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# *In silico* Study of Chromane Ring Compound Rubranonoside from *Plumeria rubra* as Anticancer Potential

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**Abstract**

Plumeriarubra is an ornamental tree of Apocynaceae family. Plumeria rubra is a flowering plant. Flowers are very fragrant, commonly red pink or purple middle rich with yellow. Cancer is characterized through uncontrolled cell division through overcoming the numerous cell signalling pathways. Thus targeting the signalling pathway at various sites can lead to development of potential anticancer molecules. With this rational CDK2 receptors involved in cell cycle pathway was selected for the purpose of *in silico* analysis. Natural Products has a renowned history in cancer therapeutics. Plant derived anticancer leads have been reported since 1950. Extensive literature survey suggested that Plumeria rubra is explored for their chemical constituents along with their anticancer potential. Reported phytoconstituent i.e. rubranonoside of this plant was subjected to docking to summarise the docking scores, hydrogen bond, electrostatic bond and various decesive factors governing the protein ligand interaction. This report can be helpful further in designing *in vitro* and *in vivo* evaluations of the anticancer activity of rubranonoside.

**Keywords:** *In silico*, Plumeria rubra, Rubranonoside, Cancer, Cell cycle, Molecular docking, Schrodinger

**Abbreviations**

**CDK2**: Cyclin dependant kinases 2
**CDK4**: Cyclin dependant kinases 4
**CDK6**: Cyclin dependant kinases 6
**NP**: Natural products

## Introduction

Natural products (NP) have a long history in cancer therapeutics and more than 60 % of currently available anticancer drugs are derived from various natural sources such as plants, microbes and marine organisms [1]. Since 1950 anticancer molecules from Natural Product were discovered and developed like Vinca alkaloids, vinblastine and vincristine, taxol, camptothecin along with the isolation of cytotoxic podophyllotoxin[2]. Plants like Plumeriarubra is being explored for their phytoconstituents and anticancer activity at extract level have been reported previously [2-6]. Over past few decades multitargeted therapeutics is being considered as upcoming strategy for treating complex diseases such as cancer and diabetes. Multi targeted therapeutics involves study of molecule which can act on multiple targets at molecular level simultaneously [7]. This strategy can be considered pharmacologically more relevant and validated as scientific models as the pharmacokinetic and pharmacodynamics of a single molecule targeted to multiple targets are more predictable than simultaneous therapy of 2 or more single targeted agents [8].

A variety of chemical compounds have been reported from P. rubra, including iridoids, triterpenoids, flavonoids, glycosides, phenolics, alkaloids, carbohydrates, amino acids, fatty acid esters, spingolipid, lignin, monoglyceride, coumarin.

About 12 flavonoids: Quercetin, quercitrin, narcissin, cyanidin-3-O-β-(2′ -glucopyranosyl-O-β-galactopyranoside, cyanidin-3-O-β-galactopyranoside, rubranonoside, plumerubroside, kaempferol-3-O-glucoside, kaempferol-3-rutinoside, kaempferol, quercetin 3-O-α-L-arabinopyranoside, rutin and 6 glycosides: Lauryl diglucoside, stearyldixylosylmethoxygallic acid, vanillic acid-4-O-tetra arabinosyl stearate, vanillic acid-4-O-hexa arabinosyl stearate, β-D-hexaglucoside, β-D-heptaglucosyl-β-D-rhamnoside have been identified from the leaves, flowers, stem bark, and whole plant of P. rubra. Cardiac glycosides were also identified in P. rubra. The chemical structure of rubranonoside is shown in **Figure 2** [9].

Cancer is characterized by uncontrolled cell division by overcoming the signalling pathways involved in normal cell proliferation leading to apoptosis, extensive angiogenesis and metastasis. If the cell cycles particular checkpoints are targeted then it may provide lead for novel method of treatment [10]. The regulatory proteins, cyclins and their effector counterparts the cyclin dependant kinases have been identified as prominent targets in cell cycle mechanism. Cyclin dependant kinases are central players and govern various events in cell cycle like the initiation, progression and completion. Inhibition of cyclin dependant kinases is proved as to be more novel strategy for the design and discovery of potent anticancer leads specifically targeting cell cycle [11]. The CDK2-cyclin E complex activity is essential for transition from G1 to S phase [12]. The CDK4 and CDK6 forms complex with cyclin D which phosphorylates the tumour suppressant retinoblastoma protein which is critical process in cell duplication hence deregulation of this pathway is considered important in most types of cancer [13].

