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IDENTIFICATION OF HUMAN cAMP DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT INHIBITORS

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ABSTRACT: Cyclic adenosine monophosphate (cAMP) dependent protein kinase plays a major role in cell signaling pathway. Over expression of extracellular cAMP dependent protein kinase catalytic subunit caused tumorgenesis in prostate. This protein may provide a base for the design of clinically applicable therapeutic strategies. In order to develop potential inhibitor of cAMP dependent protein kinase catalytic subunit, a high throughput virtual screening of zinc natural compounds database was conducted. Furthermore, QikProp, ADMET predictor and MM-GBSA was performed for ADME (Absorption, Distribution, Metabolism and Elimination), toxicity and binding energy prediction for ligands, respectively. Finally, molecular dynamics simulation was performed to get potential inhibitor. The crystal structure of cAMP dependent protein kinase was used for current study. The entire library of natural compounds was screened using HTVS for the primary level of screening. The binding of the compounds was studied using standard precision followed by Extra Precision algorithm of Glide Docking. On the basis of docking scores top ten hit compounds were selected for further analysis. The Binding affinity was further calculated using MMGBSA. The interaction studies using molecular docking and MMGBSA revealed significant docking scores and dG bind. Molecular dynamic simulation studies of zinc0B511410-protein complex for 50 ns shows the stability of system. Further experimental evaluation of the lead compounds can prove their potential as a therapeutic inhibitor.

INTRODUCTION: Various signal transduction pathways remain involved in the regulation of cell growth. The most important reversible mechanism for triggering or inhibiting the activity of specific proteins in a signalling pathway is phosphorylation. A large and diverse family of enzymes, protein kinases play a critical role in eukaryotic signal transduction [1].

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Protein substrates and protein kinases are toggled between active and inactive conformational states according to their state of phosphorylation [2] one of the simple and better-characterized members of the protein kinase family is cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA.) PKA exists as a heterotetramer in the inactive state, with a dimeric regulatory (R) subunit and two catalytic (C) subunits. Upon binding cAMP, the C subunits are released from the R subunits to interact with protein substrates. The catalytic core of the C subunit is conserved among family members and contains the basic features for binding ATP and protein substrate and for catalyzing phosphoryl transfer [3].

Conformational change is a common mechanism for kinases to orient a phosphoryl leaving group and to eliminate water from the active site [4]. Defective regulation of PKA holoenzyme activity is directly related to the progression of cancers [5]. Experimental studies revealed that over expression of cAMP dependent protein kinase catalytic subunit (PKACA) causes severe tumorgenesis in prostate and other cancers [6, 7]. Prostate cancer is the most commonly occurring form of cancer in western men, and the second leading cause of death due to cancer [8]. Prostate cancer therapies which are used these days have lots of side effects on human health. Natural compounds can be used as effective molecules in prostate cancer therapy to avoid the side effect.

Therefore, PKACA is being used as a drug target for various cancers. Few inhibitors of PKACA are under clinical trials. As drug discovery process is very expensive and time consuming process, *in silico* studies provide a cost effective platform for discovery of effective molecules. The present study was carried out to the discover the natural compounds for PKACA inhibition using high throughput virtual screening and molecular dynamics simulations technique.

MATERIAL AND METHODS:

Protein preparation and structure refinement: X-ray crystal structures for the PKACA (3AGM.pdb) with resolution of 2.0 Å was downloaded from Protein Data Bank [9]. Protein structure was prepared employing Protein Preparation Wizard of the Schrodinger Suite [10] to ensure the quality and reliability of the structure. In the protein preparation missing atoms/residues, steric clashes and format errors were checked. Determination of bond orders, partial charges/ protonation states and missing connectivity information were determined. Hydrogens were added, water molecules were removed, hydrogen bonds were optimized, disulphide bonds were created, misoriented groups were fixed and protein energy minimization was done. Protein optimization and minimization was done using OPLS (Optimized Potentials for Liquid Simulations) 2005 force field.

