Computational Molecular Bioscience, 2019, 9, 81-94
http://www.scirp.org/journal/cmb
ISSN Online: 2165-3453
ISSN Print: 2165-3445

Molecular Docking and Pharmacological Property Analysis of Phytochemicals from *Clitoria ternatea* as Potent Inhibitors of Cell Cycle Checkpoint Proteins in the Cyclin/CDK Pathway in Cancer Cells

Asad Ullah[1*], Nazmul Islam Prottoy[1], Yusha Araf[2], Sohana Hossain[1], Bishajit Sarkar[1], Ananna Saha[1]

[1]Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Dhaka, Bangladesh
[2]Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh
Email: *ullah1194@gmail.com

How to cite this paper: Ullah, A., Prottoy, N.I., Araf, Y., Hossain, S., Sarkar, B., and Saha, A. (2019) Molecular Docking and Pharmacological Property Analysis of Phytochemicals from *Clitoria ternatea* as Potent Inhibitors of Cell Cycle Checkpoint Proteins in the Cyclin/CDK Pathway in Cancer Cells. *Computational Molecular Bioscience*, **9**, 81-94.
https://doi.org/10.4236/cmb.2019.93007

Received: August 5, 2019
Accepted: September 3, 2019
Published: September 6, 2019

Abstract

Cancer comprises a group of diseases which are involved in the aberrant growth of the cells causing disruption of normal body function. Due to the lack of proper sophisticated treatments this nasty disease leads to the death of most of the patients affected with it. Moreover, treatments like chemotherapy involve other post-treatment complications which make them unfavorable for extended use. Medicinal plants possess many phytochemicals of great therapeutic value and many of them are effective in killing cancer cells. These compounds working by variety of mechanisms and in most of the cases exhibit their anticancer potentiality by inhibiting many proteins involved in cell growth and division. Molecular docking is a computational approach which facilitates the finding of the best molecule from a group which may bind with the highest affinity with the intended target by providing a virtual biological system. This process works on the basis of specific algorithm and involves scoring function to rank the molecules that fit with the target. This study has been designed to investigate the potentiality of four phytochemicals from *Clitoria ternatea*—**Kaempferol, Myricetin, P-Hydroxycinnamic acid** and **Quercetin** as inhibitors of two cell cycle checkpoint proteins—Cyclin Dependent Kinase-2 (CDK-2) and Cyclin Dependent Kinase-6 (CDK-6) in Cyclin/CDK pathway. Quercetin and Myricetin docked with higher affinity with CDK-2 and CDK-6 respectively. Drug likeness property analysis and ADME/T test impose computational approach to investigate physicochemical and pharmacological properties of candidate drug molecules. P-Hydroxycinnamic acid

performed well in both drug likeness property analysis and ADME/T than Quercetin and Myricetin. So, P-Hydroxycinnamic acid is the best finding of this experiment.

Keywords

Anticancer, ADME/T, *Clitoria ternatea*, Docking, Kaempferol, Quercetin

1. Introduction

1.1. Cancer, Its Current Status and Treatment

Cancer is a broader term reflecting a group of diseases which result in the abnormal growth and division of cells inside the human body. Subsequent to cancer development, the affected cells lose their normal function and continue to grow indefinitely spreading a larger area gradually. The notable causes to the development of cancer can be attributed to genetic heterogeneity, malnutrition, environmental hazards, etc. [1] [2]. Currently, cancer affects the people of both less and more developed country with more recorded incidents in female than in male. The increasing occurrence of cancer is subjected to increased risk factors such as, smoking, physical inactivity, overweight and changing reproductive pattern in most of the cases. Almost 14.1 million new cancer cases and 8.2 million deaths were reported worldwide only in 2012. And the trend is shifting toward the less developed country day by day [3]. Sophisticated treatments like chemotherapy, surgery, radiation therapy and stem cell therapy display a great percentile of recovery but there is always a growing demand of new medication since the available treatments are not accessible to every person due to higher cost. Moreover, these treatments often involve a range of short and long term health effects which again discourage most of the cancer patients [4]. Many natural compounds from medicinal plants have been reported to have anticancer property against variety of cell lines and they exploit this role with different mechanisms [5]. *Clitoria ternatea* is a medicinal herb that contains alkaloids, tannins, saponins, anthocyanins, cardiac glycosides, etc., which provides potential health benefits to consumers. Its major phytochemicals of potent therapeutic value include Kaempferol, Myricetin, P-Hydroxycinnamic acid, Quercetin, Beta-sitosterol, Anthoxanthin glucoside, Tannic acid, Taxaxerol, etc. Aqueous extract of seeds from this plant has already been shown to have cytotoxic activity in laboratory experiment [6] [7] [8].

1.2. Role of Cyclin/CDK Pathway in Cell Cycle and Cancer

Cyclin dependent kinases (CDKs) are specific serine/threonine kinases which contribute to the cell cycle progression by phosphorylating and inactivating Retinoblastoma (Rb) protein. Rb protein usually resides inside the cell forming a complex with a transcription activator called E2F which has at least five DNA

binding domains and takes part in progressing the dividing cell from G_1 phase to S phase (**Figure 1**). In the complex form, Rb represses the activity of E2F protein which is the case when the cell is in resting sate [9]. Several mitogens released from the upstream signaling pathway activate Cyclin D which forms complex with CDK-4/6 and helps in its activation. In the active state, CDK-4/6 phosphorylates Rb and partially inactivates it facilitating the release of E2F from the complex [10] [11]. Once E2F is released from the complex, it becomes activated and carries out the events required for the G_1 to S phase transition in the cell cycle. E2F also promotes the activation of Cyclin E-CDK-2 complex which in turn contributes in the phosphorylation of Rb in a feedback loop and thus prolongs the E2F activity [12].

