Research Article

In silico Pharmacokinetics & Molecular Dynamics Studies of JAB1/CSN5 Protein and Discovery of Small Potent Drug against Pancreatic Cancer

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ABSTRACT

Pancreatic cancer (PC) is a major problem worldwide and it is the fourth leading cause of cancer-related deaths in developed countries. The global burden of PC is shifting rapidly from developed countries to the developing countries. Treatment against its malignancy still remains a major challenge in oncology as evidenced by the unchanged mortality rate and also a death to incidence ratio of 0.99. There are numerous molecular factors involved in the chemotherapeutic resistance of pancreatic cancer tumors. Jab1 (Jun activation domain binding protein 1), integrated into COP9 signalosome complex (CSN), initially identified as a co-activator of c-jun and also induces degradation of cell cycle inhibitor p27 and tumor suppressor (p53,Smad4) and therefore a new target in cancer therapy. Here our current study involves designing the suitable inhibitors against MPN domain of this protein by insilico approach. In this, a library of designed compounds constructed and virtually screened through TOPKAT/ADMET test. Screened nontoxic drugs were docked with the Jab1/CSN5 receptor protein. Finally, docking results shows a best drug by comparative study of these ligand molecules, which had lowest Cdocker energy, was chosen against Jab1/CSN5 protein. The best molecule identified was further evaluated by molecular dynamics simulation of protein-ligand complex.

Keywords: Cdocker energy, Jab1/CSN5, MPN domain, Pancreatic cancer, TOPKAT/ADMET

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I. INTRODUCTION

Currently, treatment of pancreatic cancer is dependent majorly on surgical resection. However, only about 25% of patients' with pancreatic cancer are feasible for surgical resection due to the invasion of pancreatic cancer. Many patients with locally advanced or metastatic pancreatic cancer rely upon gemcitabine chemotherapy [1]. PC is a representative of the most highly vascularized and angiogenic solid tumors, which responds poorly to gemcitabine chemotherapy. Treatment with gemcitabine results in the median survival time of about 6.2 months [2]. Despite the worldwide decline in incidence and the major improvements in diagnosis and treatment, less than 20% of patients survive to 5 years. Thus, seeking new treatment strategies aiming to improve the anti-angiogenic capability is urgent in clinical practice in order to enhance the therapeutic efficacy and inhibit metastasis of pancreatic cancer.

A potential therapeutic target, the c-Jun activation domain-binding protein-1(Jab1), has been implicated to be involved in the tumorigenic process. Jab1 is involved in multiple protein interactions that affect many stages of tumorigenesis and, therefore, has the potential to be an effective therapeutic target. Jab1/CSN5 was originally identified as a c-Jun coactivator and subsequently discovered to be the fifth member and an integral component of the constitutivephotomorphogenic-9 (COP9) signalosome (CSN) complex, a multifunctional protein complex involved in modulating signal transduction, gene transcription, and protein stability in cells [3].

The human Jab1/CSN5 gene is located on chromosome 8 which consists of 334 amino

acids and has a molecular mass of 38 kDa [4]. CSN-associated Jab1/CSN5 is located primarily in the nucleus, free-form Jab1/CSN5 can be both cytoplasmic and nuclear [5]. Jab1/CSN5 contains an Mpr1-Pad1-N-terminal (MPN) domain metalloenzyme motif (JAMM) that is essential for the CSN isopeptidase activity responsible for the deneddylation of the cullin-RING ubiquitin ligases (CRLs) by CSN. Jab1/CSN5 plays an essential role in positively regulating cellular proliferation by functionally inactivating several key negative regulatory proteins and tumor suppressors through their subcellular localization, degradation and deneddylation, including p53, Smad 4/7, and the cyclin-dependent kinase inhibitor p27Kip1 (p27)[6-8, 21]. JAB1/CSN5 also interacts directly or indirectly with estrogen receptor α and that the increase in hormone-induced transcription and disruption of theNEDD8 pathway impairs ligand-ER_ degradation [9].Ectopic expression of Jab1 decreased endogenous Smad4 steady-state levels and its ubiquitination. In addition, Jab1 inhibited TGF-β induced gene transcription [10].

