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Research Article

INHIBITION OF CYCLIN DEPENDENT KINASE-2 AND GLYCOGEN SYNTHASE KINASE-3 BY HERBAL DERIVATIVE 1, 2-DISUBSTITUTED IDOPYRANOSE THROUGH *IN-SILICO* ANALYSIS

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ABSTRACT

Cyclin dependant kinase-2 (CDK-2) is a key regulator of cell cycle progression and glycogen synthase kinase-3 (GSK3) that plays a critical role in the regulatory pathway of serine/threonine kinase, which is being targeted for the treatment of human cancer. The natural product of 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) was isolated from the leaves of the medicinal plant, *Vitex negundo* to treat human cancer. The bioactive compound of functionalized 1, 2 disubstituted idopyranose was studied through molecular docking and evaluated for their inhibitory activity against CDK2 and GSK3 using GLIDE module and also ADME/T properties of the analog was analyzed using QikProp module. Based on the docking studies, we have identified some key features in the 1, 2 disubstituted idopyranose that is responsible for simulations of a promising lead compound for the inhibition of CDK-2 and GSK-3 inhibitory activity. The 1, 2 disubstituted idopyranose**,** which showed docked energy -33.82 kcal/mol demonstrated against CDK-2 (2c4g) and docked energy -55.94 kcal/mol demonstrated against GSK-3 (3f7z). A series of 1, 2 disubstituted idopyranose demonstrated good inhibition against CDK-2 and GSK-3 and are useful candidates as leads for the development of potential anti-carcinogenic agents.

Keywords: Cyclin dependant kinase-2, Glycogen synthase kinase-3, 1, 2 disubstituted idopyranose, *Vitex negundo,* Medicinal plant.

1. INTRODUCTION

The knowledge of the molecular basis of carcinogenesis has provided for the discovery of new, more selective and less toxic chemopreventive agents. At present, considerable attention has been focused on identifying naturally occurring substances capable of inhibiting carcinogenesis. Although a number of natural compounds have been reported to possess anticancer properties, their mechanisms of an action are undefined. In this study, a compound, 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) from the leaves of the medicinal plant, *Vitex negundo* Linn (Verbanaceae), which exhibits anticancer activity [1, 2] was studied using the Glide module [3]. To preliminarily determine the potential molecular targets as well as to support enzyme/receptor protein for anti-carcinogenic activity test of this compound, the docking simulation were performed using two different molecular targets involved in cell cycle, cell growth and DNA replication, i.e., cyclin-dependent kinase 2 (CDK-2) and glycogen synthase kinase-3 (GSK-3).

CDK-2 is the cyclin-dependent serine/threonine kinases, which plays important roles in cell cycle control, apoptosis, transcription and neuronal functions and become active only when associated with a regulatory partner (e.g., cyclins or other proteins). The cyclin dependent protein kinases are key regulators of cell cycle progression. Aberrant expression or altered activity of distinct cyclin dependent kinase (CDK) complexes results in escape of cells from cell cycle control, leading to unrestricted cell proliferation. CDK inhibitors have the potential to induce cell cycle arrest and apoptosis in cancer cells and identifying small molecule CDK inhibitors has been a major focus in cancer research [4, 5]. Glycogen synthase kinase-3 (GSK-3) is a unique multifunctional serine/threonine kinase that participates in numerous signaling pathways involved in diverse physiological processes [6]. GSK-3 mediates the addition of phosphate molecules on serine and threonine amino acids in particular cellular substrates. GSK-3 beta regulates multiple cell signaling pathways has been implicated in glucose intolerance, neurodegenerative disorders and inflammation [7]. GSK-3 has been linked to a diverse array of diseases like cancer [8, 9], chronic inflammatory process [10], bipolar mood disorder [11], schizophrenia and diabetes [12]. GSK-3 is a critical regulator of nuclear factor-kappaβ (NF-kβ) nuclear activity that suggests the inhibition of GSK-3 beta could be effective in the treatment of a wide variety of tumors

with constitutively active NF-kβ [6]. GSK-3 inhibition prevented the formation of the tumor in nude mice generated by the inoculation of human ovarian cancer cells and its activity is important for the proliferation of ovarian cancer cells, implicating this kinase as a potential therapeutic target for cancer [13].

