Anthraquinone Derivative Chrysophanol a Potent Inhibitor of CK2 Protein Kinase - A Computational Study Using DFT and Molecular Docking

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Abstract:
Anthraquinone derivatives have been identified to inhibit the CK2-dependent phosphorylation of multiple key proteins implicated with apoptosis. The optimization of anthraquinones was done using density functional theory (DFT) with the B3LYP/6-311G+(d, p) basis set to determine their frontier molecular orbitals, Mulliken charges, and chemical reactivity descriptors. According to the DFT results, Chrysophanol has the smallest HOMO-LUMO gap (2.46 Kcal/mol), as well as the highest electrophilicity index and basicity. Anthraquinones were docked into the active site cavity of CK2 to evaluate their structure-based inhibitory activity. The docking simulation studies predicted that Chrysophanol has the lowest binding energy (-6.31 Kcal/mol), which is inconsistent with the DFT calculations and suggests that it could be a powerful inhibitor of CK2 comparable to its known inhibitor viz. ellagic acid, having a binding affinity of -5.05 Kcal/mol. Anthraquinones' strong binding affinity was linked to the presence of hydrogen bonds as well as various hydrophobic interactions between the ligands and the receptor's essential amino acid residues.

Keywords- DFT, anthraquinones, CK2 inhibitor, ellagic acid, Mulliken charges, chemical, reactivity descriptors, frontier molecular orbitals

1. Introduction

Casein Kinase 2 (CK2), is an omnipresent necessary, and highly multifaceted protein kinase whose abnormally high constitutive activity is thought to underpin its harmful potential in neoplasia and other disorders[1, 2]. It is excessively high in almost all cancer types, making it one of the key contributors to the cancer cells' aggressiveness. This is primarily brought on by the phosphorylation of numerous important proteins [3] associated with apoptosis, which is CK2 dependent [4, 5]. As a result, various pharmacological inhibitors have been identified and produced, making CK2 an attractive target in the therapy of cancer [6, 7]. However, the lack of selectivity, cell permeability, metabolic stability, and appropriate pharmacokinetic properties in these inhibitors leads to a reduced therapeutic efficacy in cancer cells [8].

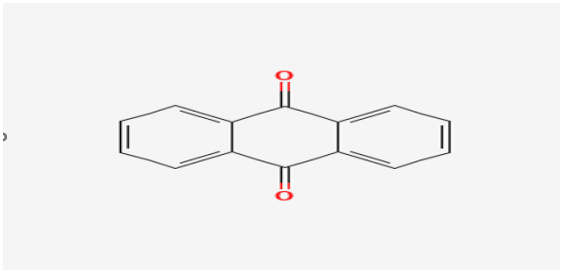
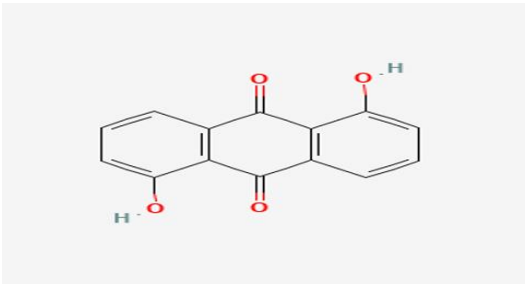
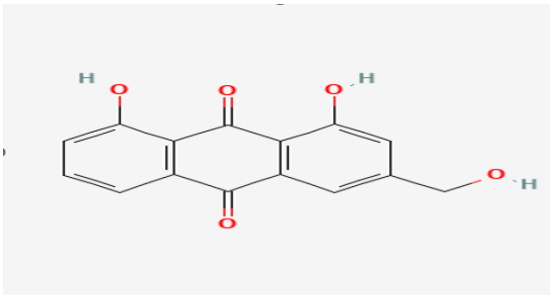
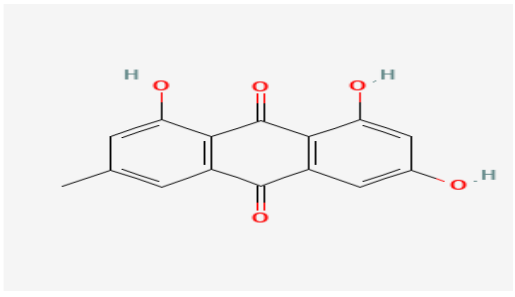
Anthraquinones have many biochemical and pharmacological properties [9]. These have a wide range of bioactivities, including cathartic, anti-inflammatory, antibacterial, diuretic, vasorelaxant, and phytoestrogen activities [10, 11)] This suggests that they may have clinical uses in treating a variety of disorders [12]. Even though anthraquinones' chemistry and biology have been better understood recently, research into their modes of action and promise as treatments for autoimmune illnesses is still in its infancy [13, 14].In this study using in silico approaches we have identified the derivatives of anthraquinone as potent CK2 inhibitors in comparison to Ellagic Acid, a tannic acid derivative [15, 16]. The crystal structure of human CK2 alpha in complex with emodin (PDB ID 3Q9W) is available at Protein Data Bank [17]. Ellagic Acid, a known inhibitor of CK2, binds in the active site and

