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In-Silico Analysis of Andrographolide against Cancer

R. Sharmila[1*], K. M. Subburathinam[2], S. Aishwarya[1], A. Anita Margret[1]

[1]*Department of Biotechnology and Bioinformatics, Bishop Heber College, Tiruchirappalli-620017, Tamil Nadu, India*
[2]*P.G. and Research Department of Zoology, Rajah Sarfoji College, Thanjavur-613005, Tamil Nadu, India*

ABSTRACT
Cancer is the uncontrolled growth of abnormal cells in the body. It is the most serious disease on which extensive research is being done all over the world. Structure based drug designing offers a computational approach to identify the potential leads which can be developed into a drug. The *In-Silico* study of the current work aimed at inhibiting the three major targets of cancer by a natural compound Andrographolide from *Andrographis paniculata*. This exhibited a minimal energy against the targets hence suggesting the stability of the compound. Comparison studies of the compound with the already available anti-cancer drugs and enzyme inhibitors, stated that andrographolide is efficient to act on the targets by exhibiting promising interactions and good scores. This was also found to obey the Lipinski's Rule of 5 and computed ADMET properties showed the drug likeliness and improved bioavailability. Since it is from a natural source the compound is non toxic and has reduced side effects.

Keywords: Cancer, Andrographolide, *In Silico,* ADMET, anti-cancer drug, Bioavailability.

INTRODUCTION

Structure-based virtual screening and post-screening analysis are emergent tasks in computer-based drug discovery. Combining these two methods to effectively reduce the false positives from a large compound database is considered as a key step to finding the lead compounds. Various signal transduction pathways are involved in regulation of cell growth, thus their impairment may be related to tumor pathogenesis. Phosphorylation is the most important reversible mechanism for triggering or inhibiting the activity of specific proteins in a signaling pathway. This fundamental process is achieved through the activity of various protein kinases. The first anticancer agent specifically targeted to a protein kinase was Imanitib, which acts as an inhibitor of the oncogenic kinase BCR-Abl and is active in the chronic myelogenous leukemia. [1] The first intracellular second messenger was described in the late Fifties as adenosine 3'5'-cyclic monophosphate (cyclic AMP, cAMP). [2]

It is present in every cell, where it is synthesized by adenylyl cyclase from ATP, and is hydrolyzed by cAMP-specific phosphodiesterases to adenosine 5'- monophosphate. The rate of cAMP production and degradation is sensitive to a wide range of extracellular and intracellular signals, such that cAMP can directly regulate a variety of cell functions, from metabolism to ion channel activation, cell growth and differentiation, gene expression and apoptosis. [3] Within each cell, cAMP may activate different proteins. PKA are ubiquitous intracellular cAMP effectors that regulate multiple processes. Their target is specified by their intracellular localization, obtained through anchoring at specific sites in macromolecular complexes, and through the expression of specific subunits.

During cancer pathogenesis, the normal cell activity is imbalanced via mutation of selected proteins, or by altering their rate of synthesis/degradation, or by affecting the activity of otherwise normal proteins. Given the involvement of PKA in several different intracellular functions, it is conceivable that pathological processes may affect the cAMP/PKA pathway. Indeed, several converging data reveal that the cAMP/PKA signaling pathway is altered in different cancers, and may be exploited for cancer diagnosis and/or therapy. [4-5] Epidermal growth factor receptor (EGFR) is a member of the receptor tyrosine kinase family, and overexpression of EGFR is associated with poor prognosis and progression of many human cancers, including oral cancer. [6]

At the molecular level, stimulation of EGFR induces intrinsic tyrosine kinase activity and cellular signaling that results in cell growth and proliferation. *Andrographis paniculata* (Acanthaceae) is one of the most valuable medicinal plant and bio-factory of diterpronidlactons which have immense value like immune stimulating, anti – inflammatory, anti-fertility, liver protection, anti-HIV and bile secrecation stimulating agent. [7]

A number of active components are reported in this plant which mainly includes diterpone lactones, flavonoides and

***Corresponding author: Mrs. R. Sharmila,**
Department of Biotechnology and Bioinformatics, Bishop Heber College, Tiruchirappalli-620017, Tamil Nadu, India; **Tel.:** +91-8012427354; E**-mail:** r_sharmi2k3@yahoo.co.in

polyphenols. [8-9] However, the most pharmacological properties present in active principle of andrographolide. The present study focused on *In-Silico* analysis of andrographolide against cancer.

