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Artificial Intelligence-Driven Assessment of Aromadendrin as *Casein-Kinase* Inhibitor against Cholangiocarcinoma

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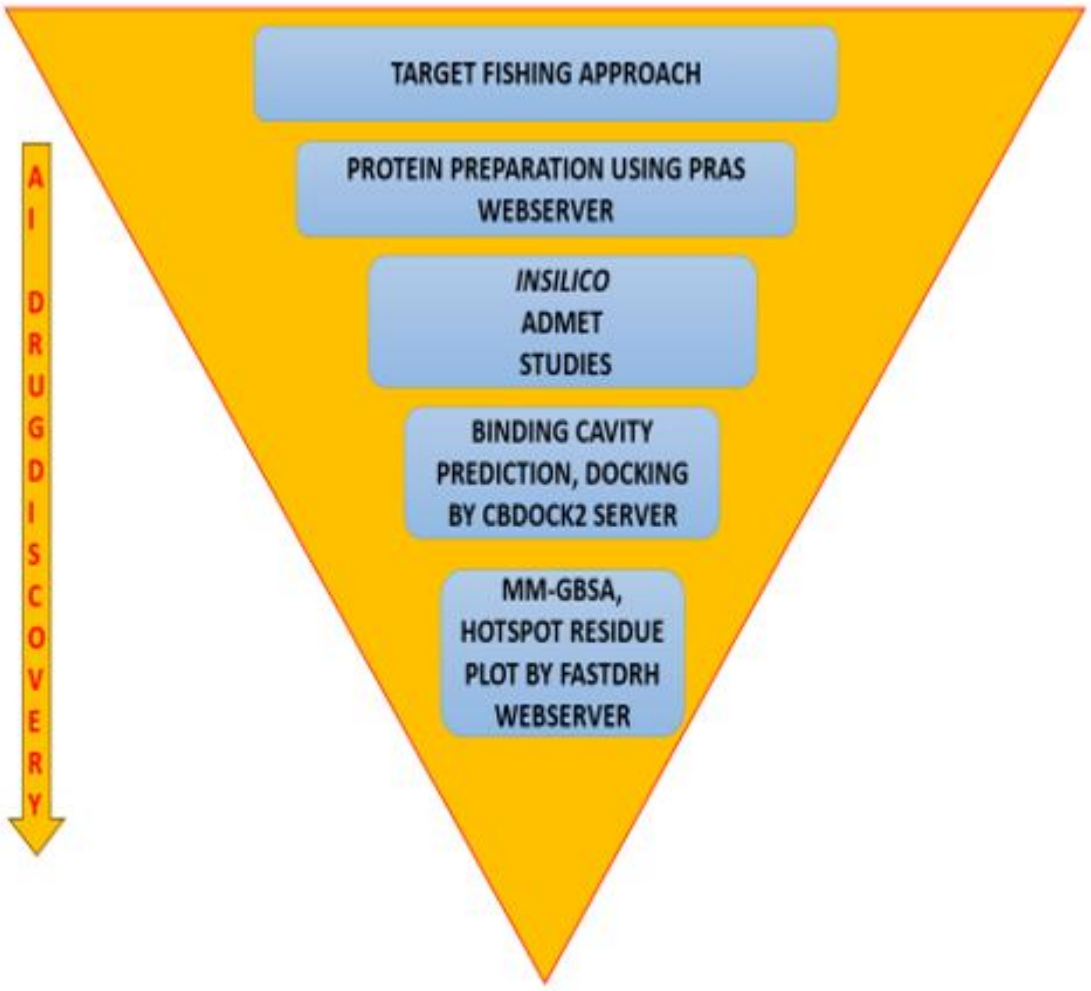
ABSTRACT