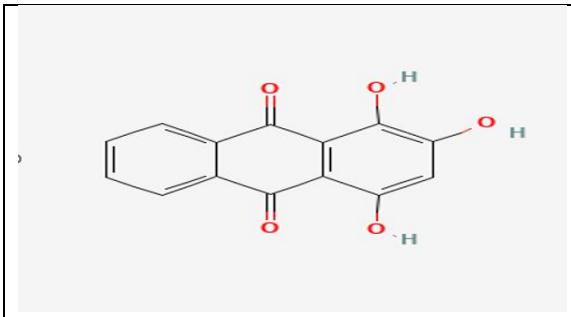
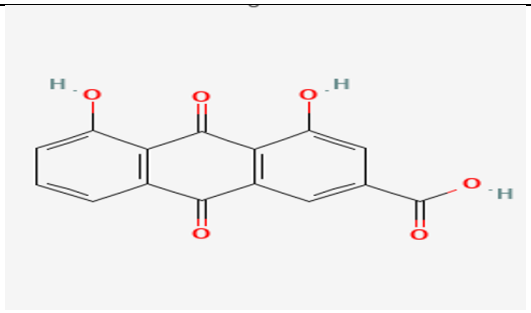
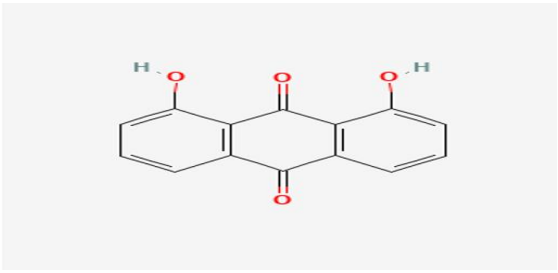
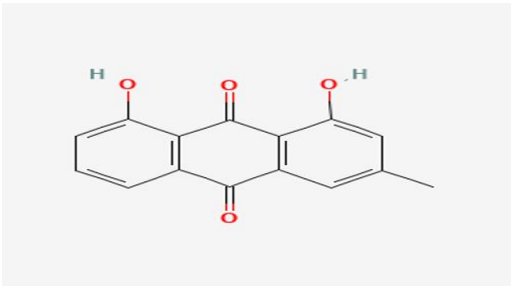
shows non-hydrophobic bonding with Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, and Asp175 [18, 19]. The mechanism of binding and stability of these anthraquinones to human CK2 were examined using molecular docking and DFT investigations. The use of molecular docking studies makes it possible to predict potential molecular interactions between ligands and enzymes that could produce important molecules and reveal subsequent molecular cross-talk within the system [20]. DFT simulations were utilized to determine the molecular structure with the lowest energy as well as the Mulliken charges, chemical reactivity parameters, and molecular orbitals. These variables are crucial in explaining how much interaction occurs in CK2's binding pocket. The HOMO of an inhibitor may transfer its electrons to less energetic amino acid residues in the active site of an enzyme because anthraquinones have the lowest HOMO-LUMO gap. These Anthraquinones can effectively bind to and inhibit the action of CK2, according to the results.

