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Identification of Ellagic acid analogues as potent inhibitor of protein Kinase CK2:A chemopreventive role in oral Cancer

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Abstract:
Over expression of Protein kinase (CK2) suppresses apoptosis induced by a variety of agents, whereas down-regulation of CK2 sensitizes cells to induction of apoptosis. In this study, we have built quantitative structure activity relationship (QSAR) models, which were trained and tested on experimentally verified 38 enzyme's inhibitors having inhibitory value IC50 in μM. These inhibitors were docked at the active site of CK2 (PDB id: 2ZJW) using AutoDock software, which resulted in energy-based descriptors such as binding energy, intermol energy, torsional energy, internal energy and docking energy. For QSAR modeling, Multiple Linear Regression (MLR) model was engendered using energy-based descriptors yielding correlation coefficient r^2 of 0.4645. To assess the predictive performance of QSAR models, different cross-validation procedures were adopted. Our results suggests that ligand-receptor binding interactions for CK2 employing QSAR modeling seems to be a promising approach for prediction of IC_{50} value of a new ligand molecule against CK2.Further, twenty analogues of ellagic acid were docked with CK2 structure. After docking, two compounds CID 46229200 and CID 10003463 had lower docking energy even lower than standard control Ellagic acid with CK2 was selected as potent candidate drugs for Oral cancer. The biological activity of two compounds in terms of IC_{50} was predicted based on QSAR model, which could be used as a guideline for anticancerous activity of compounds before their synthesis.

Keywords: Ellagic acid; Docking; CK2; AutoDock; Ellagic acid analogues; Protein kinase.

Background:
Casein kinase 2 (CK2) is a highly ubiquitous, essential, and highly pleiotropic protein kinase **[1]** that has been involved cell growth, proliferation and in suppression of apoptosis in cells. It is localized in both the nucleus and cytoplasm in normal cells, but is particularly predominant in the nuclear compartment in cancer cells. Down regulation of CK2 by chemical or molecular methods promotes apoptosis in cells. It has been reported that antisense CK2 alpha is particularly potent in inducing apoptosis in cancer cells in culture as well as in xenograft models of cancer such as oral cancer and squamous cell carcinoma of head and neck **[2].** A number of evidence suggesting that the catalytic subunits of CK2 behave as oncoproteins **[3-6]** consistent with the observation that they display an antiapoptotic effect in prostate cancer cells **[7].** CK2 subunits are more abundant in tumors as compared with normal tissues, and their overexpression is causative of neoplastic growth in animal and cellular models, giving rise to alterations in the expression levels of cellular oncogenes or tumor suppressor genes **[8].**

Nature is a rich source of anti-cancer compounds which are used as preventive and/or curative agents with general acceptance as a dietary element with a well-established safety

profile. According to one of the estimates by World Health Organization approximately 80% of the world's population relies on traditional medicine for their primary health care **[9]**. Ellagic acid is an antioxidant and an anti-proliferative phenolic constituent present in fruits, nuts and vegetables **[10].** Several research studies have identified Ellagic acid as a potent anticarcinogenic and antimutagenic compound. At present, ellagic acid represents the most potent known CK2 inhibitor (Ki = 20 nM) **[11].** Using in silico approaches, we have identified the ellagic acid analogues, as a novel, potent and selective CK2 inhibitor. 2. Experimental and predicted pIC50 value are plotted in graph **(Figure 1).**