MATERIALS AND METHODS

Target Identification: The targets were selected the 3 Dimensional structures of the target proteins Epidermal growth factor receptor complexed with erlotinib (Fig. 1 PDBID – 1M17), Human Abl kinase in complex with nilotinib (Fig. 2 PDBID- 3CS9) and Human cAMP dependent protein kinase (PDB ID-30VV) were downloaded from the online protein structure repository, Protein Data Bank (PDB). The structures were determined by Xray Diffraction method and had ligands coupled in the binding site.

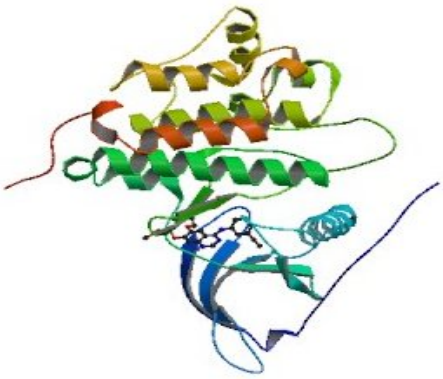


Fig. 1: The Crystal structure of the targets 1M17

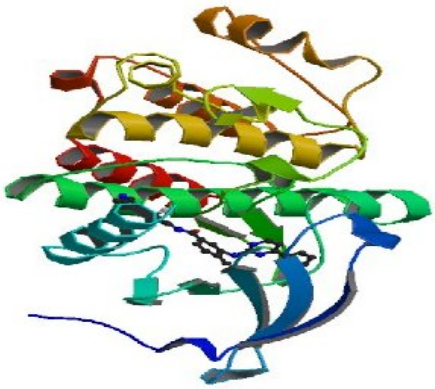


Fig. 2: The Crystal structure of the targets 3CS9

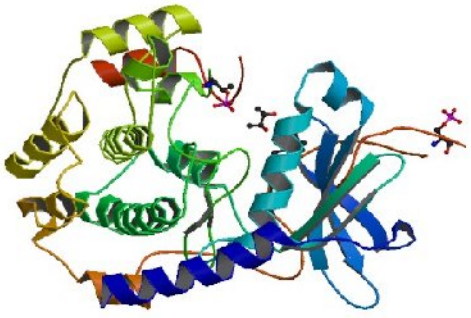


Fig. 3 The Crystal structure of the targets 3OVV

Preparation of compounds: The structures of the compounds andrographolide and three current anti-cancer drugs were downloaded from Pubchem in SDF format and converted to PDB format using the tool MARVIN SKETCH (http://www.chemaxon.com/marvin/sketch/index.php). The compound structures were energy minimized and considered for docking studies. The original ligands of the targets were also prepared similarly and taken for docking.

Molecular Docking: Graphical-Automatic Drug Design System for Docking, Screening and Post-Analysis program iGEMDOCK was used to gain the docking results of the listed compounds with the target. The binding site of the target was prepared and the energy minimized compounds were imported. The docking protocol consisted of 25 generations per ligand and the population size of 100 random individuals. All the docking conformations were performed twice using genetic evolutionary algorithm and the fitness of the docked structures were calculated. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was identified at a distance 8Å. The empirical scoring function of iGEMDOCK was estimated as: Fitness = vdW + Hbond + Elec.

Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively.

Drug likeliness and Bioavailability: It is important for any compound to obey the Lipinski's rule of five [10] in order to be formulated as a drug. The Absorption Distribution, Metabolism, Elimination and Toxicity (ADMET) parameters are much significant for a drug to be available to the system. MedChem Designer [11] is a tool that instantly generates predicted values of key ADMET properties: LogP (Hydrophillicity), LogD (permeability), Topological Polar Surface Area, Molecular weight, HBDH, HBA (N+O as M_NO), and Rule of 5. The ADMET properties of the compounds were computed and the drug likliness is evaluated.

