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Research Article

Screening of diverse phytochemicals with Aurora Kinase C protein: An *In Silico* approach

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ABSTRACT

Aurora Kinase C, a vital serine-threonine protein Kinase, is an important member of the Aurora Kinase protein family which plays an important role in mitosis is a part of Chromosomal Passenger Complex (CPC). Aurora Kinase C overexpression is found to be linked with several cancer cell lines which demonstrate its oncogenic involvement and activity. Aurora C overexpression in certain cancer types makes it an important target to be considered for cancer therapeutics. The present research work focuses on Aurora Kinase C as an important target for computational studies. The protein model of Aurora Kinase C, as a protein target on docking with 1500 natural compounds (phytochemicals) reveals the binding of the natural ligand 3-beta,23,28-trihydroxy-12-oleanene 23-cafeate belonging to the terpenoid class with highest docking score. This best-bound ligand with the protein Aurora Kinase C was chosen for further understanding their protein-ligand interactions at the molecular level using the molecular dynamics simulation approach. Stability of the protein-ligand complex and its conformation helps in disclosing the potentiality of the best-bound ligand to be further chosen as an important small molecule inhibitor that would help to play a lead role in the further drug discovery process.

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1. INTRODUCTION

According to WHO Cancer fact sheets, cancer is the second leading cause of deaths throughout the globe which have been responsible for the total of 8.8 million deaths worldwide in the year 2015 (ref link: <http://www.who.int/en/news-room/fact-sheets/detail/cancer>). The burden of cancer have been predicted to be increasing globally, up-to 15 million by the year 2020, wherein the developing countries would be at the higher risk relating to incidences of cancer morbidity and mortality¹.

Aurora Kinases, the serine-threonine protein kinases that have been evolutionarily conserved are the novel kinases which plays a vital role in normal cell mitosis events as well as tumorigenesis in cancerous cells². During mitosis, the Aurora kinase family of proteins plays an indispensable role at varied stages for cellular development. Their aberrant expression caused by genetic instability could be a cause of cancer due to its over-expression³. The three Aurora Kinases have been found to be mapped on the intrinsically unstable regions of chromosomes giving a better explanation about their aberrant expression in various cancer types such as leukemia^{4,5}. Aurora C expression is found to be normally expressed in the testis, whereas its aberrant expression is also found in certain cancer cell lines⁶.

Of all the three kinases, Aurora Kinase C has been lesser reviewed and is found to be overexpressed in cancer types such as Breast cancer, Colorectal cancer, cervical cancer, prostate cancer, liver cancer as well as Gliomas^{3,4,6}. It had also been demonstrated that the mechanism of CpG methylation helps in further regulation of the expression of Aurora Kinase C gene in cancerous cells⁷. Aurora Kinase C is considered as a proto-oncogene as it aids for amplification in centrosome and multinucleation activity while its overexpression. Its overexpression in NIH-3T3 cells also promoted tumor formation in nude mice⁸.

Overexpression of Aurora Kinase C and its prognostic role had been found to be demonstrated in the invasive breast cancer and prostate cancer cells⁹. Aurora C at the DNA level has been found to exhibit Gene amplification and thus overexpression in the breast cancer cell lines¹⁰. Increased Aurora Kinase C activity has been found to be involved in tumorigenesis by promoting the cellular proliferation activities¹⁰. Published Literature suggests the overexpression of Aurora Kinase C in diverse cell lines such as hematological, breast and prostate cancer cell lines^{5,11,12}.

Overexpression of genes related to Aurora C and Survivin has been found to play an important role in the development of colorectal cancer^{12,13}.

Aurora C overexpression which results in phenotypical expression of polyploid cells with the abnormal centrosome numbers has been found to be provoked in the absence of p53¹⁴. It had also been found that the overexpression of Aurora C caused an interference with the spindle checkpoint activation mediated by Aurora B¹⁰. This further implies that the Aurora kinase C tends to decrease the activity of Aurora kinase B. Moreover the overexpression of Aurora Kinase C protein implicated an increase in the tumorigenicity of the cancerous cells¹¹.