High throughput virtual screening: zinc biogenic compounds (Zbc), a commercially available primary and secondary metabolite database, originally includes 189466 compounds was used for high throughput virtual screening (HTVS). OPLS 2005 force field [11] was applied for database preparations Zbc compounds have been used to identify the mitogen activated protein kinase inhibitors [12]. The entire database of the zinc compounds was prepared by using LigPrep 3.5 module before the docking to obtain different stereochemical, tautomeric, and ionization conformer with minimum energy state of the ligands [13, 14].

In order to avoid excessive runtimes for high throughput virtual screening (HTVS) against entire database, prepared compounds were filtered on the basis of their drug-likeness properties, in terms of pharmacokinetic properties and, in terms of reactive functional groups to screen out unwanted compounds. Filtered database was subjected for the stepwise HTVS, followed by standard precision (SP) and extra precision (XP) docking using Glide. Prior to the HTVS, receptor grid was generated using Glide 6.8 module [15].

The prepared 3D structure of the target protein was used to generate the glide scoring grid for the successive docking calculations. Pharmacokinetic, ADME (absorption, distribution, metabolism and excretion) properties were calculated using Qikprop 4.5 module [16]. In general, the ADME descriptors are calculated at the last stage of the drug discovery process, but in the present study, the ADME properties calculations were performed in the preliminary stage to save the time as well as to curtail the cost involved in the process.

Binding free energy calculation: Generally, the calculations of binding energy are termed to be accurate when compared to the docking energy calculations [17]. Therefore, in the present study, the MMGBSA algorithm in the Prime 4.1 module was employed to determine the binding energy of XP docked compounds [18]. The equation for the binding energy calculation is:

$$G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA}$$

where, $\Delta E = E_{complex} - E_{protein} - E_{ligand}$

Above, $E_{complex}$, $E_{protein}$ and E_{ligand} and shows the minimized energy values of the protein−ligand complex, protein only, and ligand only,

respectively. The electrostatic solvation energy of the complex is represented as ΔGsolv. Similarly, the nonpolar contribution by the surface area to the solvation energy is represented as ΔGSA.

Molecular dynamics simulation: MD simulations were performed to obtain the most stable conformation of PKACA and ligand complex. Prior to MD simulation, XP docked complexes were prepared by protein preparation wizard in the same manner as prepared earlier for the virtual screening. Desmond 3.1 MD package was employed for MD simulations using OPLS-AA (All-Atom) force field parameters [19]. To immerse the prepared complex a truncated orthorhombic box of SPC water molecules was used, and the space between the peptide atoms and the boundary of the water box was not less than 10.0 Å. System was neutralized by adding Na+ ions.

Before the simulation, system was optimized in 2000 steps with constraints on heavy atoms in the system; this was followed by 2000 steps of full minimization without any constraints to remove bad contacts. Electrostatic interactions were calculated using Particle Mesh Ewald method, with a 10 Å cutoff in real space. Finally, 100 ns MD simulation in the isothermal-isobaric ensemble (the number of particles, the pressure, and temperature [NPT]) was carried out to adjust the pressure of the system. Nose- Hover chain thermostat and Martyna-Tobias-Klein barostat were used to maintain the system. A Langevin thermostat, with a collision frequency of 1.0 ps-1 was used to sustain the temperature at 300 K. The pressure was maintained at 1 atm using isotropic pressure coupling, with a relaxation time of 2 ps. MD simulation used a time step of 2 fs, and all covalent bonds involving hydrogen atoms were constrained with the SHAKE algorithm [20-22]. The coordinates of trajectories were saved every 10 ps during MD simulations.

RESULTS AND DISCUSSION:

High throughput virtual screening: The structure-based virtual screening is highly accepted for the identification of novel compounds. In this study, HTVS protocol was applied on zinc compounds to identify the PKACA inhibitors. Molecular possessing of useless compounds and generation of false-positives was avoided by calculating the pharmacokinetic properties. OPLS-2005 force field was applied to optimize the compounds. In addition, Lipinski rule of five was calculated applying cut-off range of the removal of the outliers [23]. After the filtration with defined criteria the prepared zinc database was compressed to 164530 compounds, which were then subjected to a stepwise HTVS protocol, followed by SP and XP docking to calculate the Glide score and Glide energy [24]. **Fig. 1** illustrates the 2D conformation of the ligands with the top-ten highest scores obtained after XP docking. The docking studies indicated that all top-ten hit compounds binds with the best efficacy with active site of receptor. MM/GBSA score analysis was performed to determine the binding efficiency using the pose viewer file of the protein-ligand complex generated from XP docking.

