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ANTICANCER POTENTIALS OF QUASSINOIDS FROM *SIMAROUBA GLAUCA* – DOCKING AND ADME ANALYSIS

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ABSTRACT: The objective of this study was to analyze the inhibitory activity of selected quassinoids from the plant *Simarouba glauca* using docking studies against Phosphoinositide 3-kinases (PI3Ks). *In silico* technique was applied to screen abilities of the quassinoids as a potent inhibitor of PI3Ks. The 3D structure of the protein was obtained from PDB database and of the ligands from PUBCHEM database. Docking analysis of the compounds was performed using Ligpep 2.3, Schrodinger Suite 2009. The comparison of the docking score indicated that the compounds exhibited better binding affinity similar to that of the known drug Idelalisib. Further analysis of the drug likeness by means of ADME properties were predicted using Swissadme online server. None of the compounds violated Lipinski's parameters, making them potentially promising agents for biological activities. Finally, the results indicated that these compounds are potential inhibitor of PI3K and expected to be effective in cancer treatment.

KEYWORDS: *Simarouba glauca*, Quassinoids, ADME Properties, Phosphoinositide 3-kinases (PI3Ks), Anti – Cancer.

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1. INTRODUCTION

Simarouba glauca, commonly known as Laxmitaru or paradise tree belongs to the family Simaroubaceae. Common names for *S.glauca* are bitter ash, bitter damson princess tree and Simarouba. This tree is also known as "Tree of solace of cancer" as it is widely used in cancer treatment [1]. It produces bright green leaves of 20-50 cm length, yellow flowers and oval elongated

purple colored fleshy fruits. It is suited for temperature range of 10-40ºC, with pH of the soil to be 5.5-8.0 [2]. The bark and leaf extracts of *Simarouba* is well known for its different types of pharmacological properties such as haemostatic, anthelminthic, antiparasitic, antidysentric, antipyretic, anticancerous[3], antimicrobial, antiherpetic, antiprotozoal [4], antiamoebic, antimalarial, antifungal, antioxidant and antiulcer[5] activities along with hepatoprotective property. The main group of chemicals in *Simarouba glauca* is quassinoids, which belong to the triterpene family. This includes: ailanthinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, tirucalla etc. [3]. After the initial discovery of the antileukemic activity of bruceantin, a quassinoids, these molecules gained much attention [6]. Based on the chemical structures and biological properties nearly 150 quassinoids have been isolated and classified. A wide range of inhibitory effects has been shown by quassinoids which includes anti-inflammatory, antiproliferative effects on tumor cells types [7]. Considering the future in generating anticancer agents with more active and less toxic compounds, natural quassinoids represent a promising source of small molecules. From the quassinoids listed, four were selected (Glaucarubine, Glaucarubolone, Glaucarubinone, Melianone) based on the PASS (Prediction of Active Spectra for Substances) online server prediction and target hunter databases which are used to find the biological activities of the particular compounds. In order to understand the biological activity of the compounds, structural scaffold of the ligands was taken into consideration. The selected ligands were allowed for their prediction against novel targets for which PASS prediction server is used [8] (www.way2drug.com). This server predicts more than 300 pharmacological factors and biochemical mechanisms on the basis of the structural formula of the compounds. The given ligands were predicted to interact with 15 targets, among which the anticancer target PI3K was the most profound target for all the ligands. Smiles format of the ligands were used as input for the PASS online server. From 1980's the family of lipid kinases termed Phosphoinositide 3-kinases (PI3Ks) has been found to play key regulatory roles in many cellular processes including cell survival, proliferation and differentiation [9]. The discovery of PIK3CA gene which encodes p110α confirmed the importance of PI3Ks in cancer, as these genes are frequently mutated in most of the common human tumors [10, 11, 12]. PI3Ks are divided into three classes, based on their structural characteristics and substrate specificity [13, 14]. Class IA PI3Ks are heterodimers consisting of p110 catalytic subunit and p85 regulatory subunit. There are three isoforms of p110 which include p110α, p110β which are expressed ubiquitously and p110δ which is restricted to immune system [15]. PI3Kδ is selectively expressed in leukocytes which made them a therapeutic target for diseases in which there is pathological activation of the Akt pathway in hematopoietic cells [16]. PI3K/Akt signaling pathways are activated in hematological malignancies of B cells which include indolent non-Hodgkin lymphoma, chronic lymphocytic leukemia and mantle cell lymphoma which responds to pathway