The aim of the present study is to explore the inhibitory activity of the herbal derivative, 1, 2 disubstituted idopyranose from the leaves of *Vitex negundo* on cancer proteins by molecular docking simulations and analyse the pharmacokinetics and pharmacodynamics of the compound for drug like candidates by using the Schrodinger software 9.0 and hence it would serve as to design drug alternative to cancer.

2. MATERIAL AND METHODS

Molecular docking studies were performed with 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) (Fig. 1) using Glide 5.5 module of Schrodinger suite.

2.1. Computational methods with Glide Version 5.5

All computational studies were carried out using Glide version 5.5, installed in a single machine running on Intel Core 2 Duo processor with 1GB RAM and 160 GB hard disk with Red Hat Linux Enterprise version 5.0 as the operating system.

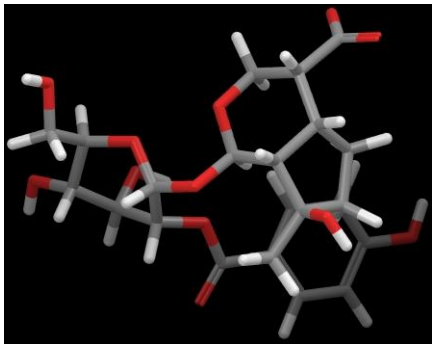


Fig. 1: 1, 2 disubstituted idopyranose

2.2. Protein preparation

The structure of the proteins, cyclin dependent kinase-2 (CDK-2) and glycogen synthase kinase-3 (GSK-3) were obtained from the Research collaboratory for structural bioinformatics (RCSB) Protein data bank (PDB). After evaluating numbers of entries, the best proteins were selected by analyzing the protein with Ramachandran Plot and ProCheck using structure analysis verification server (SAVS) based on ligand and number of disallowed regions [14, 15, 16]. After selection, protein preparation wizard of Schrodinger suite used to prepare protein. The proteins were preprocessed separately by removing the substrate cofactor as well as the crystallographically observed water molecules (water without H bonds), correcting the mistakes in PDB file, optimizing hydrogen bonds. After assigning charge and protonation state finally energy minimization was done using OPLS2001 force field.

2.3. Validation of the docking protocol in Glide

The most suitable method of evaluating the accuracy of a docking procedure is to determine, how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the docking of proteins with their already presented ligand was performed to test the reliability and reproducibility of the docking protocol for our study. The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of the ligand by Glide (3 Å) was analyzed. This indicates the reliability of the docking method in reproducing the experimentally observed binding mode for target proteins.

2.4. Ligand Preparation

The structure of the compound, 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) was drawn by using ChemSketch (ACDLABS 12.0) and converted to 3D structure with the help of 3D optimization tool. By using the LigPrep (2.3) module [17], the drawn ligand was geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field with the steepest descent followed by truncated newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field. The LigPrep is a utility in Schrodinger software suite that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers and steric isomers and geometry minimization of ligands. Finally, 32 poses were prepared with different tautomeric and steric features for docking studies.

2.5. Docking Studies

2.5.1. Grid generation and Ligand Docking

The Docking studies were done for all the prepared proteins separately. Docking studies on LigPrep treated compounds were carried out in the active site of the protein. Receptor Vander Waals scaling for the non polar atoms was set to 0.9 which makes the protein site "roomier" by moving back the surface of non-polar regions of the protein and ligand. This kind of adjustments emulate to some extent the effect of breathing motion to the protein site, it is a kind of giving breathing to the receptor, this approach softens the active site region of the receptor making it flexible [18]. The prepared

protein and the ligand were employed to build energy grids using the default value of protein atom scaling (1.0 Å) within a cubic box of dimensions, centered around the centroid of the X-ray ligand pose. After Grid generation, the ligand was docked with the protein by using Glide 5.5 module, [19] in Extra precision mode (XP) which uses MCSA (Monte Carlo Based Simulated Algorithm) based minimization. The best docked pose (with lowest Glide Score value) obtained from Glide [20, 21, 22 & 23] was analysed. The binding energy was calculated by Liaison module [24].