Natural tubulin inhibitors which disrupt the normal functioning of mitotic spindle apparatus have proven to be best class of cancer therapeutic drugs [14]. With this rational protein CDK2 (PDB ID: 1DI8) which is involved in G1 phase of cell cycle were selected for present *in silico* studies. Thus here the docking simulations were performed using CDK2 protein involved in cell cycle and apoptosis pathways.

Molecular docking is most widely used technique for *in silico* drug design and drug discovery which involves structure-based virtual screening for identification of new compounds towards a particular target protein [15]. With this context the present study aims to find molecules from selected natural source which can be developed as a cell cycle and apoptosis pathway inhibitor.

## Materials and methods

### Ligand selection

Plants have proven as a potential source of anticancer agents like vincristine, vinblastine, taxane, podophyllotoxin and camptothecin derivatives which are in clinical practice [16]. The ethanolic extract of flowers of Plumeriarubra was tested for its anti cancer activity against liver cancer HePG2 cell line [17,18]. The isolated compounds Rubranonoside were selected for molecular docking analyses.

### Protein selection

The eukaryotic cell division involves various stages from G1 phase that is resting state to cell division M phase. The control of eukaryotic cell growth and division occurs at specific checkpoints like G1, G2/ M and meta phase to anaphase transitions [19]. Cycle dependant kinase (CDK) member of the family of heterodimeric kinases play a significant role in regulation of cell cycle progression at every check point [20]. The cell dependant kinases are present as inactive monomers which are activated by binding to cyclin with phosphorylation by CDK activating kinases. Hence any overexpression, amplification, or mutation of the cyclin or CDK can lead to cancerous growth of cells [21,22]. Therefore CDK are considered as biomarkers of proliferation and fascinating pharmacological targets for development of anticancer leads. For transition of G1 phase into S phase activity of CDK 2-Cyclin E complex is essential in cell cycle pathway. CDK 6 in conjunction with CDK 4 acts as a switch signal for the control of G1 to S phase [23-27].

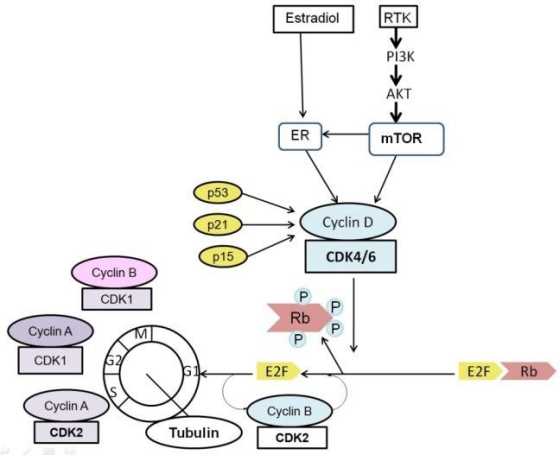



**Figure 1** Schematic representation of cell cycle signalling pathway (Self created with software ChemBioDraw Ultra 14.0. PerkinElmer, Waltham, Massachusetts, United States.)

A schematic representation of the cell cycle signalling pathway and key receptor proteins and enzymes involved in it is shown in **Figure 1**.

With this rational proteins CDK2 (PDB ID: 1DI8) which is involved in G1 phase of cell cycle, was selected for present *in silico* studies.

**Molecular docking**

The Schrodinger Maestro 9.1 software package was utilized for conducting the molecular docking experiments of selected ligands. The 4 selected receptors were prepared using Protein preparation wizard. The crystal structure of CDK2 (PDB ID: 1DI8) enzyme and receptor proteins was taken from Protein Data Bank. The protein structure was preprocessed by protonation and removal of water molecules except the ones at active site. The structures of the compounds were drawn using ChemDraw software. The ligands were prepared using Ligand preparation wizard application. OPLS-2005 force field was applied so as to minimize the geometries of compound and structure of proteins. Ligand docking was performed by the generation of receptor grid. The Glide (Schrodinger Inc. U.S.A.) software was used for ligand docking studies in to the CDK2 (PDB ID: 1DI8) binding pocket, with the XP mode.