Inside the cell both types of CDKs are repressed by inhibitors comprising proteins from INK4 (inhibitor of CDK-4) and CKI (cyclin-dependent kinase inhibitor) families and this contributes to the decision of the cell to undergo cell division or not. CDK-4/6 is inactivated by inhibitors like p15/p16 and CDK-2 is inactivated by the p21/p27 inhibitory proteins [13]. The dysregulated hyperactivity of CDK due to the mutation in CDKs or their inhibitor encoding genes can lead to uncontrolled cell growth which characterizes the cancer. Different types of CDKs have been reported to be associated with different forms of cancer in which they lack the ability to bind inhibitor and ultimately become resistance [14] [15].

1.3. In Silico Molecular Docking and ADME/T

Computational drug design is a widely accepted technique for new lead discovery. Virtual screening technique reduces both time and cost of the drug discovery expenditure. More than 50 drugs have been designed and repurposed with the aid of these computational simulation tools and many of them received FDA approval for marketing like Raltegravir, Saquinavir, Nelfanavir, Itraconazole etc. Molecular docking tries to predict the pose, interaction and conformation of a ligand molecule within the binding site of a target molecule, usually a large macromolecule. After estimating the type of interactions, the software assigns scoring function to each of the bound ligands with specified algorithm which reflects the binding affinity. The lowest score of binding represents the most favorable interaction between ligand and receptor molecule [16] [17] [18].

The safety and efficacy testing of a candidate drug molecule is a major concern in clinical and preclinical trial. *In silico* approaches to assess the drug features has enabled the test to be carried out in a much simpler way where *in vitro* and *in vivo* assessment of safety and toxicity is time consuming and costly. ADME/T testing provides information regarding the drug feature like adsorption, distribution, metabolism, excretion and toxicology information inside human body. Moreover, these approaches help in generating data about the extent of drug absorption inside the body, blood brain barrier permeability, susceptibility to biodegradation, mutagenicity, carcinogenicity etc. [19] [20].

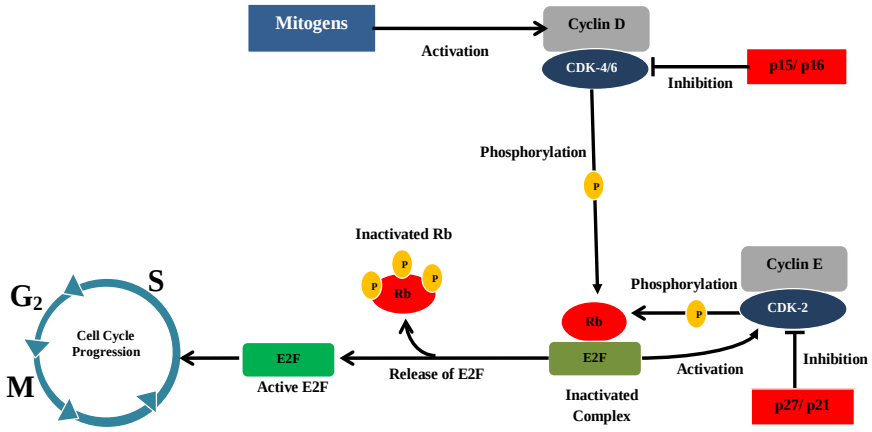

Figure 1. Signaling through Cyclin/CDK pathway in cell cycle. Both CDK-4/6 and CDK-2 contribute in the progression from G1 phase to S phase of cell cycle. CDK-4/6 becomes activated when it forms heterodimer with D type cyclin which is already activated by mitogens from upstream signaling pathway. CDK-2 becomes activated in complex form with E type cyclin. Both of the kinases phosphorylate and inactivate Rb to release E2F which then promotes cell cycle progression. Both CDK-4/6 and CDK-2 become inactivated by different inhibitory proteins like p27, p15 etc. which makes sure that cell cycle progresses in very tightly regulated way.

This study has been designed to investigate the inhibitory potentiality of four phytochemicals: **Kaempferol, Myricetin, P-Hydroxycinnamic acid** and **Quercetin** (Figure 2) from *Clitoria ternatea* against CDK-2 and CDK-6 (Figure 3) in cancer cell and to assess their physicochemical, pharmacokinetic and pharmacodynamic properties inside biological system.

2. Materials and Methods

Ligand preparation, Grid generation, Glide docking and 2D and 3D representation of ligand receptor interaction were obtained using Maestro Schrödinger Suite 2018 (Figure 4 and Figure 5). The chemical structures of ligands were refined using ChemSketch (Figure 2). Discovery Studio Visualizer was used for the visualization of the structures (Figure 3) [21] [22] [23].

2.1. Protein Preparation

Three dimensional structures of CDK-2 (PDB Id: 3EZV) and CDK-6 (PDB Id: 1XO2) were downloaded in PDB format from protein data bank (www.rcsb.org). The protein was then processed and refined utilizing the Protein Preparation Wizard in Maestro Schrödinger v11.8. Bond orders were assigned, hydrogens were added to heavy atoms. All the waters were deleted from the molecules and selenomethionines were converted to methionines. Finally, the structure was optimized and then minimized using built-in default force field OPLS_2005. Minimization was performed setting the greatest substantial particle RMSD (root-mean-square-deviation) to 30 Å and any outstanding water under 3H-bonds to non water was again erased during the minimization step.