2.6. ADME/T property analysis

The above mentioned prepared ligands were then neutralized and checked for their ADME/T properties using Qikprop 2.3 module [25]. Qikprop helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Predicted significant ADME/T properties such as Molecular weight (MW), permeability through MDCK Cells (QPlogMDCK), QikProp predicted log IC_{50} value for blockage of K^+ channels (QPlogHERG), QikProp predicted gut-blood barrier (QPPCaco) and violations of the Lipinski's rule of five (LROF) are reported.

3. RESULTS AND DISCUSSION

The docking simulation technique was performed by using Glide module (Schrodinger suite). 2c4g for CDK-2 (Fig. 2) and 3f7z for GSK-3 (Fig. 3) were selected after evaluating number of geometries from Protein data bank (PDB) for docking studies. For validating the software, the proteins were redocked with the already bound ligand. By docking the known ligand PHA533514 with CDK-2 and Oxadiozole with GSK-3, the Root Mean Square Derivatives (RMSD) is 1.37 Å and 1.50 Å respectively. Glide RMSD value below 3 Å indicates the reliability of docking method in reproducing the experimentally observed binding mode of the proteins.

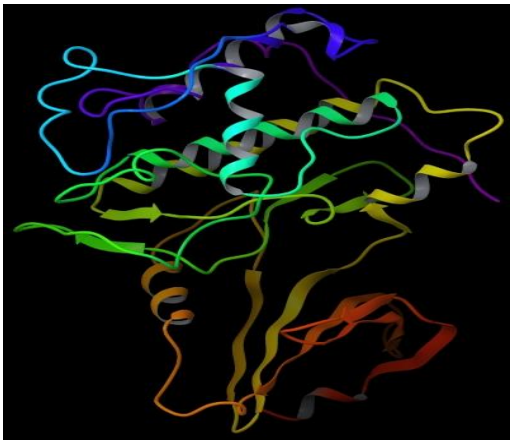


Fig. 2 Cyclin dependent kinase-2

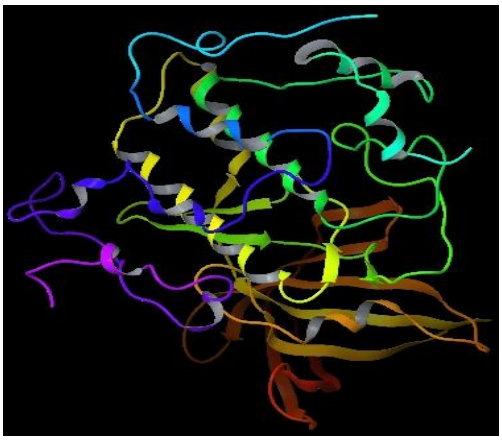


Fig. 3: Glycogen synthase kinase-3

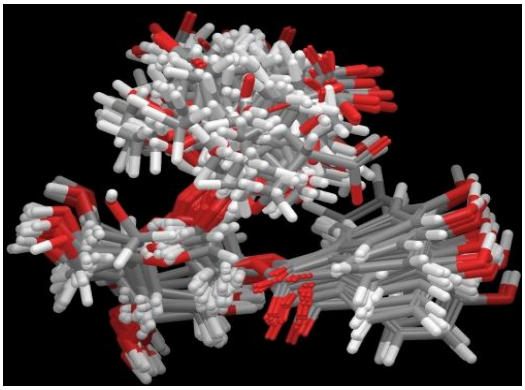


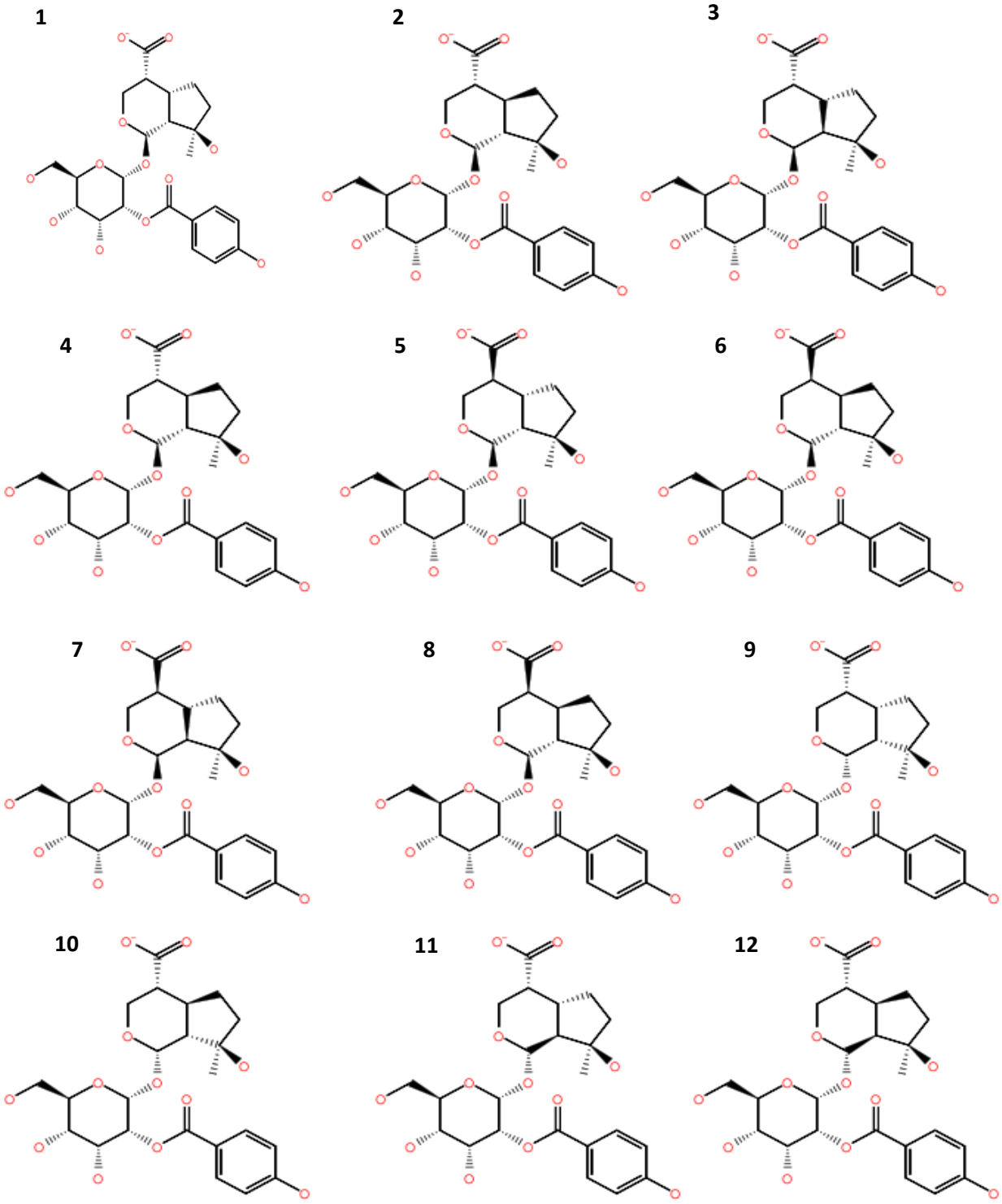
Fig. 4: 1, 2 disubstituted idopyranose-32 poses