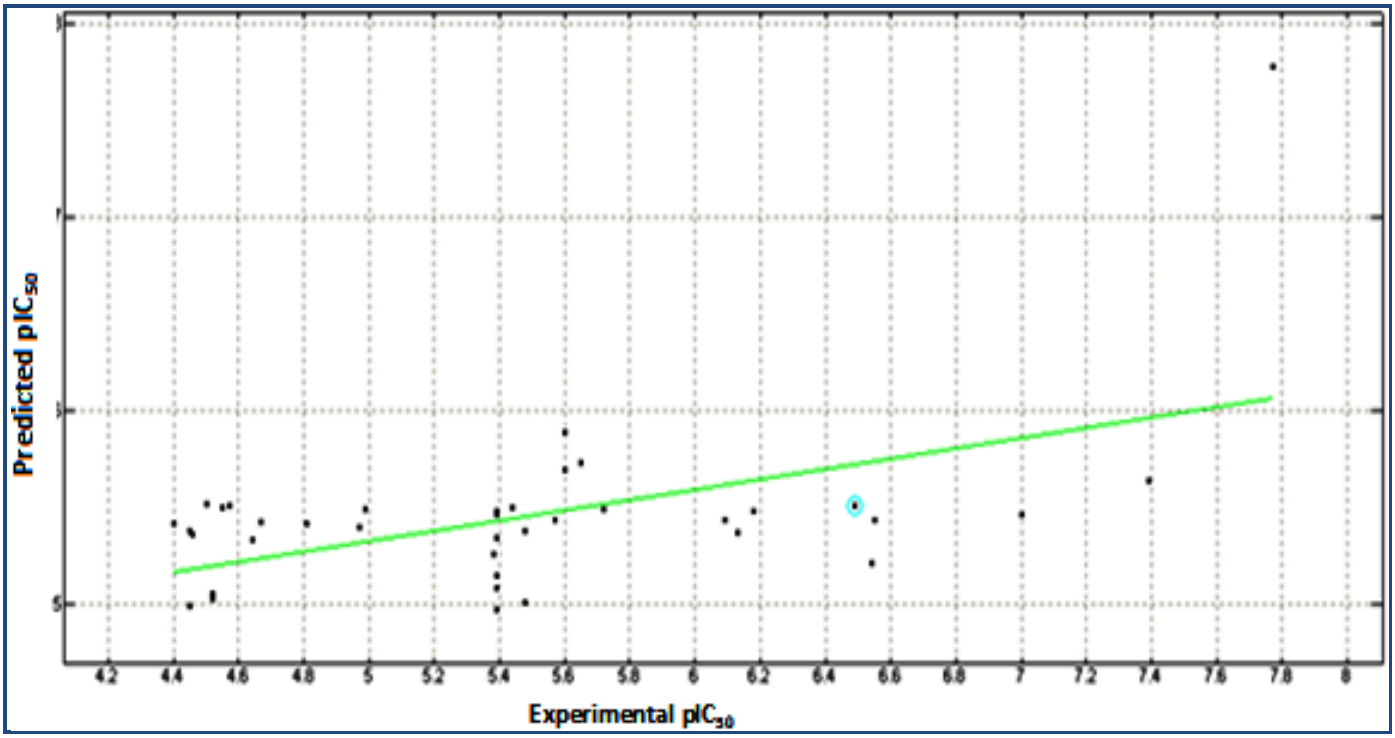

Figure 1: Depict the experimental and predicted pIC50 value in X and Y direction respectively with r2 value 0.4645 using LINEAR MODEL**.**

Methodology:

Protein preparation

The 3D coordinates of the crystal structure of human CK2 alpha complex with ellagic acid (PDB id: 2ZJW) was retrieved from Protein Databank (http://www.rcsb.org/) and taken as the receptor model in flexible docking program. Human casein kinase II (CK2) was optimized by chimera tool **[12].** Before docking heteroatom Ellagic acid was removed from coordinate file of CK2 protein by charge method AMI-BCC using chimera. After removing the water molecule, hydrogen atom were added to protein.

Active site analysis

The active site residues of human casein kinase II (CK2) was taken from the PDBSUM entry of 2ZJW having binding site residues ASP175, PHE113, LYS68, ILE174, ILE95, VAL66, VAL53 and LEU45 for inhibitor Ellagic acid (2,3,7,8-tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione).

Inhibitors Dataset

The data regarding the experimentally known 38 coumarin inhibitors, classified as potent, moderate and slightly weak, was obtained from the literature **[13].** The 3D structures of known 38 inhibitors were downloaded in .sdf format from pubchem compound database. They were later converted in .pdb format by the help of open babel **[14]** software. All the ligands were subjected to energy minimization and molecular dynamics using the HyperChem software **[15].** Energy calculations were carried out using the AMBER force field. Molecular structure optimization of ligands were carried out using conjugate gradient method Polak-Ribiere until the maximum energy derivative was lower than 0.1kcal/Å mol in order to obtain a correct geometry.

Molecular docking

Docking of 38 inhibitor screened from literature against CK2 structure was done using molecular docking program AutoDock **[16].** Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool **[17]**. Kollman charges and the solvation term were then added to the protein structure using the same. We have made the grid and adjusted the number of points in X, Y, Z-axis so that the entire active site residues (ASP175, PHE113, LYS68, ILE174, ILE95, VAL66, VAL53 and LEU45) of the human casein kinase II (CK2) are covered.The Lamarckian genetic algorithm implemented in Autodock was used. Docking parameters were as follows: 30 docking trials, population size of 150, maximum number of energy evaluation ranges of 25,0000, maximum number of generations is 27,000, mutation rate of 0.02, cross-over rate of 0.8, Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock program.