2. Materials and Methods

The Anthraquinones selected randomly for investigation include anthroquinone, anthrarufin, rhein, purpurin, emodin, aloe-emodin, chrysazin and chrysophanol. The two-dimensional structure of the anthraquinones has been extracted from PubChem (**Fig. 1).**

Fig. 1 The two-dimensional structure of the anthraquinones

Anthraquinone 	Anthrarufin 
Aloe-emodin 	Emodin 
Purpurin	Rhein

	
Chrysazin	Chrysophanol
	

2.1 DFT Calculations

In this study, computational calculations were implemented by using density functional theory (DFT) with the hybrid functional B3LYP [21, 22]by using 6-311G+(d, p) basis set in the gas phase in Gaussian09 program package [23-25].

2.2. Molecular Docking

The interaction of anthraquinones with CK2 was investigated using computational docking techniques. The docking of anthraquinones with CK2 was carried out using AutoDock 4.2.6 [20,25]. AutoDock calculates the binding free energy of a small molecule to a macromolecule using a semi empirical free energy force field. The coordinates of CK2 were obtained from the RCSB database from the crystal structure of human CK2 bound to emodin (PDB ID: 3Q9W)[17, 26, 27]. By deleting heteroatoms, as well as adding explicit hydrogen molecules and corresponding Kollman charges (16.0), using AutoDock 1.5.6 a receptor molecule was created and saved in.pdbqtfile format. Herein, eight compounds Anthraquinone, Anthrarufin, Aloe-emodinEmodin, Purpurin, Rhein, Chrysazin and Chrysophanol were used for the docking studies with CK2. Ellagic acid, a recognised inhibitor of CK2, was docked as a positive control and its binding affinity scores were compared to those of anthraquinone and its derivatives. Gauss View 5.0 [28, 29] was used to draw the 3D structures of all the Anthraquinones. The ligands were created by combining hydrogen atoms and Gasteiger charges before being saved in.pdbqt format. The torsional degrees of freedom of a ligand molecule were specified using ligand flexibility. Lamarckian genetic algorithm and grid aided energy evaluation approach were used for docking. The pose with the highest binding affinity score and the associated interactions were chosen and visually viewed and analysed in LigPlot [30, 31].

3. Results and discussion

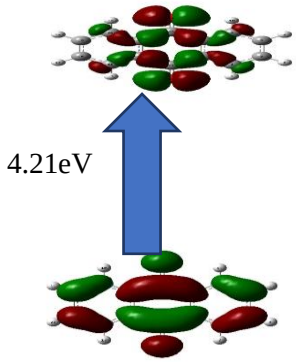
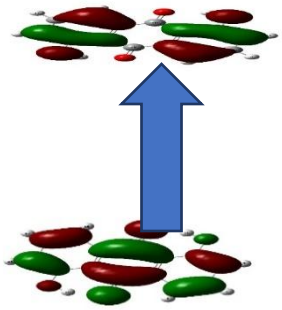
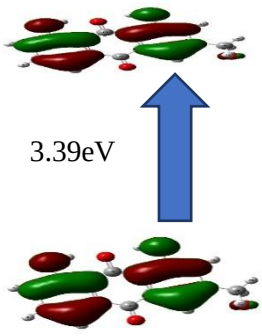
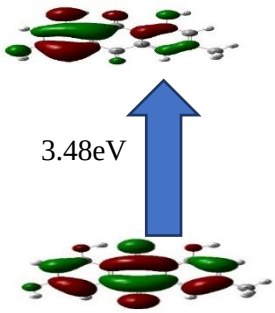
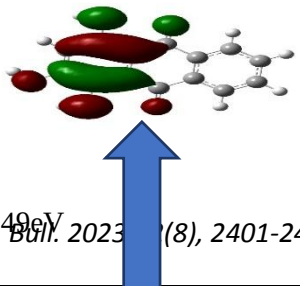
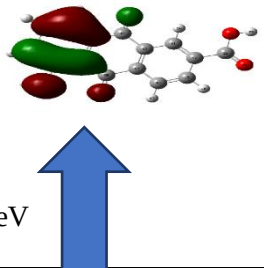
3.1. DFT calculation studies