The ligand, 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) prepared with 32 poses using LigPrep (Fig. 4 & 5) were docked with two cancer causing/inducing proteins, cyclin dependent kinase-2 (PDB ID: 2c4g) and glycogen synthase kinase-3 (PDB ID: 3f7z) separately. In that 32 poses, the best 10 poses (1 to 10) were selected according to the Glide XP score and lowest energy docked conformation and subjected to the energy minimization using Liason module. The docking results were listed in Table 1.

According to the docking result, the best ligand was selected with best dock score (-10.061360), glide energy (-60.84865 kcal/mol) and low bound energy -93.593 kcal/mol for CDK-2 and the dock score (-9.56), glide energy (-51.00 kcal/mol) and low bound energy -93.116 kcal/mol for GSK-3. The ligand pose 3 had the good result compared to other poses. Comparing this result with already presented ligand (Glide Score, -8.70 and Glide Energy, -33.82 kcal/mol) for CDK-2 (2c4g) and glide score, -8.80 and glide energy, -55.94 kcal/mol for GSK-3 (3f7z), the idopyranose possess better score than the previously bound one (Table 2). It forms five hydrogen bond interactions (Glu81, Lys33, Leu83, Gln131

and Asp145) with the protein CDK-2 (Fig. 6) and four hydrogen bond interactions (Gln185, Gln185, Arg141 and Pro136) with GSK-3 (Fig. 7). The liaison values (-93.593 for CDK-2 and -93.116 for GSK-3) confirmed the good bound result (Table. 1).