RESULTS AND DISCUSSION

Docking simulations of andrographolide with three different targets for cancer resulted in better interactions than the commercially available cancer drugs.

Docking of andrographolide with the target 3CS9

The original ligand bound to the target exhibited a fitness score of -80.44 and interacted with the active site residues Glu266, Asp381, Glu282, Lys285, Lys266, Arg362 and Asp361. The compound andrographolide exhibited a fitness score of -76.06 and interacted with the residues Ser500, Lys285, Glu296, Val289, Asp381 and Arg362 at the active site. This shows good interaction and efficient score than the available drugs (Table I).

Docking of andrographolide with the target 3OVV

The original ligand 1SB bound to the target 3OVV exhibited a fitness score of -60.06 and interacted with the active site residues Thr51, Asn20 and Tyr330. The compound andrographolide ehibited a fitness score of -71.35 and interacted with the residues Thr51, Asn20, Tyr330 Arg93, Trp30, Arg93, Arg190 and Lys192 at the active site of 3OVV. The compound exhibits minimum energy than the ligand and interacts well at the binding pocket. This also shows good interaction and efficient score than the available drugs (Table II).

Docking of andrographolide with the target 1M17

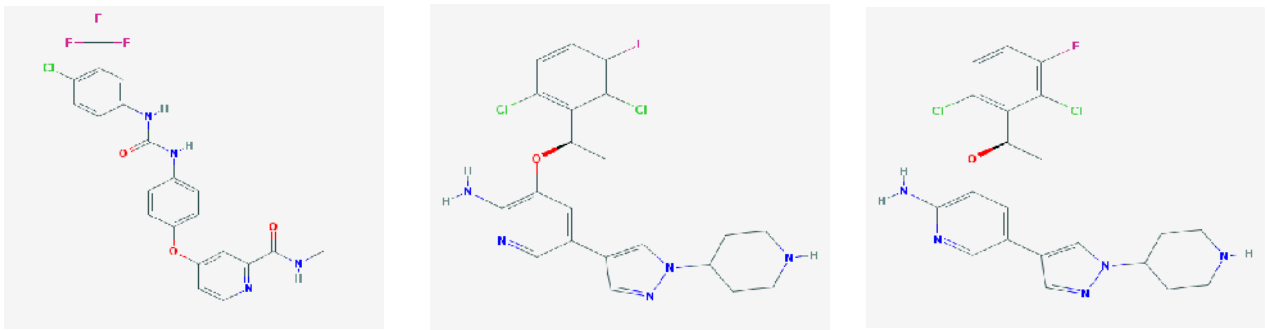
The original ligand erlotinib bound to the target 1M17 exhibited a fitness score of -62.37 and interacted with the active site residues Tyr740, Leu838, Glu673 and Ala674.

Table I: Interaction of andrographolide and the available drugs with 3CS9

S. No	Compound	Energy	vdW	H Bond	Elec	Interacting residues
1	Ligand-Nilotinib	-80.44	-71.22	-9.22	0	Glu266 Asp381 Glu282 Lys285 Lys266 Arg362 Asp361 His361
2	Drug-sorafenib	-88.02	-79.09	-8.93	0	Asp381 Lys285 Glu286 Val289 His361 Asp381
3.	Drug-imatinib	-77.56	-65.06	-12.5	0	Lys285 Glu286 Val289 Lys271 Glu232 His361 Asp381
4.	Drug-crizotinib	-65.69	-60.5	-5.44	0	Lys285 Glu286 Val289 Ser500 Asp361 Ser500
5.	Andrographolide	-76.06	-63.97	-12.09	0	Lys285 Glu296 Val289 Asp381 Arg362

Table II: Interaction of andrographolide and the available drugs with 3OVV

S. No	Compound	Energy	vdW	H Bond	Elec	Interacting residues
1	Ligand-1sb	-63.06	-50.88	-12.18	0	Thr51 Asn20 Tyr330
2	Drug-sorafenib	-71.28	-64.58	-6.7	0	Thr51 Ala21 Gly52 Tyr330
3.	Drug-imatinib	-71.17	-55.69	-15.48	0	Asp75 Asn115 Glu334 Gly55 Arg56
4.	Drug-crizotinib	-59.66	-55.4	-4.25	0	Asp75 Tyr235 Arg137 His260 Thr51
5.	Andrographolide	-71.35	-59.43	-6.7	0	Asn20 Tyr330 Arg93 Trp30 Arg93 Arg190 Lys192