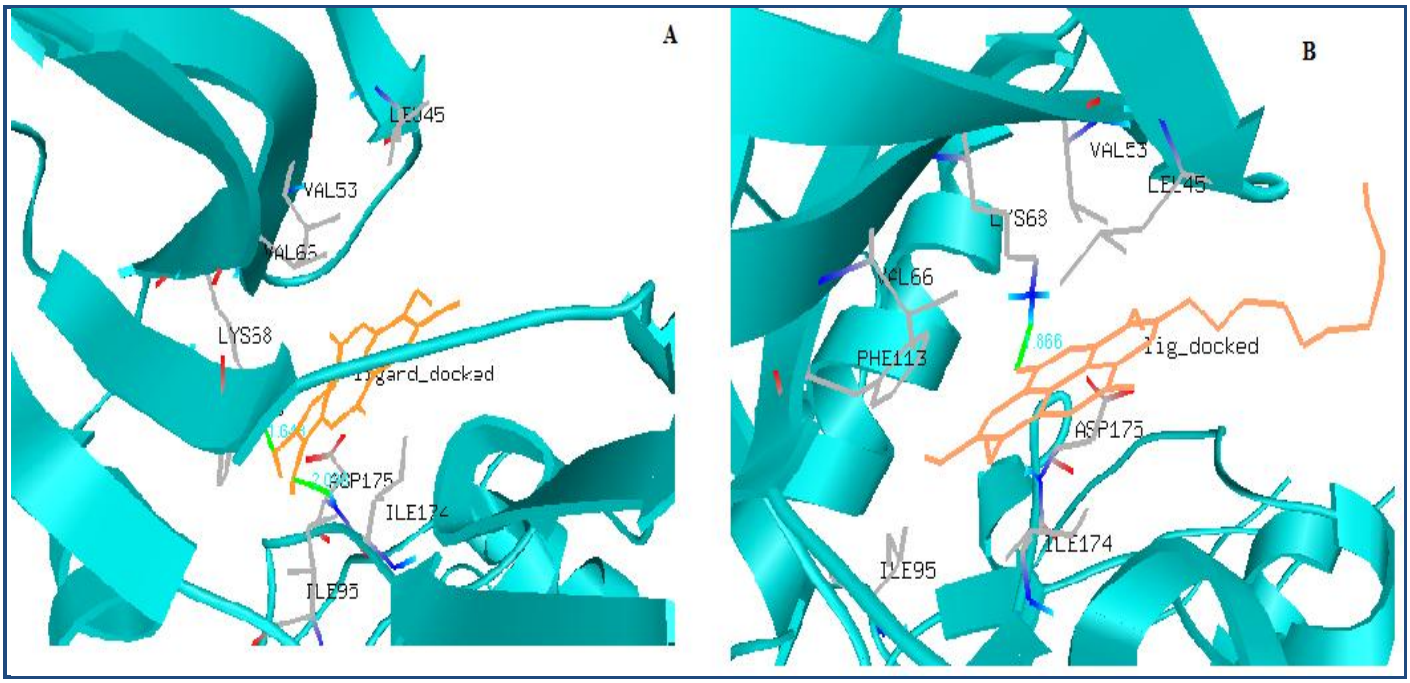

Figure 2: (A): The docking poses of complex between CK2 protein and compound CID 46229200. Two H-bonds is formed between amino acid LYS68 (HZ3) and ASP175 (HN) of protein with compound CID 46229200(O), respectively; **(B)** The docking pose of complex between CK2 protein and compound CID 10003463. One H-bond is formed between amino acid LYS68 (HZ3) of protein with compound CID 10003463(O). Hydrogen bond between inhibitor and residue is represented by green line in each case. Compound is colored in orange (in stick drawing) and amino acids involved in hydrogen bonds color by atom type.

2D QSAR study

Thirty eight known coumarin inhibitors of CK2 **[13]** were used for 2D QSAR studies. Using MLR, the QSAR model was developed with five types of energy based independent variables such as binding energy (EBind), intermol energy (EInterMol), torsional energy (ETors), internal energy (EEnt) and docking energy (DE) and one dependent variable activity (IC_{50}) with the help of different cross-validation procedures values.