Cancer is a disease characterized by abnormal gene expression. Abnormal gene expression results from different types of mutations within genes and/or chromosomes. A diverse category of cancers known as cholangiocarcinomas (CCA) can develop from the Hering canals and spread to the main bile duct. Computer-aided drug design has grown in favour and demonstrated its ability to significantly reduce the time and resources needed, particularly in the early phases of the pipeline for drug discovery and development. **Materials and methods:** The SuperPred 3.0 platform is used for finding the suitable enzyme target. *In-silico* ADMET evaluation is done with the *SwissADME* and the *Osiris property explorer*. Protein preparation is done for the *human casein kinase 2 alpha* retrieved from RCSB databank with PDB ID:6TLS using the *Protein repair and analysis server (PRAS)*. Active site prediction and docking are done using the *CB-DOCK2 webserver*. Results of docking are visualized using the *Biovia discovery studio*. Molecular dynamics and re-scoring calculations are performed using the *Cabsflex 2.0* and the *fastdrh server* respectively. **Results and discussion**: The docking results showed a binding affinity of -8.7 kcal/mol against the *human casein kinase 2 alpha*.MD simulation shows that the protein-ligand complex is stable to withstand the realistic environment. **Conclusion:** From the current research, we found that the Aromadendrin can inhibit effectively against *human casein kinase 2 alpha* as an orally bioavailable, non-toxic ligand in the area of cancer drug discovery. Hence, the compound Aromadendrin may be considered as leads in cancer drug design. We also understood that AI-guided drug design fastens the drug discovery process.

GRAPHICAL ABSTRACT



INTRODUCTION

Cancer is a disease characterized by abnormal gene expression. Abnormal gene expression results from different types of mutations within genes and/or chromosomes. Mutations are usually somatic, which means they have acquired mutations in a diploid cell. Loss of control of the cell cycle is a critical step in cancer development. Cells become abnormal and processes regulating normal cell division are disrupted[1]. A diverse category of cancers known as cholangiocarcinomas (CCA) can develop from the Hering canals and spread to the main bile duct. CCAs are uncommon tumours with an incidence of less than 2/100,000 and make up about 3% of gastrointestinal tumours overall[2].

After hepatocellular carcinoma, they are the second most prevalent primary hepatic cancers (HCC). About 20% of hepatobiliary cancer fatalities, which account for 13% of all cancer deaths globally, are attributable to CCA[3].In the latter stages of the illness, patients with CCA frequently exhibit vague symptoms such cholangitis, weight loss, or painless jaundice. The prognosis becomes uncertain because these malignancies are challenging to identify and treat; in fact, over half of patients who do not receive treatment may away in 3–4 months because of local tumour growth and bile duct[4].

The phrase "computer-aided drug design," or "CADD," is more general and is frequently used to describe the use of computational methods and techniques in the methodical and logical design of novel treatments. CADD has grown in favour and demonstrated its ability to significantly reduce the time and resources needed, particularly in the early phases of the pipeline for drug discovery and development[5]. Molecular docking, molecular dynamic simulation, similarity search, pharmacophore mapping, quantitative structure-activity relationship (QSAR), and scaffold hopping are a few of the well-liked CADD tools[6].

Chionanthus retreatus is the source of the flavonoid aromadendrin, which is found to exhibit wide Pharmacological properties, such as antiproliferation, anti-cardiac hypertrophy, anti-inflammation, anti-oxidant, and antihyperglycemia[7-9]. The present study also aims to evaluate the Aromadendrin as inhibitor of *human casein kinase 2 alpha* against the cholangiocarcinoma through the *In-silico* approach.

MATERIALS AND METHODS

2.1 TARGET FISHING AND DATA CURATION

Superpred 3.0 platform is used for finding the suitable target for screening (https://prediction.charite.de/)[10]. The structure of the Aromadendrin was retrieved from the *PubChem* database in the structure data file (SDF) format for the screening process. (https://pubchem.ncbi.nlm.nih.gov/).

2.2 ADMET EVALUATION

ADMET properties are of utmost importance in determining the drug action. In the current study, using the *SWISSADME*, the drug-likeness profiling based on adsorption, distribution, metabolism, and excretion is done. The SMILES notation obtained from the *Pubchem* database was given as data input in the *SWISSADME (http://www.swissadme.ch/index.php)*[11]. *Osiris property explorer* is used to predict the toxic effects of the flavonoid. (https://openmolecules.org/propertyexplorer/).

2.3 DOCKING STUDIES

2.3.1 PROTEIN RETRIEVAL AND PREPARATION

The 3D structure of the *human casein kinase 2 alpha* (PDB CODE: 6TLS) was retrieved from the RCSB protein data bank. Then it is given as input in the *Protein repair and analysis server* (*PRAS*- https://www.protein-science.com/) for checking the missing heavy atoms, residues and the hydrogen atoms and are fixed[12].