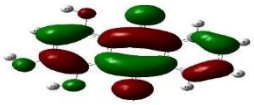
The theoretical DFT calculations were carried out using the Gaussian09 software on the basis set B3LYP 6-311G+(d,p). The structural geometry was optimised by minimising its energy in comparison to all geometrical variables while avoiding any molecular symmetry constraints. GaussView 5.0 was used to depict the molecular structure of the optimised anthraquinones **(Fig. 2).**

Fig. 2The optimized structures of the Anthraquinones&Ellagic Acid

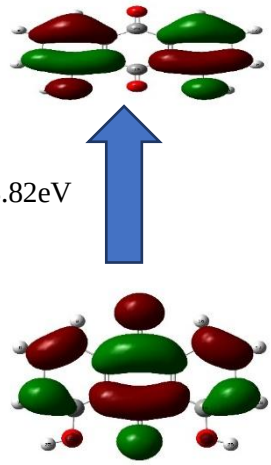
Anthraquinone 	Anthrarufin 
Aloe emodin 	Emodin 
Purpurin 	Rhein 
Chrysazin 	Chrysophanol 

Ellagic acid

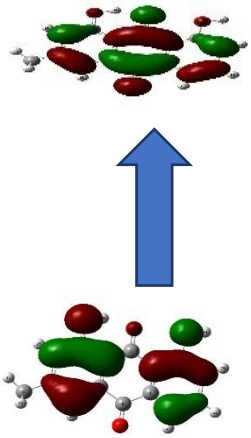
Anthraquinone  4.21eV	Anthrarufin  3.38eV
Aloe emodin  3.39eV	Emodin  3.48eV
Purpurin  3.49eV	Rhein  3.77eV



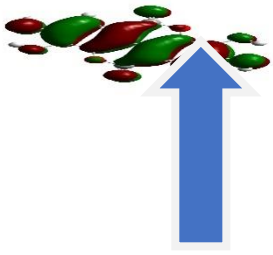
Chrysazin

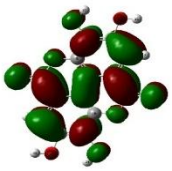


Chrysophanol



Ellagic acid





3.1.1. Frontier molecular orbitals

The Molecular orbital (MO)energies of the highest occupied molecular orbital (HOMO)and the lowest occupied molecular orbital (LUMO) were calculated and from those the gap in energy between the HOMO and the LUMO energies (GAP) was calculated**(Fig.3).**

Fig. **3Frontier Molecular Orbitals (FMOs) of Anthraquinones and ellagic acid**

HOMO and LUMO are very important quantum chemical parameters to determine the reactivity of the molecules and are used to calculate many important parameters. The HOMO and LUMO of the studied anthraquinones were calculated using DFT method at B3LYP 6-311G+(d,p) basis set and are tabulated (**Table 1**).

Table 1: HOMO, LUMO, gap, hardness (η), softness (δ), electronegativity (χ), electrophilicity index (ω), ionization potential (I) and electron affinity (A) of all the compounds.