Results & Discussion:

Result of molecular docking

Docking studies predicted the interaction of ligands with protein and residues involved in this complex. For such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best autodock score were used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock program. The docking results of the known inhibitors with CK2 were shown in **Table 2 (see supplementary material).**

Result of 2D QSAR studies

After docking 38 inhibitors screened from literature **[13]** against CK2 structure, five types of energy values namely binding energy (EBind), intermol energy (EInterMol), torsional energy (ETors), internal energy (EEnt) and docking energy (DE), based descriptors were then used as independent variables for QSAR modeling. Biological activity was expressed in terms of pIC50, the logarithm of measured IC_{50} (μM) against CK2 enzyme. Using linear regression analysis, a QSAR based model was generated having correlation coefficient r^2 value 0.4645. The equation was obtained for the inhibitory activities represented as pIC_{50} values, using the five types of energy values as variable descriptors. A model with the correlation coefficient (r^2) of 0.4645 was obtained for 38 compounds is shown in equation 1.

Predicted pIC_{50} = 4.68369 - 0.36245 (DE) -0.07374 (EBind) + 0.32636 (EInterMol) -0.06069 (ETors) + 1.19649 (EEnt)… (1)

Evaluation of QSAR models

To assess the predictive performance of QSAR models, different cross-validation procedures were adopted. First, in leave-one-out strategy (LOOCV), one molecule was removed from the dataset as a test compound and the remaining 37 molecules were used to build the model. This process was repeated 38 times with each inhibitor as a test molecule. Once a regression model was constructed, goodness about the fit and statistical significance was assessed using the statistical parameters.

Prediction of activity of newly designed CK2 inhibitors based on QSAR model

A total of 20 analogues of Ellagic acid were screened from PubChem Compound Database (http://www. ncbi. nlm.nih.gov/ pccompound) using the criteria (Compounds having similarity value >=95%) for docking studies with CK2 structure. The docked compounds were selected for calculation of biological activity on the basis of QSAR model is shown in **Table 1 (see supplementary material).** The docked complexes were validated and enumerated based on the AutoDock scoring function to pick out the best compounds based on

docked energy. Thus from the 20 compounds which were Docked, we got best 2 (CID 46229200, CID 10003463) of them with optimal energy. These compounds CID 46229200 (-9.73 kcal/mol) and CID 10003463 (-9.97 Kcal/mol) had lower docking energy even lower than standard control Ellagic acid (-8.73 kcal/mol).Further the two best-docked complexes were analyzed through Python Molecular Viewer software **[18]** for their interaction studies and were shown in (**Figure 2a & 2b).** Thus from the Complex scoring and binding ability it's deciphered that these compounds could be promising inhibitors for CK2.

Conclusion:

A QSAR model was developed using IC_{50} value of thirty eight known coumarin inhibitor with CK2 as dependent variable and five energy based descriptors namely binding energy, intermol energy, torsional energy, internal energy and docking energy as independent variable having correlation coefficient r^2 is 0.4645. Further twenty analogues of ellagic acid were screened from database and docked with CK2 and found two compounds CID 46229200 and CID 10003463 as potent candidate drugs for oral cancer.

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Supplementary material:

Table 1: Predicted activity of newly designed analogues of Ellagic acid based on QSAR model.

No.	CID No.	Predicted pIC50	Binding energy (Kcal/mol)	Docking energy (Kcal/mol)	Intermol energy (Kcal/mol)	Torsional energy (Kcal/mol)	Internal energy (Kcal/mol)
1.	56611575	5.54	-8.52	-8.92	-8.83	0.31	-0.09
2	11290032	5.69	-7.72	-7.83	-8.03	0.31	0.2
3	15006880	5.56	-7.44	-7.66	-8.69	1.25	1.03
4	53946689	6.12	-7.58	-8.07	-8.83	1.25	0.76
5	5319108	4.90	-3.02	-3.45	-3.34	0.31	-0.12
6	5316858	5.76	-7.89	-8.23	-8.51	0.62	0.28
7	5491816	5.82	-7.49	-7.73	-8.11	0.62	0.39
8	11580745	5.71	-6.84	-7.12	-7.46	0.62	0.34
9	1167459	5.26	-6.68	-7.02	-7.62	0.93	0.6
10	54122653	4.68	-3.12	-3.74	-3.74	0.62	0.0
11	46229200	5.74	-9.72	-9.73	-9.72	0.0	-0.02
12	18504424	5.53	-8.14	-8.19	-8.14	0.0	-6.05
13	10003463	5.39	-6.9	-9.97	-10.01	3.11	0.04
14	16049230	5.17	-4.5	-5.43	-5.43	0.93	0.01
15	53768212	5.13	-4.44	-5.39	-5.37	0.93	-0.02
16	11545697	5.05	-1.85	-2.56	-2.79	0.93	0.23
17	14503023	3.88	7.43	6.75	6.81	0.64	-0.05
18	54746813	4.74	-1.56	-2.32	-2.18	0.62	-0.13
19	44519391	5.80	-7.34	-7.57	-7.96	0.62	0.39
20	10400911	5.51	-7.69	-7.72	-7.69	0.0	-0.03

Table 2: The experimental and predicted pIC50 values for the training and test set molecules docked with CK2 structure. Experimentally known 38 coumarin inhibitors were obtained from the literature [13].