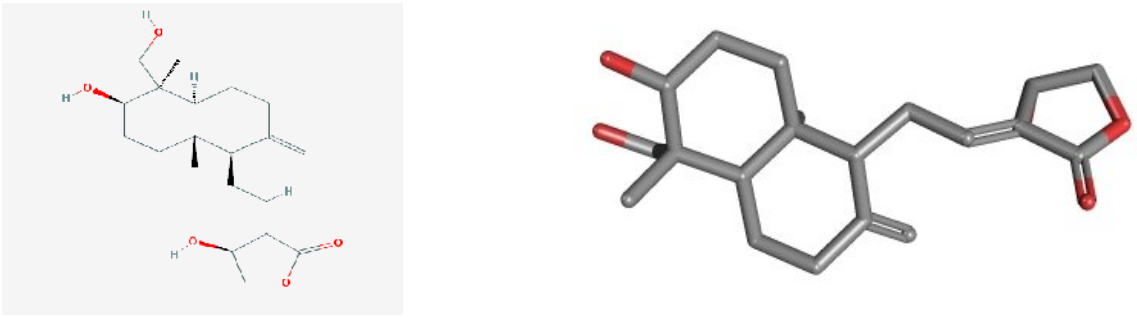


(a) **(b)** **(c)**

Fig. 4: 2D structures of anti-cancer drugs – (a) Sorafenib, (b) Imatinib and (c) Crozitinib

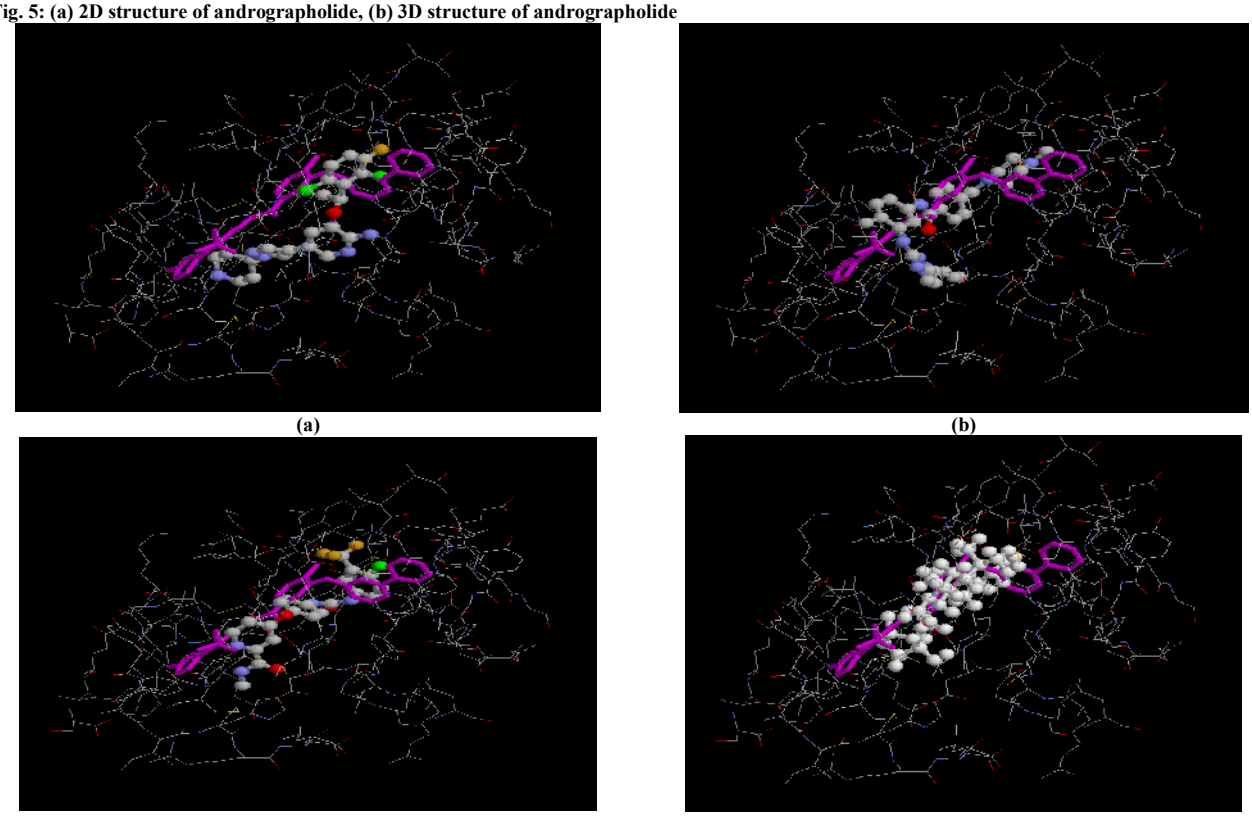
Table III: Interaction of andrographolide and the available drugs with 1M17