2.2. Ligand Preparation

The 3D conformations of Kaempferol (PubChem CID: 5280863), Myricetin (PubChem CID: 5281672), P-Hydroxycinnamic acid (PubChem CID: 637542) and Quercetin (PubChem CID: 5280343) were downloaded from PubChem (https://www.pubchem.ncbi.nlm.nih.gov/). These structures were then processed prepared using the LigPrep of Maestro Schrödinger. Minimized 3D structures of ligands were generated using Epik2.2 and within pH 7.0 ± 2.0 in the suite. Minimization was again carried out using OPLS_2005 force field which generated maximum 32 possible different rearranged spatial conformations (stereoisomers) depending on available chiral centers for each of the ligand molecules.

2.3. Receptor Grid Generation

Grid usually restricts the active site to specific area of the receptor protein for the ligand to dock specifically within that area. In Glide, a grid was generated using default Van der Waals radius scaling factor 1.0 and charge cutoff 0.25 which was then subjected to OPLS_2005 force field for the minimized structure. A cubic box was generated around the active site (reference ligand active site) of target molecules. Then the grid box volume was adjusted to 14 × 14 × 14 for docking to be carried out.

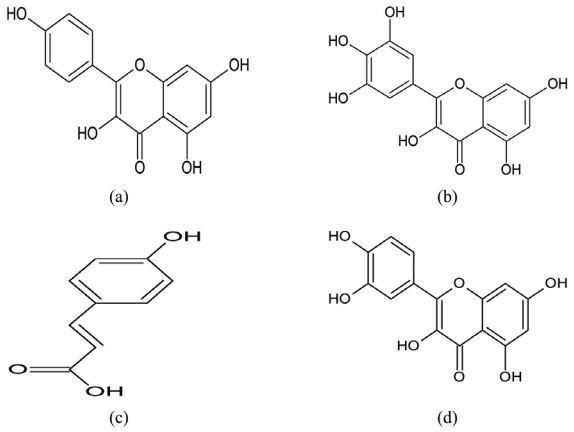

Figure 2. Chemical structures of (a) Kaempferol (PubChem CID: 5280863), (b) Myricetin (PubChem CID: 5281672), (c) P-Hydroxycinnamic acid (PubChem CID: 637542) and (d) Quercetin (PubChem CID: 5280343).

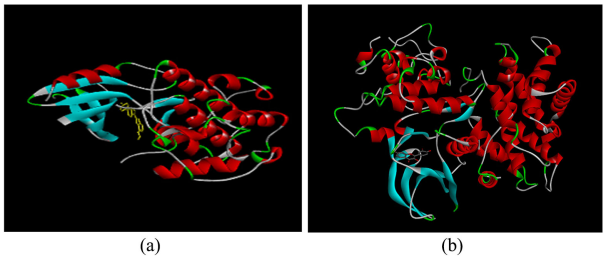

Figure 3. 3D crystallographic conformation of target molecules (a) CDK-2 (PDB Id: 3EZV), (b) CDK-6 (PDB Id: 1XO2) in ligand bound form. Ribbons represent the target molecule backbone and ligands are represented in stick style.