Our study involves the design of a suitable blocker to bind to the JAB1/CSN5 protein domain and inhibit the cell proliferation and it may potentially decrease the risk of cancer.By binding to MPN domain may inhibit the ubiquitination of tumour suppressor protein (TSP) such as p27, p53 and Smad4 and thereby inhibit the degradation of TSP. Curcumin and emodin, two natural plant-derived compounds, have been identified as Jab1/CSN inhibitors as they potently inhibit the kinase activity of Jab1/CSN5 and have been shown to enhance the stability of the p53 protein [1, 20, 11]. Similarly, thymoquinone isolated from *Nigella sativa* have shown to inhibit carcinogen induced formation of pancreatic tumors by suppressing AKT & ERK signalling pathway [12] and Mucin4 [13] and many antitumor activity has been reported [17,18]. GFER (Growth factor erv1-like) and Serine 10- phosphorylated form of p27Kip1 are the molecules have the ability to inhibit the Jab1/CSN5 mediated degradation of p27Kip1and Jab1 coactivation of c-jun respectively [6, 23].

In present study, appropriate modifications in the molecular structure of thymoquinone have been done to increase the effect and safer drugs for the treatment of neoplastic tumors. Docking studies indicate the not only interactions but also indicate the potential effect on the target. The Pharmacophore analysis was further carried out in order to provide a new insight to design novel molecules that can enhance or inhibit the function of the target. The computational analysis was carried out using Discovery Studio (DS) 2.5 (Accelrys Software Inc., San Diego; http://www.accelrys.com)

II. EXPERIMENTAL SECTION

2.1 Target Identification

The amino acid sequence of human COPS5 (Q92905) retrived from Uniprot database [19] was used as the query sequence for further studies. Sequence analysis of query protein with 16 different kinds of model organism such as vertebrates *(Homo sapiens, Bosgrunniesmutus, Bostaurus, Musmusculus, Daniorerio, Acyrthosiphonpi-sum)*, invertebrates *(Drosophila yatuba, Drosophila melanogaster)*, plants *(Zea mays, Arabidopsis thaliana, Solanumlycopercicum)* and microorganisms *(Dictyostelium-discoideum, chlamydomonasreinhardi, Zymo-saptoriatritici, Schizosaccharomyce-spomb, Saccharomyces cerevisiae)* on different parameters (Expected threshold, Blosum series and Word size) have been done using NCBI-BLAST [14]. Phylogenetic analysis of the JAB1/CSN5 protein of query and these model organisms have been done by SDSC Biology Workbench with the help of ClustalWand ClustalW distance matrix algorithm. Structure validation of query protein was done using the sequence from NCBI protein database against the proteomic tools in Expasy. Common structure from tertiary analysis tools (CPHModels, HHPred and GENO3d) having high score value and less E value have been taken for docking. The X-ray structure of unliganded human COPS5 was used in the present study (PDB code: 4F7O) [16].

2.2 Chemical Library

Lead molecule thymoquinone were then designed by introducing suitable modification in the scaffold molecule. This was done using **ACD/ChemSketch**

Freeware. The molecules basic function was maintained and no modifications were made to their functional group. The molecular properties of the designed lead molecules were then calculated using the 'General Purposes' protocol in Discovery Studio software. The molecular properties include chemical structure, molecular formula, weight, logP, hydrogen bond donor, hydrogen bond acceptor, characteristics of the compound etc. This was done to check, if the molecules satisfied Lipinski's rule of five. The compounds were then subjected to Toxicity Prediction (TOPKAT) in the ADMET protocol. TOPKAT computes a probable value of toxicity for a submitted chemical structure from a Quantitative Structure Relationship (QSTR) equation. Compounds which were found to have low and intermediate probability values were selected for further analysis by ADMET descriptors in the 'ADMET' protocol. High absorption level and low hepatotoxicity level were the criteria for further screening of compounds.