Aurora Kinase C protein, which thus serves as a promiscuous target for cancer therapy have been further explored through computational approaches such as protein modeling and have been chosen as a Protein target receptor. The library of 1500 naturally occurring compounds with proven anti-cancer activity from the NPACT database have been docked with the Aurora Kinase C protein structure which helped in exploring the binding efficacy of best ligand from the large pool of natural ligands, that could further help in the pathophysiological conditions such as cancer arising from its overexpression.

2. MATERIALS AND METHODS

Aurora C Protein Modeling and protein preparation

The Aurora Kinase C protein structure model had been predicted through its amino acid sequence available in Uniprot (<http://www.uniprot.org/>) with uniprot ID: Q9UQB9¹⁵. Using automated I-Tasser (The iterative threading assembly refinement) Server (<http://zhanglab.ccmb.med.umich.edu/I-TASSER>), the 3d atomic models of the Protein structure have been built through the iterative threading assembly simulations technique^{16,17,18}.

A meta threading program LOMETS which consists of multiple threading algorithms, the given amino acid query sequence identification of the structural templates is done. Full-length topology model is constructed by the reassembling process of the continuously aligned protein fragment structures from the templates¹⁹. The unaligned regions in the structures are built by the *ab initio* procedure of folding which is based on the replica-exchange Monte Carlo simulations^{19,20}. Trajectories are then constructed by the clustering approach based program, SPICKER for two

rounds in order to produce a refined structural model^{21,22}. Another round of structural reassembly was performed for the refinement of structural models. Conformations having lower free-energy further refined by full atomic simulation process by the help of ModRefiner and FG-MD^{22,23}.

Preparation of protein prior to docking was done by deleting water molecules and cleaning the protein in YASARA Structure followed by Energy minimization using the YAMBER force field²⁴.

Natural ligands dataset and ligand preparation

NPACT (Naturally occurring plant-based Anti-Cancer compound activity Target Database) is an important database related to the plant derived natural anticancer compounds²⁵. It consists of around 1500 natural ligands which were further compiled into data-sets and used for the docking purpose. These ligands were cleaned and the hydrogens were added to the ligands for further use using Marvin Sketch tool.

Molecular Docking

YASARA Structure (version 17.8.15) have been utilized for the protein-ligand docking purpose²⁶. It is based on the Autodock Vina algorithm and it utilizes the following formula for calculating the docking score.

$$\Delta G = \Delta G_{(vdw)} + \Delta G_{(HBond)} + \Delta G_{(elec)} + \Delta G_{(tor)} + \Delta G_{(desolv)}.$$

Wherein, $\Delta G_{(vdw)}$ is the component energy terms related to van der wals bond, $\Delta G_{(HBond)}$ is the component energy term related to Hydrogen bonds, $\Delta G_{(elec)}$ is the component energy terms related to electrostatics, $\Delta G_{(tor)}$ is the component energy term related to the ligand's torsional free energy and

$\Delta G_{(desolv)}$ is the component energy term related to the desolvation for the empirical calculation of the docking/binding energy for a protein-ligand complex. The Higher docking score represents the better protein-ligand binding whereas, the negative score represents no binding between the Protein and the ligand. The protein-ligand interactions were further visualized in 3D and 2D using the Accelrys Discovery Studio Visualizer.

Molecular Dynamics Simulation

The protein-ligand complex with highest binding energy have been selected for the Molecular Dynamics simulations and was simulated for the 30ns production period time with the constant number of atoms at the constant pressure and temperature using the AMBER14 force field^{27,28}. The molecular dynamics simulation had been performed at 1 bar pressure, 298 K temperature, along with 0.9% NaCl at physiological pH of 7.4 at solvent density 0.997, time steps of 1 fs with periodic boundaries and all atoms in mobile state^{29,30}. The default water model TIP3P (Three-site Transferable Intermolecular Potential) had been chosen for the Molecular dynamics simulation³¹. Estimation of the binding affinity in terms of time vs energy along with time vs RMSD were further calculated to decipher the stability of the protein-ligand complex.