S. No	Compound	Energy	vdW	H Bond	Elec	Interacting residues
1	Ligand-erlotinib	-62.37	-54.35	-8.02	0	Tyr740 Leu838 Glu673 Ala674
2	Drug-sorafenib	-68.97	-59.06	-9.91	0	Asp813 Asp831 Phe699 Val702
3.	Drug-imatinib	-67.53	-60.26	-7.27	0	Met769 Asp831 Leu694 Val702
4.	Drug-crizotinib	-59.63	-52.93	-6.7	0	Pro890 Asp892 Ser787 Leu838
5.	Andrographolide	-58.19	-3.9	-19.19	0	Glu673 Gln788 Tyr789 Asp950 Glu961



(a) (b)

Fig. 5: (a) 2D structure of andrographolide, (b) 3D structure of andrographolide

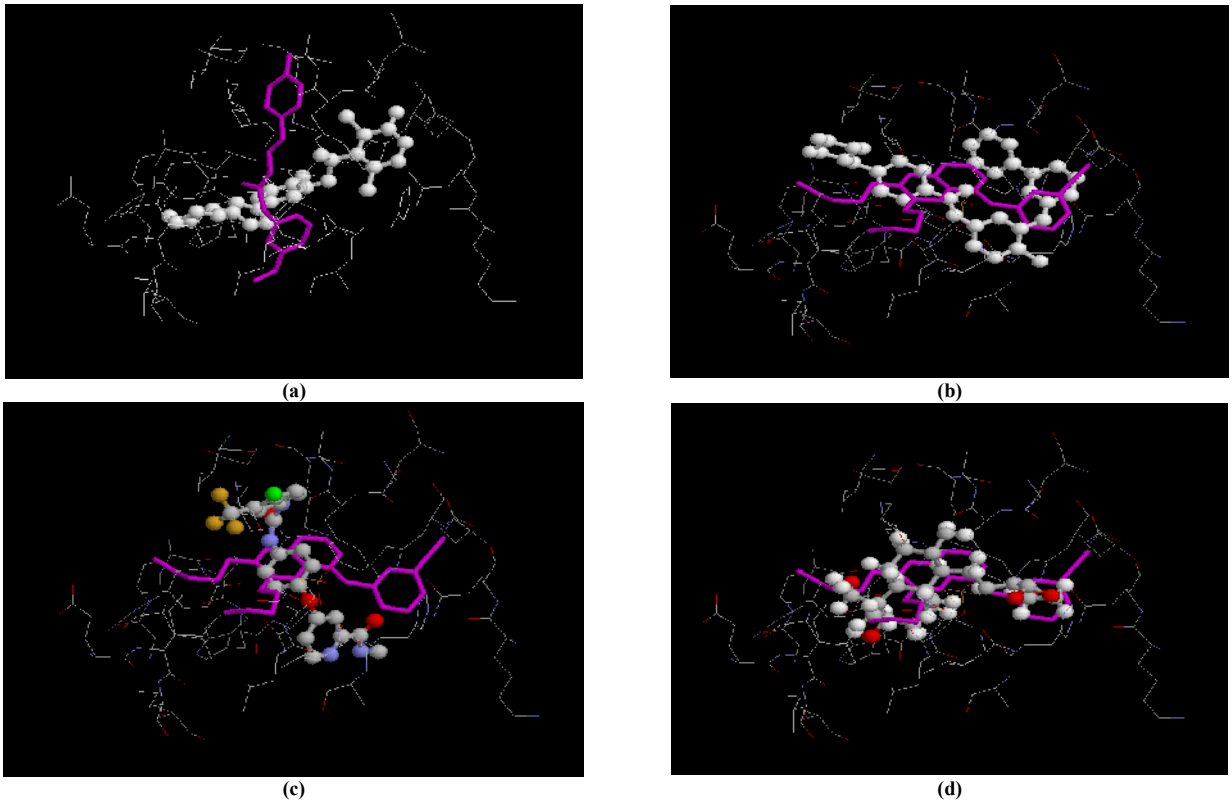


(a) (b)

(c) (d)