2.3 Receptor and Ligand Preparation

To make the protein to be stable, we minimized the energy of both protein (receptor 4F7O) and screened compounds by applying the forcefield '**CHARMm**' (Chemistry at HARvard Macromolecular Mechanics) and algorithm **conjugate gradient** with the help of Discovery studio. The steps are increased by 200 until the energy is equal.

2.4 Molecular Docking and Pharmacophore analysis

Docking is frequently used to predict the binding orientation of drug candidates to our protein target (receptor) in order to predict the affinity and activity of the drug molecule. CDOCKER, powerful CHARMm based docking method in Accelyrs Discovery studio 2.5, that have been shown to generate highly accurate docked poses, was used to dock the minimized receptor and ligands. The interactions for the pose with the lowest CDOCKER energy were studied and intermolecular hydrogen bonds were further examined.

Pharmacophore incorporates a complete definition of 3D chemical features (such as H bond donors, acceptors, lipophillic areas, positively and negatively ionizable chemical groups) that describe the interaction of a bound small organic molecule (ligand) and the surrounding binding site of the macromolecule. To create pharmacophore from protein-ligand complex, **Ligand Scout v2.0** software was used.

III. RESULTS

Evolutionary related species (phylogenetic analysis, phylogemetic tree-dendogram) from ClustalW and ClustalW distance matrix, in which sequences were taken from Blast result, so we knew which species, was closely related to the concerned protein or which were distinct. From this analysis, the query protein sequence was found to be evolutionarily closely related to vertebrates because they come under the same branch as well as close numerical distance in light year between the organismsin distance matrix and Microorganisms are distantly related with our query sequence, because they have long horizontal branch and large numerical distance in distance matrix [**Fig 1 A & B**]. From structure validation of query protein, PDB id 4F7O having highest score value of 394; least e-value of 1e-109 (CPHModels analysis [15]) and also the least e-value 4e-74, identity100% and high score 535.9 (HHPred analysis).Thus, PDB id 4F7O assumes to be as a template for docking analysis.

3.1 Virtual screening

The virtual screening of potential inhibitors against JAB1/CSN5 was carried out using Accelyrs Discovery Studio 2.5. Prior to docking to the ligand binding pocket of JAB1/CSN5, each subset of compounds was filtered by certain criteria as follows: For a total of 78 lead-like compounds, the criteria were hydrogen bond donors <5, hydrogen bond acceptors <10, Molecular weight <500 Da, AlogP <5 (Lipinski's Rule Of 5), benzene rings <4 and rotatable bonds <8 (Molecular properties shown in (**Table 1**) and their toxicity, ADMET tests were also done to screen the best molecule for further analysis.

A report of TOPKAT was analyzed for each compound and compounds having; NTP Carcinogenicity Call (Male Mouse) (v3.2) < 0.5, FDA Carcinogenicity Female Mouse Single vs Multi (v3.1) <0.5, Developmental Toxicity Potential (DTP) (v3.1) <0.5 and Aerobic Biodegradability (v6.1) <0.5 were

filtered out. Only compounds which indicate green signals for TOPKAT test were investigated for pharmacokinetic properties. (**Fig 3A**) indicates TOPKAT results of designed drug molecule 5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl) cyclohexa-2,5-diene-1,4-dione.