The ADME/T prediction of 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$) shows good result with least number of stars and least number of violations (Table. 3).



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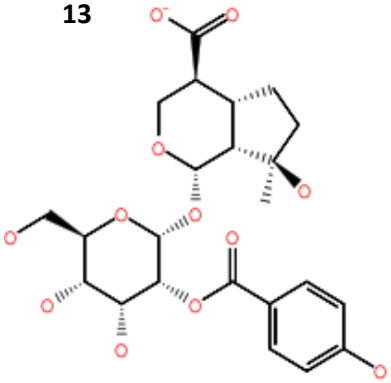

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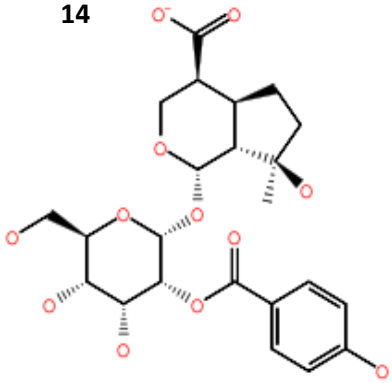

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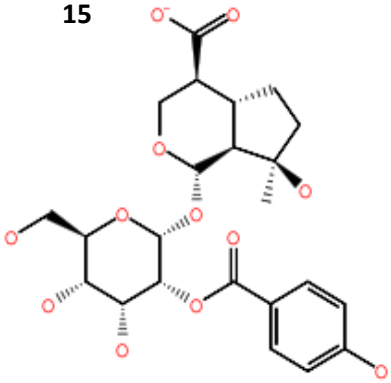

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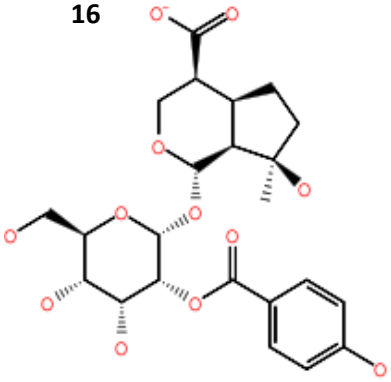

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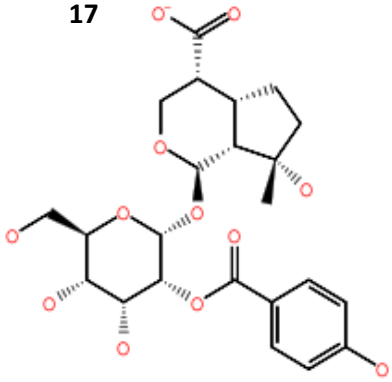

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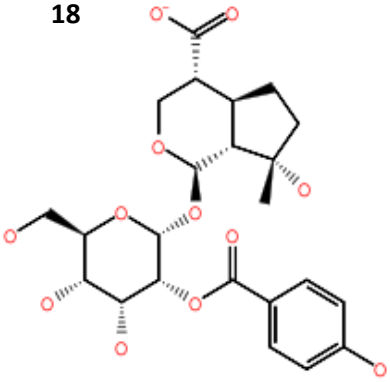

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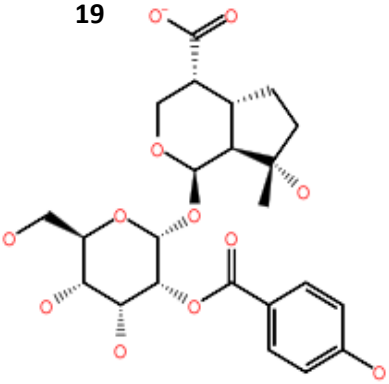

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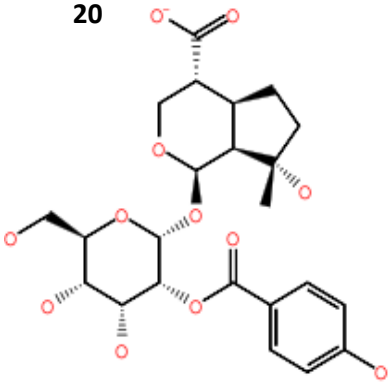

