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Structure Prediction and Drug Designing of aTarget Protein CPE-B4on *In-Silico* Platform for Angiogenesis and Chronic Liver Disease

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Abstract

In recent years, it has become increasingly evident that pathological neoangiogenesis processes occur in chronic inflammation and Chronic Liver Diseases (CLD). The challenge for the upcoming years is the characterization of the molecular basis and pathways of angiogenic disorders in an integrated manner. If the currently uncertain Patho-genetic role of angiogenesis in CLD can be demonstrated, it could have a prognostic value in the evaluation of disease progression. The current work involves the identification of the protein CPEB4, which is the main cause of CLD& angiogenesis.

We haveanalyzed its role in the disease by using bioinformatics tools. The sequence structure and functional analysis of the concerned protein has been studied through BLAST and Phyre2.0. Since the precise structure of CPEB4proteinis not yet available, therefore, theoretical model for the query protein was generated using Easy Modeler 4.0. The protein-ligand docking was done with use of Autodock software by drugs downloaded from Drug Bank.Ligand N-[4-(6-Carbamoyl-7-methoxy-4-quinolyl) oxy-2-trifluoromethylphenyl]-N'-methylureashowed an impressive binding energy of -14.3 kcal/mol,in comparison with the already proved drugsNateglinide&Lenvatinib(binding energy: -8.8 kcal/mol& -8 kcal/molrespectively). Overall result suggests that this inhibitor could represent a promising moleculefor the effective treatment of CRC.

Keywords: Angiogenesis; Chronic Liver Diseases; CPEB4-protein Inhibitor Therapy

Introduction

Chronic Liver Diseases (CLDs) are one of the major causes of death, which are still increasing year-on-year[1].Therefore, knowledge about the pathophysiology of CLDs and its complications is of uttermost importance. There are clear role of angiogenesis in the disease progression of various liver diseases[2,3].Looking closer at the Pathophysiology of Portal Hypertension (PH)[4], fibrosis[5,6], cirrhosis[7],Non-Alcoholic SteatoHepatitis(NASH)[8]and Hepatocellular Carcinoma (HCC)[9]. We find that angiogenesis is a recurring factor in the disease progression. The close relationship between the progression of CLDs and angiogenesis emphasizes the need for anti-angiogenic therapy as a tool for blocking or slowing down the disease progression[3]. The fact that angiogenesis plays pivotal role in CLD give rise to new opportunities for treating CLDs and its complications[3].Angiogenesis in liver is characterized by intrahepatic vascular remodeling with capillarization of the sinusoids and development of intrahepatic shunts, which lead to increased hepatic resistance and decreased effective hepatocytes perfusion[3]. It has become increasingly clear that angiogenesis occurring during chronic wound healing and fibro genesis provides a key contribution to disease progression and complications[3].

The progression from fibrosis to cirrhosis, the end-point of CLDs, is distinguished by a prolonged inflammatory and fibrogenic process that leads to an abnormal angioarchitecture distinctive for cirrhosis[6]. Several mechanisms are responsible for the angiogenic switch during the pathogenesis of CLDs[10,11]. First, CLDs are characterized by chronic inflammation[11]. Increased intrahepatic vascular resistance is primarily caused by anatomical changes, such as fibrotic scar tissue compressing portal and central venues[12]. In addition, the formation of fibrotic septa, as well as sinusoidal capillarization, can result in an increased resistance to blood flow and oxygen delivery[6]. This results in hypoxia and the transcription of hypoxia-sensitive pro-angiogenic genes, usually modulated trough HIF[7]. Also, an increased contribution of the hepatic artery to the sinusoidal blood flow leads to sensitization of the hepatocytes to abnormal high-oxygen conditions[1].