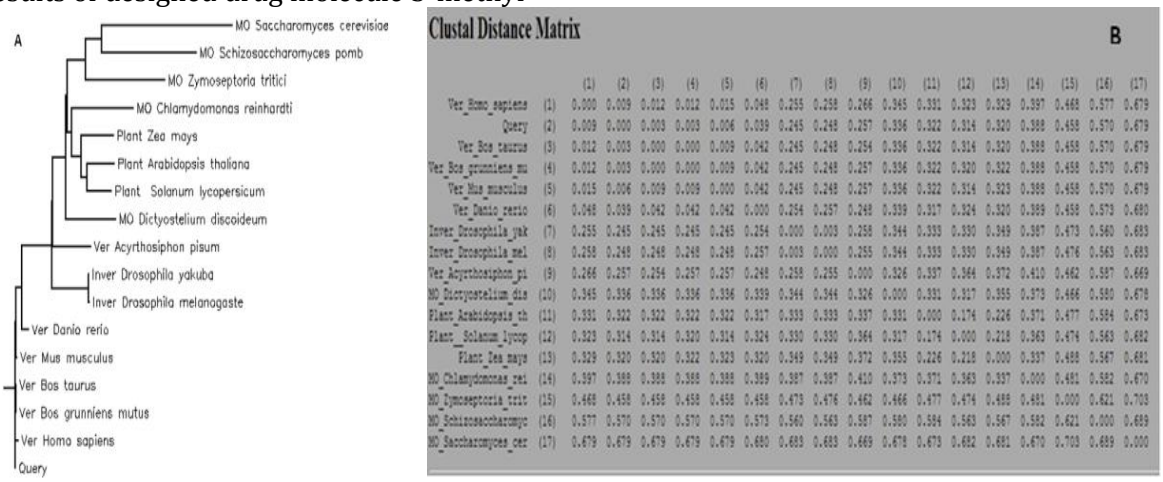

Figure 1: Phylogenetic relationship of query with different model organisms* (A) Dendogram showing evolution of query protein from different model organisms (B) Clustal Distance Matrix showing the numerical distance in light year between the organisms. *Ver-Vertebrates,Inver-Invertebrates, MO-Microorganisms.

The ADME (absorption, distribution, metabolism and excretion) values influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug.If the dot is in between the circles which indicate good ADMET values of the compound. The ADMET profiles of the 5 designed molecules are shown in (**Table 3**).

3.2 Molecular Docking

Based on the drug-likeness and pharmacokinetic properties these lead molecules were selected for docking and the interactions were studied by docking simulations. The designed molecules are minimized and are docked with the active minimized site of the receptor molecule whose Pdb id is 4F7O and the CDocker energy and CDocker interaction energy values are shown in (**Table 4**). The designed molecule 5MCD-5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl)cyclohexa-2,5-diene-1,4-dione docked with the lowest CDocker energy of -15.8545 kcal/mol (**Table 4**) interacts with residues TYR120, GLY128, ARG129 and LEU130 within the MPN domain by Intermolecular Hydrogen bonds (**Fig. 3 A**). This interaction/affinity plays an important role to inhibit the function of MPN domain. Docking the best drug molecule **5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl) cyclohexa-2,5-diene-1,4-dione,** which having a least Cdocker energy in Cdocker, has also been done by Hex (6.3) to study the pharmacophore analysis (**Fig. 3B**). E max = -230.83 and E.min =-115.92 values found out from docking with Hex 6.3.

3.3 Pharmacophore Analysis

The docked compound with binding pocket of receptor can be easily visualized on four feature of pharmacophore model using LigandScout v2.0. Binding pocket of ligand with receptor as well as aromatic ring (yellow), hydrophobic region (blue), hydrogen bond acceptor (red) and hydrogen bond donor feature (green) have also shown in (**Fig. 4**).

DISCUSSION

Growth inhibition and induction of apoptosis constitute the major mechanisms of action of most chemotherapeutics during cancer. Unfortunately, Pancreatic Cancer is inherently resistant to apoptosis in all conventional cancer therapeutic agents, which poses a great challenge to clinicians for the treatment of patients diagnosed with Pancreatic Cancer [22].