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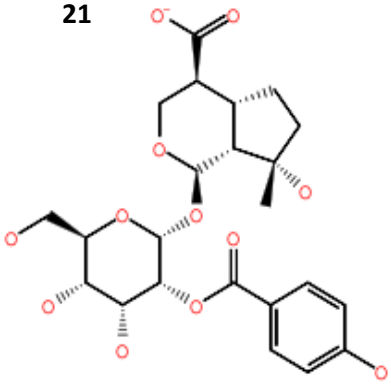

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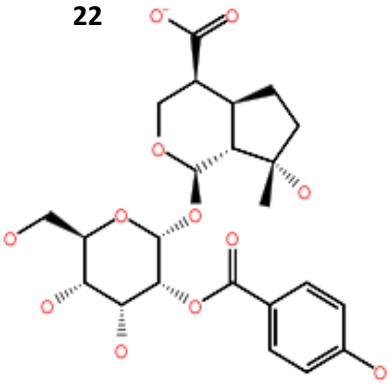

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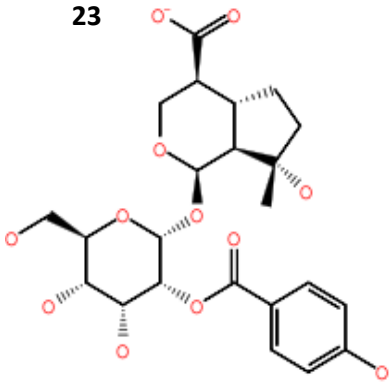

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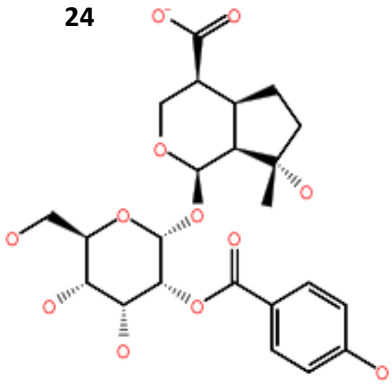

