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DOCKING OF SECONDARY METABOLITES DERIVED FROM MARINE FUNGI WITH Hsp90a PROTEIN IN CANCER TREATMENT

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ABSTRACT

The molecular docking studies of marine derived fungal secondary metabolites with protein involved in cancer cells is an upcoming area to determinate the alternative fungal secondary metabolites to the potential targets. The Epoxyphamalin A, Conidiogenone C, Ustusorane E, Peribysin H and Aspergiolide B compounds shown potential targets for the heat shock protein (hsp90alpha). Hsp90a is an effective and safer target for Anti cancer therapies because normal cells do not secrete Hsp90α in the absence of stress, drugs that target F-5 should be more effective and less toxic in treatment of HIF-1α–positive tumors in humans. Molecular docking calculations and binding interactions studies inferred that these cytotoxic compounds taken for study can be potential leads for developing chemotherapeutic drugs. This study indicates the importance of cytotoxic secondary metabolites from marine fungi in regards to their use as antitumor agents. Further work can be extended towards experimental studies and evaluation of their antitumor activity as the findings suggest that these compounds could be developed as lead compounds for designing of anti-cancer drugs with novel targets and mechanisms of action.

Key words: Hsp90a protein, LeadIt, Hex, PROCHECK, Molecular docking and E value

[I] INTRADUCTION

Docking' is the process by which two molecules fit together in 3D space. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized [1]. The computing methods to find the probable target proteins for active

compounds, natural products or old drugs. This searching of drug targets is called "*Insilico* Target fishing" or "Inverse Virtual Screening/Reverse Docking". There are a number of drug target databases and reverse docking servers developed for the purpose of performing target fishing [2]. These tools generate a tractable set of target proteins for experimental validation. In our own previous study we have first tested 30 compounds against Lipinski rule and toxicity analysis to check their druggability. Out of them 7 compounds obeyed Lipinski rule and were found to be druggable. These compounds were submitted to PharmMapper – a web server that uses pharmacophore mapping approach for potential drug target identification and top 3% to 5% of proteins reported by PharmMapper were collected as potential targets and their cancer relevance. Further molecular docking studies and binding site predictions were performed to predict the affinity and binding sites of the cytotoxic compounds with their potential targets as determined by PharmMapper. Five of the seven compounds had heat shock protein (Hsp90alpha) as one of the potential targets reported in top 3 to 5% ranking list. Four compounds were predicted to have estradoil 17-beta-hydroxysteroid dehydrogenase 1 as one of the potential targets. Molecular docking calculations and binding interactions studies inferred that the cytotoxic compounds taken for study can be potential leads for developing chemotherapeutic drugs.

[II] MATERIALS AND METHODS

2.1 Target preparation and validation:

3D structure of Hsp90 alpha [PDBID: 1OSF] was downloaded from Protein Data Bank [3] and prepared for docking using DockPrep tool of UCSF Chimera [4]. The procedure involved selecting and deleting the complexed ligand 17DMAG from the structure and then using DockPrep tool to delete solvent molecules, Change selenomethionine (MSE) residues to methionine (MET), add hydrogen and amber charges and finally write a mol2 file of the optimized structure. A pdb file was also created by stripping hydrogens from the receptor structure. The structure obtained from Chimera was validated using PROCHECK [5] PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing its overall and residue-by-residue geometry.

2.2. Molecular Docking:

The ligand receptor interactions were studied for Hsp90 alpha and the 5 compounds. LeadIt [6] and Hex softwares were used for the purpose of docking [7]. LeadIt is the state of the art tool for docking and scoring. LeadIt modules help to investigate possible binding conformations of the receptor ligand complex using state of the art docking software –"FlexX". The receptor structure is uploaded as a .pdb file and is prepared for docking in the protein definition wizard.

2.2.1. Docking using FlexX:

Docking is one of the key features of LeadIt. A library of molecules can be docked using the FlexX docking algorithm. The docking dialog is opened using the docking icon on the toolbar. The molecules to be docked are loaded into the library. MarvinSpace of ChemAxon suite was used to prepare the ligand library. The number of receptor poses to be generated per docked molecule is set to 10. Hybrid strategy of FlexX is selected for docking and scoring. The hybrid strategy is a versatile way of placing ligand fragments in binding site.This hybrid strategy combines the best of both the classical Flex triangle approach and the single interaction scan.
OK option is selected to save the above settings for all the docking tasks. Apply and Dock option is selected to start the docking run. Results of the docking calculation are shown in the solutions table. The results can be browsed in 2D and 3D by clicking on the complex. The first pose

reported in docking solutions was exported to a file and saved. The pose was also exported for affinity assessment using Hyde.