inhibition [17]. Idelalisib is a potent selective inhibitor of p110δ kinases activity. This drug along with rituximab was clinically approved by the United States and European Union and is now recommended for treatment of patients with relapsed chronic lymphocytic leukemia and as a monotherapy for patients with relapsed follicular B cell non-Hodgkin lymphoma and small lymphocytic leukemia [18]. In the present study, comparative analysis of the selected quassinoids from the plant *Simarouba glauca* with the known drug Idelalisib was carried out using molecular docking studies and ADME properties which analyzed the drug likeness of the ligands.

2. MATERIALS AND METHODS

2.1. Target and Binding site

The three dimensional structure of Phosphoinositide 3-kinases (PI3Ks) complexed with known drug Idelalisib (PDB ID: 4XE0) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein Data Bank [29]. Binding sites of the protein were identified by FT site server [19]. Binding site identification has a wide range of applications which includes structure-based prediction of function, the elucidation of functional relationships among proteins, protein engineering and drug design. FT Site Server describes an accurate method of binding site identification [20].

2.2. Ligands preparation

The ligands are small molecules which bind to the Protein's binding sites. The SDF (Structure-Data Format) files of all compounds i.e. Glaucarubine, Glaucarubolone, Glaucarubinone, Melianone were obtained from Pubchem database [21] and analyzed by Marvin view [30]. The compounds were converted to 3D structure (PDB) using PyMol version 1.3 [31] (The PyMol Molecular Graphics System). The physicochemical properties of the ligands are estimated by Pubchem open chemistry database (www.pubchem.com).

2.3. Analysis of drug likeness of the ligands

The drug likeness prediction of the ligands were carried out by Lipinski filter, according to which an orally active drug should fulfill a minimum of four out of five criteria for drug likeness namely molecular mass, cLogP, hydrogen donor, hydrogen acceptor and molar refractive index [22]. These properties were analyzed by SWISS ADME (http://www.swissadme.ch), which is reported as a convenient tool in drug discovery [23]. Also, the properties of ligands with respect to adsorption, distribution, metabolism and excretion (ADME) were analyzed by SWISS ADME. Gastrointestinal absorption and brain access are two pharmacokinetic behaviors crucial to estimate various stages of the drug discovery processes. The Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules [24].

2.4. Molecular Docking Procedures

The aim of molecular docking is to predict the binding modes of ligand and thus define the orientation of molecule with respect to the active or binding site. In this method according to the affinity score in terms of (kcal/mol), ranking all the binding poses of the molecule inside the catalytic site of an enzyme is being done .The selected compounds were taken for minimization using Ligprep module of Schrodinger 09 where probable tautomeric and ionization states at pH = 7 ± 1 followed by energy minimization with OPLS 2005 force field [25, 32] was carried out. The protein preparation of the target (PDB ID 4XE0) was performed using Protein preparation wizard of Schrodinger 09 where missing hydrogen bond order were assigned followed by energy minimization.

2.4.1 Molecular Docking

The receptor grid was prepared keeping cocrystal (Idelalisib) ligand on PI3Ks (PDB ID 4XE0) at the center of grid with 20 Å edges bearing catalytic site. Initially docking study on the cocrystal was performed on prepared receptor grid for cross-validating the binding mode with respect to X-ray crystal structure binding mode. Further, molecular docking was performed for given ligands against PDB ID using Glide XP 5.8 Program [26, 27, 28]. The top analogs based on docking score as well as binding interaction with catalytic residues were allowed for induced fit docking and results were compared with the cocrystal after Glide XP. The docked conformation corresponding to the lowest free energy (or highest score) provided by Glide program was selected as the most probable binding pose of top compound.

2.4.2. Structural features of the complex

Once the docking was performed, best poses for hydrogen bonding, Hydrophobic and π interactions were analyzed using PyMol version 1.3 (The PyMol Molecular Graphics System).

3. RESULTS AND DISCUSSION

Owing to the important functions of PI3Ks and challenges in designing specific inhibitors for kinase proteins because of highly conserved active site architecture, there is big demand for structure analysis to identify new inhibitors targeting various diseases. Together with the above mentioned fact, the usefulness of the *Simarouba glauca* plant against many illness and important compounds were selected to screen their affinity towards PI3Ks. This selection was guided by PASS prediction server.