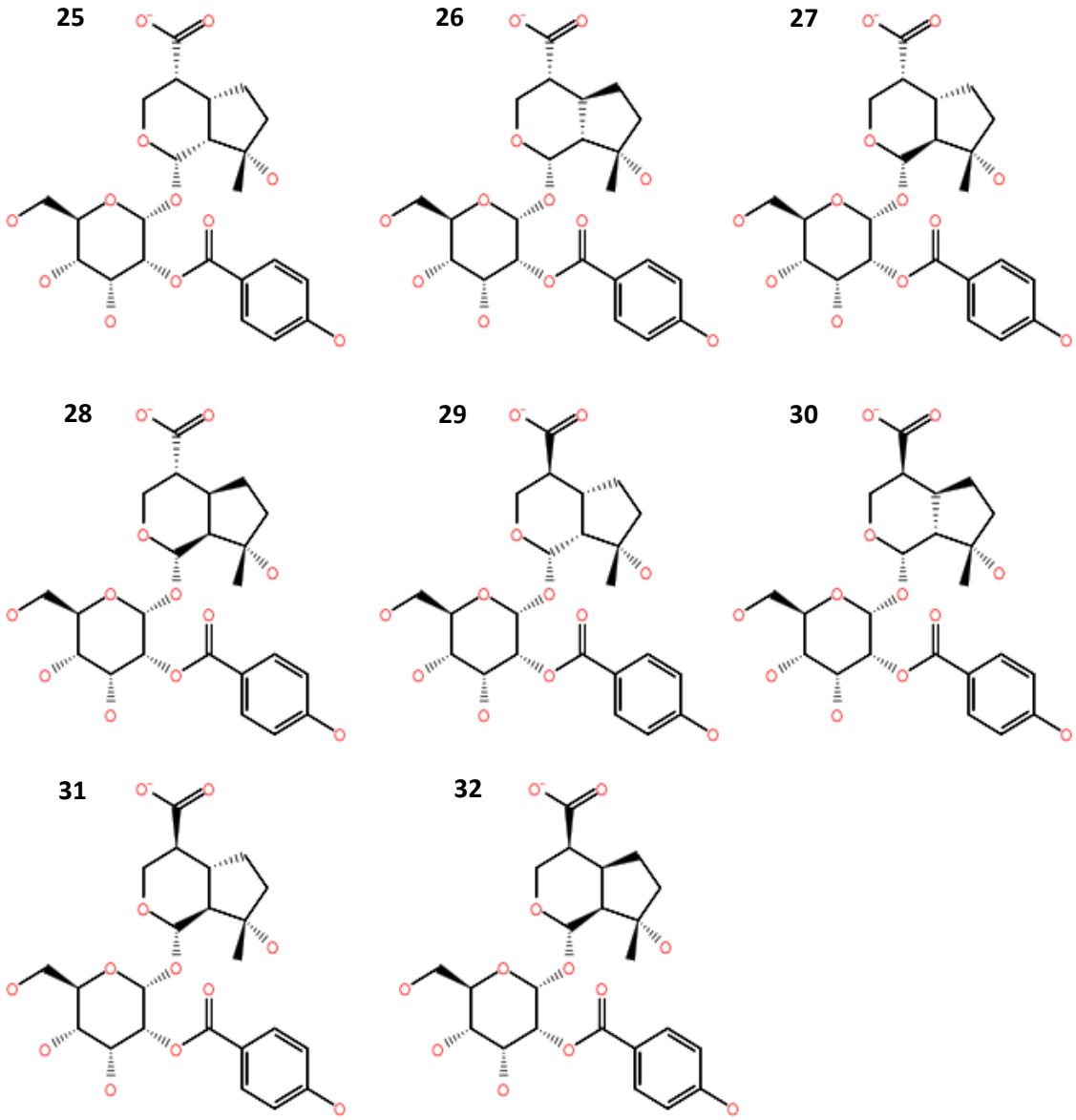

Fig. 5(1-32): 1, 2 disubstituted idopyranose with 32 Poses

Table 1: Docking result of the ligand, 1, 2 disubstituted idopyranose with 32 Poses

Ligand Poses	CDK-2 (2C4G)			GSK-3 (3F7Z)		
	Glide Score (XP)	Glide Energy (kcal/mol)	Liaison<Uele>	Glide Score (XP)	Glide Energy (kcal/mol)	Liaison<Uele>
1.	-10.66	-51.65	-82.36	-10.26	-48.86	-55.16
2.	-10.25	-49.82	-75.51	-9.66	-41.16	-55.82
***3.**	**-10.06**	**-60.84**	**-93.11**	**-9.56**	**-51.00**	**-99.47**
4.	-9.56	-50.14	-81.11	-9.55	-52.81	-77.12
5.	-9.35	-50.93	-76.94	-9.27	-39.79	-75.58
6.	-9.31	-53.54	-64.89	-9.22	-52.95	-78.75
7.	-9.22	-60.54	-79.41	-9.16	-49.72	-58.83
8.	-9.21	-51.26	-106.22	-9.04	-51.22	4.27
9.	-9.17	-53.30	-9.96	-8.90	-54.53	-47.9

Continued…

10.	-9.03	-52.48	-100.23	-8.89	-47.75	-15.97
11.	-9.03	-48.03	-76.88	-8.76	-44.62	-10.13
12.	-9.01	-58.87	-83.89	-8.72	-48.40	-26.66
13.	-9.00	-51.92	-57.47	-8.71	-53.48	-42.95
14.	-8.79	-49.38	-125.91	-8.67	-46.66	-39.38
15.	-8.64	-47.50	-109.14	-8.38	-46.48	-36.68
16.	-8.61	-50.04	-111.26	-8.19	-48.74	-77.04
17.	-8.57	-54.69	-115.36	-8.11	-46.84	18.09
18.	-8.55	-50.33	-66.19	-8.10	-46.36	-35.41
19.	-8.46	-54.35	-84.44	-8.09	-43.08	-90.7
20.	-8.33	-44.24	-73.94	-8.07	-48.70	9.85
21.	-7.84	-51.90	-56.16	-7.97	-49.62	9.02
22.	-7.84	-51.71	-78.29	-7.89	-47.73	-41.67
23.	-7.81	-49.93	-92.00	-7.80	-42.88	-78.55
24.	-7.75	-54.75	-88.69	-7.66	-52.88	-58.86
25.	-7.74	-51.72	-88.61	-7.65	-44.84	-17.98
26.	-7.71	-50.94	-82.03	-7.63	-50.65	3.73
27.	-7.47	-50.66	-66.00	-7.61	-46.76	20.12
28.	-7.32	-53.43	-70.43	-7.58	-43.77	-38.48
29.	-7.31	-53.95	-61.13	-7.41	-48.49	9.99
30.	-6.87	-53.06	-72.60	-7.30	-49.76	-34.04
31.	-6.72	-50.82	-44.32	-7.02	-48.97	-23.26
32.	-6.40	-43.77	-50.28	-6.93	-41.15	21.96