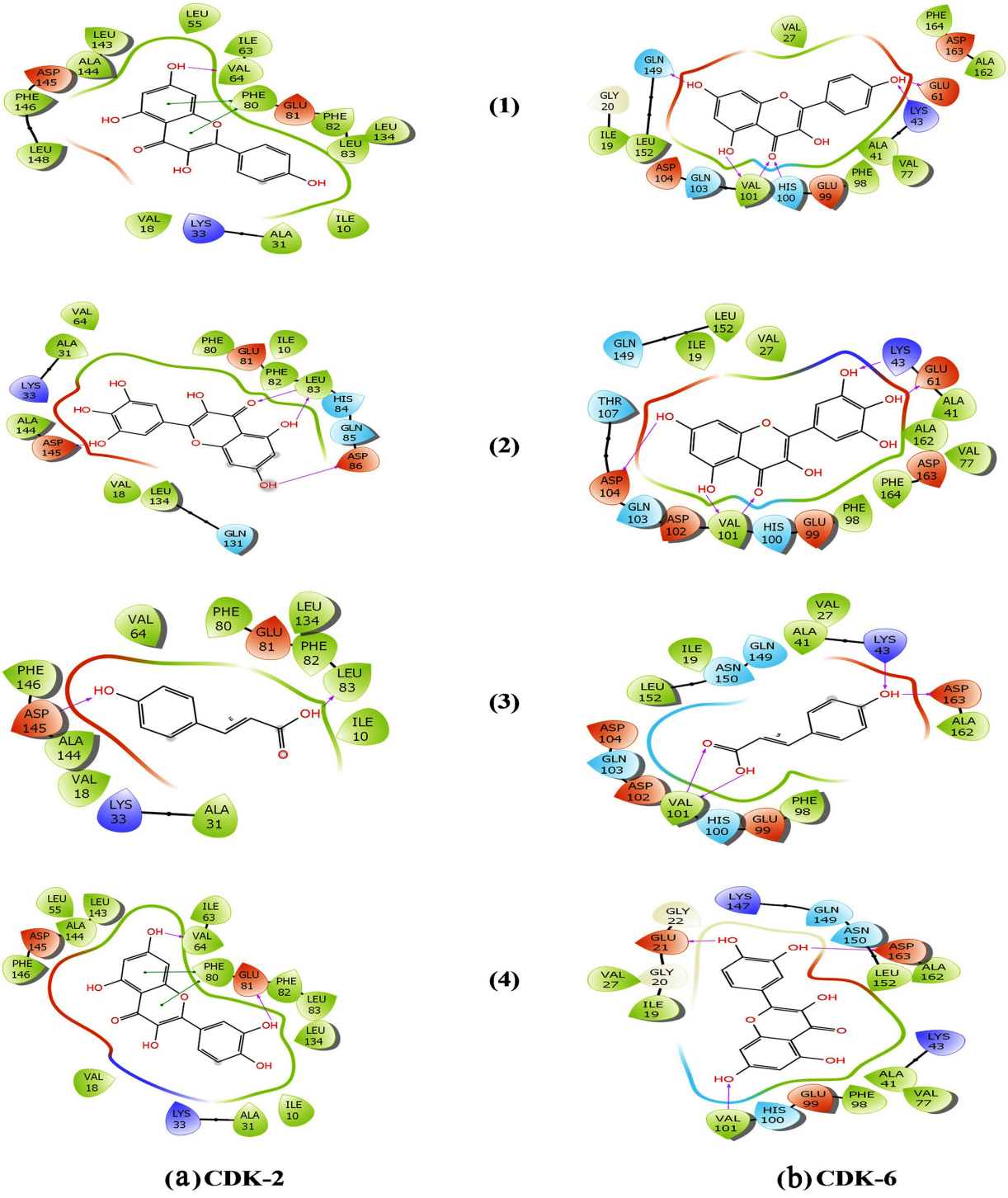

Figure 4. 2D representation of interaction between ligand molecules (1) Kaempferol, (2) Myricetin, (3) P-Hydroxycinnamic acid, (4) Quercetin and target molecules (a) CDK-2and (b) CDK-6. The minimized ligand structure is represented in protonated form. Color spheres indicate the residue types in the target: Red-Acidic (Asp, Glu), Blue-Polar (Ser, Thr, Gln, Asn, His), Green-Hydrophobic (Ala, Val, Leu, Ile, Tyr, Trp, Phe, Met, Cys, Pro), Purple-Basic (His, Lys, Arg), Darker gray-metal atom, Lighter gray-Other (Gly, water). Interactions are represented as color lines-Solid pink-H-bond in target, Dotted pink-H-bond between receptor and ligand, Green line-Pi-Pi stacking interaction. Ligands exposed to solvent are represented by grey sphere. The protein pocket for the ligand is marked with the color line according to the nearest atom (Blue and orange line represents salt bridge). Interruption of line indicates opening of the pocket.