Out of all the ten hit compounds, zinc0B511410 showed the best dG binding (-96.776 kcal/mol) along with good glide score (-10.165 kcal/mol) and low glide energy (-88.207 kcal/mol) (Table 1). The interaction study reveals that good binding affinity was due to hydrogen bonding and hydrophobic interactions. **Fig. 2** shows the one hydrogen bond was forming between the zinc08511410 and Phe 54 of receptor. Phe 34, Leu 49, Val 57, Ala 70, Leu 74, Val 104, Met 120, Tyr 122, Val 123, Leu 173 and Phe 327 showed a strong hydrophobic interaction suggesting the strong ligand-protein interaction.

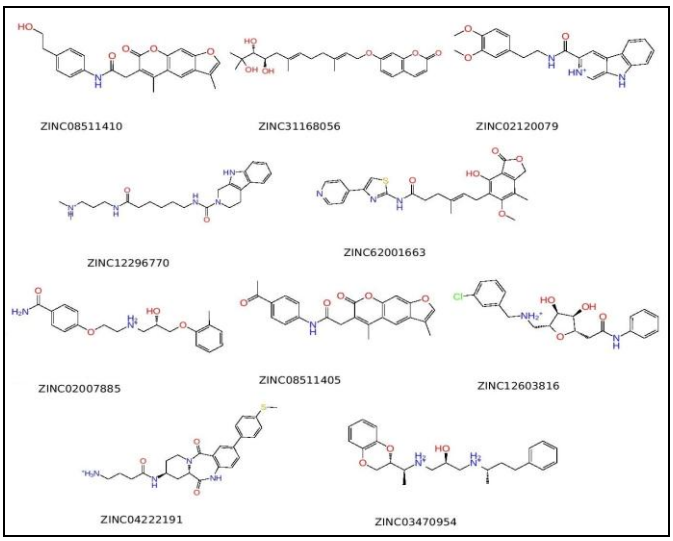

FIG. 1: REPRESENTING TOP 10 HIT COMPOUNDS OBTAINED FROM VIRTUAL SCREENING, 2D STRUCTURES AND THEIR CORRESPONDING ZINC IDs

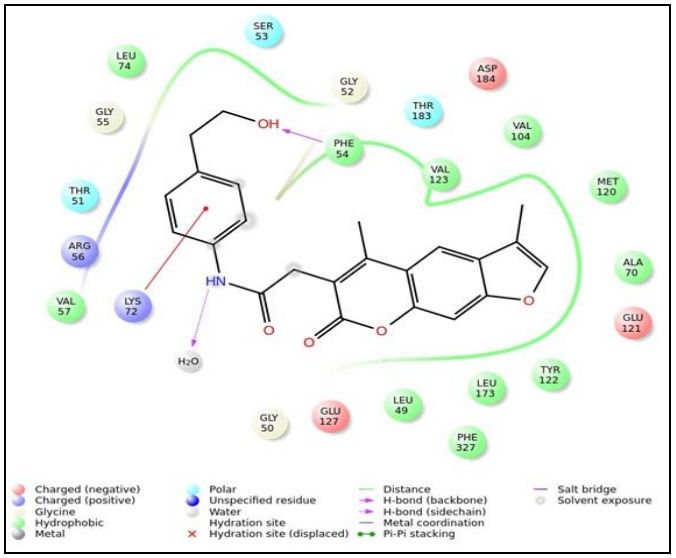

FIG. 2: 2D-INTERACTION MAP OF cAMP DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT OBTAINED THROUGH XP DOCKING OF ZINC08511410.