3.1. Macromolecule

The target Phosphoinositide 3-kinases (PI3Ks) complexed with known drug Idelalisib (PDBID:4XE0) was used for docking studies (**Figure 1).**

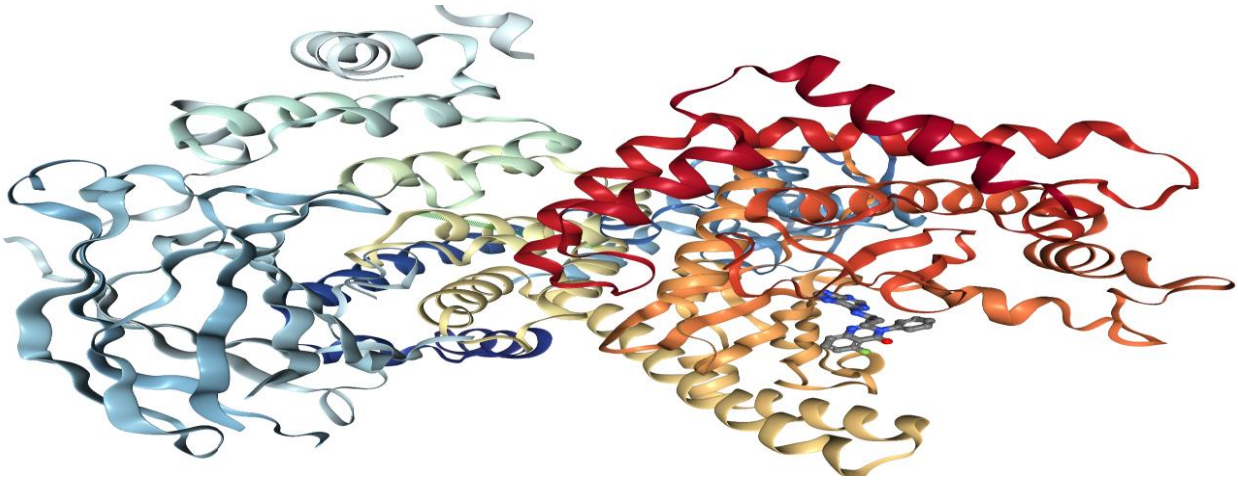
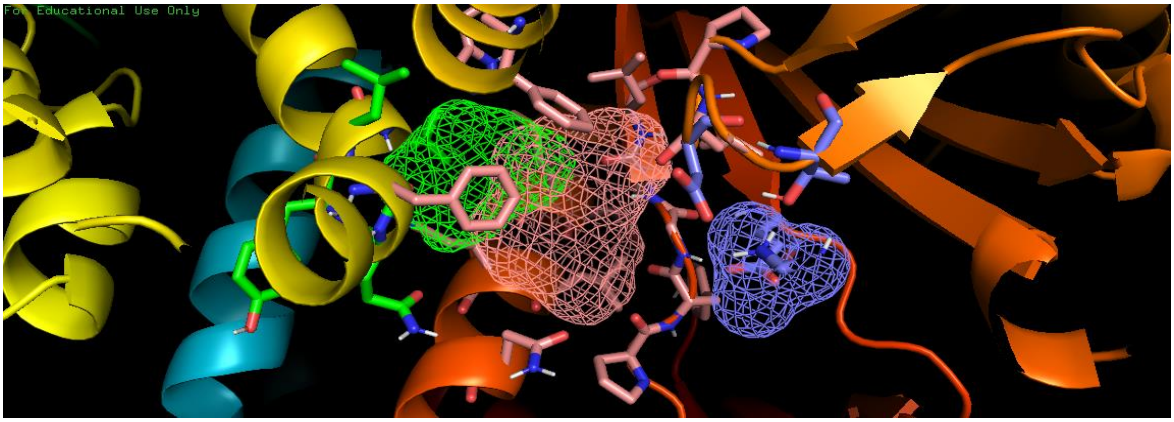


Fig 1. Cartoon representation of PI3K (PDB Code: 4XE0). Idelalisib is shown as solid ball and stick.