Table 1: Molecular properties of 5 designed molecules; a molecule satisfies Lipinski's Rule of 5

Name of the Drug	Molecule ID	Molecular Formula	Mol. weight	AlogP	Num_ H Acceptor	Num_H Donors	Num_ Rotatable bonds	Num_ Aromatic rings
5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl)cyclohexa-2,5-diene-1,4-dione	5MCD	C13H16O2	204.265	3.256	2	0	3	0
2-[(2R)-hex-5-en-2-yl]-5-methylcyclohexa-2,5-diene-1,4-dione	2RMD	C13H16O2	204.265	3.266	2	0	4	0
2-[(2S)-hex-5-en-2-yl]-5-methylcyclohexa-2,5-diene-1,4-dione	2SMD	C13H16O2	204.265	3.266	2	0	4	0
3-ethenyl-5-methyl-2-(propan-2-yl)cyclohexa-2,5-diene-1,4-dione	3ECD	C12H14O2	190.238	2.8	2	0	2	0
2-methyl-5-(propan-2-yl)-3-(prop-2-en-1-yl)cyclohexa-2,5-diene-1,4-dione	2MCD	C13H16O2	204.265	3.256	2	0	3	0

Table 2: Probability values of the TOPKAT prediction indicate all molecules are non-carcinogenic and have high level of aerobic biodegradability

S.No	Molecule ID	NTP carcinogenicity Call (Male mouse) (v3.2)	FDA carcinogenicity female mouse single vs multi (v3.1)	Developmental Toxicity Potential (DTP) (v3.1)	Rat oral LD50 (v3.1) (in mg/kg)	Skin irritation (v6.1)	Probability of Bio degradability (v 6.1)
1	5MCD	0.040	0.000	0.000	77.1	0.879	0.000
2	2RMD	0.000	0.000	0.000	165.1	0.997	0.000
3	2SMD	0.000	0.000	0.000	165.1	0.997	0.000
4	3ECD	0.316	0.001	0.002	174.8	0.999	0.000
5	2MCD	0.005	0.000	0.000	80.7	0.939	0.000

Table 3: ADMET Descriptors; indicate that the lead compounds are predicted to be easily absorbed, low probability of causing hepatotoxicity and are non – inhibitors of CYPD26 enzyme

Molecule ID	ADMET BBB	Absorption level	Solubility	Solubility level	Hepato toxicity	Hepatotoxicity probability	CYP2D6 Probability	AlogP28
5MCD	0.305	0	-3.92	3	0	0.238	0.089	3.256
2RMD	0.308	0	-3.76	3	0	0.35	0.099	3.266
2SMD	0.308	0	-3.76	3	0	0.35	0.099	3.266
3ECD	0.164	0	-3.56	3	0	0.198	0.009	2.8
2MCD	0.305	0	-3.91	3	0	0.158	0.099	3.256

Table 4: The Docking Results with their C-Docker Interaction Energy to active site of target receptor

Molecule ID	Lowest CDOCKER Energy (kcal/mol)	CDOCKER Interaction energy (kcal/mol)	Pose number
5MCD	-15.8545	21.6982	10
2RMD	-3.0177	9.0827	10
2SMD	-0.869	8.6710	10

