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Structure based Pharmacophore modeling, Virtual screening and Molecular Docking of Potential Phytochemicals against HSP70

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ABSTRACT

Objectives: Heat shock protein 70(HSP 70) forms the central components of cellular network of molecular chaperones and is highly conserved. It is a potential anticancer target as it influences many signaling pathways. Hence the study was designed to identify the novel Phytochemicals against the substrate binding domain of HSP70 by *in silico* analysis.

Materials and Methods: Structure based pharmacophore modeling was performed , followed by virtual screening from a library of diverse Phytochemicals to identify the potential inhibitors of HSP70, in specific ,to the substrate binding domain .Further validation of the hit compounds was done by Molecular docking and ADMET analysis in Discovery studio V4.0.

Results: Pharmacophore modeling resulted in the generation of 5 feature and 6 feature pharmacophore models that identified Eleutheroside E and calamistrin D as the lead binders to the substrate binding domain of HSP70 exhibiting best fit values and high binding energy . Also, both the compounds were non mutagenic, non carcinogenic, non hepatotoxic and showed good blood brain barrier penetration efficiency by ADMET analysis.

Conclusion: Thus, the identified lead Phytochemicals can be proposed for further *in vitro* and *in vivo* evaluation on the expression of HSP70.

INTRODUCTION

Molecular chaperones are proteins which recognize and selectively bind non native proteins to form stable complexes. They comprise several highly conserved families of unrelated proteins and are ubiquitous. Heat shock proteins, both the constitutive and inducible protein has the ability to bind to solvent exposed hydrophobic segments of nonnative polypeptides permitting folding, transport and assembly of the polypeptide through a cycle of binding and release (Hartl and Hayer-Hart 2002; Giuseppina *et al*., 2011). Heat shock protein 70, a molecular chaperon regulates the protein misfolding and aggregation. HSP70 proteins are the central components of the cellular network of molecular chaperones and folding catalysts. Also it acts as a chaperon to many other HSPs besides exhibiting a broader spectrum of cellular functions and is also found to be highly conserved. HSP70 proteins are found in almost intracellular compartments in contrast to other high molecular weight HSPs (HSP 90).Chaperones of the HSP70 family acts by holding nascent and newly synthesized chains in a state competent for folding i.e., preferentially binds the unfolded or partially folded proteins (Slepenkov and Witt 2002; Bukau *et al*., 2006).

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HSP 70 contains two major domains such as N-terminal ATPase domain that binds to ATP and hydrolyzes it to ADP and the other domain is the Substrate binding domain that can interact and transiently associate with short linear peptide segments of folding intermediates(Lund 2001). Heat shock proteins are induced in a variety of pathological states including cerebral Ischemia, neurodegenerative disease, epilepsy and trauma and have also been detected in neurons, glia and endothelial cells (Foster and Brown, 1997). Expression and activation of HSP70 is found to present or reduce protein aggregation in neurodegenerative diseases (Lund 2001; Mayer and Bukau 2005)

Also heat shock protein plays a major role in the inhibition of apoptosis and acts as a cochaperon to HSP90 in delivering the misfolded proteins to ubiquitin proteosome system for degradation[11]. Over expression of HSP70 has been associated with carcinogenesis of the oral epithelium, breast, ovary and lung carcinomas and also been described as an important molecule in the assembly and trafficking of steroid receptors (Schlecht *et al*., 2013; Stefan *et al*., 1997).

Recent studies indicate that medicinal plant extracts and phytochemicals such as curcumin, celastrol, gambogic acid, Withafin A, carsonic acid had shown to induce expression of HSPs (Asea *et al*.,2013; Chow *et al*., 2013; Davenport *et al*., 2011; Grogan *et al*., 2013, Kato *et al*., 1998, Ma *et al*., 2013; Zhang & Sarge 2007).Thus, structure based pharmacophore modeling was performed to explore more novel Phytochemicals against HSP70 and that was further validated by molecular docking.

MATERIALS AND METHODS

Generation of Structure based Pharmacophore model and Validation

Structure based pharmacophore method uses the known or suspected active site of a protein to select compounds that could bind with that site and hence the structure of heat shock protein 70 substrate binding domain with covalently linked novolactone was retrieved from RCSB (PDB ID: 4WV7). The defined active site was first analyzed to create an interaction map of features that the ligand was expected to satisfy for a reasonable interaction with the protein. The Binding site was the substrate Binding domain of HSP70 which was defined and the input site sphere was kept as 9 Å. The interaction map was generated consisting of hydrogen bond acceptors, hydrogen bond donors and hydrophobic features within the receptor active site sphere. The other parameters were kept as default.