This protein comprised of 939 residues, out of which 815 were observed under electron density. The kinase domain and ATP binding sites are well defined by the electron density whereas certain fractions of the protein have weak electron density and correspondingly high temperature factors. The drug Idelalisib binds in the ATP binding pocket. The inhibitor forms hydrogen bonds to the hinge region that is similar to those made by ATP. The 2.4A˚ crystal structure of the Idelalisib-PI3Kδ complex showed the inhibitor binding in the ATP site and revealed the specific and entirely noncovalent interactions between protein and inhibitor [18].

3.2. Active sites of the Protein molecule

In this study, FT Site Server predicted the active sites of the targeted protein PI3K. Three active sites are projected in this protein (**Figure 2**). The active sites comprises of amino acid residues are Active site 1 : PHE 609, PHE 646, HIS 650, PRO 734, LEU 735, ASP 787, MET 788, LEU 791, GLN 792, GLN 795, PRO 812, TYR 813, GLY 814, CYS 815, LEU 816, Active site 2: PHE 609, GLN 610, TYR 611, LEU 612, LEU 613, PHE 646, HIS 650, MET 788, GLN 792, Active site 3: ARG 246, ASP 736, THR 739, GLU 826.



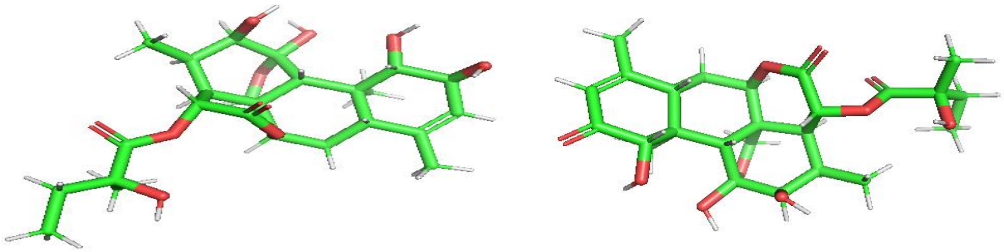
Pink: Active site 1, Green: Active site 2, Blue: Active site 3 (as predicted by FT Site Server)

Fig 2. Active Sites of PI3K

3.3. Ligands

The physicochemical properties of the ligands those 3D structures shown in **Figure 3** are mentioned in table 1.

3a: Glaucarubine **3b: Glaucarubinone**



3c: Glaucarubolone **3d: Melianone** **3e: Idelalisib (Cocrystal)**

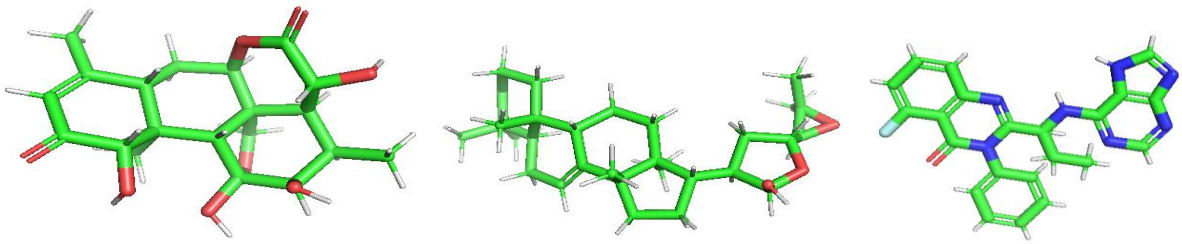


Figure 3. 3D structures of the Ligands

Table 1: Physicochemical Properties Of The Ligands

Sr. No.	Ligand	Molecular formula	Molecular weight	Monoisotropic mass	Heavy atom count	Topological Polar Surface Area
1	Glaucarubine	$C_{25}H_{36}O_{10}$	496.553 g/mol	496.231 g/mol	35	163 $A^{\circ 2}$
2	Glaucarubinone	$C_{25}H_{34}O_{10}$	494.537 g/mol	494.215 g/mol	35	160 $A^{\circ 2}$
3	Glaucarubolone	$C_{20}H_{26}O_8$	394.42 g/mol	394.163 g/mol	28	134 $A^{\circ 2}$
4	Melianone	$C_{30}H_{46}O_4$	470.694 g/mol	470.34 g/mol	34	59.1 $A^{\circ 2}$
5	Idelalisib (Cocrystal)	$C_{22}H_{18}FN_7O$	415.432 g/mol	415.156 g/mol	31	99.2 $A^{\circ 2}$

Idelalisib's exposed polar surface area (PSA) is 99.2 $A^{\circ 2}$ which is less than the selected compounds except Melianone whose PSA is 59.1 $A^{\circ 2}$ though the mass is 470 g/mol.