S. No.	Ligand	HOMO	LUMO	ΔE	χ	η	δ	ω	I	A
1	Ellagic Acid	-6.54	-2.33	4.21	4.43	2.10	0.47	4.67	6.54	2.33
2	Anthraquinone	-7.40	-3.19	4.21	5.29	2.10	0.48	6.65	7.40	3.19
3	Anthrarufin	-6.90	-3.52	3.38	5.21	1.69	0.59	8.04	6.90	3.52
4	Aloe emodin	-6.90	-3.51	3.39	5.20	1.69	0.59	7.97	6.90	3.51
5	Emodin	-6.61	-3.14	3.48	4.87	1.74	0.58	6.83	6.61	3.14
6	Purpurin	-6.30	-2.81	3.49	4.55	1.75	0.57	5.94	6.30	2.18
7	Rhein	-7.08	-3.32	3.77	5.20	1.88	0.53	7.18	7.08	3.32
8	Chrysazin	-6.77	-2.94	3.82	4.86	1.91	0.52	6.17	6.77	2.94
9	Chrysophanol	-8.76	-6.30	2.46	7.53	1.23	0.81	23.07	8.76	6.30

In this analysis, Ellagic Acid has the HOMO-LUMO gap (4.2eV) whereasChrysophanol (2.46ev) shows the lowest energy gap. Because adding electrons to a higher LUMO and taking them away from a lower HOMO are both energetically advantageous in some reactions, big energy gaps are associated with high kinetic stability and poor chemical reactivity, while small energy gaps reflect low chemical stability [32, 33].

3.1.2.Thermodynamic properties

The optimized geometrical parameters are used in the calculation of the energy of HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital), energy gap (ΔE), dipole moment (μ) and free energy (**Table 2**).

Table 2: Free energy and dipole moment of Ellagic Acid and all the Anthraquinones

S. No.	Phytochemicals	Free energy (AU)	Dipole moment (Debye)
1	Anthraquinone	-688.954	0.0000

2	Anthrarufin	-839.473	0.0019
3	Aloe emodin	-954.036	0.7583
4	Emodin	-954.031	4.8811
5	Purpurin	-914.676	4.9033
6	Rhein	-952.827	4.1344
7	Chrysazin	-839.429	0.3163
8	Chrysophanol	-878.780	2.5805
9	Ellagic acid	-1139.246	0.0000

The initial geometry of anthraquinones was taken from an online chemical information resource named PubChem and further modified in Gaussian 09 software using GaussView5. The absence of imaginary frequency confirmed that phytochemicals were fully optimized. Chemical reactivity descriptors such as hardness (η), softness (δ), electronegativity (χ) and electrophilicity index (ω) of all the phytochemicals were also calculated from the energies of frontier HOMOs and LUMOs [30, 31] and hardness (η), electronegativity (η) and softness (δ) is calculated by the equations as-

$$\eta = -1/2(E_{HOMO} - E_{LUMO}) \quad (1)$$

$$\chi = -1/2(E_{HOMO} + E_{LUMO}) \quad (2)$$

$$\delta = 1/\eta \quad (3)$$

$$\omega = \chi 2 /2\eta \quad (4)$$

The χ value predicts the molecule's ability to attract electrons, i.e., Lewis acid, whereas lower values of (χ) indicate a suitable base. The global hardness (η) of a molecule is a measure of its ability to prevent charge transfer; meanwhile, the global softness (δ) characterises its ability to take electrons. Soft molecules have a small energy difference between border molecular orbitals and are more reactive than harder molecules because electrons can be quickly transferred to acceptors. The electrophilicity (ω), which is computed from electronegativity and chemical hardness, is a sign of a reduced energy difference due to the greatest electron movement between the acceptor, LUMO, and the donor, HOMO. Chrysophanol has a high basicity (χ =7.53) and an electrophilicity index (ω =23.07) when compared to other Anthraquinones.