2.2.2. Hyde Assessment of Hsp90α complex:

The first docked pose was exported to Hyde using the context menu. Hyde scoring function gives a predicted binding affinity for the docked complex and also labels the ligand with an intuitive colouring scheme to show us at a glance where positive and negative score contributions arise. Hyde runs automatically and when Hyde is finished we get an assessment of Binding efficiency, Ligand efficiency and Ki. Regions of the complex that contribute favorably to score are colored green and regions that contribute unfavourably are colored red while white indicates neutral. Colors shown on the ligand atoms reflect contribution of both ligand atoms and its nearest binding site neighbours. Score is made up of the desolvation cost plus the saturation gain. The atom contribution table can be opened to examine score more closely.

2.2.3. Docking using Hex:

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can also calculate protein - ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. Default parameters were used for the docking process and Energy values (E values) of each docking were obtained.

[III] RESULTS

3.1. Target preparation and validation:

Hsp90 alpha pdb structure file was prepared using UCSF Chimera and validated using PROCHECK tool. A snapshot of the structure obtained from Chimera is shown in figure 1. PROCHECK analysis produced Ramachandran plot (figure 2). The Φ and Ψ distributions for non-glycine non-proline residues are summarized in table 1

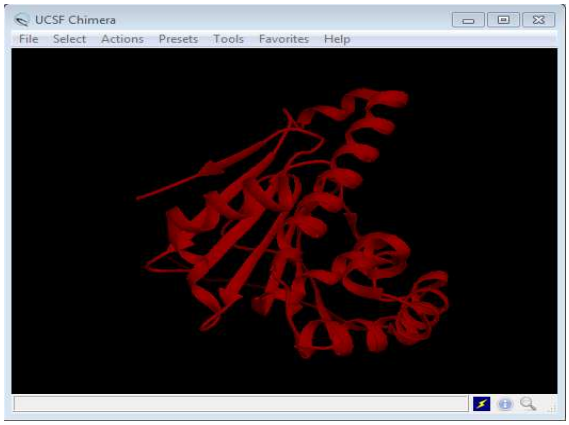

Fig 1: Hsp90 α viewed in UCSF Chimera

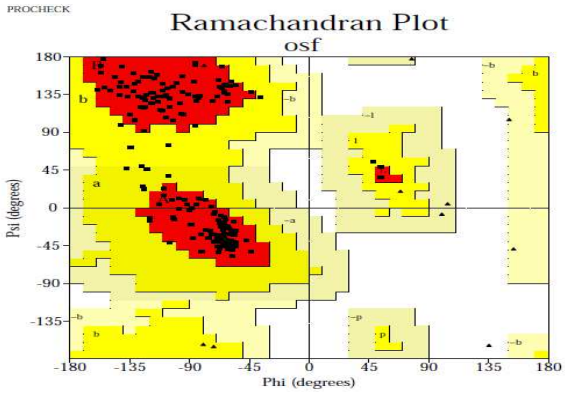

Fig 2: Ramachandran Plot for Hsp90α

Residues in most favored regions [a,b,l]	179	92.7%
Residues in additional allowed regions [a,b,l,p]	14	7.3%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	193	100.0%
Number of end-residues [excl. Gly and Pro]	3	-
Number of Glycine residues [shown as triangles]	15	-
Number of proline residues	5	-
Total number of residues	216	-

Table 1: Ramchandran plot statistics

3.2. Molecular Docking:

Molecular docking was performed with hsp90 alpha as target and ligands namely Epoxyphamalin A, Conidiogenone C, Ustusorane E , Peribysin H , Aspergiolide B are the inhibitors of hsp90 alpha. Docking using LeadIt gave free energy of binding and affinity score by Hyde assessment while Hex program reported the

energy values of the docked complexes (table 2). From docking study we infer that Epoxyphomalin A and Ustusorane E are the best ligands towards the target receptor Hsp90 alpha.