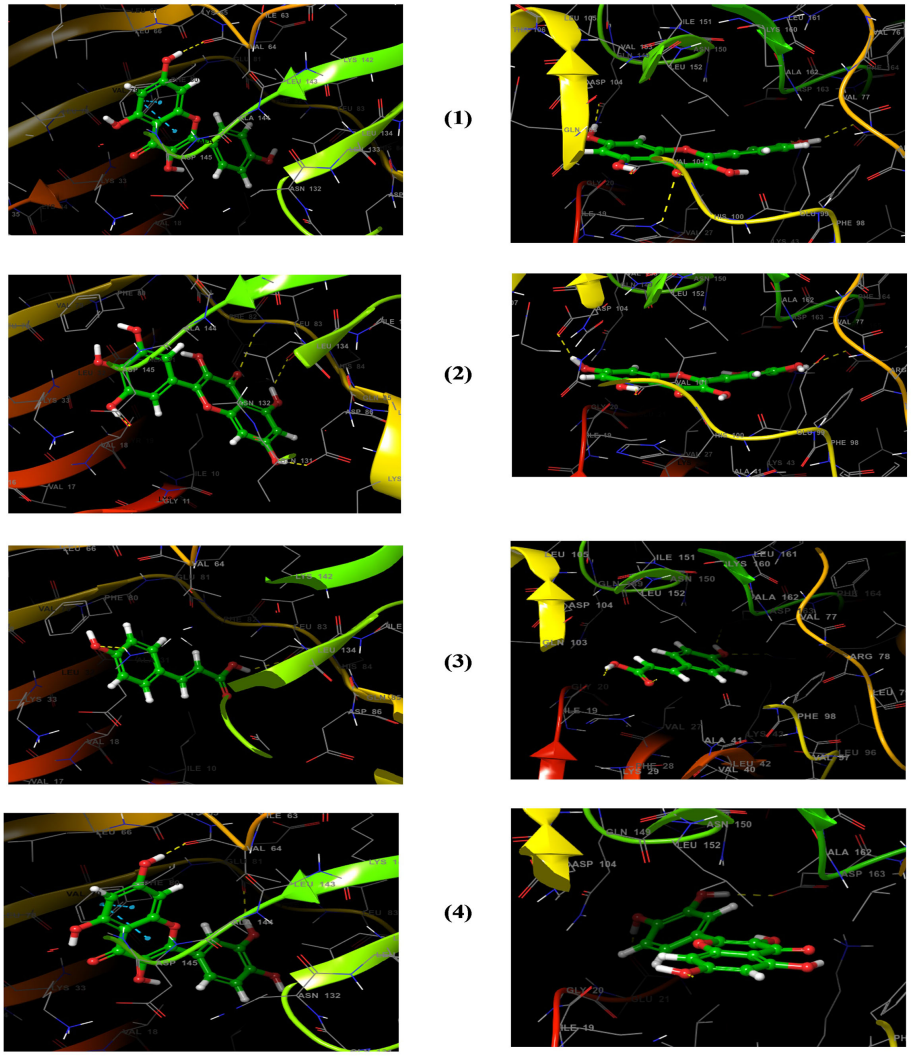

Figure 5. 3D representation of best possible pose of ligands within binding site, type of interactions between ligand molecules (1) Kaempferol, (2) Myricetin, (3) P-Hydroxycinnamic acid, (4) Quercetin and target molecules (a) CDK-2 and (b) CDK-6. Target molecules are represented in ribbon backbone and ligands are represented in ball and stick style. Dotted lines represent interactions-Yellow-Hydrogen bonds, Light blue-Pi-Pi stacking interactions. The colored ribbons of target molecule represent secondary structures according to different chains. In the ligand backbone, white, green and red colors represent hydrogen, carbon and oxygen atoms respectively.

2.4. Glide Standard Precision (SP) Ligand Docking

SP adaptable glide docking was carried out using Glide in Maestro Schrödinger. The Van der Waals radius scaling factor and charge cutoff were set to 0.80 and 0.15 respectively for all the ligand molecules under study. Final score was assigned according to the pose of docked ligand within the active site of the receptor molecules. The docking result is summarized in Table 1. 2D and 3D representation of ligand-receptor interaction are summarized in Figure 4 and Figure 5 respectively.

Table 1. Result of molecular docking between ligands and receptors.

Compound Name	PubChem CID	CDK-2			CDK-6		
		Binding Energy (Kcal/mol)	Hydrogen Bonds, Distance (Å)	Interacting Amino Acids	Binding Energy (Kcal/mol)	Hydrogen Bonds, Distance (Å)	Interacting Amino Acids
Kaempferol	5280863	−7.872	Val64, 2.22.	Val64, Ala144, Asp145, Leu134, Phe146, Phe80, Ala31, Val18.	−9.012	Lys43, 2.33; Glu61, 2.14; His100, 2.78; Val101, 2.25; Val101, 2.21; Gln149, 1.71.	Ile19, Val27, Ala41, Lys43, His100, Gln103, Val101, Glu61, Ala162, Leu152, Gln149.
Myricetin	5281672	−8.137	Leu83, 2.13; Leu83, 2.73; Asp86, 1.64; Asp145, 1.89.	Ile10, Val18, Asp86, Ala31, Leu83, Leu134, Phe80, Ala144, Asp145.	−9.622	Lys43, 2.78; Glu61, 2.51; Val101, 1.83; Val101, 2.08; Asp104, 1.81.	Ile19, Val27, Ala41, Lys43, His100, Glu99, Val101, Glu61, Gln103, Ala162, Asp163, Leu152, Asp163, Gln103.
P-Hydroxycinnamic Acid	637542	−7.149	Leu83, 2.06; Asp145, 2.16.	Val18, Ala31, Phe82, Leu93, Val64, Ala144, Asp145.	−7.103	Lys43, 2.63; Val101, 2.00; Val101, 2.06; Asp163, 2.39.	Val27, Lys43, His100, Val101, Asp163.
Quercetin	5280343	−8.298	Val64, 1.92; Glu81, 1.99.	Val18, Asp145, Ala144, Leu134, Ala31, Leu83, Glu81, Val64, Phe80.	−8.559	Glu21, 1.70; Val101, 2.56; Asp163, 1.88.	Lys147, Leu152, Ala162, Asp163, Val101, Glu21, Val27, Ala41, His100.

2.5. Ligand Based Drug Likeness Property and ADME/T Prediction

The molecular structures of every ligands were analyzed using SWISSADME server (http://www.swissadme.ch/) in order to confirm whether the ligands follow Lipinski's rule of five or not. Physicochemical properties of ligand molecules were calculated using OSIRIS property explorer (https://www.organic-chemistry.org/prog/peo/). The result of drug likeness property analysis is summarized in Table 2.

The ADME/T for each of the ligand molecules was carried out using an online based server ADMET-SAR (http://lmmd.ecust.edu.cn/admetsar1/predict/) to predict their various pharmacokinetic and pharmacodynamic properties including blood brain barrier permeability, human abdominal adsorption, AMES toxicity, Cytochrome P (CYP) inhibitory capability, carcinogenicity, mutagenicity, Caco-2 permeability etc. The result of ADME/T for all the ligand molecules is represented in Table 3.

3. Result

3.1. Binding Energy

All the selected ligand molecules docked successfully with both CDK-2 and CDK-6. Kaempferol, Myricetin, P-Hydroxycinnamic acid and Quercetin docked with CDK-2 with −7.872 Kcal/mol, −8.137 Kcal/mol, −7.149 Kcal/mol and

−8.298 Kcal/mol binding energies respectively (Table 1). Kaempferol formed total 1 hydrogen bond withVal64, Myricetin formed total 4 hydrogen bonds-2 with Leu83, 1 with Asp86 and another 1 with Asp145, P-Hydroxycinnamic Acid formed 2 hydrogen bonds with Leu83 and Asp145 and Quercetin also formed 2 hydrogen bonds with Val64 and Glu81 within the binding site of CDK-2 structure backbone. Kaempferol, Myricetin, P-Hydroxycinnamic acid and Quercetin interacted with 8, 9, 7 and 9 amino acid residues respectively in total within the binding pocket of CDK-2 target molecule.