TABLE 1: TOP-10 HIT COMPOUNDS OBTAINED FROM VIRTUAL SCREENING AGAINST cAMP DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT ALONG WITH dG BINDING, DOCKING SCORE AND GLIDE ENERGY

Title	MM/GBSA dG bind	G-score (kcal/mol)	glide energy (kcal/mol)
zinc08511410	-96.776	-10.165	-88.207
zinc31168056	-95.893	-10.549	-76.875
zinc02120079	-92.365	-9.901	-76.052
zinc12296770	-90.805	-9.893	-91.935
zinc62001663	-87.227	-10.255	-101.822
zinc02007885	-86.605	-10.526	-88.579
zinc08511405	-86.429	-9.523	-89.938
zinc12603816	-85.43	-9.814	-83.448
zinc04222191	-84.735	-9.627	-85.417
zinc03470954	-82.841	-9.58	-81.176

***In silico* pharmacokinetic evaluation:** Pharmacokinetic properties which are critical for estimation of absorption and distribution of drugs within the body, drug metabolism and its access to biological membranes were predicted by QikProp. Molecular weight, violations of the Lipinski's rule of five (LROF) hydrogen bond donors, hydrogen bond acceptors, water solubility (QPlogS), logP (water/octanol), permeability through Madin-Darb y Canine Kidney Cells (QPlogMDCK), Qik Prop predicted log IC50 value for blockage of K+ channels (QPlogHERG), QikProp predicted gut-blood barrier (QPPCaco), water partition coefficient (QPlogPo/w), were calculated. All these values for zinc08511410 were well within the acceptable range for a drug with good pharmacokinetic properties (**Table 2**).

TABLE 2: PHARMACOKINETIC PROPERTIES OF ZINC08511410

Properties	Values
QPlogPw	13.056
% Human Oral Absorption	92.023
QPlogHERG	-6.234
QPlogPo/w	3.175
CNS	-2
QPlogBB	-1.358
QPPMDCK	181.567
donorHB	2
accptHB	7.2
QPlogS	-5.373
mol MW	391.423
QPPCaco	395.604
LROF	0

Protein−ligand complex simulation: The dynamic properties of zinc0B511410-system were analysed by molecular dynamics simulation studies for 50 ns. zinc0B511410-cAMP dependent protein kinase catalytic subunit complex having best MM/GBSA score was considered for the simulation study. The system was monitored using the root mean-square deviation (RMSD) values of C-alpha atoms. **Fig. 3** shows the RMSD values of zinc0B511410 system for 50 ns MD simulation.

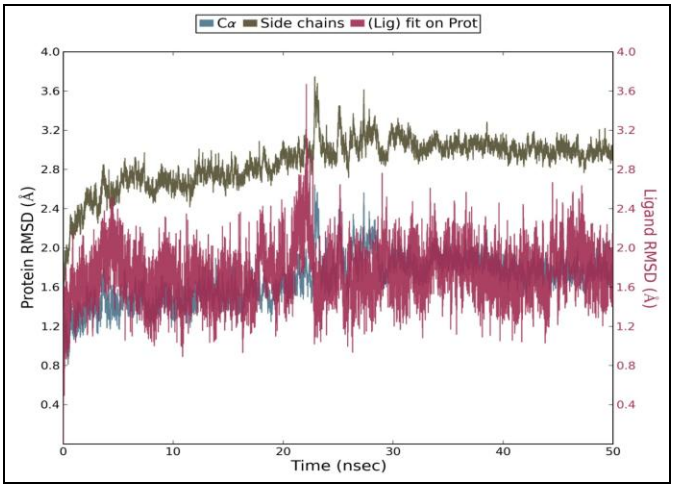

FIG. 3: ILLUSTRATING RMSD PLOT OBTAINED FROM 50 NS MD SIMULATION RUN. X AND Y AXES REPRESENT TIME (NS), AND ROOT MEAN SQUARE DEVIATION (RMSD), RESPECTIVELY

Trajectories analysis revealed that zinc0B134104 system was stable and well within the binding pocket of cAMP dependent protein kinase catalytic subunit. Lower root mean-square fluctuation (RMSF) values were depicted by the residues of the

active site and alpha helix regions, suggested the stability of the regions (**Fig. 4**). The energy of the system was relatively consistent during 50 ns MD simulations run.