Fig. 6: Results of the docked structures with the target

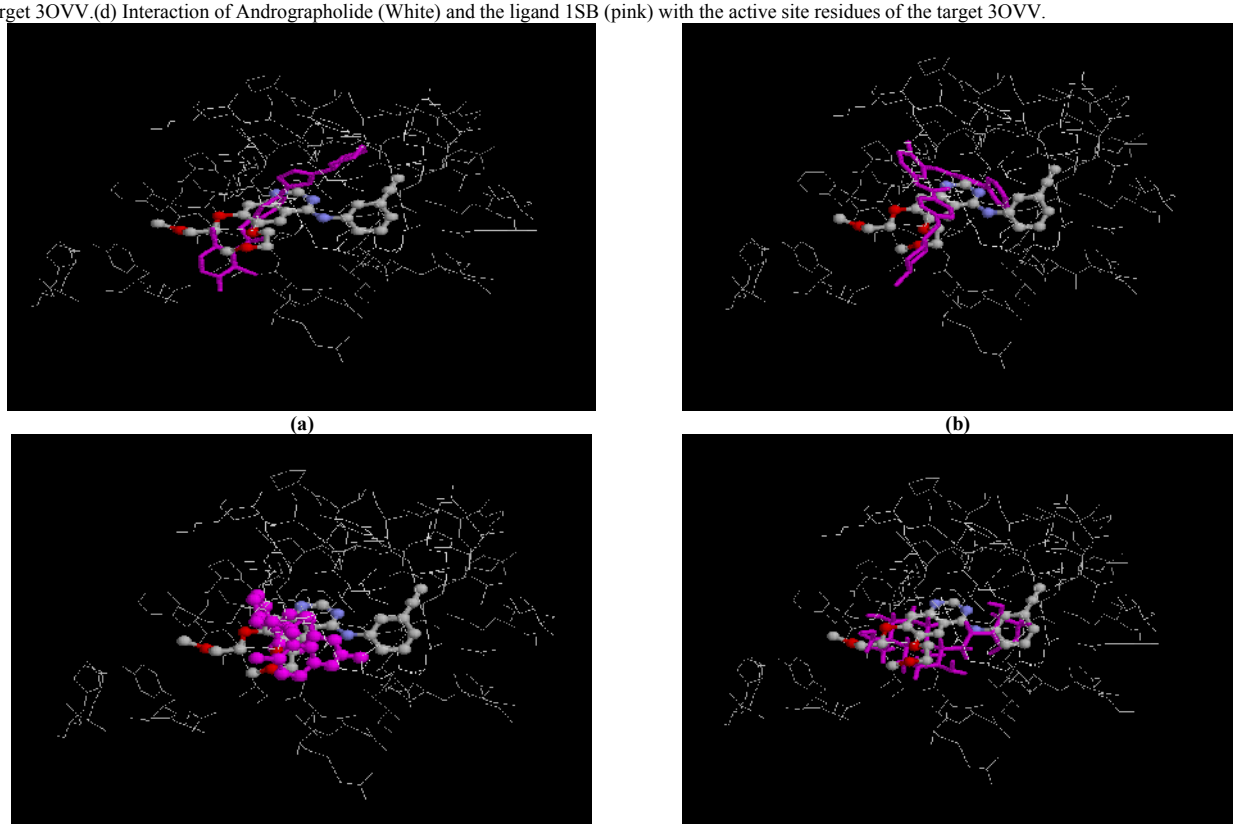
(a) Interaction of Crizotinib (CPK) and the ligand nilotinib (pink) with the active site residues of the target 3CS9. (b)Interaction of Imatinib (CPK) and the ligand nilotinib (pink) with the active site residues of the target 3CS9. (c)Interaction of Sorafenib (CPK) and the ligand nilotinib (pink) with the active site residues of the target 3CS9.(d)Interaction of Andrographolide (White) and the ligand nilotinib (pink) with the active site residues of the target 3CS9.