All the selected molecules exhibited a slightly lower binding energy and hence higher affinity for CDK-6 than CDK-2. Kaempferol, Myricetin, P-Hydroxycinnamic acid and Quercetin docked with CDK-6 with −9.012 Kcal/mol, −9.622 Kcal/mol, −7.103 Kcal/mol and −8.559 Kcal/mol binding energies respectively (Table 1). Kaempferol formed 6 hydrogen bonds-2 with Val101, and 1 with Lys43, Glu61, His100 and Gln149 each in the binding site of CDK-6. Myricetin formed 5 hydrogen bonds-2 with Val101 and 1 with Lys43, Glu61 and Asp104 each. P-Hydroxycinnamic formed 4 hydrogen bonds (2 with Val101 and 1 with Lys43 and Asp163 each) and Quercetin formed 3 hydrogen bonds with Glu21, Val101 and Asp163 within the binding site of CDK-6. Moreover, Kaempferol, Myricetin, P-Hydroxycinnamic acid and Quercetin interacted with 11, 14, 5 and 9 amino acid residues respectively in total within the binding pocket of CDK-6 target molecule.

3.2. Drug-Likeness Property

Kaempferol, P-Hydroxycinnamic acid and Quercetin followed Lipinski's rule of five with respect to with respect to molecular weight (acceptable range: ≤500), number of hydrogen bond donors (acceptable range: ≤5), number of hydrogen bond acceptors (acceptable range: ≤10), lipophilicity (expressed as LogP,

Table 2. Drug-likeness properties of selected ligand molecules.

Drug Likeness Properties	Kaempferol	Myricetin	P-Hydroxycinnamic Acid	Quercetin
Molecular Weight	286.2 g/mol	318.24 g/mol	164.16 g/mol	302.24 g/mol
LogP	1.84	1.08	0.95	1.63
LogS	−3.31	−3.01	−2.02	−3.16
H-bond Acceptor	6	8	3	7
H-bond Donor	4	6	2	5
Molar Refractivity	76.01	80.06	45.13	78.03
Heavy Atoms	21	23	6	22
TPSA	107.2	151.59	57.53	127.4
Rotatable bonds	1	1	2	1
Drug Likeness Score	0.90	0.75	0.58	1.6
Drug Score	0.46	0.46	0.75	0.30

Table 3. Result of ADME/T test of selected ligand molecules.

Properties	Kaempferol	Myricetin	P-Hydroxycinnamic acid	Quercetin
Blood-Brain Barrier	BBB+	BBB−	BBB+	BBB−
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+
Caco-2 Permeability	Caco2−	Caco2−	Caco2+	Caco2−
P-glycoprotein Substrate	Substrate	Substrate	Substrate	Non-substrate
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 1A2 Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Inhibitor
CYP450 2C9 Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Inhibitor
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity
AMES Toxicity	Non AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Biodegradation	Not ready biodegradable	Not ready biodegradable	Ready biodegradable	Not ready biodegradable
Acute Oral Toxicity	II	II	III	II
Carcinogenicity (Three-class)	Non-required	Non-required	Non-required	Non-required

acceptable range: ≤5) and molar refractivity (40 - 130) without any violation (Table 2) [24]. But, myricetin violated the rule of hydrogen bond donors (6) exceeding the acceptable range. Myricetin possesses the largest polar surface area or topological polar surface area (151.59) and p-hydroxycinnamic acid possesses lowest area (57.53). Kaempferol and quercetin encompass a moderate topological polar surface area 107.2 and 127.4 respectively. Kaempferol has the lowest LogS value of −3.31 whereas p-hydroxycinnamic acid shows the largest value of −2.02 among all the ligand molecules. Myricetin and quercetin exhibit slightly similar LogS values of −3.01 and −3.16 respectively. Both quercetin and myricetin showed satisfied drug likeness and drug score than other two ligand molecules.

3.3. ADME/T Test

The result of ADME/T test of selected ligand molecules is summarized in Table 3. Kaempferol and P-Hydroxycinnamic acid are capable of penetrating blood brain barrier but other two ligands are not. All the ligand molecules are highly absorbable in human intestinal tissue. Only P-Hydroxycinnamic acid is

biodegradable in biological medium. No ligand molecules showed mutagenicity and AMES toxicity and hence carcinogenicity test is not required. P-Hydroxycinnamic acid might induce type III acute oral toxicity whereas others may capable of inducing type II. Both myricetin and quercetin are inhibitors of Cytochrome CYP450 1A2 and CYP450 3A4. Kaempferol inhibits another 2 cytochromes-CYP450 C9 and CYP450 C19 in addition to CYP450 1A2 and CYP450 3A4. However, P-Hydroxycinnamic acid is non-inhibitors of every cytochromes that are summarized in **Table 3**.

4. Discussion

Molecular docking estimates the best possible pose of a ligand molecule within the constraint of binding site of a receptor molecule and calculates binding energy. Higher binding energy contributes to lower affinity binding and vice versa [25]. Quercetin exhibited the strongest binding with CDK-2 target molecule with lowest binding energy (−8.298 Kcal/mol) and as a result interacted with most number of amino acids (9) in the target molecule backbone. On the other hand, Myricetin bound with CDK-6 with lowest binding energy (−9.622 Kcal/mol) and interacted with most number of amino acids (14) inside the binding pocket than other ligand molecules (**Table 1**). Hydrogen bonding between ligand and receptor increases the specificity of the interaction and hence contributes to the molecular recognition and strength of interaction [26]. All the ligand molecules formed significant amount of hydrogen bonds within the binding site of the receptor molecules depending on the strength of binding.