2.3.2 LIGAND PREPARATION

The ligand is subjected to the energy minimization step by using the *MM2 force field* tool of the *Chem3d* module in the *chemBioOffice software package*.

2.3.3 ACTIVE SITE PREDICTION

The Binding cavity is predicted using the *CB-DOCK2 web server* which utilizes a structure-based approach to predict cavities by clustering the solvent accessible surface. It offers valuable information regarding the center, size, and volume of the predicted cavities. (https://cadd.labshare.cn/cb-dock2/index.php)[13].

2.3.4 DOCKING AND VISUALIZATION STUDY

Docking is performed using the *Autodock vina* module in the *CB-DOCK2 webserver* and the binding interactions are visualized using the *Biovia Discovery Studio software 2021*.

2.3.5 MOLECULAR DYNAMICS (MD) SIMULATION

The *cabs flex 2.0 webserver* is used to simulate the protein. The maximum RMSF value indicates greater flexibility, while the minimum value indicates that the system was constrained throughout the simulation run. (https://biocomp.chem.uw.edu.pl/CABSflex2)[14].

2.3.6 MOLECULAR MECHANICS – GENERALIZED BORN SURFACE AREA SOLVATION (MM- GBSA) CALCULATION

A free, publicly accessible web server called *fastDRH* has been launched to forecast and examine protein–ligand interaction structures. Structure-truncated MM/PB(GB)SA free energy calculation processes and various poses based per-residue energy decomposition analysis were well integrated into a user-friendly and versatile web platform in this server. It includes the docking protocol based on user defined selection of *AutoDock Vina*, and *AutoDock*-GPU docking engines[15]. (http://cadd.zju.edu.cn/fastdrh/overview)

3. RESULTS AND DISCUSSION

3.1 TARGET FISHING

SuperPred software predicted the target, *human casein kinase 2 alpha* with a probability score of 88.92% and model accuracy of 99.23%. The indication of the predicted target is found to be cholangiocarcinoma. The results of predicted targets and predicted indications are given in the fig.no 1 and fig no.2.

Target Name	ChEMBL-ID	UniProt ID	PDB Visualization	TTD ID	Probability	Model accuracy
Tyrosyl-DNA phosphodiesterase 1	CHEMBL1075138	Q9NUW8	6N0D	Not Available	99.64%	71.22%
Endoplasmic reticulum-associated amyloid beta-peptide-binding protein	CHEMBL4159	Q99714	2O23	Not Available	97.93%	70.16%
DNA-(apurinic or apyrimidinic site) lyase	CHEMBL5619	P27695	6BOW	T13348	97.62%	91.11%
DNA topoisomerase II alpha	CHEMBL1806	P11388	6ZY5	T17048	92.24%	89%
Transcription intermediary factor 1-alpha	CHEMBL3108638	O15164	4YBM	Not Available	91.6%	95.56%
Bloom syndrome protein	CHEMBL1293237	P54132	4O3M	Not Available	90.12%	70.06%
Monoamine oxidase A	CHEMBL1951	P21397	2Z5Y	Not Available	89.1%	91.49%
Casein kinase II alpha/beta	CHEMBL3038477	P67870	6TLS	T51565	88.92%	99.23%
Cathepsin D	CHEMBL2581	P07339	4OD9	T67102	87.9%	98.95%
Pregnane X receptor	CHEMBL3401	O75469	6TFI	T82702	86.15%	94.73%
Transthyretin	CHEMBL3194	P02766	6SUG	T86462	85.3%	90.71%
Thyroid hormone receptor alpha	CHEMBL1860	P10827	3ILZ	T79591	84.42%	99.15%
LSD1/CoREST complex	CHEMBL3137262	O60341	5L3D	Not Available	82.82%	97.09%
Dual specificity protein kinase CLK4	CHEMBL4203	Q9HAZ1	6FYV	Not Available	82.41%	94.45%
Arachidonate 12-lipoxygenase	CHEMBL3687	P18054	3D3L	Not Available	81.71%	75.57%
Kruppel-like factor 5	CHEMBL1293249	Q13887	Not Available	Not	80.91%	86.33%