(a) **(b)** **(c)** **(d)**

Fig. 7: Results of the docked structures with the target

(a) Interaction of Crizotinib (white) and the ligand 1SB (pink) with the active site residues of the target 3OVV.(b) Interaction of Imatinib (white) and the ligand 1SB (pink) with the active site residues of the target 3OVV. (c)Interaction of Sorafenib (CPK) and the ligand 1SB (pink) with the active site residues of the target 3OVV.(d) Interaction of Andrographolide (White) and the ligand 1SB (pink) with the active site residues of the target 3OVV.



(a) **(b)** **(c)** **(d)**

Fig. 8: Results of the docked structures with the target 1M17

(a) Interaction of Crizotinib (CPK) and the ligand erlotinib (pink) with the active site residues of the target 1M17.(b)Interaction of Imatinib (CPK) and the ligand erlotinib (pink) with the active site residues of the target 1M17.(c) Interaction of Sorafenib (CPK) and the ligand erlotinib (pink) with the active site residues of the target 1M17.(d)Interaction of Andrographolide (CPK) and the ligand erlotinib (pink) with the active site residues of the target 1M17.

Table IV: The drug- like parameters of Andrographolide

S. No	Properties	Value
1	Slog P	1.699
2	SlogD	1.699
3	mlogP	1.801
4	Molecular weight	350.458
5	Hydrogen Bond Donor	3.000
6	Hydrogen Bond Donor (M_No)	5.000
7	Topological polar Surface Area (T_PSA)	86.990
8	Rule of 5	0
9	Violation of Rule of 5	Nil

The compound andrographolide ehibited a fitness score of -58.19 and interacted with the residues Leu838, Glu673, Ser787, Gln788, Tyr789, Asp950 and Glu961 at the active site. Andrographolide also shows good interaction and efficient score than the available drugs (Table III).

Drug likeliness and ADMET prediction

Lipinski's rule of 5 states that a drug should be below 500 Da in its molecular weight; Sholud have log P (Ratio of drug concentration in octanol: water) <5; H Bond donor < 5 and H bond acceptor <10.

From the Table IV, its inferred that Andrographolide had a molecular weight of 350.458 Da, the ratio of concentration of Andrographolide in octanol:water (mlog P) is 1.801. The compound has 3 Hydrogen Bond donors and 5 Hydrogen Bond acceptors (M_No). Andrographolide was found to obey all the parameters of Lipinski's Rule of 5 and has drug likeliness. The T-PSA (Topological Polar Surface Area) is 86.990. Hence its accomplished that Andrographolide can efficiently act as drug.

The *In-Silico* studies of the current work proves the inhibition of 3 major targets of cancer by a natural compound Andrographolide. Human cAMP dependent protein kinase (PDB ID-30VV) was efficiently inhibited by the compound andrographolide. This showed minimal energy on docking against the target hence suggesting the stability of the compound. It is also found to interact well at the active sites of the target. When compared with the already available anti-cancer drugs and enzyme inhibitors, andrographolide was found to be efficient against the enzyme kinases. This was also found to obey the necessary parameters to act as drug especially no violations in the Lipinski's Rule of 5. The interactions and fitness score of the compound and computed ADMET properties suggest that Andrographolide can be formulated as an anti cancer drug.

Further studies can be extended to analyse the pharmacokinetics and pharmacodynamics of andrographolide in cancer survivors.

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