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Embelin as ATM kinase, Checkpoint Kinase-2 modulator: An *In silico* analysis

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Abstract

Embelin is a natural benzoquinone from *Embelia ribes Burm.*, is an Ayurvedic medicine for various therapeutic applications. Embelin is the effective against various type of cancer. Embelin can upregulate checkpoint kinase-2 (Chk2) a key factor involved in the cancer metabolism and it enhance chemosensitizing effects in cancer treatment. In the present study, *in silico* analysis is used to assess the potentiality of embelin as ATM (Ataxia telangiectasia mutated) kinase inhibitors. Structures of embelin retrieved from Pubchem. Preliminary screening of compounds was done in accordance with Lipinski's rule of five. A docking simulation was carried out using a predictive docking tool Autodock Vina to obtain model structures of ATM kinase and Checkpoint Kinase-2 embelin complex. The binding energies of embelin with ATM kinase was found to be -7.0 Kcal/mol with 2 hydrogen bonds, and binding affinity of embelin with Chk2 kinase protein was found to be -13.6 Kcal/mol with 2 hydrogen bonds.

Key words : Embelin, ATM kinase, Chk2 kinase, Autodock Vina, Lipinski's Rule.

Cancer is considered as most important health issue in developed countries[13]. Lack of Early diagnosis, and delayed therapies has resulted in a substantial progression of cancer survival. Metastasis is one of the prime factor which includes extensive proliferation, degradation of the surrounding extracellular matrix, migration and even vascularization[16].

Embelin is a benzoquinone derived from a woody shrub *Embelia ribes* Burm. Embelin has traditionally been used in Chinese medicine as anticancer agent as well as a contraceptive[4]. Recent studies have demonstrated that embelin by binding XIAP, blocks XIAP from interfering with and supressing caspase-9. It has been demonstrated that embelin has

an extensive antitumor role and is able to restrain the growth of various tumor cells, including those of breast, colon, prostate and pancreatic cancer[1,5,7,14]. In the present study, our aim was to determine a novel embelin molecule as potential target for ATM kinase and Chk2 kinase signalling with lesser toxicity.

Drug likeliness of ligands :

The structure of embelin were obtained from Pubchem. Biological activities as well as bioavailability of compounds are considered as chief indicators for development of probable drug candidates. Molecular descriptors of embelin was analysed with Lipinski's rule[12]. Rule of five has become a computational approach for the estimation of solubility and permeability of new drug candidates. In accordance with the rule, drug candidates must have molecular mass not more than 500 g/mol, the number of acceptors of hydrogen bonds not more than 12, and the number of hydrogen-bond donors not more than 5 and partition coefficient log P value less than 5. The drug likeliness of embelin thus obtained were deduced with online cheminformatics software Molinspiration (http://www.molinspiration.com).

Preparation of ligands for docking :

The Ligand preparation includes a series of steps that perform energy minimisation, apply corrections to the structure, conversions from 2D to 3D. The structure of embelin was obtained from pubchem, the compounds in 2D structure format were retrieved in SDF format from Pubchem database and energy of each molecule was minimised using Dundee prodrg online server[3,17]. The ligand molecules were used as an input for AutoDock Vina, to perform docking simulation[18]. 2D structure of embelin was converted to 3D structures using PyRX software[6].

Preparation of Protein structure for docking:

The 3-Quinoline Carboxamides inhibitor of Pi3K crystal structure was retrieved from RCSB Protein Data Bank (http://www. rcsb.org) under the PDB ID: 5G55 with a resolution of 2.45 Å. The 3D coordinates of the crystal structure of hinge region of ATP Chk2-binding site (PDB ID: 4BDJ) at a resolution of 3.01 Å was retrieved from Protein DataBank (PDB). Ligands, water molecules and heteroatoms were detached from the 3D structure of ATM kinase and ChK2 kinase and were converted to PDBQT format with PyRX software.