Hepatic Stellate Cell motility and migration promote coverage of HSC around sinusoids, causing sinusoidal constriction and contributing to the hepatic resistance in cirrhosis[2]. The characteristic fenestrated phenotype of the sinusoidal ECs is lost and an organized basement membrane is established, which leads to an impairment of oxygen diffusion even though the increased arterial flow provides a high supply of oxygenated blood[6].The deposition of collagen in the space of Disseaccentuates the narrowing and distortion of the sinusoidal lumen, further restricting micro vascular blood flow[6].This is aggravated by leucocytes, either mechanically trapped in the narrowed sinusoids or adhering to the endothelium, as a result of activation of a hepatic micro vascular inflammatory response[6]. The hypoxic liver tissue causes an up-regulation of VEGF, Ang and their receptors in HSCs, not only enhancing the hypoxia-induced angiogenesis but also stimulating activation and migration of HSC[13]. As stated previously, activated HSCs induce an inflammatory response and enhance angiogenesis[14].New vessels themselves can significantly contribute to the perpetuation of the inflammatory response by expressing chemokines and adhesion molecules promoting the recruitment of inflammatory cells[15]. Furthermore, angiogenesis, early in the course of a CLD, may contribute to the transition from acute to chronic inflammation[16].This work explores the potential usefulness of angiogenesis inhibitor N-[4-(6-Carbamoyl-7-methoxy-4-quinolyl)-oxy-2-trifluoromethylphenyl]-N'-methylureain the treatment of CLD using in silico platform.

Methodology

Sequence Alignment

The FASTA sequence of CPEB4 query protein was obtained from online source of National Centre for Biotechnology Information, NCBI (http://www.ncbi.nlm.nih.gov) and aligned by using BLASTp sequence alignment tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Chain a of H1N1 influenza A virus (PDB ID: 4F15) has showed maximum identity with the query protein. Hence, it has been selected as a template protein for structure prediction of the targeted protein.

Structure Prediction

The three-dimensional secondary structure of CPEB4 protein has been predicted with the help of online server 3DPSSM and Phyre2. Ten protein templates have been selected showing maximum identity with our query protein structure. The PDB file format of the selected templates have been obtained from Protein Data Bank (PDB), a computational database, having resolution <3.0, R-Value <0.5 and X-Ray crystallographic structures. With the help of the software EASY MODELLER (version 4.0), the 3-dimensional model of query protein has been created by submitting PDB file formats of these template protein to the software program.

Validation of Predicted Model (Loop Modelling)

Structural Analysis and Verification Server, SAVES (Prochek) is an online bioinformatics tool which has been used in our work for validation of the query protein model (Ramachandran Plot Analysis) of the query protein. The model query protein has been rebuilt into its secondary structure by further submitting it into the RAMPAGE. The final secondary structure obtained after validation has been viewed using the Swiss PDB Viewer (SPDB 4.10).

Prediction of the Active Site

The query protein, having 704 amino acid residues, has been submitted to online DogSiteScorer, an Active site prediction and Analysis server. Prediction of active site is necessary to identify the ligand binding site, present on the query protein.

Drug Library Search

Drug Bank database has been used to obtain the structure of drug molecules (in MOL format) already available for the treatment of swine influenza. The MOL files of these structures were then converted into PDB format with the help of software Open-Babel. These molecules were used as ligand during molecular docking[17].

Molecular Docking

Molecular docking has been performed using Auto Dock software against the active site of the query protein with the available ligand[17]. The ligand molecule (that showed minimum free energy) has been used as a base molecule for designing in silico structure of drug molecule against Swine Influenza.A computational pipeline was developed for large-scale molecular docking of drugs to protein targets. Briefly, we collected all 3D structures available for each drug target, determined binding pockets in the structures, and docked drugs to each pocket. Results were collected and thresholds were applied to select the top predicted interactions, which were then visually inspected.

Finding Similar Drug Molecule of Best Docked Ligand

With the help of Pubchem server, the in-silico drug molecules were obtained. The molecule that showed minimum free energy when binding with the active site of the query protein has been selected as the final drug molecule.