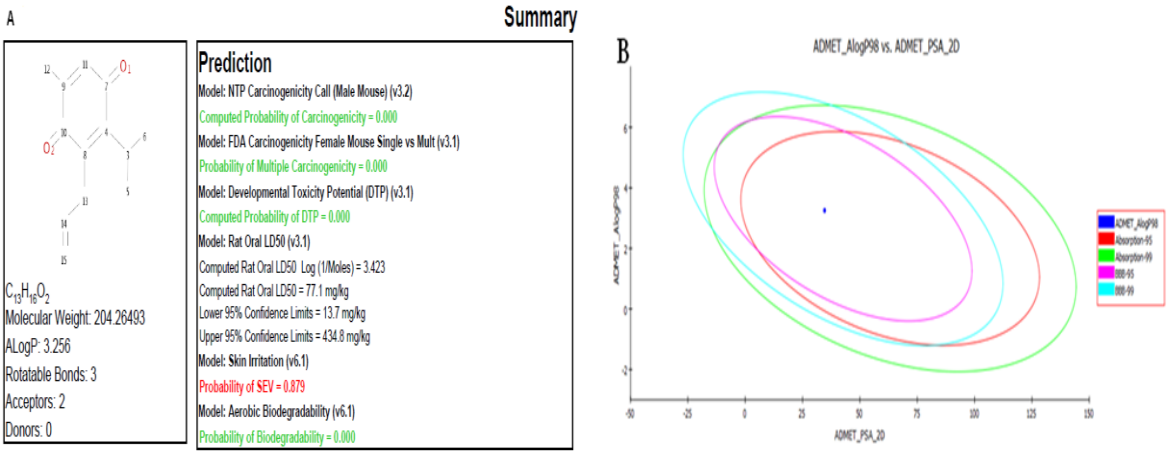

Figure 2: TOPKATand ADMET Results of Designed Drug Molecule 5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl) cyclohexa-2,5-diene-1,4-dione

(A) Showing Carcinogenicity, Biodegradability, Rat Oral LD50, Skin Irritation and Aerobic Biodegradability Properties of5MCD (B) Point-point plot of indicating the good absorption (inside <95% ellipsoid- red) and high Brain Concentration/Blood concentration (inside <95% ellipsoid- pink)

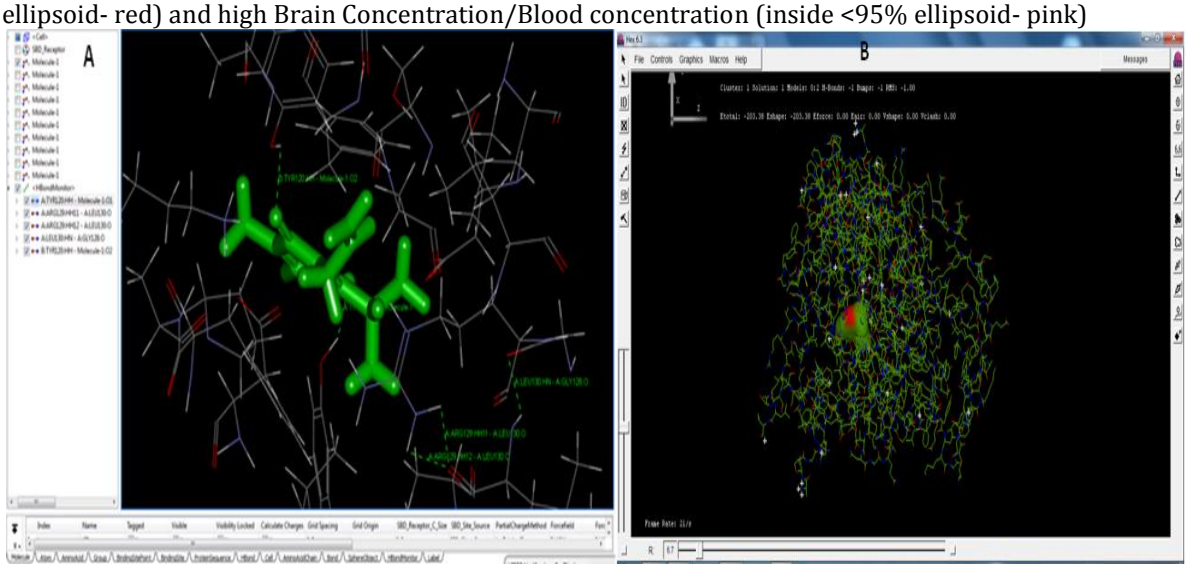

Figure 3: Docking of 5MCD with target Protein

(A) Intermolecular Hydrogen bond between 5MCD (O1 and O2) and TYR120 of target JAB1/CSN5 domain (B) Docking by Hex showing E max and Emin values