3.1.3.Mulliken atomic charges

The Mulliken atomic charges of the estimated ligands were calculated with DFT using B3LYP as a method at a 6-311G+(d,p) basis set, the data were tabulated in **Table 3.** The positively charged centers are the most susceptible sites for nucleophilic attacks i.e., electron donation. However, the most negatively charged centers are the most susceptible sites for electrophilic one. However, the most negatively charged atoms in Chrysophanol are O16 and C6 while their corresponding positively charged atoms are O18 and C2 respectively.

Table 3: Mulliken charges with hydrogen summed into heavy atoms

Aloe-emodin		Emodin		Purpurin		Rhein		Chrysazin		Chrysophanol		Anthraquinone		Ellagic acid		Anthrarufin	
1C	-0.1517	1C	0.0477	1C	-0.2894	1C	0.1065	1C	-0.2707	1C	-0.0924	1C	0.3440	1C	0.4563	1C	0.1310
2C	0.7459	2C	0.4490	2C	-0.2963	2C	0.2054	2C	-0.3072	2C	0.9088	2C	-0.1992	2C	0.6334	2C	-0.1820
3C	-0.1627	3C	-0.0416	3C	0.3564	3C	-0.2779	3C	0.2740	3C	0.1135	3C	-0.1992	3C	-0.2186	3C	0.5059
4C	0.2476	4C	0.8377	4C	0.1372	4C	-0.0812	4C	-0.1454	4C	0.6564	4C	0.3440	4C	-0.3278	4C	-0.0152
5C	-0.2875	5C	-0.1776	5C	0.2053	5C	-0.1491	5C	0.7096	5C	-0.1473	5C	0.1488	5C	-0.2091	5C	0.2294
6C	0.1182	6C	-0.6423	6C	0.2673	6C	0.8509	6C	0.0036	6C	-0.6288	6C	0.1488	6C	-0.2555	6C	-0.2773
10C	-0.2468	9C	0.1326	11C	0.7928	10C	-0.0059	10C	0.7096	7C	-0.3218	11C	0.3440	8C	-0.3278	10C	-0.0152
11C	-0.2034	10C	-0.0069	12C	0.5069	11C	0.1876	11C	-0.1454	8C	-0.3345	12C	0.1488	9C	-0.2091	11C	0.2294
12C	-0.1355	11C	-0.6713	13C	-0.1862	12C	0.1149	12C	0.2741	9C	0.0330	13C	0.1488	10C	-0.2555	12C	-0.2773
13C	0.2283	12C	0.3265	14C	-0.0109	13C	0.3624	13C	-0.3071	10C	0.4149	14C	0.3440	11C	0.4563	13C	0.1310
14C	-0.1982	13C	-0.1773	15C	-0.4524	14C	0.1027	14C	-0.2708	11C	0.0765	15C	-0.1992	12C	0.6334	14C	-0.1820
15C	0.6384	14C	0.6904	16C	-0.4551	15C	-0.3832	15C	0.0036	12C	-0.1298	16C	-0.1992	13C	-0.2186	15C	0.5059
18C	0.1850	17C	0.0855	18C	-0.1467	19C	-0.6192	19C	0.1453	13C	-0.1368	21C	-0.3533	15O	-0.0340	19C	-0.1229
19C	-0.4782	18C	0.0299	19C	-0.1139	20C	-0.3571	20C	-0.4221	14C	-0.2856	22C	-0.3533	160	0.0411	20C	-0.1229
20C	0.2673	19C	0.0259	20C	-0.2171	21O	0.4823	21O	0.0958	15C	0.2670	23O	-0.2338	17O	-0.0340	21O	-0.2946
21O	-0.3987	20O	0.0641	21O	-0.2324	22O	-0.2142	22O	-0.2144	16O	-0.2856	24O	-0.2338	18O	0.0411	22O	0.0256
22O	-0.2155	27O	0.1897	22O	0.0826	23O	-0.2001	23O	0.0958	17O	-0.2208			19O	-0.1635	23O	-0.2946
23O	0.0415	28O	-0.6458	24O	-0.0383	24O	-0.2997	24O	-0.2282	18O	0.0838			20O	-0.1635	24O	0.0256
24O	-0.0377	29O	-0.2950	26O	0.0899	25O	0.0815			19O	0.0294			25C	0.3575		
27O	0.0436	30O	-0.2213			26O	0.0931										