Fig no.1: Predicted targets for the Aromadendrin using *SuperPred server*

Target Name	ChEMBL-ID	Indication	Probability	Model accuracy
DNA-(apurinic or apyrimidinic site) lyase	T13348	Glioma [ICD-11: 2A00.0]	97.62%	91.11%
DNA-(apurinic or apyrimidinic site) lyase	T13348	Melanoma [ICD-11: 2C30]	97.62%	91.11%
DNA-(apurinic or apyrimidinic site) lyase	T13348	Ocular cancer [ICD-11: 2D00-2D07]	97.62%	91.11%
DNA-(apurinic or apyrimidinic site) lyase	T13348	Solid tumour/cancer [ICD-11: 2A00-2F9Z]	97.62%	91.11%
DNA topoisomerase II alpha	T17048	Solid tumour/cancer [ICD-11: 2A00-2F9Z]	92.24%	89%
Casein kinase II alpha/beta	T51565	Cholangiocarcinoma [ICD-11: 2C12.10]	88.92%	99.23%
Casein kinase II alpha/beta	T51565	Solid tumour/cancer [ICD-11: 2A00-2F9Z]	88.92%	99.23%
Cathepsin D	T67102	Hypertension [ICD-11: BA00-BA04]	87.9%	98.95%
Cathepsin D	T67102	Multiple sclerosis [ICD-11: 8A40]	87.9%	98.95%
Pregnane X receptor	T82702	Arteriosclerosis [ICD-11: BD40]	86.15%	94.73%
Transthyretin	T86462	Amyloidosis [ICD-11: 5D00]	85.3%	90.71%
Transthyretin	T86462	Cardiomyopathy [ICD-11: BC43]	85.3%	90.71%
Transthyretin	T86462	Hereditary amyloidosis [ICD-11: 5D00.2]	85.3%	90.71%
Thyroid hormone receptor alpha	T79591	Congestive heart failure [ICD-11: BD10]	84.42%	99.15%

Fig no.2: Predicted indications for the Aromadendrin using *SuperPred server*

3.2 ADMET RESULTS

Aromadendrin obeys the Lipinski rule and is also found to show good GI absorption. It does not inhibit cytochrome enzymes like CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. It exhibits a bioavailability score of 0.55. Drug likeness filters such as Lipinski, Ghose, Veber, Egan and Muegge are obeyed which makes evident that the Aromadendrin is potentially safe as orally active drug.

On the scale of synthetic accessibility ranging from 0 to 10, it lies at 3.42 which tells it can be synthesized easily. The BBB permeation is not present, avoiding the deleterious effects in the CNS. The table no.1 shows the ADMET results obtained using the *SWISSADME* and *Osiris property explorer. Osiris property explorer* has predicted the compound 'Aromadendrin' to be non-toxic for toxic endpoints such as mutagenicity, tumorigenic, irritation, and reproductive toxicity. Thus, Aromadendrin can be safe for medicinal use. Additionally, a drug-score value of 0.86 adds credit for the compound to be a lead molecule against the cancer. The fig.no 3 and 4 shows the ADMET results of the Aromadendrin.

Table no.1 ADMET results using the *SWISSADME* and *Osiris property explorer*

S.NO	COMPOUND	M.W	LOGP	TPSA	GI ABSORPTION	LIPINSKI RULE	SYNTHETIC ACCESSIBILITY	TOXICITY
1.	AROMADENDRIN	288.25	1.42	107.22	HIGH	OBEY	3.42	NO