3.4. Drug likeness analysis

Results of Lipinski filter analysis is tabulated **(Table 2a)** which explains the rigidity of all compounds to be considered for structure based drug design. Also **table 2b** list out the properties of compounds with relevance to their usage as explained by ADME properties.

Table 2a: Lipinski Filter Analysis

Ligand	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	cLogP	Molar Refractivity
Glaucarubine	496.553 g/mol	5	10	0.32	120.27
Glaucarubinone	494.537 g/mol	4	10	0.23	119.30
Glaucarubolone	394.42 g/mol	4	8	-0.02	93.95
Melianone	470.694 g/mol	1	4	4.57	135.70
Idelalisib (Cocrystal)	415.432 g/mol	2	7	3.15	115.95

Criteria: log *P*≤5.0, molecular weight in the range of 150–500, H-bond donor's ≤5, and H-bond acceptors ≤10.

The results from the above table shows that the ligands selected were found to be in the acceptable range defined for human use which reveals their potential drug like property.

Table 2b: Admesar Analysis

Properties	Glaucarubine	Glaucarubinone	Glaucarubolone	Melianone	Idelalisib (Cocrystal)
Blood-Brain Barrier Human Intestinal Absorption	BBB- HIA+	BBB- HIA+	BBB- HIA++	BBB- HIA+++	BBB- HIA++
Log S(scale Insoluble < -10<Poorly<-6< Moderately <-4<Soluble<-2Very<0< Highly)[Water solubility]	-2.76	-3.04	-1.41	-6.70	-5.11
Permeability – glycoprotein Substrate	Substrate	Substrate	Substrate	Non-Substrate	Substrate
Carcinogens	Non-Carcinogens	Non-Carcinogens	Non-Carcinogens	Non-Carcinogens	Non-Carcinogens
AMES mutagenicity	Non toxic	Non toxic	Non toxic	Non toxic	Toxic
Acute Oral Toxicity(II – LD 50 – LD 500, LD III – LD 500 – LD 5000)	III	III	III	III	III
Synthetic accessibility [from 1 (very easy) to 10 (very difficult)]	6.80	6.70	6.01	6.58	3.86

Interestingly, the known drug show some toxicity which justify our efforts to screen natural

compounds.

3.4.1. Brain Or IntestinaL EstimateD permeation method (BOILED-Egg)

The prediction also reveals that Melianone has high GI absorption followed by the known drug Idelalisib which is then followed by Glaucarubolone. Glaucarubine and Glaucarubinone have low GI absorption **(Figure 4).**

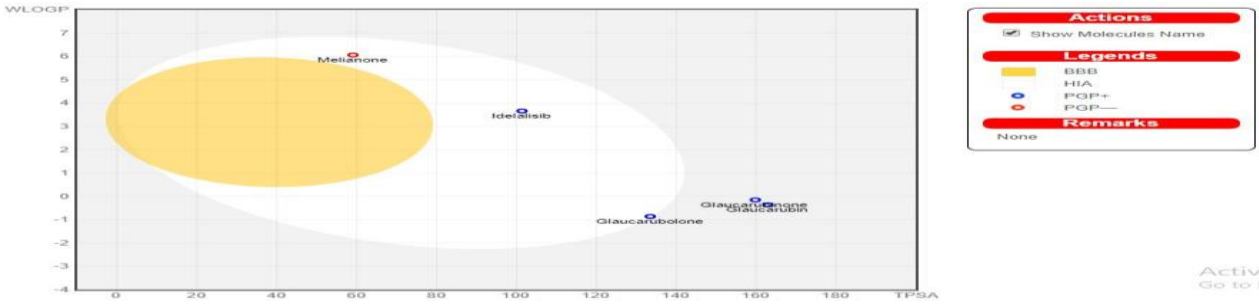


Figure 4. BOILED EGG Model: The white region is the physicochemical space of molecules with highest probability of being absorbed by the gastrointestinal tract, and the yellow region (yolk) is the physicochemical space of molecules with highest probability to permeate to the brain.