Toxicity Prediction

The toxicity of the selected ligand has been checked with the help of an online server ACD Labs ILabs. This online server gives the information regarding the physical and chemical properties of any molecule by following the five rules of Lipinsky, Health effects and ADME properties.Quantitative Structure-Activity Relationships (QSAR) is quantitative models which relate the variation in measures of activity in a series of chemical compounds to the variation in chemical structure between compounds in the series. The use of QSAR in the study and identification of potentially toxic chemicals is receiving increased attention, as demonstrated by several recent reviews (for example, McKinney, 1985; Hansch, 1985; Enslein, 1984; Birge and Cassidy, 1983).

Energy Minimization of Protein Drug Complex

Energy was minimized byGromacs software on Linux operating system of theprotein-drug complex structure,so that our complex will be strong enough to be stable.Minimization provides information that is complementary to that obtained from molecular dynamics or Monte Carlo. Ensembles of dynamics or Monte Carlo structures are useful for calculating thermodynamic averages and estimating entropy, but the large number of structures involved makes detailed microscopic analysis cumbersome. Minimized structures in some sense represent the underlying configurations about which fluctuations occur during dynamics and, as such, provide a convenient and meaningful basis for structural analysis (Hagler, 1985; Struthers et al., 1984).

Results

The FASTA sequence of CPEB4 (Accession No.: AAI43960) obtained from NCBI has showed maximum identity of 32% with Chain A of Crystal Structure of a Heterogeneous Nuclear Ribonucleoprotein A/b (hnrpab) From Homo Sapiens At 2.15 A Resolution (PDB: 3S7R) (Figure 1).

With the help of 3DPSSM and PHYRE 2 online servers, secondary structure of the query protein has been obtained showing the minimum dope score (Figure 2).

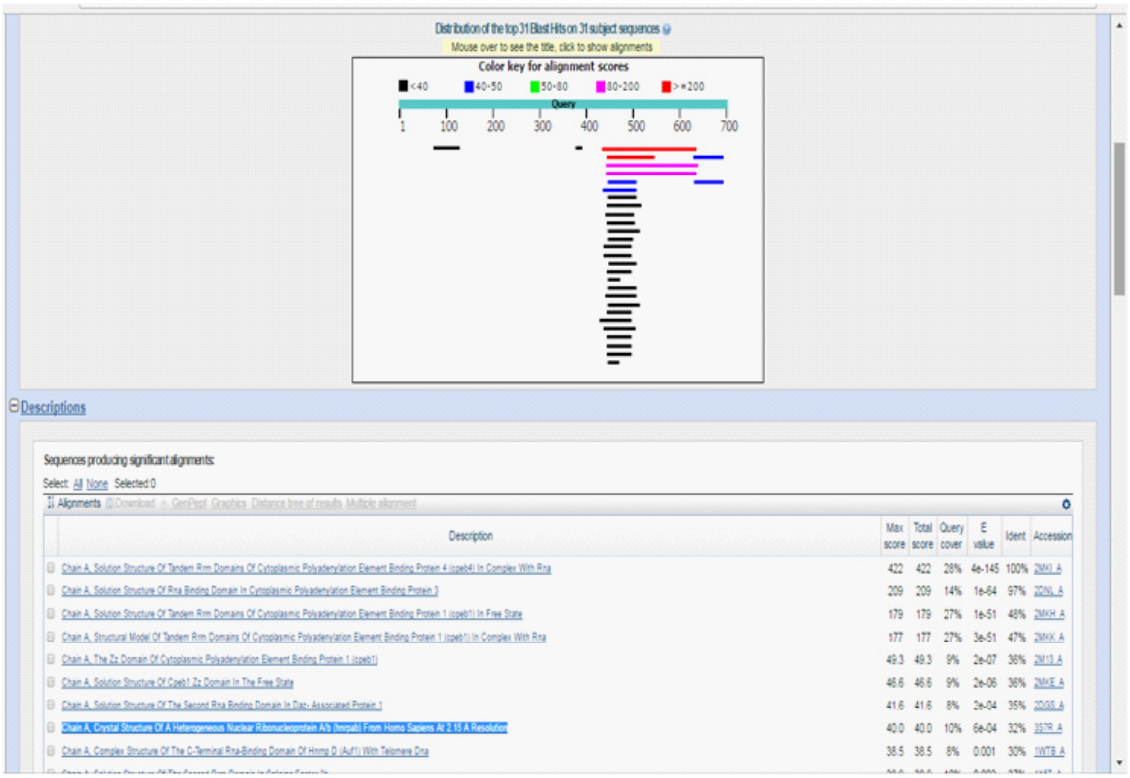

Figure 1: Maximum Identity value of CPEB4 after running BLASTp.

