
		Interactions observed	Bond length(A °)		Energies (kcal/mol)
1.	Quercetin	π–6	6.423	ARG 255	-14.28
		π–6	5.262	VAL 249	
		π–6	6.423	VAL 239	
		Hydrogen bonds	6.330	ASP 237	
			3.066	SER 253	
			2.637	GLU 256	
			2.736	GLU 256	
2.	Combretastatin	π–6	6.826	ARG255	-12.27
		Hydrogen bond	5.452	ASP 237	
		Hydrogen bond	7.454	SER 253	

By observing the docking positions and binding energy the stilbene analogues ie., combretastatin shows a good affinity towards the antiprotozoal protein 1T5F (Arginase I-OH complex).

In vitro antiprotozoal activity for quercetin and combretastatin were performed. The drug was diffused into nutrient agar medium which contains the rhizopoda (protozoa). The zone of inhibition was observed after 48 h of incubation at 37 °C and it was found to be 6 mm

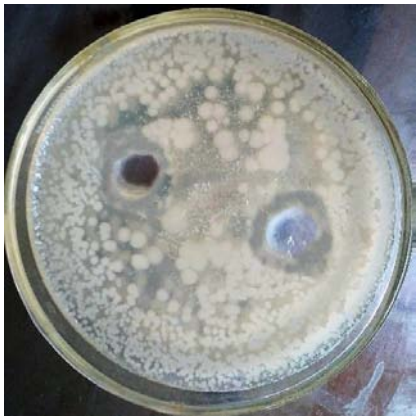

Fig. 5: Zone of inhibition of combretastatin against Rhizopoda

Invitro anti protozoal activity for quercetin and combretastatin were performed. The drug was diffused into nutrient agar medium which contains the rhizopoda (protozoa). The zone of inhibition was observed after 48 h of incubation at 37 °C and it was found to be 3 mm respectively.

CONCLUSION

Virtual screening of selected pharmacophore was successfully performed, the stilbene analogues combretastatin was chosen for the study. Insilico docking studies of stilbene analogue, Combretastatin was successfully performed. Insilico docking studies shown that the stilbene analogues have a least binding affinity towards 1T5f (Arginase I-OH complex). A significant correlation was observed between the silicon and *in-vitro* studies of selected analogues. Combretastatin showed the antiprotozoal activity. Further establishment of combretastatin as antiprotozoal can be done by *in-vivo* evaluation.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest

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