Fig no.3: *SwissAdme* results for the Aromadendrin

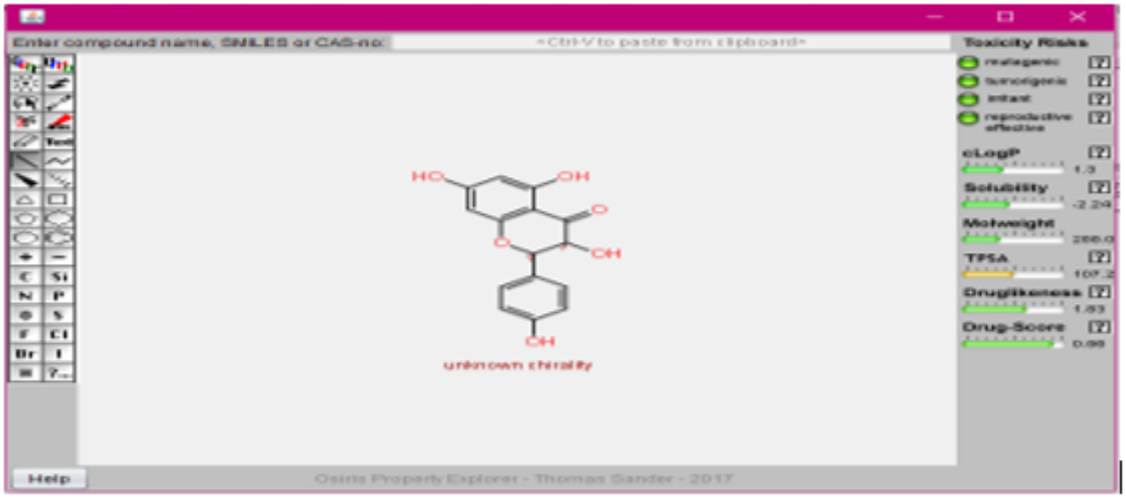


Fig no.4: Toxicity profile of Aromadendrin by the *OSIRIS software*

3.3 DOCKING RESULTS

The protein repaired using the *PRAS server* and the Validation of protein structure is carried out using the *PROCHECK* software tool. More than 90% residues of the amino acid lie in the most favorable region of the Ramachandran plot and the protein is used in further docking study[16]. The Ramachandran plot for the Protein Id: 6TLS is shown in the fig.no.5. It is found that 91.7% residues lie in the most favored region,7.9% residues in the additional allowed region. The table no.2 shows the details about the binding cavity volume, size, docking scores and docking interactions.