3. RESULTS AND DISCUSSION

Aurora C- Protein modelling

The predicted protein model with the C- Score (confidence score) of -0.93 had been selected for further computational studies wherein, the C- score with higher value depicts the model with high confidence.

Figure 1(A): Protein model of Aurora C protein kinase obtained by threading. (B.) Ramachandran plot for the Aurora C protein kinase obtained after energy minimization

After protein structure minimization the number of residues in the favoured region, allowed region and outlier regions are 92.2 %, 7.2 % and 0.7 % respectively. The Ramachandran plot for the protein model has been shown in the figure 1.

The predicted normalised B-factor values for the protein are shown in the figure 2. The B-factor value shows an indication

of the extent of inherent thermal mobility of the amino acid residues/ atoms in the protein structure. Normalised B-factor values are inferred from the template proteins known while threading process.

Figure 2: Normalised B- factor values for the protein Aurora Kinase C

Molecular Docking

The molecular docking studies reveals the binding affinity of the natural ligands towards the Aurora C protein model. Various types of interactions such as hydrogen bond interactions, pi-sigma bond, alkyl bond and pi- alkyl bond were observed in the protein- ligand interaction complex.

Key contacting receptor residues were also noted. The top three protein-ligand complexes with the respective binding energies, dissociation constants and the number of hydrogen bonds are noted in the table-1. Better binding of the ligand towards the protein chain is indicated by the higher positive binding energy, whereas the negative binding energies indicate no binding.

Table 1: Top 3 Molecular Docking Results of phytochemicals with Aurora Kinase C.

Sr. No.	Ligand Name	Binding energy [kcal/mol]	No. Of H-bonds	Contacting receptor residues
1	3-beta,23,28-trihydroxy-12-oleanene 23-cafeate.	9.171	3	LEU 49, LYS 51, GLY 52, LYS 53, PHE 54, VAL 57, ALA 70, LYS 72, LEU 104, LEU 120, GLU 127, LYS 130, ASP 166, LYS 168, GLU 170, ASN 171, LEU173, ALA 183 ,ASP 184, TRP 187, GLY 201, THR 202, LEU 203.
2	3-beta-trans-(3,4-dihydroxycinnamoyl-oxy)olean-12-en-28-oic acid	9.133	2	GLU 136, LYS 137, LEU 138, ASP 139, GLU 140, TYR 230, LEU 233, VAL 234, GLY 235, TYR 236, GLU 240, ARG 250, ARG 257, PHE 258, PRO 259, SER 261, MET 262, PRO 263, TRP 292.
3	Gitoxin	9.035	6	GLU 136, LYS 137, LEU 138, ASP 139, GLU 140, TYR 230, LEU 233, VAL 234, GLY 235, TYR 236, PRO 237, PRO 238, GLU 240, ARG 250, ARG 257, PRO 259, SER 261, MET 262, PRO 263, TRP 292.

Figure: 3 2D and 3D Protein ligand complex representation of Aurora C protein with ligand 3-beta_23_28-trihydroxy-12-oleanene 23-cafeate.

Figure 4: 2D Protein ligand complex representation of Aurora C protein with ligand 3-beta-trans-(3,4-dihydroxycinnamoyl-oxy) olean-12-en-28-oic acid

Figure 5: 2D and 3D Protein ligand complex representation of Aurora C protein with ligand Gitoxin

Molecular Dynamics Simulations

The best bound protein-ligand complex of Aurora C with the terpenoid 3-beta₂₃-trihydroxy-12-oleanene 23-caffeate had been subjected to Molecular dynamics simulations using AMBER 14 force Field in NPT ensemble wherein, the number of atoms, pressure and temperature are constant. The Time (ns) vs Energy (kJ/mol) plot demonstrated the fluctuations

of the simulated complexes wherein, the energy values ranged from -3698145.074 kJ/mol to -2827450 kJ/mol. The average energy value had been observed to be -2834566.119 kJ/mol. The major peaks with energy values in kJ/mol were observed to be -2827768.52, -2827450.13, -2827547.37, -2830373.04 and -2830576.36 at 1.7 ns, 8.2 ns, 12.2 ns, 12.9 ns and 23.6 ns respectively.