Filename	MOL pdf	DOPE score	GA341 score
query.B99990001.pdb	33402.42578	-43397.82031	1.00000
query.B99990002.pdb	31461.16797	-44809.94531	1.00000
query.B99990003.pdb	32744.52539	-43974.26953	0.99976
query.B99990004.pdb	28798.55273	-45995.11719	1.00000
query.B99990005.pdb	33979.85938	-43749.45703	1.00000
query.B99990006.pdb	32087.38281	-43740.45312	1.00000
query.B99990007.pdb	31686.76172	-44783.13672	0.99999
query.B99990008.pdb	36510.32812	-42603.83203	0.99998
query.B99990009.pdb	34452.33594	-42844.48047	0.99996

Figure 2: Minimum Dope Score showed by the template protein molecules after Homology Modeling and Running Easy Modeler.

By using the secondary structure, 3-dimensional tertiary structure of the query protein has been created by using software Modeler. The obtained three-dimensional computational model of query protein has been allowed for validation by following Ramachandran Plot analysis, using RAMPAGE, an online server. It has been viewed under Swiss PDB Viewer (Figure 3-5).

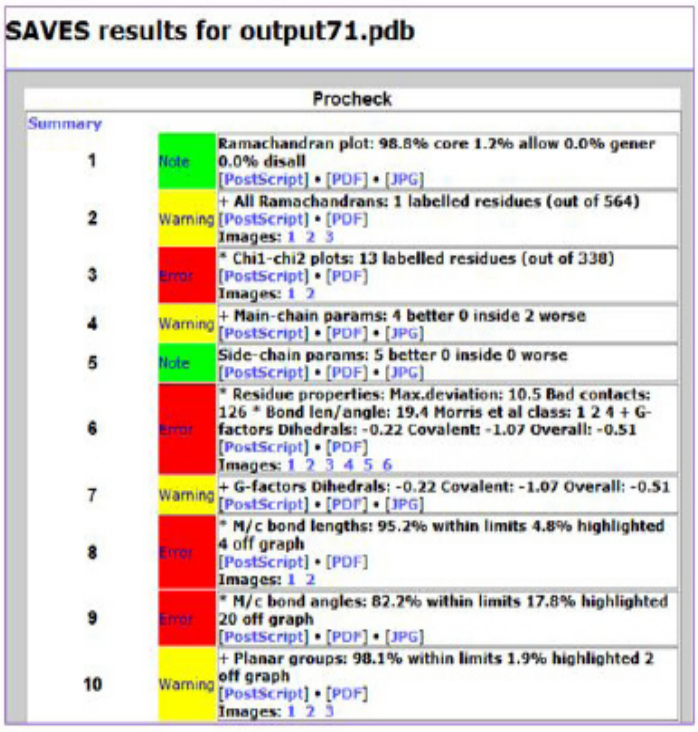

Figure 3: Validation with RAMPAGE Server.