The pharmacophore query was created from a Ludi interaction map .The acceptor, donor and Hydrophobe features were selected separately using cluster features and with the help of Dendogram, the 12 cluster feature were selected corresponding to the residues of the active sites ,therefore, avoiding the remaining features that are too far from the relevant active site residues. The parameters were kept as default except by changing the selection mode to replace; scope to amino acid; radius kept to 2.00 A; required features group to true and by changing the maximum features to 8 in the input Pharmacophore Parameter group. This parameter setting signifies that the screened ligands must fit at least five features of the sixteen features of the edited pharmacophore. The generated pharmacophore models were used to screen the in-house library of Phytochemicals. Before screening the pharmacophore, best hydrophobe characteristic of methylpodocarp and ethylene groups were selected. The pharmacophore model was validated by pharmprint frequency and also based on the discriminating efficiency of the models to distinguish the set of ATP ase inhibitors of HSP70 from substrate binding domain molecules.

Lipinski rule and ADMET

Molecular properties and drug likeness of the phytochemical compounds were examined on the basis of "Lipinski's Rule of Five in Discovery Studio 4.0. ADME and Toxicity studies was performed by considering the parameters such as Atom based Log P98 (A LogP 98), polar surface area (PSA), Blood Brain Barrier (BBB), Cytochrome P450, Hepatotoxicity. Compounds that passed the screening experiments were retained for Virtual screening and molecular docking.

Virtual screening

The generated different featured Pharmacophore models were used as a 3D structural query for retrieving potent molecules from the in house phytochemical database. The best pharmacophore model was then used to screen the databases for those compounds that could fit the minimum feature of five of the selected 16 features required. Hit compounds were used further for flexible Docking analysis in DS V4.0.

Molecular Docking

Molecular docking was performed in DSV4.0 by flexible docking analysis. The protein structure of HSP70 was retrieved from PDB (ID: 4WV7). The ligand and crystallographic water molecules were removed from the protein and the Chemistry of the protein was corrected for missing hydrogen by the software. Also loop refinement was carried out to screen the violations and determination of disallowed amino acids in the protein. Ligand molecules were prepared and energy was minimized using CHARMm force field in DS .Active site was predicted followed by the binding site definition. The predicted active site in both chain A and chain B are Glu 444,Leu 456,Ile 480,Gly 484,ILE 485,Leu 486,Asn 505 and Leu 510. The similarity analysis was carried out for the best hit compounds using Pubchem search.

RESULTS AND DISCUSSION

Structure based pharmacophore Modeling

Structure Based Pharmacophore modeling was performed in this study based on the interaction of the natural Phytochemical Novolactone with the binding site of the HSP70. The features selected using cluster feature analysis were HA features: A10, A34, A50, A100, A145, A208 and HD features

:D14, D19, D90, D170, D179, D219, D295, D352, D403 and Hydrophobe Y4,Y15,Y23,Y31,Y39,Y49,Y61. These features represent the inclusion and correspond to the residues of the active site (Figure 1).

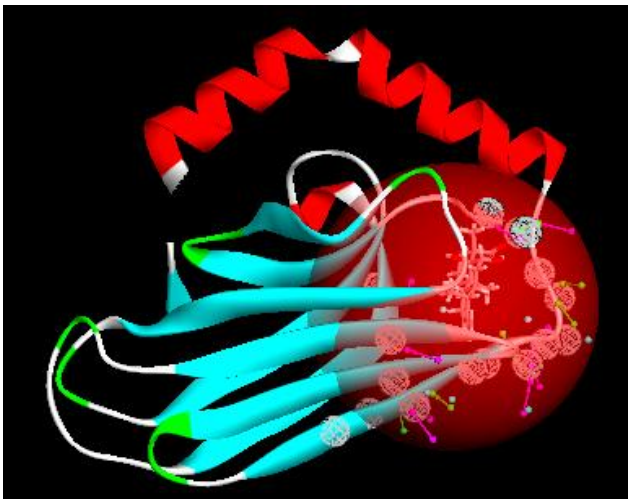


Fig. 1: 3D structure of the substrate binding domain protein showing hydrogen bond donor, hydrogen bond acceptor and hydrophobe features