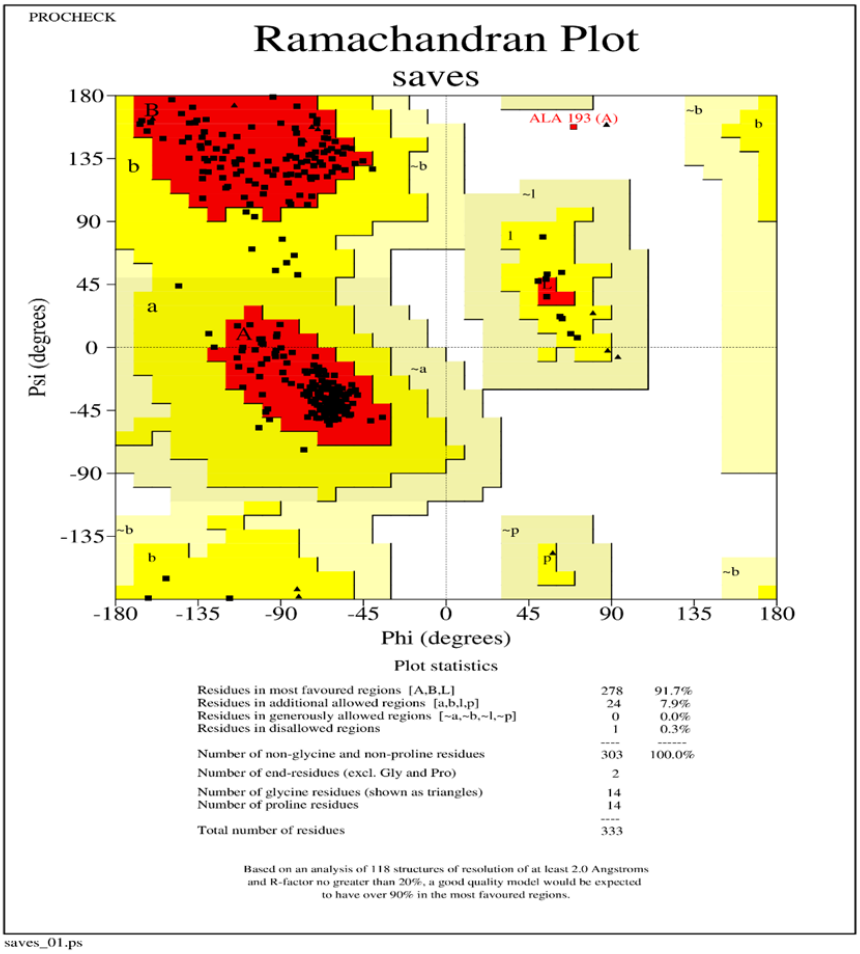


Fig no.5: Ramachandran plot for the PDB ID:6TLS

Table no. 2 Docking results

Attributes	**Details**
Docking score	-8.7 kcals/mol
Binding cavity details	Cavity volume :2222 A^3 Center (x,y,z) :63,14,21 Cavity size :27, 18, 24
Conventional Hydrogen bond interactions	ARG A:47, LYS A:68, ASP A:175
Pi-alkyl interactions	VAL A:53, ILE A:95, VAL A:66, ILE A:174
Vander-Waals	PHE A:113
5- fluorouracil – standard anticancer agent	-4.5 kcals/mol

From the docking study, Aromadendrin forms a stable complex with docking score of -8.7 kcal/moles. Aromadendrin outperformed the docking score of 5-Fluorouracil, a standard drug against the cancer. The fig.no.6 represents the docking pose, interactions etc., It forms the

hydrogen bonds with ARG A:47, LYS A:68 and ASP A:175. The Pi-alkyl interactions are seen with the amino acid residues VAL A:53, ILE A:95. It is important to note that the Aromadendrin forms crucial Vander-Waals, electrostatic and hydrogen bonding interactions.

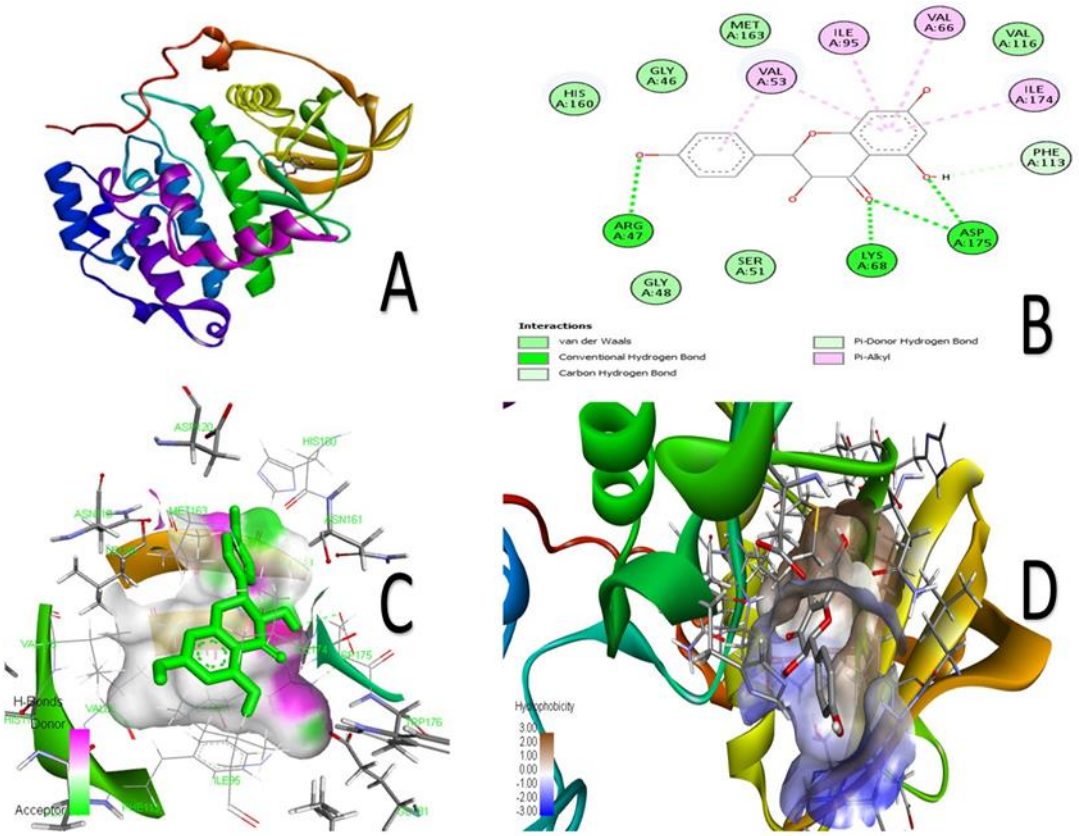


Fig no.6: A.3D structure of Aromadendrin against the *human casein kinase 2 alpha* enzyme (PDB: 6TLS).