Evaluation of drug likeness property aims in improving the drug discovery and development process. Molecular weight and topological polar surface area (TPSA) influence the permeability of the drug molecule through the biological barrier. Higher molecular weight and TPSA reduce the permeability and lower ones increase permeability. LogP is expressed in the context of lipophilicity and conferred as the logarithm of partition coefficient of the candidate molecule in organic and aqueous phase. Lipophilicity affects the absorption of the drug molecule inside the body. Higher LogP is associated with lower absorption and vice versa. LogS value influences the solubility of the candidate molecule and the lowest value is always preferred. The number of hydrogen bond donors and acceptors outside the acceptable range again influences the ability of a drug molecule to cross membrane bilayer. Increased number of rotatable bonds is concerned with oral bioavailability and it is assumed to be within 10 as acceptable range [24] [27] [28]. All the ligand molecules in this experiment followed standard rule of drug-likeness property except myricetin which violated the rule of hydrogen bond donors which may lead to reduced permeability of the molecule as a drug (**Table 2**).

ADME/T test assesses pharmacological and pharmacodynamic properties of a candidate drug molecule inside biological system and thereby it is a crucial determinant of the success of a drug discovery approach. Blood brain barrier per-

meability is crucial for those drugs that target primarily the brain cells. Oral delivery system is the most commonly used route of drug administration so it is appreciated that the drug is highly absorbed in intestinal tissue. P-glycoprotein in the cell membrane facilitates the transport of many drugs inside the cell and therefore its inhibition may affect the drug transport. *In vitro* study of drug permeability test utilizes Caco2 cell line and its permeability reflects that the drug is easily absorbed in the intestine. Orally absorbed drugs travel through the blood circulation and deposits back to liver where it is degraded by group of enzymes of Cytochrome P450 family and excreted as bile or urine. So, inhibition of any of enzymes of this family might affect biodegradation of the drug molecule [29] [30]. Taking all the parameters into consideration, P-Hydroxycinnamic acid performed well in ADME/T test (**Table 3**).

All the ligand molecules might have both CDK-2 and CDK-6 inhibitory potentiality since all of them docked successfully with both target molecules. Although Myricetin docked with CDK-6 with lowest binding energy (−9.222 Kcal/mol) but its violation of Lipinski's rule may eliminate its choice as an anticancer drug. Again, Quercetin docked with higher affinity with CDK-2 but its ADME/T test performance was poor. Kempferol also docked well with both target molecules but again its ADME/T test result was not satisfactory. On the contrary, P-Hydroxycinnamic acid docked successfully with both CDK-2 and CDK-6 although with slightly higher binding energy than other ligand molecules but its drug likeness property and ADME/T result was satisfactory (**Tables 1-3**). And therefore, P-Hydroxycinnamic acid can be considered as a best natural dual inhibitor of both CDK-2 and CDK-6 in Cyclin/CDK pathway of cancer cell. However, further laboratory experiment might be required to confirm its inhibitory effect.

5. Conclusion

Four phytochemicals from *Clitoria ternatea* were used in this experiment to explore anticancer activity. P-Hydroxycinnamic acid is the best inhibitor for CDK-2 and CDK-6 considering the pharmacokinetic and pharmacodynamic properties. However, other ligand molecules can also be investigated further as they also performed well in the docking experiment. Hopefully, this study will raise research interest among the researchers.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Burrell, R.A., McGranahan, N., Bartek, J. and Swanton, C. (2013) The Causes and Consequences of Genetic Heterogeneity in Cancer Evolution. *Nature*, **501**, 338-345. https://doi.org/10.1038/nature12625

[2] Hanahan, D. and Weinberg, R.A. (2000) The Hallmarks of Cancer. *Cell*, **100**, 57-70.

https://doi.org/10.1016/S0092-8674(00)81683-9

[3] Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J. and Jemal, A. (2015) Global Cancer Statistics, 2012. *CA*: *A Cancer Journal for Clinicians*, **65**, 87-108. https://doi.org/10.3322/caac.21262

[4] Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer Statistics, 2016. *CA*: *A Cancer Journal for Clinicians*, **66**, 7-30.

[5] Desai, A.G., *et al.* (2008) Medicinal Plants and Cancer Chemoprevention. *Current Drug Metabolism*, **9**, 581-591. https://doi.org/10.2174/138920008785821657

[6] Jacob, L. and Latha, M.S. (2012) Anticancer Activity of *Clitoria ternatea* Linn. against Dalton's Lymphoma. *International Journal of Pharmacognosy and Phytochemical Research*, **4**, 207-212. https://ijppr.com

[7] Al-Snafi, A.E. (2016) Pharmacological Importance of *Clitoria ternatea*–A Review. *IOSR Journal of Pharmacy*, **6**, 68-83. http://iosrjournals.org/IOSR-PHR.html

[8] Lijon, M.B., Meghla, N.S., Jahedi, E., Rahman, M.A. and Hossain, I. (2017) Phytochemistry and Pharmacological Activities of *Clitoria ternatea*. *International Journal of Natural and Social Sciences*, **4**, 1-10. https://ijnss.org/