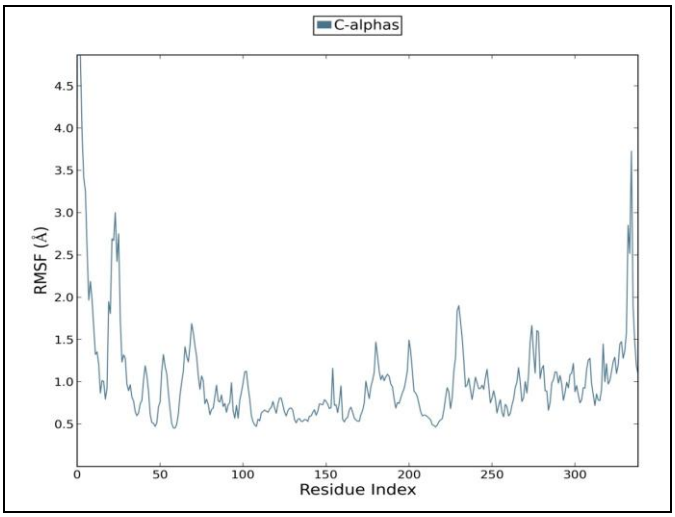

FIG. 4: ILLUSTRATING RMSF PLOT MD SIMULATION RUN FOR 50 NS. X AND Y AXES REPRESENT RESIDUE INDEX AND RMSF, RESPECTIVELY

Post-processing interaction was done to examine the ligand-protein binding interactions by implementing the trajectory frames generated from the MD simulation. The system stability was maintained mainly due to the hydrogen bond interaction with the Phe 54, and hydrophobic interactions of Val 57, Ala 70, Lys 72, Leu 173 and Phe 327 (**Fig. 5**).

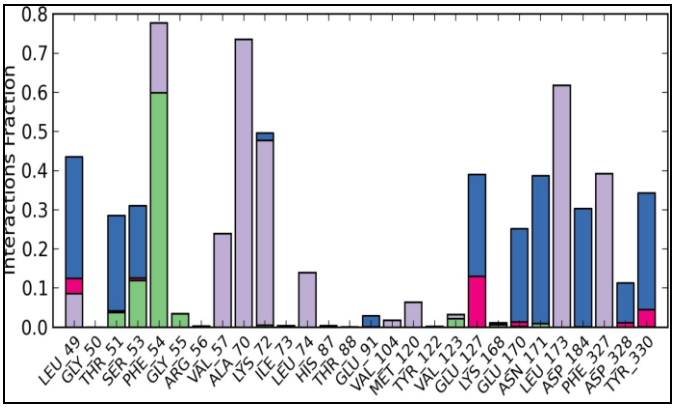

FIG. 5: PROTEIN-LIGAND CONTACT PLOT FOR 50 NS MD SIMULATION. PROTEIN -LIGAND CONTACT PLOT SHOWS THE BINDING INTERACTIONS, HYDROGEN BOND (GREEN), IONIC INTERACTION (PINK), WATER BRIDGE (BLUE) AND HYDROPHOBIC INTERACTION (PURPLE)

Leu 49,Gly 127, Glu 170, Asn 171 and Asp 184 were engaged in the formation of water bridges to maintain the stability of the system which shows that this complex is more stable in physiological conditions. Analysis of RMSD, RMSF and hydrogen bonding interactions of docking complex were depicted the steady nature of the lead compound.

Finally, the results of molecular docking and MD simulations proved that zinc0B511410 was having better binding orientations, RMSD, RMSF, potential energy and follow good pharmacokinetic properties. Therefore this lead compound can be considered for further studies against prostate cancer.

CONCLUSION: PKACA is a potential target for drug discovery. Molecular docking can act as a potential step towards the development of new therapy against prostate cancer. Novel inhibitors were identified by *in silico* screening of biogenic compounds from the zinc database using high throughput virtual screening. The selected compound zinc0B511410 was further validated by MD simulations. Finally, the results of molecular docking and MD simulations proved that zinc0B511410 was having better binding orientations, RMSD, RMSF, potential energy and follow good pharmacokinetic properties. Therefore this lead compound can be considered for further studies against prostate cancer.

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CONFLICT OF INTEREST: No conflict of interest.

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