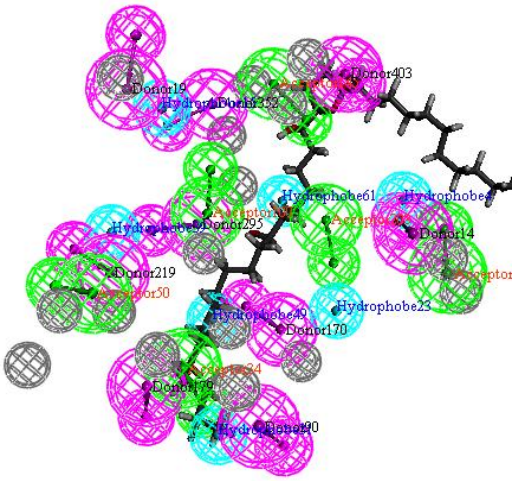
The remaining features may be too far from the relevant active site residues or have vectors pointing away from the putative binding site. These features were selected predominantly based on interactions generated solely from protein structure information. In particular, hydrophobe 15 and 31 corresponded well to the methylpodocarp and ethylene groups of the bound ligand Novolactone. Thus these sixteen features represented a pharamacophoric structural model of the protein active site. This resulted in the generation of 2 best featured model viz 5 feature pharmacophore and 6 feature pharmacophore model and further validation with test compounds qualified them as best models which showed best alignment with the test set of compounds.

Lipinski filter and Virtual screening

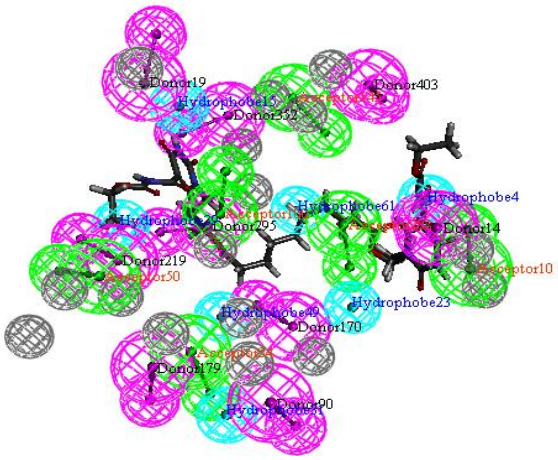
5550 Phytochemicals were subjected for Lipinski rule of five and ADMET prior to subjecting the Pharmacophore model for the Virtual screening. Among those, 240 compounds qualified the drug likeliness properties and screening resulted in the identification of 2 leads with higher fit value in comparison to the active compound subjected in the dataset. Both the models screened the same set of compounds with slighter variation in their fit values

The screened compounds Calamistrin D,Eleutheroside E mapped all the features with their respective pharmacophore features of the models and scored a fit value greater than 2 (Figure 2).The mapped features of calamistrin D are HA 145,HD 403,Y31,Y49 and Y61 whereas the mapped features of Eleutheroside E are HA 208,HD 90,HD 295,Y 23 and Y 61.

Both the compounds were found to be non mutagenic, non carcinogenic and had shown good blood brain barrier penetration efficiency without hepatoxicity as predicted by ADMET analysis. Finally, both the hit compounds were docked against the active site of the B chain of the substrate binding domain of HSP70.



A. Calamistrin D



B. Eleutheroside E

Fig. 2: Identified Phytochemical compounds showing alignment with the generated hydrophobe models. Green represents the Hydrogen Bond Donor and Magenta represents the Hydrogen bond acceptor pharmacophoric features of generated models.