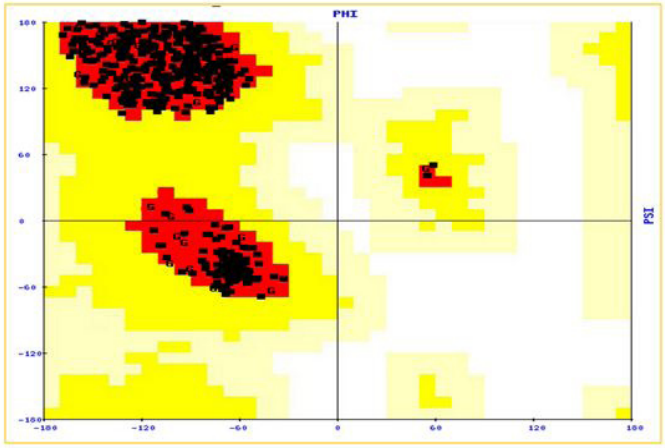

Figure 4: Ramachandran Plot Analysis with RAMPAGE.

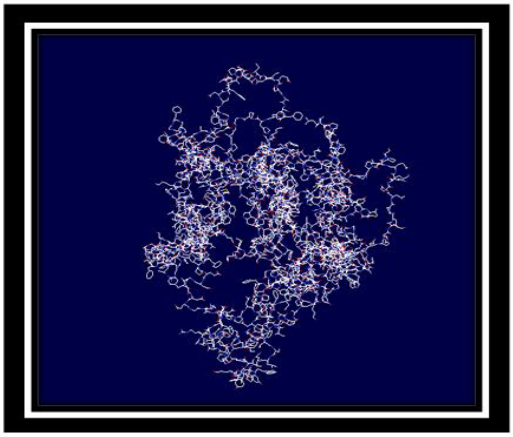

Figure 5: Three-dimensional model of CPEB4 protein viewed under Swiss PDB Viewer.

The active site of the query protein obtained from Dog Site Scorer (Figure 6), has been allowed to molecular docking and a minimum free energy of (binding energy: -8.8 kcal/mol& -8 kcal/mol, NateglinideLenvatinib simultaneously) (Figure 7) selected as a base molecule for further synonyms drugs. The novel drug molecule obtained by us from pubchem server, possess a free energy of -14.3 kcal/mol. IUPAC name of the novel drug stands N-[4-(6-Carbamoyl-7-methoxy-4-quinolyl)oxy-2-trifluoromethylphenyl]-N'-methylurea (Figure 8).

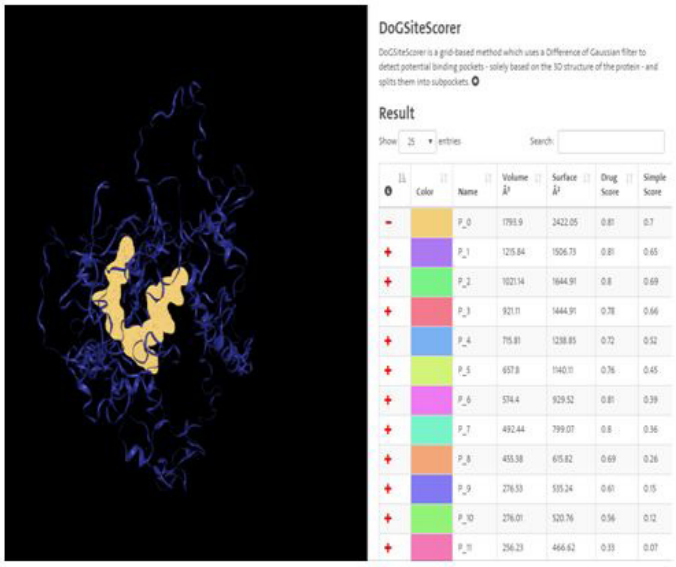

Figure 6: Active Site of Query Protein obtained by Dog Site Scorer (in yellow colour).

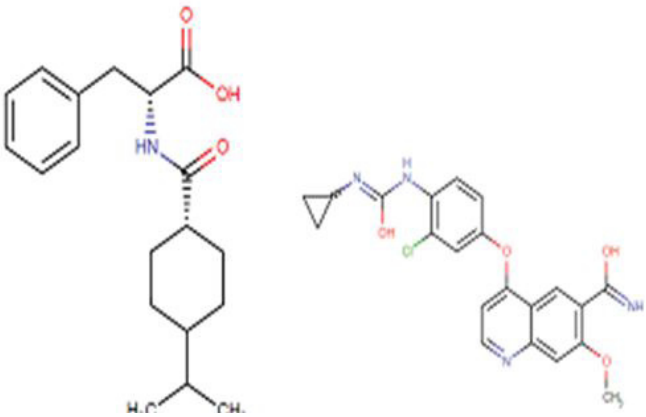

Figure 7: Chemical Structures ofNateglinide&Lenvatinib simultaneously.