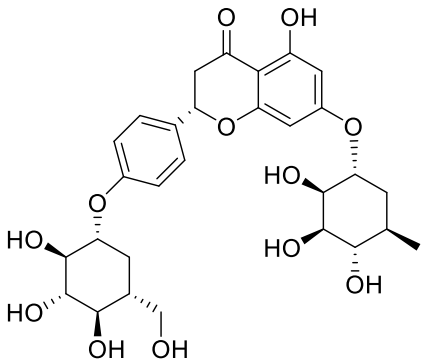
**ADME properties**

ADME properties have a major share for conversion of a potent lead molecule to a drug. It is essential to understand the absorption, distribution, metabolism and excretion parameters of the lead molecule for its effective usage in humans. Hence ADME properties of selected molecules were predicted using QikProp application of Schrodinger Maestro 9.1. Properties like overall CNS activity, MDCK and Caco-2 cell permeability, log BB and log Khsa for human serum albumin binding were predicted.

**Results and discussion**

The docking simulations highlighted peculiar role of various factors like hydrogen bonds lipophilic interactions, metal interactions, pi-pi interaction and pi-cation interaction in the protein ligand interaction profile. In the field of computational chemistry and molecular modelling scoring functions are fast approximate mathematical methods used to predict the strength of non covalent interaction between 2 molecules after they have been docked. Docking score is the physics based molecular mechanics force fields that estimate the energy of the pose within the binding site. It takes into consideration solvent effect, conformational changes in proteins and ligands, free energy due to the protein ligand interaction, internal rotations, association energy of ligand and receptor to form a single complex and free energy due to changes in vibrational mode. The selected molecules (**Figure 2**) with docking score of protein have been identified. Rubranonoside with docking score was considered for detail discussion (**Table 1**) and thier interaction with different amino acids on the receptor.

The compounds of *Plumeria rubra* i.e. rubranonoside showed interaction with CDK2 (-9.745) receptor, indicating a scope for these molecules for further *in vitro* studies. The interaction of this compound with CDK2 with LEU83, LYS33, and ASP145 amino acids of the active site. LYS was the common amino acid site of interaction across selected proteins for molecule. GLU and ASP interactions were seen from CDK2.




**Rubranonoside**

**Figure 2**Structures of selected chromane chemical constitute of *Plumeriarubra* i.e. rubranonoside (Self created with software ChemBioDraw Ultra 14.0. PerkinElmer, Waltham, Massachusetts, United States).

Lack of reasonable chemical and biological properties of compounds is the limitation in the development of new drug candidate for further lead optimization. Hence, it becomes important to predict the pharmacokinetic profile of compounds.

**Table 1** Rubranonoside and their interaction with amino acids.

| Protein | Ligand name | Docking score | Interacting amino acids |
|---|---|---|---|
| CDK2 | Rubranonoside | −9.745 | LYS20, LEU83, ASN132, ASP143 |

The pharmacokinetic properties of the selected molecules with the respective receptors were also studied *in silico* and given in **Table 2**.

It is very essential to understand the pharmacokinetic properties of molecules to make it more potential lead in drug discovery. These properties with their acceptable ranges have been established. Volume is defined as the estimated number of hydrogen bonds that would be accepted by the solute from water (500 - 2,000) and the range for molecular weight is 130-725 kDa. The number of hydrogen bond donors and acceptors shoud be <5 and <10 respectively. The predicted partition coefficient of octanol/gas, denoted as Q P log Poct(8.0-35.0). Q P log Pw, the predicted partition coefficient of water /gas (4.0-45.0) and P log Po/w, the predicted octanol/water partition coefficient log p (range −2.0 to 6.5), Q log S the predicted aqueous solubility and S inmol/L (acceptable range −6.5 to 0.5).