** Ligand pose showing high Glide score and low binding energy*

Table 2: Comparison of best ligand score with already presented ligand

PDB ID	1, 2 disubstituted idopyranose			Already Presented Ligand		
	Glide Score (XP)	Glide Energy (kcal/mol)	No of Hydrogen bond interactions	Glide Score (XP)	Glide Energy (kcal/mol)	No of Hydrogen bond interactions
2C4G	-10.06	-60.84	5	-8.70	-33.82	1
3F7Z	-9.56	-51.00	4	-8.80	-55.94	3

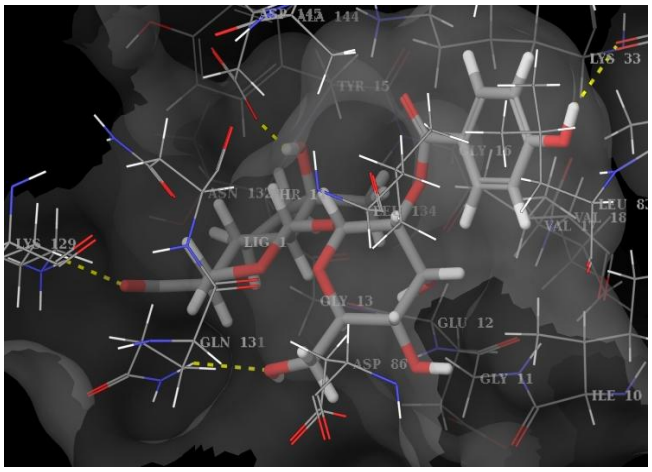

Fig. 6: 1, 2 disubstituted idopyranose bound with CDK-2

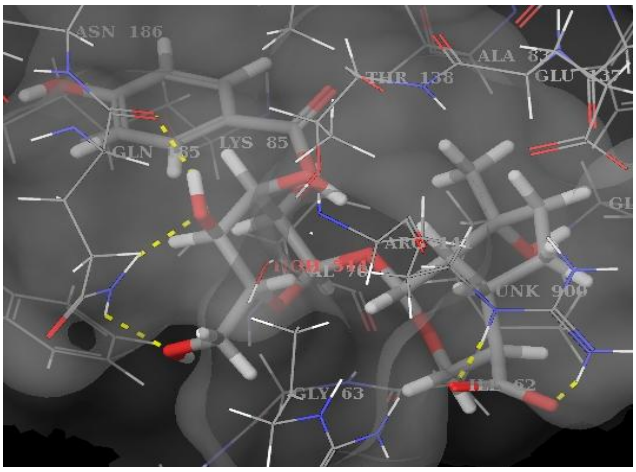

Fig. 7: 1, 2 disubstituted idopyranose bound with GSK-3

Table 3: ADME/T properties of 1, 2 disubstituted idopyranose with 32 Poses

Descriptors/Properties	Value
Mol_MW	498.483
QPlogMDCK	1.921
QPlogHERG	-3.135
LROF	2
QPPCaco	4.709
Stars	2
QPlogKp	-5.288
QPlogS	-2.779
QPlogBB	-2.889
QPpolrz	42.933

4. CONCLUSION

In conclusion, the molecular docking was applied to explore the binding mechanism and to correlate its docking score and energy with activity of herbal derivative like 1, 2 disubstituted idopyranose ($C_{23}H_{28}O_{12}$), which possessed good inhibitory activity against cyclin dependent kinase-2 and glycogen synthase kinase-3. To best of our knowledge, this is the first study aimed at deriving docking studies for 1, 2 disubstituted idopyranose derivative. The docking studies provided good insights into the binding of 1, 2 disubstituted idopyranose derivatives at the molecular level and thereby better human cyclin dependent kinase-2 and glycogen synthase kinase-3inhibitors.

5. REFERENCES

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