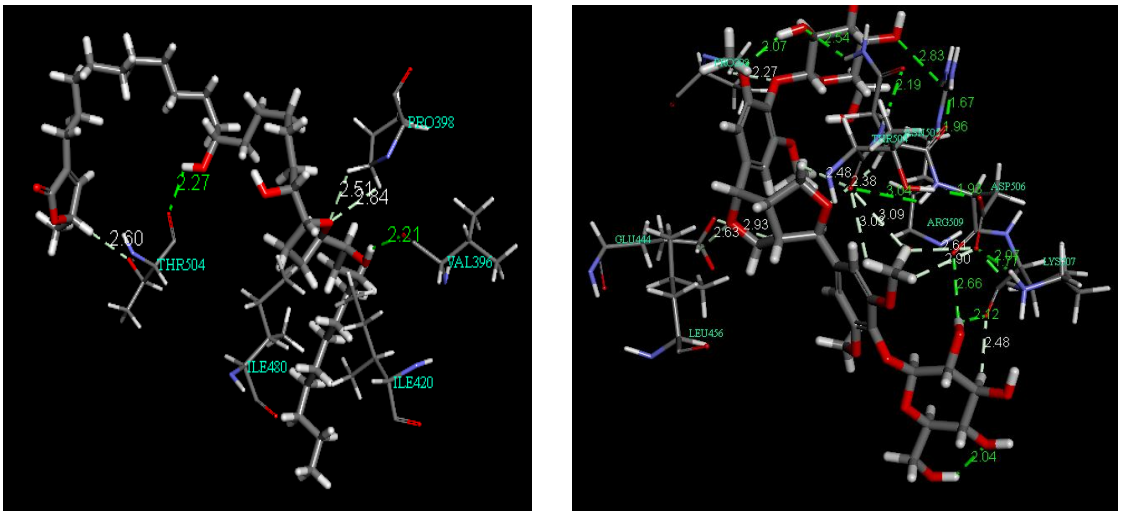
Molecular Docking

Flexible docking was utilized in DS both in the Validation and Post docking filter standpoint to predict the binding orientation of the hit compounds.

Flexible docking followed by LibDock score analysis ,binding energy calculation and hydrogen bond interaction revealed that the compound Eleutheroside E showed higher binding energy of -106.22;C Dock score of 53.29 and extended hydrogen bond interactions at the aminoacid positions Lys 507,Asp 506,Glu 444,Thr 504,Asn 505 ,Pro 398,Arg 509 .The compound Novolactone interacted with the active site region at Glu 444,Leu 456,Ile 480,Leu 510 and thus shared the common interacting aminoacid as Glu 444 with lead compound Eleutheroside E (Table 1).

Table 1: Best leads showing the interaction energy and hydrogen bond interaction with the fit value.

Name	Fit Value	Libdockscore	Binding Energy	Hydrogen Bond Interaction	Distance Å
Calamistrin D	2.86079	81.743	-102.089	C-H…O VAL396	2.21
				C-H…O THR 504	2.27
				PRO 398 C-H…O	2.51
				C-H…O THR 504	2.60
				PRO 398 C-H…O	2.84
Eleutheroside E	2.06003	53.29	-106.222	C-H…O LYS 507	2.48
				C-H…O LYS 507	2.12
				C-H…O ASP 506	2.66
				C-H…O ASP 506	2.61
				C-H…O ASP 506	2.9
				GLU 444 C-H…O	2.63
				C-H…O GLU 444	2.93
				C-H…O THR 504	3.03
				C-H…O THR 504	3.09
				ASN 505 C-H…O	2.38
				C-H…O THR 504	2.48
				ASN 505 C-H…O	2.54
				PRO 398 C-H…O	2.27
				ARG 509 C-H…O	2.83



A) Calamistrin D **B). Eleutheroside E**

Fig. 3: Molecular Interaction of substrate Binding Domain of HSP70 with the hit Phytochemcials.

Likewise, Calamistrin D showed higher binding energy of -102.08 and the libdock score of -81.743 with the hydrogen bond interactions observed at Val 396, Thr 504,Pro 398 (Figure 3).

Similarity analysis of the compounds and the novelty of non existence of the earlier reports of the compounds on HSP70 interaction revealed that calamistrin D and Eleutheroside E was found to be novel and has not been studied for HSP70 activity.Hence so far no specific interaction or direct evidence on the activities of the hit compounds Calamistrin D and Eleutheroside E on the expression of HSP70 has been reported however few reports exists on the extracts of *Eleutherococcus senticosus* possessing both *in vitro* and *in vivo* neuroprotective activity and Adaptogenic substances(ADAPT-232) derived from *Eleutherococcus senticosus* root extract, *Schisandra chinensis* berry extract, Rhodiola rosea root extract induced expression of Hsp70 from isolated human neurolgia cells (Abbas K.Samadi,2014; Ahmed *et al*., 2012) Thus further *in vitro* and *in vivo* examination of the natural compound Eleutheroside E and Calamistrin D on the expression of HSP70 activity could provide better understanding of the mechanism .