No.	CID No.	Exp. pIC50	Predicted pIC50	Binding energy (Kcal/mol)	Docking energy (Kcal/mol)	Intermol energy (Kcal/mol)	Torsional energy (Kcal/mol)	Internal energy (Kcal/mol)
1.	54732502	4.40	5.42	-6.48	-6.44	-6.48	0.0	0.03
2	54682226	4.55	5.50	-7.02	-6.97	-7.02	0.0	0.05
3	44456889	6.52	5.20	-2.73	-2.76	-3.05	0.31	0.28
4	44456746	5.44	5.50	-7.41	-7.41	-7.41	0.0	0.0
5	44456745	5.48	5.00	-1.68	-1.83	-2.0	0.31	0.17
6	44456744	5.57	5.43	-7.24	-7.28	-7.24	0.0	-0.05
7	44456718	5.39	5.46	-1.82	-2.11	-2.13	0.31	0.17
8	44456717	5.38	5.25	-6.29	-6.75	-6.6	0.31	0.03
9	44456698	4.97	5.40	-6.16	-6.13	-6.16	0.31	-0.14
10	44456697	4.99	5.48	-7.37	-7.38	-7.37	0.0	-0.01
11	44456695	4.67	5.42	-6.89	-6.91	-6.89	0.0	-0.01
12	44456694	4.69	778	-7.3	-7.77	-7.61	0.31	-0.16
13	44456692	4.64	5.33	-7.5	-8.32	-8.13	0.62	-0.19
14	44456668	4.57	5.50	-6.72	-6.92	-7.04	0.31	0.11
15	44456667	4.50	5.51	-7.25	-7.21	-7.25	0.0	0.04
16	24799273	5.39	5.07	-1.57	-1.57	-1.85	-0.31	0.28
17	24799272	5.48	5.37	-6.93	-7.33	-7.25	0.31	-0.08
18	24799271	5.60	5.88	-6.35	-6.36	-6.98	0.62	0.62
19	24799269	5.39	5.47	-6.9	-6.86	-6.9	0.0	0.04
20	24799087	6.49	5.50	-6.87	-6.9	-6.87	0.0	-0.03
21	24799086	6.55	5.43	-6.78	-6.77	-6.78	0.0	0.01
22	24799081	4.81	5.42	-6.46	-6.43	-6.46	0.0	0.03
23	24799080	5.39	4.97	-1.54	-1.7	-1.85	0.31	0.15
24	24799079	5.60	5.69	-7.21	-7.67	-7.52	0.31	-0.14
25	20144512	5.65	5.73	-6.13	-6.13	-6.13	0.0	0.0
26	15686455	6.13	5.36	-6.2	-6.2	-6.2	0.0	0.0
27	15445625	6.18	5.48	-7.45	-7.48	-7.45	0.0	-0.03
28	13881279	5.72	5.49	-7.11	-7.08	-7.11	0.0	0.03
29	11087876	4.45	4.99	-1.22	-1.32	-1.53	0.31	-0.05

30	5795340	7.00	5.46	-6.86	-6.83	-6.86	0.0	0.03
31	5417177	5.39	5.34	-6.44	-6.8	-6.75	0.31	-0.05
32	5415301	6.09	5.42	-6.64	-6.62	-6.64	0.0	0.02
33	5385113	4.52	5.02	-1.27	-1.46	-1.58	0.31	0.12
34	5376327	5.39	5.14	-1.32	-1.5	-1.63	0.13	0.13
35	5354284	4.45	5.37	-6.06	-6.03	-6.06	0.0	0.03
36	5324654	4.46	5.36	-5.85	-5.81	-5.85	0.0	0.04
37	5291935	4.52	5.06	-1.4	-1.44	-1.71	0.31	0.27
38	5281855	7.39	5.63	-8.72	-8.73	-8.72	0.0	-0.01