3.5. Docking Results:

Results obtained from induced fit docking of compounds of the active site of PI3K (4XE0) are reported in **Table 3.** The molecular docking study of the compounds with PI3K receptor shows that, all the compounds are showing better docking score than that of the known drug Idelalisib which predicts that the compounds chosen have the better binding affinity to the receptor than the cocrystal. The hydrogen binding interactions of the compounds with PI3K is shown in **figure 5 (both 2D and 3D interactions)**. This prediction leads us to believe that, the compounds will possibly be suitable for cancer treatment.

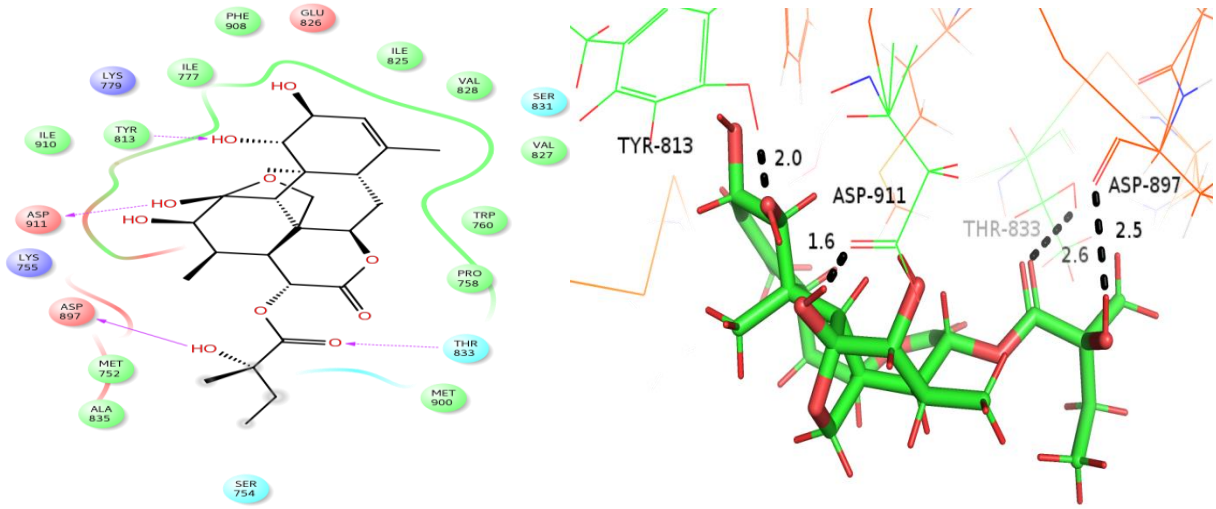
Table 3: Docking Results

Ligand	Docking Score	Glide Energy (Kcal/mol)	H – Bond	Bond length (A°)	Hydrophobic Interactions
Glaucarubine	-12.307	-78.917	(O-H---O) Asp 911 Tyr 813 (O-H---O) Thr 833 (O-H---O) (O-H---O) Asp 897	1.6 2.0 2.6 2.5	Pro 758, Trp 760, Val 828, Ile 825, Ile 777, Met 752
Glaucarubinone	-9.313	-71.535	Thr 813 (H---O-H) (O-H---O) Asp 911 (O-H---O) Asp 911 (O-H---O) Asp 897	2.1 1.9 2.2 1.8	Met 900, Trp 760, Met 752, Val 827, Ile 825, Phe 908, Pro 758, Ile 777,