CONCLUSION

The study identified Eleutheroside E and Calamistrin D as the best leads interacting with the substrate binding domain of HSP 70. Also, both the compounds were non mutagenic, non carcinogenic, non hepatotoxic and showed good blood brain barrier penetration efficiency by ADMET analysis .Thus, upon further *in vitro* and *in vivo* evaluation of the two Phytochemicals on the expression of HSP70 can be proposed as potential HSP70 inhibitors .

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REFERENCES

Abbas K.Samadi .Targeted activation of Heat shock proteins by Natural Bioactive compounds to prevent Neurodegenerative diseases. J Anc Dis Prev Rem, 2014;2:e113.

Ahmed K, Furusawa Y, Tabuchi Y, Emam HF, Piao JL. Chemical inducers of heat shock proteins derived from medicinal plants and cytoprotective genes response. Int J Hyperthermia, 2012; 28: 1-8.

Asea A, Kaur P, Panossian A, Wikman KG .Evaluation of molecular chaperones Hsp72 and neuropeptide Y as characteristic markers of adaptogenic activity of plant extracts. Phytomedicine, 2013; 20: 1323-1329.

Bukau B , Weissman J and Horwich A. Molecular chaperones and protein quality control. Cell 2006 ; 125(3) :443–451.

Chow AM, Tang DW, Hanif A,Brown IR . Induction of heat shock proteins in cerebral cortical cultures by celastrol. Cell Stress Chaperones, 2013; 18: 155-160.

Davenport J, Manjarrez J R, Peterson L, Krumm B, Blagg BS .Gambogic acid, a natural product inhibitor of Hsp90. J Nat Prod, 2011; 74: 1085-1092.

Foster JA and Brown IR . Differential induction of heat shock mRNA in oligodendrocytes, microglia, and astrocytes following hyperthermia. Molecular Brain Research, 1997;45(2) : 207–218.

Giuseppina Turturici, Gabriella Sconzo, and Fabiana Geraci. Hsp70 and Its Molecular Role in Nervous System Diseases. Biochemistry Research International, 2011 doi:10.1155/2011/618127.

Grogan PT, Sleder KD, Samadi AK, Zhang H, Timmermann BN . Cytotoxicity of withaferin A in glioblastomas involves induction of an oxidative stress-mediated heat shock response while altering Akt/mTOR and MAPK signaling pathways. Invest New Drugs,2013; 31:545-557.

Hartl FU and Hayer-Hartl M. Protein folding. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science, 2002; 295(5561): 1852–1858

Kato K,Ito H, Kameim K, Iwamoto I .Stimulation of the stress-induced expression of stress proteins by curcumin in cultured cells and in rat tissues in vivo. Cell Stress Chaperones ,1998;3: 152-160.

Lund PA .Molecular chaperones in the cell. Oxford University Press, Oxford.2001

Ma QL, Zuo X, Yang F, Ubeda OJ, Gant DJ. Curcumin suppresses soluble tau dimers and corrects molecular chaperone, synaptic, and behavioral deficits in aged human tau transgenic mice. J Biol Chem, 2013; 288: 4056-4065.

Mayer MP and Bukau B .Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci, 2005; 62:670-684.

Schlecht R, Scholz SR, Dahmen H, Wegener A, Sirrenberg C. Functional Analysis of Hsp70 Inhibitors. PLoS ONE, 2013; 8(11): e78443. doi:10.1371/journal.pone.0078443

Slepenkov SV and Witt SN. The unfolding story of the Escherichia coli Hsp70 DnaK: is DnaK a holdase or an unfoldase? Molecular Microbiology 2002; 45(5) : 1197–1206.

Stefan Rüdiger, Alexander Buchberger and Bernd Bukau .Interaction of Hsp70 chaperones with substrates. Nature Structural Biology, 1997; 4:342-349.

Zhang YQ, Sarge KD. Celastrol inhibits polyglutamine aggregation and toxicity through induction of the heat shock response .J Mol Med (Berl), 2007; 85: 1421-1428.

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