B. Binding interaction of *casein kinase alpha -aromadendrin* complex.

C. Hydrogen bond surface of *human casein kinase 2 alpha.*

D. Hydrophobic surface of *human casein kinase 2 alpha enzyme* cavity with aromadendrin complex.

3.4 MOLECULAR DYNAMICS (MD) SIMULATION

MD simulation is performed for the apoprotein and the protein-ligand complex in the *Cabs flex 2.0 webserver*. The number of cycles and trajectory frames was set to 50, with a global weight of 1.0 and a temperature of 1.4. RMSF (Root mean square fluctuation) values are

compared among them and a significant decrease in the levels of the fluctuations indicates the stability of the protein-ligand complex. It is notable that all the residues in the protein show fluctuations below the 4 angstroms. The fig.no.7 and 8 represents the RMSF plot of the *human casein kinase alpha apoprotein* and the RMSF plot of the *human casein kinase alpha -aromadendrin* complex.

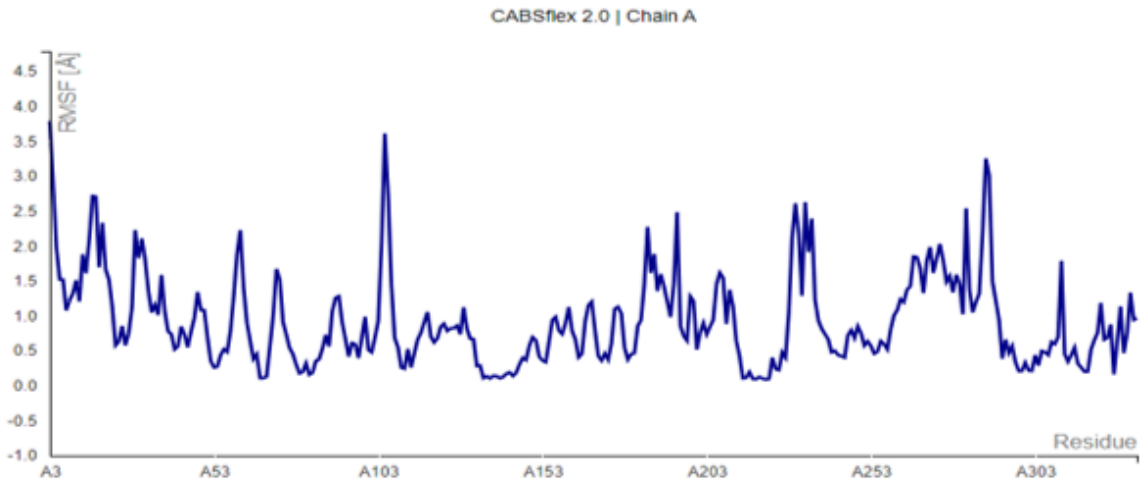


Fig no.7: RMSF plot of the *human casein kinase 2 alpha apoprotein*

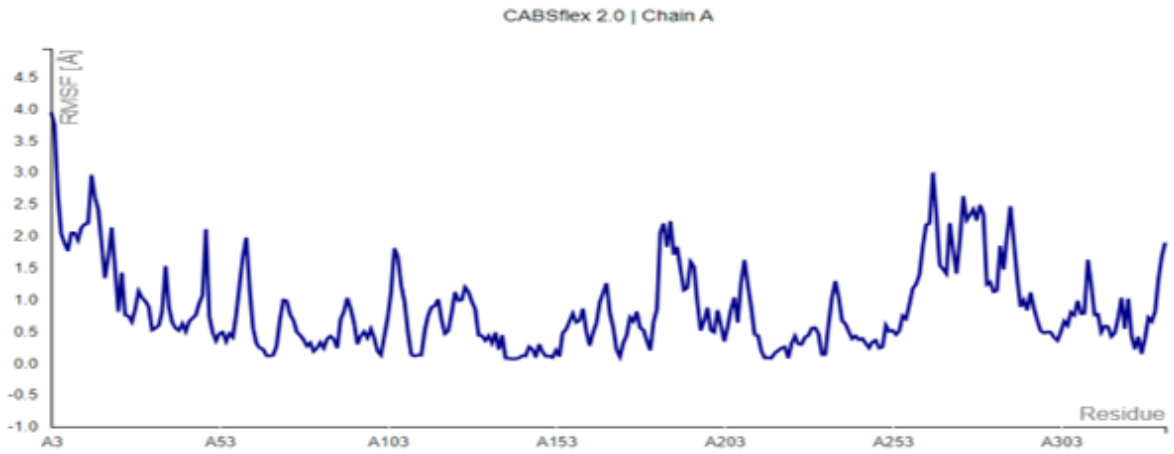


Fig no.8: RMSF plot of the *human casein kinase 2 alpha* -aromadendrin complex

3.5 MM-GBSA ANALYSIS