Molecules Rubranonoside showed acceptable pharmacokinetics with all the properties as discussed above falling within the ranges of acceptable criteria. Compounds showed hydrogen bond donar and hydrogen bond acceptor value more than the specified range properties exceeding the acceptance value and shall be modified accordingly in order to increase its pharmacokinetic profile. The molecular weight, the predicted octanol/water partition coefficient and Q log S the predicted aqueous solubility and S inmol/L is acceptable range. The predicted partition coefficient of octanol/gas is also in the range of not acceptable.

**Table 2** *In silico* pharmacokinetic study of rubranonoside on selected receptor.

| Protein | Compound | Molecular weight | Dipole | Volume | H-bond donor | H-bond acceptor | QPlogPoct | QPlogPw | QPlogPo/w | QPlogSmol/L |
|---|---|---|---|---|---|---|---|---|---|---|
| 1D18 | Rubranonoside | 580.541 | 4.9 | 1,596.666 | 7 | 19.3 | 38.554 | 32.002 | −1.549 | −3.522 |

Rubranonoside has very poor human oral absorption hence either their structural modification is required to increase the absorption or a different route of administration of drug should be followed. Thus pharmacokinetic and pharmacodynamics studies may prove extremely beneficial for further designing of experimental models and necessary modifications to ligand in order to increase its drug like properties (**Table 3**).

**Table 3** Drug like properties by Qikprop (Schrodinger Maestro 9.1).

| Protein | Compound | Q P log Po/w | Q P log HERG | QPP Caco (nm/sec) | Q P log BB | QPP MDCK (nm/sec) | QP log Kp | QP log Khsa | Percent human oral absorption |
|---|---|---|---|---|---|---|---|---|---|
| 1D18 | Rubranonoside | −1.549 | −6.288 | 2.828 | −4.527 | 0.874 | −6.506 | −1.206 | 0 |

Q P log Po/w (−2.0 to 6.5) Predicted octanol/water partition co-efficient log p (−0.20 to 6.5); Predicted $IC_{50}$ values for blockage of HERG $K^+$channels ( above −5.0); QPP Caco-Predicted Caco-2 cell permeability in nm/sec. Q P log BB Predicted brain/blood partition coefficient (−3 to 1.2) ; QPP MDCK cell permeability in nm/sec ( < 25 -poor, > 500 -great) ; Q P log KP Predicted skin permeability ( −8.0 to −0.1) ; QP $log_{hsa}$Predicted the binding to Human Serum Albumin ( −1.5 to 1.5); Percentage of human oral absorption (<25% -poor and >80 % -high).

Rubranonoside (CDK2, PDB ID: 1DI8)