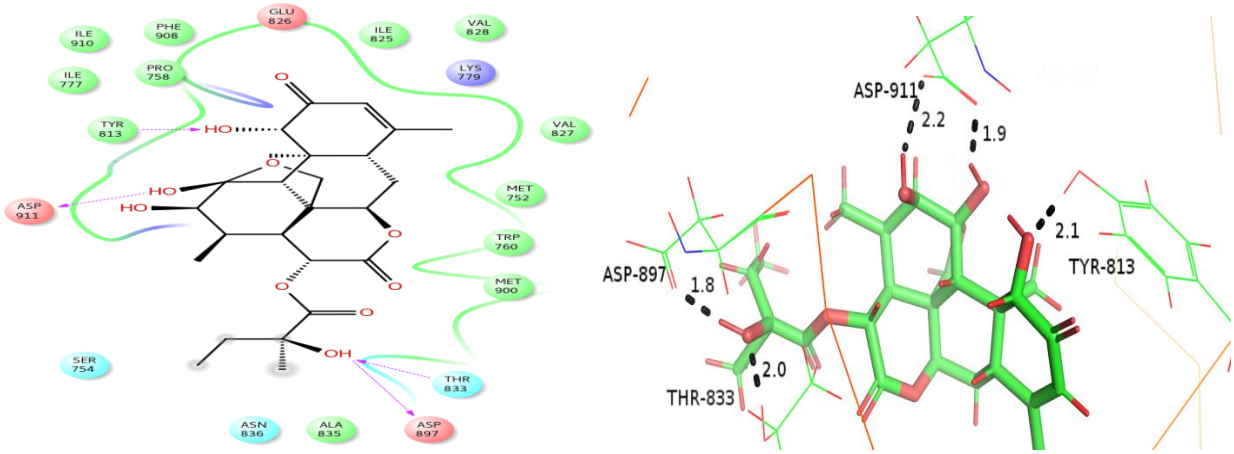
			Thr 833 (H ---O-H)	2.0	
Glaucarubolone	-11.31	-67.601	Lys 779 (N-H---O)	2.7	Val 828, Tyr 813,
			(O-H---H) Asp 911	2.4	Phe 908, Ile 825
Melianone	-11.193	-80.162	Val 828 (N-H---O)	2.5	Ala 835, Met 752,
			(O-H---O) Val 828	1.8	Leu 829, Val 827,Ile
			(O-H---O) Ser 754	1.8	777, Ile 825, Tyr
					813, Trp 760
Idelalisib	-10.242	-76.018	Val 828 (N-H---N)	2.8	Tyr 813, Trp 760,
(Cocrystal)					Val 827, Ile 825, Ile
					777, Pro 758, Phe
					751.

The docking results mentioned in the above table clearly shows that all the compounds selected for docking study displayed excellent binding affinity when compared to the known drug Idelalisib. Melianone showed best glide energy (-80.162) followed by Glaucarubine (-78.916) which is much better than the cocrystal used Idelalisib (-76.018). The hydrogen bond interactions along with the bond distance (A˚) are shown in the PyMol interactions (Figure 5). The hydrophobic interactions are mentioned in table 3.

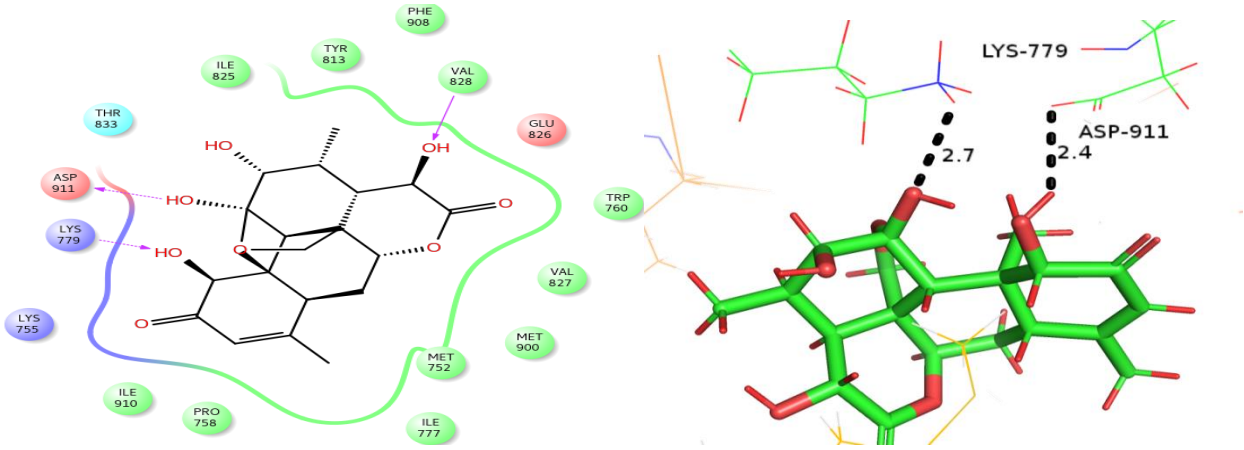
Figure 5. 2D and 3D interactions of the compounds