Setting grid map parameters :

The grid box was set using the graphical 'MGL tools' so that it surrounds the active pocket of the macromolecule. In this study active pocket was designed based upon the amino acids present in interaction of ATM Kinase (5G55) and Chk2 inhibitor (4BDJ) with protein retrieved from PDB. For ATM Kinase, the grid was centered at the region including all the 13 amino acid residues (Met 804, Trp 812, Thr 887, Met 953, Ile 881, Val 882, Ile 963, Glu 880, Tyr 867, Asp 964, Ile 879, Ile 831, Pro 810) that surround active site either with hydrophobic interactions or hydrogen bonds. The grid volume was set to 60, 60 and 60 Å for x, y, and z dimensions, respectively,

and the grid center was set to 46.44, 14.699, and "6.035 for x, y, and z center, respectively, which surrounded all the 11 amino acid residues in the present active pocket. For Checkpoint Kinase-2, the grid was centered at the region including all the 13 amino acid residues (Leu226, Leu354, Leu301, Glu308, Val234, Thr367, Ala247, Leu303, Glu302, Met304, and Lys249) that surround active site either with hydrophobic interactions or hydrogen bonds. The grid volume was set to 60, 60 and 60 Å for x, y, and z dimensions, respectively, which surrounded all the 11 amino acid residues in the present active pocket.

Docking studies :

Auto Dock Vina was used to determine the best conformation between protein and ligand. In course of docking procedure, a maximum of 10 conformers for each ligand molecule was considered. The docking was conducted in Intel Core i5 processor with 4GB DDR3 RAM. AutoDock Vina was conducted and compiled with Windows 10 system. The softwares Lig Plot+ [11] and PyMol[8] was used to determine the 2D and 3D interactions of embelin with ATM and Chk2 protein.

In this study molecular docking approach was used to analyze interaction and affinities of embelin with ATM Kinase and Chk2 kinase domain. The chosen molecules were docked onto ATM kinase and Chk2 kinase active pocket to investigate and to understand similar interactions as that of known ATM and Chk2 inhibitors. Preliminary screening of molecule was done in accordance with Lipinski's rule of five. Embelin did not show any violation to Lipinki's rule of 5. The molecular properties of Embelin are shown in Table-1. The data obtained from the present study predicts Embelin as lead molecule with lower toxicity and higher bioavailability.

A docking simulation was carried out using a predictive docking tool Autodock- Vina to obtain model structures of ATM kinase and Chk2 kinase Embelin complex. The docking program Auto Dock 4.2 based on Lamarckian genetic algorithm was used in the study. Embelin were docked onto the active sites of ATM and Chk2 kinase Domain and their binding affinities are presented in Table 2. Embelin showed better binding energies with a maximum binding affinity of '-7.0 Kcal/mol for ATM kinase' with 2 hydrogen bonds, and a binding affinity of '-13.6 Kcal/mol with 2 hydrogen bonds.

Narayanaswamy *et al.*,[15], performed the docking studies and binding free energy calculations of embelin with Nitric oxide synthase enzyme (NOS), in which embelin exhibited the higher interaction energy (-7.46 kcal/mol) with Asn370 and Gly371 amino acid residues, whereas quercetin (reference compound) that showed least interaction energy (-3.91 kcal/mol). Embelin demonstrated binding energy of 7.7 kcal/Mol and 7.0 kcal/Mol with COX_1 and COX_2 with two hydrogen bonds[10]. Embelin exhibited better binding affinities with the mutated BRAF protein[2]. *In-silico* study revealed binding mode of Embelin with ER alpha and HER2 receptors of breast cancer cells indicating it as a potential modulator against ER positive and HER2 positive breast cancers[9].

Figures :