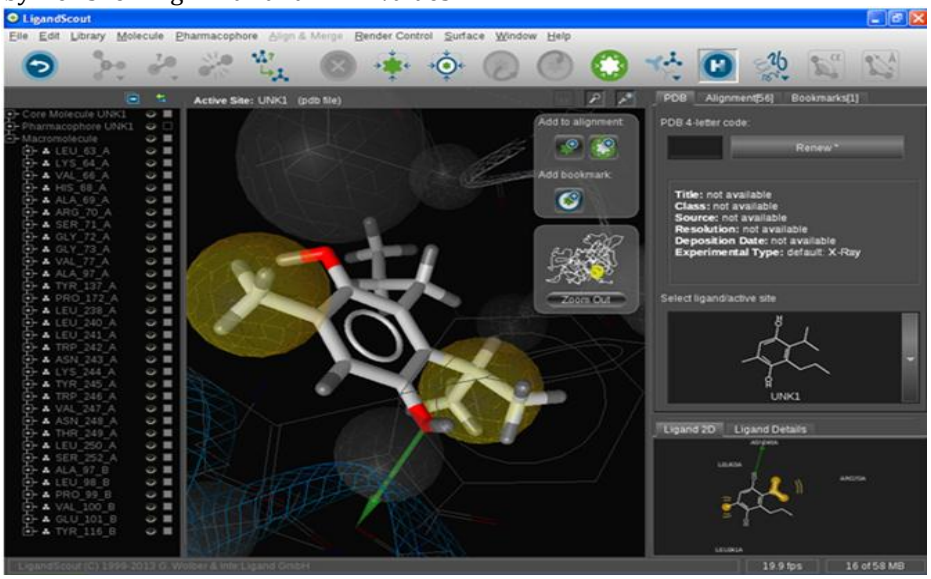

Figure 4: The designed compound with binding pocket of target receptor

The effects of Jab1/CSN5's multiple protein interactions are generally oncogenic in nature, and overexpression of Jab1/CSN5 in cancer provides evidence that it is involved in the tumorigenic process [4]. In this study, we used the crystal structure of JAB1/CSN5 protein and thymoquinone as scaffold to construct a computer model of the predicted antagonist form of target protein and used this model as atemplate to discover *in silico* small molecule inhibitors of JAB1/CSN5. Using the most active ACD/Chemsketch, we generated a small virtual library that can be easily and rapidly synthesized. Five designed compounds indicated in (**Table 1**) when subjected to Toxicity Prediction (TOPKAT) and ADMET Description protocol were predicted as non-carcinogenic, non-hepatotoxic, non-inhibitors of CYPD26 enzyme with good absorption levels and aerobic biodegradability, thus indicating low risk of side effects. Molecular docking analysis of the target and designed molecules provides evidence of effective binding of the compounds and interacting residues have been examined. Pharmacophore analysis generated a number of hydrogen bond donors, hydrogen bond acceptors and hydrophobic regions for the potential inhibitors. Based on binding energy, and hydrogen bond formed, docking results were analyzed to find out the best ligand **5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl) cyclohexa-2,5-diene-1,4-dione** which had high values to potentiate the target among all ligands. Thus the in-silico method adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of Pancreatic Cancer. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness. Further studies must be done to evaluate its therapeutic value in preclinical models.

CONCLUSION

No study has been published yet that evaluates the effect of thermoquinone derivatives in the expression of any Jab1/CSN5 protein by insilico approach. Follow-up studies include the analysis of derivatives of thermoquinone in the expression of other subunits of COP9 protein complex that are aberrantly expressed in tumors and evaluate its therapeutic and chemosensitizing effect in combination with other natural-derived drugs. Overall, **5-methyl-2-(propan-2-yl)-3-(prop-2-en-1-yl) cyclohexa-2,5-diene-1,4-dione**, which has a role in Jab1/CSN5 down regulation among other anti-tumorigenic properties, has potential for the development of novel therapies against pancreatic cancer. It is noteworthy to point out that since the binding affinity of JAB1/CSN5 with each selected compound is not measured experimentally, considering that virtual screening may yield false-positive results, further experiments are needed in the future to evaluate these JAB1/CSN5–compound interactions.

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