[9] Nevins, J.R., Leone, G., DeGregori, J. and Jakoi, L. (1997) Role of the Rb/E2F Pathway in Cell Growth Control. *Journal of Cellular Physiology*, **173**, 233-236. https://doi.org/10.1002/(SICI)1097-4652(199711)173:2<233::AID-JCP27>3.0.CO;2-F

[10] Sherr, C.J. and Roberts, J.M. (1999) CDK Inhibitors: Positive and Negative Regulators of G1-Phase Progression. *Genes & Development*, **13**, 1501-1512. https://doi.org/10.1101/gad.13.12.1501

[11] Ho, A. and Dowdy, S.F. (2002) Regulation of G1 Cell-Cycle Progression by Oncogenes and Tumor Suppressor Genes. *Current Opinion in Genetics & Development*, **12**, 47-52. https://doi.org/10.1016/S0959-437X(01)00263-5

[12] Boonstra, J. (2003) Progression through the G1-Phase of the on-Going Cell Cycle. *Journal of Cellular Biochemistry*, **90**, 244-252. https://doi.org/10.1002/jcb.10617

[13] Harper, J.W. and Elledge, S.J. (1996) Cdk Inhibitors in Development and Cancer. *Current Opinion in Genetics & Development*, **6**, 156-164. https://doi.org/10.1016/S0959-437X(96)90011-8

[14] Hall, M. and Peters, G. (1996) Genetic Alterations of Cyclins, Cyclin-Dependent Kinases, and Cdk Inhibitors in Human Cancer. *Advances in Cancer Research*, **68**, 67-108. https://doi.org/10.1016/S0065-230X(08)60352-8

[15] Cicenas, J. and Valius, M. (2011) The CDK Inhibitors in Cancer Research and Therapy. *Journal of Cancer Research and Clinical Oncology*, **137**, 1409. https://doi.org/10.1007/s00432-011-1039-4

[16] Zoete, V., Grosdidier, A. and Michielin, O. (2009) Docking, Virtual High Throughput Screening and *in Silico* Fragment-Based Drug Design. *Journal of Cellular and Molecular Medicine*, **13**, 238-248. https://doi.org/10.1111/j.1582-4934.2008.00665.x

[17] Schneidman-Duhovny, D., Nussinov, R. and Wolfson, H.J. (2004) Predicting Molecular Interactions in Silico: II. Protein-Protein and Protein-Drug Docking. *Current Medicinal Chemistry*, **11**, 91-107. https://doi.org/10.2174/0929867043456223

[18] Phillips, M.A., Stewart, M.A., Woodling, D.L. and Xie, Z.R. (2018) Has Molecular Docking Ever Brought Us a Medicine? In: *Molecular Docking*, IntechOpen, London.

[19] Cheng, F., Li, W., Liu, G. and Tang, Y. (2013) In Silico ADMET Prediction: Recent Advances, Current Challenges and Future Trends. *Current Topics in Medicinal*

Chemistry, **13**, 1273-1289. https://doi.org/10.2174/15680266113139990033

[20] Moroy, G., Martiny, V.Y., Vayer, P., Villoutreix, B.O. and Miteva, M.A. (2012) Toward in Silico Structure-Based ADMET Prediction in Drug Discovery. *Drug Discovery Today*, **17**, 44-55. https://doi.org/10.1016/j.drudis.2011.10.023

[21] Release, S. (2018) 2: Maestro, version 11.8. Schrödinger, LLC, New York. https://www.schrodinger.com/maestro

[22] Visualizer, D.S. (2012) Release 3.5. Accelrys Inc., San Diego, CA. https://www.3dsbiovia.com

[23] Spessard, G.O. (1998) ACD Labs/LogP dB 3.5 and ChemSketch 3.5. *Journal of Chemical Information and Computer Sciences*, **38**, 1250-1253. https://doi.org/10.1021/ci980264t

[24] Lipinski, C.A. (2004) Lead-and Drug-Like Compounds: The Rule-of-Five Revolution. *Drug Discovery Today*: *Technologies*, **1**, 337-341. https://doi.org/10.1016/j.ddtec.2004.11.007

[25] Yuriev, E. and Ramsland, P.A. (2013) Latest Developments in Molecular Docking: 2010-2011 in Review. *Journal of Molecular Recognition*, **26**, 215-239. https://doi.org/10.1002/jmr.2266

[26] Hubbard, R.E. and Kamran Haider, M. (2001) Hydrogen Bonds in Proteins: Role and Strength. e LS. https://doi.org/10.1038/npg.els.0003011

[27] Sander, T. (2001) OSIRIS Property Explorer. Organic Chemistry Portal. https://www.organic-chemistry.org/prog/peo/

[28] Pollastri, M.P. (2010) Overview on the Rule of Five. *Current Protocols in Pharmacology*, **49**, 9-12. https://doi.org/10.1002/0471141755.ph0912s49

[29] Li, A.P. (2001) Screening for Human ADME/Tox Drug Properties in Drug Discovery. *Drug Discovery Today*, **6**, 357-366. https://doi.org/10.1016/S1359-6446(01)01712-3

[30] Guengerich, F.P. (1999) Cytochrome P-450 3A4: Regulation and Role in Drug Metabolism. *Annual Review of Pharmacology and Toxicology*, **39**, 1-17. https://doi.org/10.1146/annurev.pharmtox.39.1.1