3.2. Molecular docking

Molecular docking is a popular computational method for validating the interaction of an appropriate orientation of a small molecule with a receptor protein. The findings of this study revealed that because the benzene rings of anthraquinone are structurally similar to the known inhibitor, ellagic acid, they are likely to imitate the binding mechanism at the active site of CK2 [34, 35]. As indicated in **Table 4**, anthraquinones have a binding energy in the range of 5.64 to 6.60 kcal/mol, which is comparable to ellagic acid (5.05 kcal/mol). As shown in **Fig. 4 and Table 5**, these anthraquinones occupied the active site cavity, which included residues such as Phe131, Val166, Ile95, Asp175, Val116, Leu45, Val153, Met163, Asn118, and Ile174.

Table 4: Binding affinity of the Anthraquinones

S. No.	Phytochemicals (Anthraquinones)	Binding energy (kcal/mol)
1	Ellagic Acid	-5.05
2	Anthraquinone	-6.60
3	Anthrarufin	-5.83
4	Aloe-emodin	-5.79
5	Emodin	-6.19

6	Purpurin	-5.75
7	Rhein	-5.64
8	Chrysazin	-5.97
9	Chrysophanol	-6.31

Fig.4 Amino acid residues in the protein binding pocket

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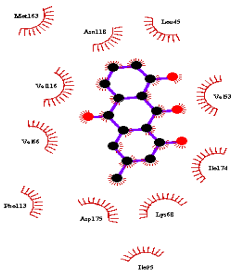
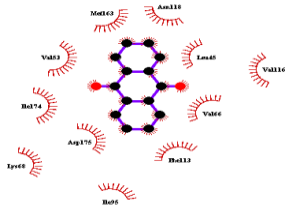
  </td><td>Anthraquinone
  </td></tr>
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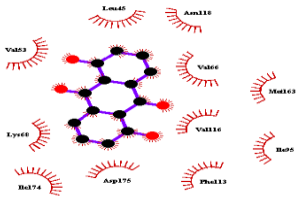
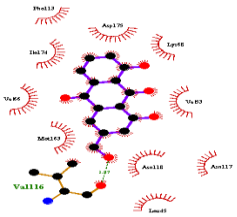
  </td><td>Aloe-emodin
  </td></tr>
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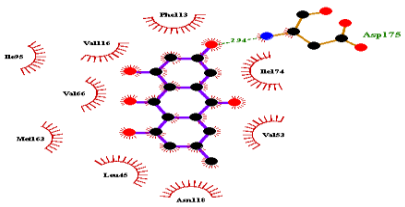
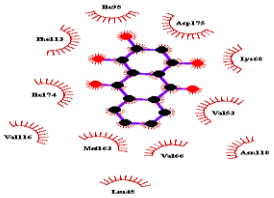
  </td><td>Purpurin
  </td></tr>
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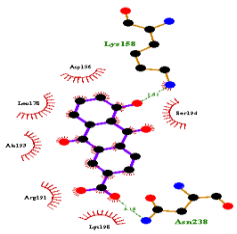
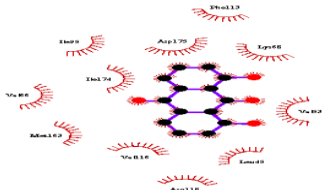
  </td><td>Chrysazin
  </td></tr>
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Ellagic acid