Figure 6: Time (ps) vs Energy (kJ/mol) of the best bound protein-ligand complex

The time vs RMSD plot aided further in revealing the stability of the protein-ligand complex. The minimum and the maximum RMSD observed were 0.459 Å and 13.576 Å

respectively. Average RMSD of 8.230 Å had also been observed during the production time period of 30ns.

Figure 7: Time (ps) vs RMSD (Å) of the best bound protein-ligand complex.

Molecular dynamics simulations results significantly explains about the stability of the protein-ligand complex. The time vs RMSD and time vs Energy fluctuations result demonstrates the binding affinity of the protein-ligand

complex along with the conformational changes associated with protein-ligand complex for the production period of 30ns.

Figure 8: Changes in Protein-ligand complex observed after every 2.5 ns of the molecular dynamics simulation (30 ns).

At every 2500 ps step interval the trajectory was exported for analysis of the dynamic interaction profile of the the Aurora C Protein with the top bound ligand 3-beta,23,28-trihydroxy-12-oleanene 23-cafeate (figure 9).

Initially the Hydrogen bond interactions between the ligand 3-beta,23,28-trihydroxy-12-oleanene 23-cafeate and the Aurora C protein were observed with the residues Asp 184, Lys 53, Glu 127, Gly 201, Leu 203 and Lys 51 at the distance of 1.63 Å, 1.97 Å, 2.89 Å, 2.16 Å, 2.40 Å, 1.70Å respectively. Furthermore, the ligand the alkyl bonds bonds with the residues Trp187, Ala 183, Val 57, and Leu 173 were observed at the distances of 5.42 Å, 5.13 Å, 4.64 Å and 4.57 Å respectively. It had been observed that the Hydrogen bond interaction of the ligand with the Lys 53 residue was found to be conserved for the 20 ns time interval. Hydrogen bond interaction of the ligand with the residue Asp184 and Lys 51 along with the alkyl bond interaction with the residue Als 183 and Lys 53 were also been found to be conserved for the 30 ns time interval.

4. CONCLUSION

Aurora Kinase C is overexpressed in many cancer types and can be considered as a target for anti-cancer therapy. Screening of varied phytochemicals with the protein Aurora C Kinase have been performed using the Molecular docking approach wherein the ligand 3-beta,23,28-trihydroxy-12-oleanene 23-cafeate, a terpenoid, 3-beta-trans-(3,4-dihydroxycinnamoyl-oxy)olean-12-en-28-oic acid, a steroid and Gitoxin, a steroidal glycoside has been observed to be the interacting with the protein Aurora Kinase C with the binding energies of 9.171 kcal/mol, 9.133, kcal/mol and 9.035 kcal/mol, respectively. The protein-ligand complex of Aurora Kinase C with the topmost ligand 3-beta,23,28-trihydroxy-12-oleanene 23-cafeate were further subjected to molecular dynamics simulations for 30 ns time interval. The time (ps) vs RMSD (Å) and Time (ps) vs Energy (kJ/mol) plots were plotted for the same and the time (ps) vs energy (kJ/mol) plot showed nearly stable energy conformation throughout the production period of 30 ns. The hydrogen bond interaction of the ligand with the protein residue Asp 184 and Lys 51 was found to be conserved for the 30 ns time interval. The molecular hits identified in the study can be further tested *in vitro* before claiming its inhibitory potential.

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