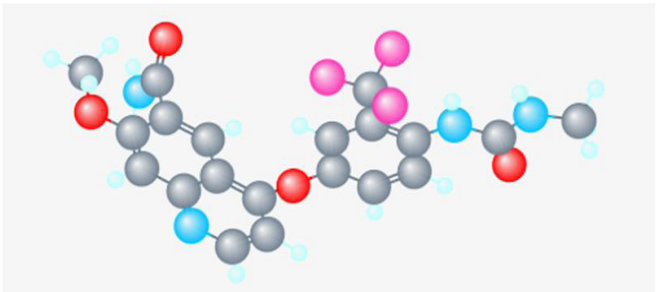

Figure 8: Chemical Structure ofN-[4-(6-Carbamoyl-7-methoxy-4-quinolyl)oxy-2-trifluoromethylphenyl]-N'-methylurea.

The obtained molecule is the most suitable with consideration of the Lipinsky's rule for the toxicity of the chemical compounds in vivo (Table 1).

Molar Refractivity	123.90 ± 0.3 cm3
Molecular Weight	434.375 g/mol
No. of Hydrogen Bond Donors	3
No. of Hydrogen Bond Acceptors	8

Table 1: Various chemical parameters ofN-[4-(6-Carbamoyl-7-methoxy-4-quinolyl)oxy-2-trifluoromethylphenyl]-N'-methylurea.

Therefore, this molecule has been chosen for the best possible drug against angiogenesis and chronic liver disease.Energy was minimized by Gromacs software on Linux operating system of the protein-drug complex structure (Figure 9) so that our complex will be strong enough to be stable.

```
gmx mdrun -v -deffnm em
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Steepest Descents converged to Fmax < 1000 in 178 steps
Potential Energy  = -5.0933919e+05
Maximum force     =  9.1495343e+02 on atom 1266
Norm of force     =  5.1248310e+01
```

Discussion

The in-silico identification of the biological targets of small organic molecules has attracted substantial attention from biologists, chemists and pharmacologists.In the present work we have found that angiogenesis and chronic liver disease is caused by CPEB4 protein. Through homology modeling and loop modeling we have got the final structure of our protein and after its docking with the best result, from Pubchem server, we haveobtained a suitable ligand having minimum free energy when docked with the active site of the query protein. In vivo toxicity of the novel drug has been checked. The de-novo structure based drug designing approach has led to a potential ligand (drug) molecule that blocks the active site of CPEB4 protein preventing the responsible gene of angiogenesis and chronic liver disease[18,19].Energy was minimized by Gromacs software on Linux operating system of the protein-drug complex structure so that our complex will be strong enough to be stable.Ourin-silico analysis shows that N-[4-(6-Carbamoyl-7-methoxy-4-quinolyl) oxy-2-trifluoromethylphenyl]-N'-methylurea could offer an improved treatment CLD patients. Further prospective pre-clinical and clinical studies are still needed to confirm our findings.The active site of the query protein obtained from Dog Site Scorer (Figure 6), has been allowed to molecular

docking and a minimum free energy of (binding energy: -8.8 kcal/mol& -8 kcal/mol, Nateglinide&Lenvatinib simultaneously) (Figure 7) selected as a base molecule for further synonyms drugs. The novel drug molecule obtained by us from Pubchem server, possess a free energy of -14.3 kcal/mol. IUPAC name of the novel drug stands N-[4-(6-Carbamoyl-7-methoxy-4-quinolyl)oxy-2-trifluoromethylphenyl]-N'-methylurea.

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