The MM-GBSA calculations done in the *FastDRH server* portray the binding affinity of -26.38 kcal/mol indicating the stability of the complex. The MM-GBSA scores of the various poses of the Aromadendrin are tabulated in the table.no.3. Hotspot residue analysis (Per

residue decomposition) results as mentioned in fig no.9 show that the best pose forms interactions with the amino-acid residues VAL A: 116, MET A:163, and LYS A:170.

Table no.3 - MM-GBSA calculations

POSE	MM GBSA SCORE
POSE001	-26.38
POSE002	-22.56
POSE003	-22.89
POSE004	-21.5
POSE005	-19.52
POSE006	-18.06
POSE007	-22.61
POSE008	-22.78
POSE009	-20.83
POSE010	-20.44

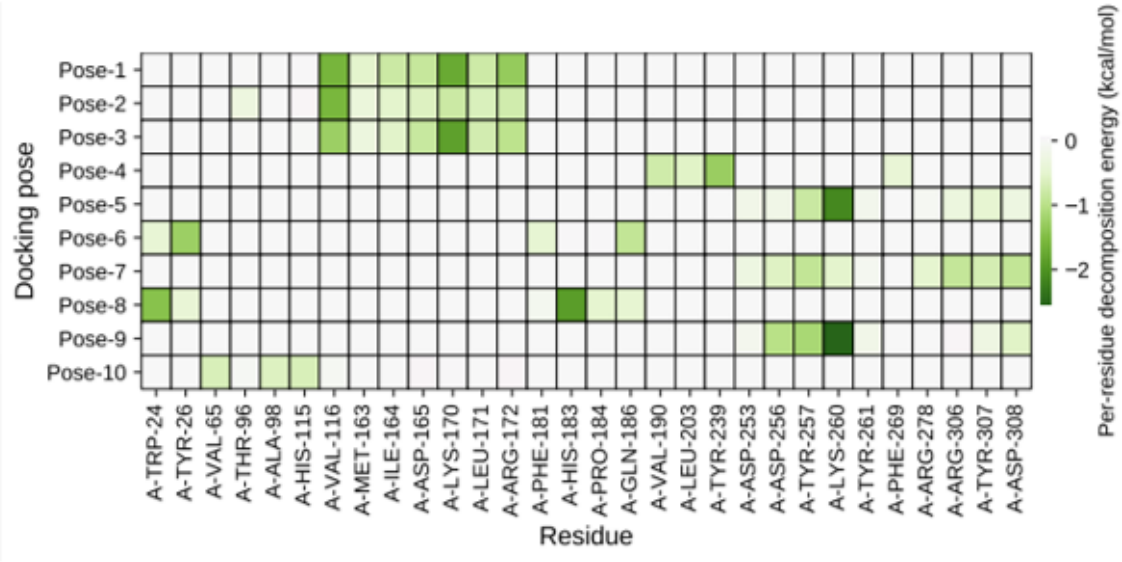


Fig no.9: Hotspot residues predicted by using the *Fastdrh server*

CONCLUSION

From the current research, we found that Aromadendrin is capable of being effective against *human casein kinase 2 alpha* as an orally bioavailable, non-toxic ligand in anticancer drug discovery. Hence, the compound Aromadendrin may be considered as a lead in treatment of cancer drug design. We also understood that AI-guided drug design fastens the drug discovery process.

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