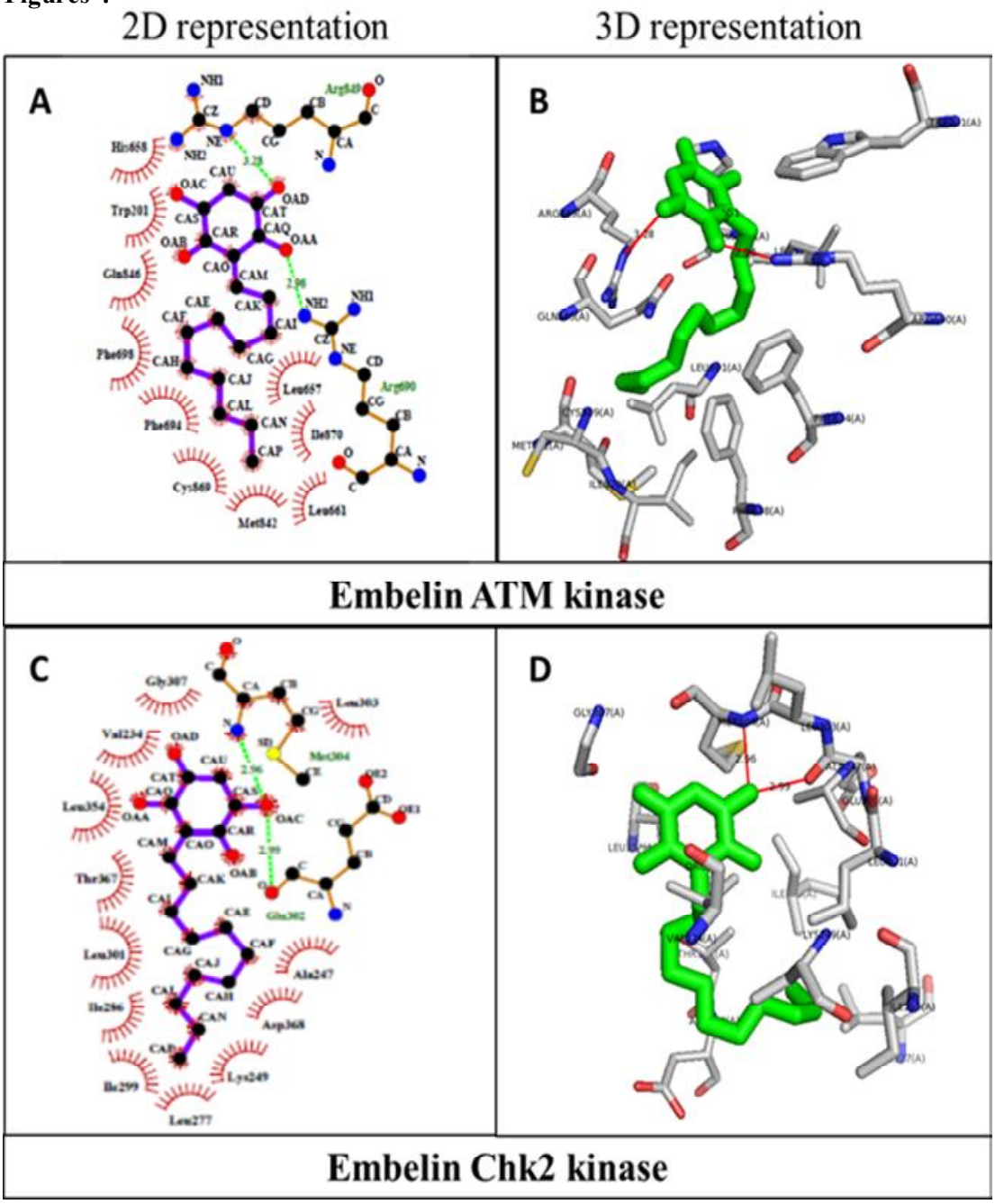

Figure 1: **A** and **B** depicts 2D and 3D representations of Embelin ATM kinase. **C** and **D** depicts 2D and 3D representation of Embelin chk2 kinase.

Table-1. Drug likeliness of Embelin used in the study.

Sl. No	Compound	Pubchem ID	Hydrogen bond donor	Hydrogen bond acceptor	Log P	Molecular Weight g/mol	No. of viola-tions
1	Embelin	3218	2	4	4.313	228.20	0

Table-2. Binding affinity (Kcal/mol), number of Hydrogen-bonds, Hydrogen -bond length and Hydrogen -bond formation of embelin with ATm kinase and Chk2 kinase.

Sl No.	PDB Id	Proteitn Target	Hydro-gen Bonds	Bond Length in A°	H-Bond With	Binding Energy (Kcal/mol)
1.	5G55	ATM kinase	2	3.28	Drg:O(AD)::Arg 549 NE	-7.0
				2.96	Drg:O(AA)::Arg 690 NH2	
2.	4BDJ	Chk2 kinase	2	2.96	Drg:O(AC)::Met 304 NH	-13.6
				2.99	Drg:O(AC)::Glu 302 CO	

The results of *in silico* binding studies showed promising results in terms of binding energies. Theoretically, Embelin was found to be bound to Checkpoint kinase-2 and ATM Kinase with specific orientation encompassing the active pocket.

Declaration of interest

Authors declare no conflict of interest.

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