Compound	Free energy of binding (kJ/mol)	Docking score (kJ/mol)	E value
Epoxyphomalin A	**-11**	**-12.3700**	**-238.19**
Conidiogenone C	-4	-14.4545	-199.80
Ustusorane E	**-18**	**-15.8516**	**-200.68**
Peribysin H	1	-16.4235	-178.35
Aspergiolide B	-1	-26.0958	-267.28

Table 2: Docking Calculations

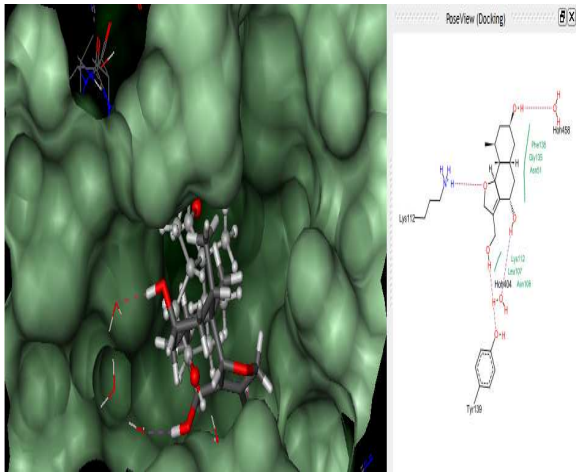

Fig 10: Hsp90 docked with perbysin H in LeadIt

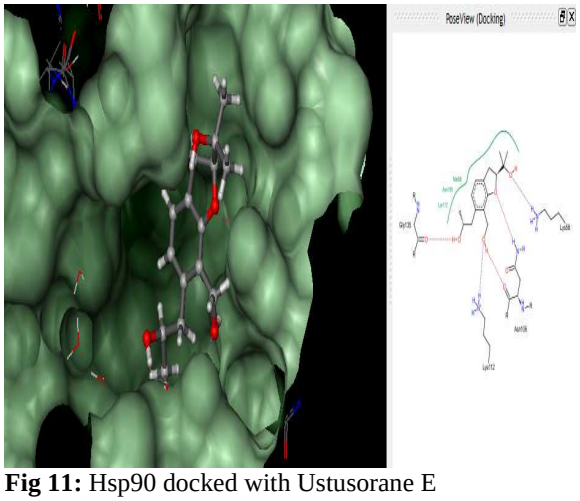

Fig 11: Hsp90 docked with Ustusorane E

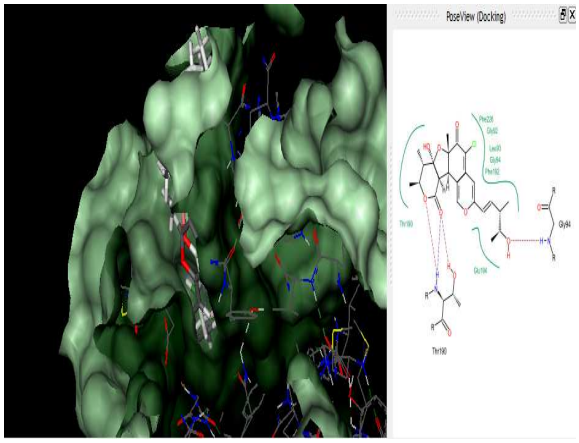

Fig12: Hsp90 docked with Conidiogenone C

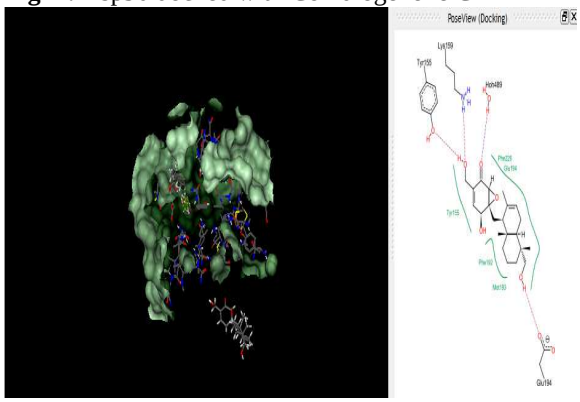

Fig 13: Hsp90 docked with Epoxyphomalin A

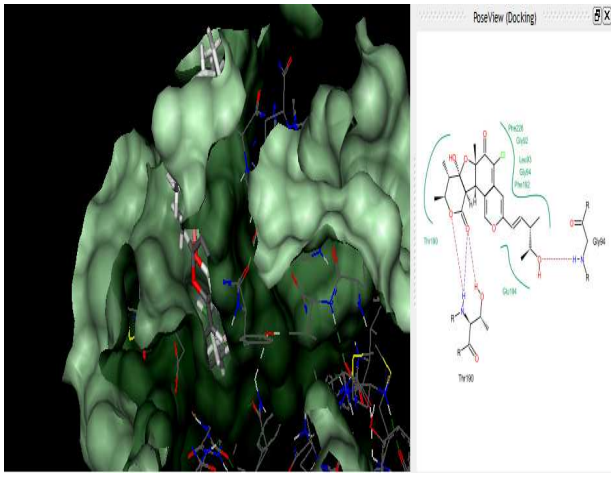

Fig 14: Hsp90 docked with Aspergiolide B

[IV] DISCUSSION

The five compounds namely Peribysin H, Ustusorane E, Conidiogenone C, Epoxyphomalin A and Aspergiolide B to have Heat shock protein Hsp90 aplha as a common predicted anti cancer target. The molecular chaperone Hsp90 (heat shock protein 90) is a promising target in cancer

therapy. Preclinical and clinical evaluations of a variety of Hsp90 inhibitors have shown antitumor effect as a single agent and in combination with chemotherapy. HSP90 plays a pivotal role in the acquisition and maintenance of the malignant phenotype. Its expression in malignant cells is 2- to 10- fold higher than in normal cells [8]. These higher expression levels are coupled to multiple fundamental oncogenic pathways and indicate a crucial role associated with the development and maintenance of the malignant phenotype as well as the acquisition of drug resistant phenotypes [9]. Hsp90 alpha is a potential chemotherapeutic target in cases of Breast cancer and HIF1 induced tumors. Docking activity was performed with hsp90 as receptor and 5 compounds along with known inhibitors of hsp90 as ligands. Prior to molecular docking, receptor structure was preprocessed and prepared by adding the missing hydrogen, deleting ligand and solvent molecules using UCSF Chimera software and the model was validated using procheck showed that model has a quality factor of 97% and is suited for docking calculations. Molecular docking using LeadIt and Hex programs showed that the binding energies and E values of the compounds are with known hsp90 inhibitors. This work shows that the five secondary metabolites studied here have the potential to become druggable candidate molecules or leads for anti cancer therapy.