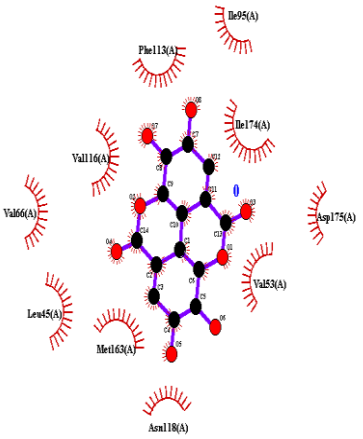

Table 5: Amino acid residues in the CK (PBD 3q9w) binding pocket

S. No.	Ligand	Type of interactions		Number of bonds		Common residues
		H-bond residues	Hydrophobic bond residues	H-bonds	Hydrophobic bonds	
1	Ellagic acid	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175	0	10	100
2	Anthraquinone	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175, Lys68	0	11	100
3	Anthrarufin	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175	0	11	100
4	Aloe emodin	Va116 (2.87 Å)	Phe113, Va166, Met163, Leu45, Asn118, Val153, Ile174, Asn117, Lys68	1	9	80
5	Emodin	Asp175 (2.94 Å)	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174	1	9	100
6	Purpurin	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175, Lsy68	0	11	100
7	Rhein	Lsy158 (2.82 Å), Asn238(3.15 Å)	Asp156, Ala193, Arg191,Lsy198,Leu178, Ser194	3	6	0
8	Chrysazin	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175, Lsy68	0	11	100
9	Chrysophanol	-	Phe113, Va116, Va166, Ile95, Met163, Leu45, Asn118, Val153, Ile174, Asp175, Lsy68	0	11	100

Nonbonding interactions, in addition to regular hydrogen bonding, are often used terms to describe the form and behaviour of molecules. These findings imply that all of the anthraquinones examined can readily bind in the active region of CK2. Furthermore, Chrysophanol appears to be a powerful CK2 inhibitor. As a result, Chrysophanol's inhibition of CK2 can diminish phosphorylation.

The negative Mulliken charges on oxygen atoms in anthraquinones, as previously mentioned from DFT simulations, could be exploited for hydrogen bond interactions with protein receptors. The energy levels of HOMOs range from -6.30 eV to -8.76 eV, while LUMOs range from -2.81 eV to -6.30 eV depending on conjugation and the presence of polar groups. Furthermore, Chrysophanol's low FMO energy gap (E=2.46), high basicity (=7.53), and high electrophilicity index (=23.07) compared to others may have an effect on binding affinity. Furthermore, docking experiments revealed that Chrysophanol binds to CK2 with the lowest binding energy (-6.31 kcal/mol), supporting the DFT investigations. All of these parameters may interact to varying degrees to greatly influence the degree of binding affinity of these anthraquinones with the active protein sites.

Conclusion

The phosphorylation of multiple essential proteins is linked to apoptosis, which is controlled and triggered by Casein Kinase 2 (CK2). CK2 levels are abnormally high in almost all cancer forms, making it one of the major contributors to the aggressiveness of cancer cells. Anthraquinones are readily available phytochemicals that are being studied as effective CK2 enzyme inhibitors using DFT and Molecular docking models. This computational analysis demonstrates that the binding affinities of Chrysophanol-CK2 complexes are similar to those of a known inhibitor-protein combination, namely Ellagic acid-CK2. As a result, anthraquinone and its derivatives can bind to CK2 effectively and limit its action in the protein phosphorylation pathway. The focus of our research is on the inhibitory effects of Anthraquinones on CK2 activity. It might be concluded that these characteristics interact with each other in varying degrees and influence the degree of binding affinity of these Anthraquinones with active protein sites to provide a particular level of inhibition.

Declaration of Competing Interest

The authors affirm that they have no known financial or interpersonal conflicts that might have looked to have influenced the research presented in this study.

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CRediT authorship contribution statement

S. Anamika: Writing - original draft. T.Nikita: Formal analysis. M.Anil: Writing - review & editing. G. Monika: Supervision, Writing - review & editing

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