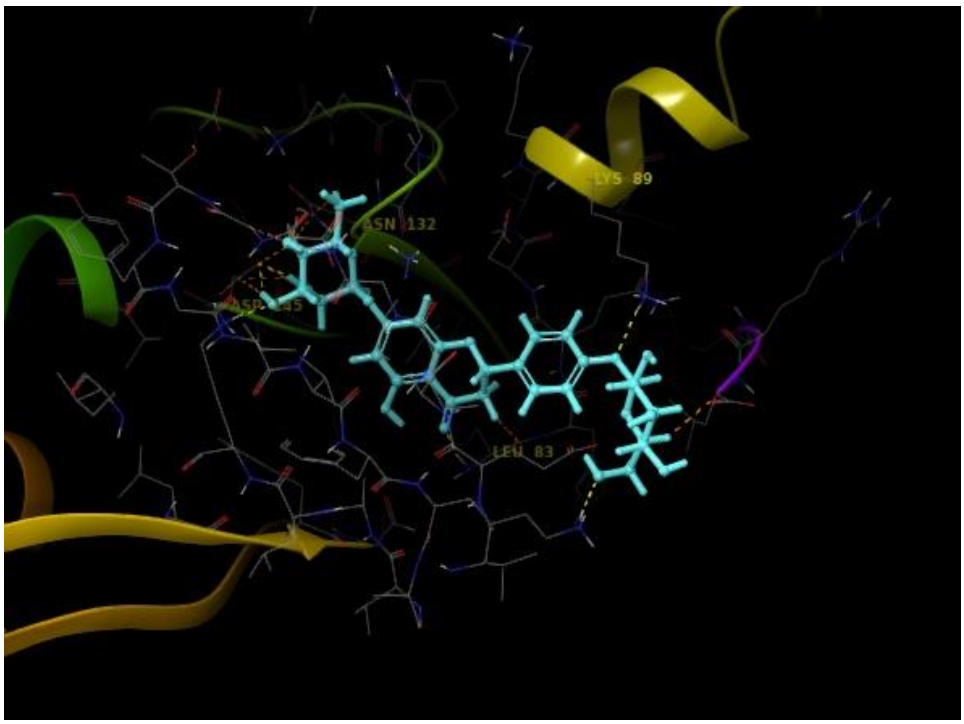



**Figure 3**(A) Rubranonoside 3D ligand interaction shows hydrogen bond as yellow dotted line, polar interaction as blue dotted line.

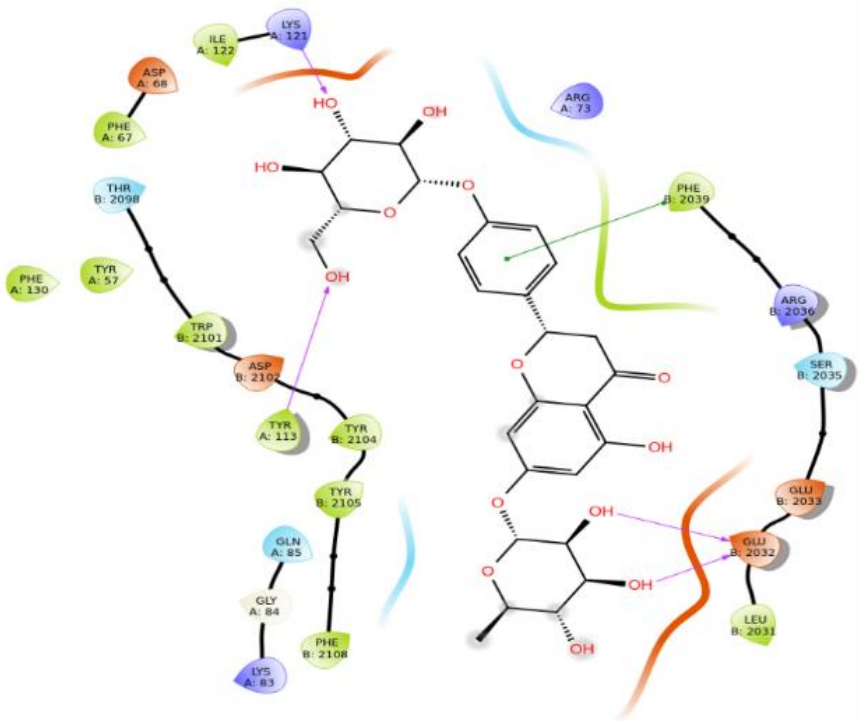



**Figure 3**(B) 2D view of ligand interaction with CDK2 (1DI8) obtained using the Schrodinger Glide SP program; essential amino acid residues at the binding site are circled. The purple (+ve) and brown (−ve) circles show amino acids involved in electrostatic, the green circle amino acids involved in hydrophobic interactions, the blue circle as polar interactions, the purple arrow represents the hydrogen bond, respectively.

## Conclusions

Investigation of reported phytoconstituents of compounds of Plumeriarubra i.e. rubranonoside is selected anticancer plants as the potential for multitarget inhibition in cell cycle pathway was carried out with the help of molecular docking simulation technique. Binding efficiency of rubranonoside on selected receptor proteins and enzymes was analysed. Compouds having best docking free energy score against CDK2 has been reported in present study. Their pharmacokinetic properties have also been enlisted. Results of this study can be beneficial in understanding the molecular mechanism of these phytoconstituents as a potential anticancer drug leads. The current findings can further be validated by *in vitro* and *in vivo* studies.

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