[V] CONCLUSION

The development of novel chemotherapeutic agents would play a key role in the treatment of refractory or relapsing cancer patients. Nowadays, the chemical, biological and ecological diversity of the marine ecosystem has contributed immensely potent antitumor compounds. In this study we demonstrated that the secondary metabolites of marine fungi are potential leads to evolve novel anti cancer drugs and they are found by PharmMapper screening to target many of the experimentally validated targets relating to various cancers. This study indicates the importance of cytotoxic secondary metabolites from marine fungi in regards to their use as antitumor agents. Further work can be extended towards experimental studies and evaluation of their antitumor activity as the findings suggest that these compounds could be developed as lead compounds for designing of anti-cancer drugs with novel targets and mechanisms of action.

REFERENCES

1. Maria Antony, Dhivyan JE and Anoop MN(2012), Virtual Screening And Lead Optimization To Identify Novel Inhibitors For Hdac-8, School of Health and Life Sciences, Edinburgh Napier University, Edinburgh, United Kingdom.
2. Rong Zheng , Tuan-Sheng Chen and Tun Lu (2012),A Comparative Reverse Docking Strategy to Identify Potential Antineoplastic Targets of Tea Functional Components and Binding Mode, International Journal Of Molecular Sciences,12:5200-5212
3. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov and P.E. Bourne (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242.
4. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE (2004), UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem.25 (13):1605-1612.
5. R. A. Laskowski, M. W. MacArthur, D. S. Moss and J. M. Thornton (1993), PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Cryst.26: 283-291.
6. Rarey M., Kramer B., Lengauer T., and Klebe G. (1996), A fast flexible docking method using an incremental construction algorithm J Mol Biol., 261(3):470-89.
7. Ritchie D.W., Hex 6.3 User Manual Protein Docking Using Spherical Polar Fourier Correlations Copyright 1996-2010.
8. Bagatell R and Whitesell L. (2004), Altered Hsp90 function in cancer: a unique therapeutic opportunity. Mol CancerTher. 3:1021-30.
9. Yonghong Liu (2012) , Renaissance of Marine Natural Product Drug Discovery and Development, J Marine Sci Res Development , 2:2-2