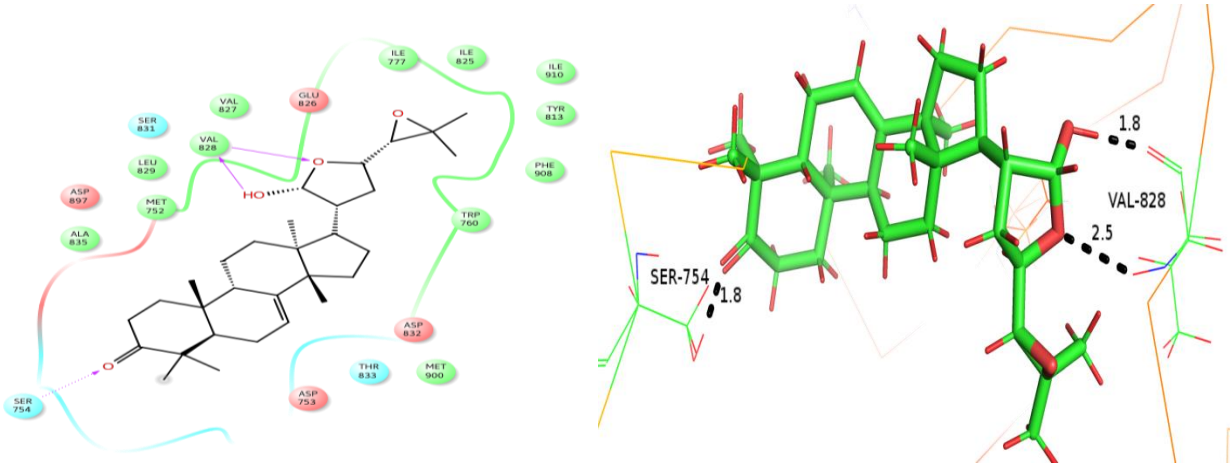
5a: Glaucarubine



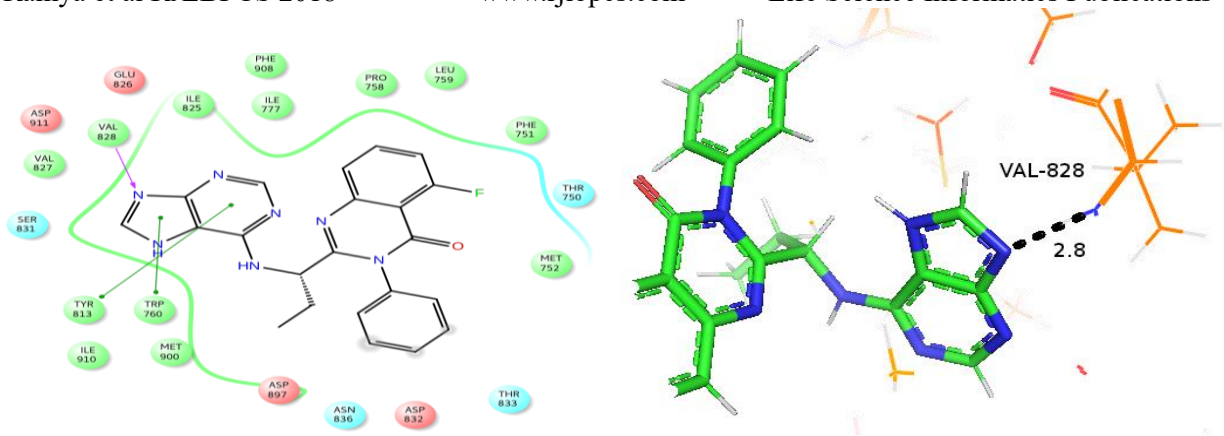
5b: Glaucarubinone



5c: Glaucarubolone



5d: Melianone



5e: Idelalisib (Cocrystal)

4. CONCLUSION

The *in silico* studies of the selected quassinoids from the plant *Simarouba glauca* showed favorable results for using as an inhibitor for PI3K. The compounds bound with more competencies to the binding sites similar to the known drug Idelalisib. The chosen compounds showed better results in *in silico* analysis with better binding efficiency in terms of glide energy compared to Idelalisib. Based on the ADME predictions Melianone has high absorption when compared to the known drug Idelalisib. On the other hand Idelalisib is AMES toxic and the ligands from the plant *Simarouba glauca* selected were AMES non-toxic. Hence, it has been predicted that all the compounds can possibly act as new leads for the treatment of cancer. These results may, in future be used in *in vivo* experiments to test their effects on the abilities for the treatment of cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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