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Research Article

GLYCOGEN SYNTHASE KINASE-3 BETA PROTEIN INHIBITION BY SELECTED PHYTOCOMPOUNDS *IN SILICO*

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ABSTRACT

Objectives: Bioactive phytocompounds are a rich source of chemopreventive substance. In the present investigation, docking study was performed for the selected bioactive phytocompounds such as oleanolic acid, ecdysterone, betaine, stigmasterol acetate, and cinnamic acid to evaluate their affinity to glycogen synthase kinase-3 beta (GSK-3β) protein, a wound-healing biomarker. 2-chloro-5-[4-(3-chloro-phenyl)-2, 5-dioxo-2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid was used as an inhibitor for GSK-3β with minimum binding energy (−31.5 kcal/mol).

Methods: Molecular docking study was conducted using AutoDock 4.2 version and the visualization result using Discover Studio 4.5.

Results: The docking analysis ranked the selected phytocompounds that have high theoretical scores to bind to the proteins. The binding mode of the phytocompounds that bound to all the target proteins with high affinity was studied. The simulation demonstrated that the protein-ligand complex stabilized by multiple hydrogen bonds (H-bonds) was preferentially formed at the catalytic site. The results highlighted in this study reveals that among the selected lead phytocompounds that docked into the active site of GSK-3β, ecdysterone showed acceptable 6 H-bond interactions with residues LYS85, TYR134, ARG141, GLN185, ASP200, PRO136 when compared to the reference compound with 5 H-bond interactions.

Conclusion: Thus, based on the docking score ecdysterone could be considered as a novel compounds that can be used for experimental studies for the inhibition of GSK-3β kinase. These results can be helpful for further design of novel GSK-3β inhibitors.

Keywords: Phytocompounds, Molecular docking, Simulation, Receptor, Ligand, Inhibition.

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INTRODUCTION

Plants constitute major phytocompounds which serve as a source of drugs for prevention and spread of a wide range of pathogenic organisms and also treating various diseases of human beings. Nowadays, people prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects. Over thousands of years, medicine and natural products (NPs) have been closely linked through the use of traditional medicines and natural poisons [1,2]. Despite competition from other drug discovery methods, NPs are still providing their fair share of new clinical candidates and drugs. In search of novel active compounds from plant origin, and to assess the efficient therapeutic properties with minimum side effects, application of advanced methods like computational techniques play a crucial role in designing and development of drug of interest.

Thus, the need for new drugs has increased the use of computational prediction of potential drugs by docking methods which helps to investigate the intermolecular interactions between the ligand and the target protein. Computational biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs but also of changing the way drugs are designed. It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually protein [3]. Molecular docking is a key tool in structural biology and computer-aided drug design [4]. Molecular docking is a great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of protein. As a result, novel ligands for receptors of known structure were designed, and their interaction energies were calculated using the scoring functions. The three dimensional structure of the protein-ligand composite could be served as a considerable source of understanding the way of proteins interact with one another and perform biological functions. Drug-likeness was analyzed as per "Lipinski Rule of 5" [5].

Glycogen synthase kinase-3 (GSK-3) was first identified over 20 years ago as a consequence of its phosphorylation activity toward glycogen synthase, the rate-limiting enzyme of glycogen biosynthesis [6]. GSK-3 exists in two isoforms, namely GSK-3α and GSK-3β, but each isomer functionality is different and involves in the phosphorylation process. GSK-3β is a serine/threonine kinase that plays a key role in the regulation of numerous signaling pathways. As GSK-3β plays a crucial role in several human diseases, it is being considered as one of the potential therapeutic targets for diseases such as cancer, diabetes, cardiac, Alzheimer's and other central nervous system disorders [7]. Various researches on GSK-3β have reported different inhibitors to treat different disease conditions.

Hence, the present work was carried out to perform the molecular docking analysis of potential phytocompounds into the active site of GSK-3β receptor. The binding mode and the intermolecular interactions between ligands and the GSK-3β kinas receptor were examined by performing Ligand Fit Module of Discovery Studio 3.5.

METHODS

Selection of phytocompounds

The phytocompounds with various pharmacological properties such as oleanolic acid, ecdostyrene, cinnamic acid, beatine, and stigmasterol acetate were selected from the various literatures. The 2D structure of the selected compounds was drawn using ACD Chemsketch. The structures were then converted to 3D; their geometries were optimized and saved in "MDL mol file" format (Fig. 1).

Ligand preparation and optimization

Extensive literature survey was done to select the lead bioactive phytocompounds with pharmacological activity. The structures of the ligands were obtained from the Pubchem database (http://pubchem.ncbi.nlm.nih.gov/). The ligand preparation included 2D-

3D conversions, correcting structures, generating variations of these structures, verifying and optimizing the structures. All these tasks were performed using Marvin Sketch [8]. Marvin was used for drawing, displaying and characterizing chemical structures, substructures, and reactions. Ligand optimization was carried out with CHARMm and Merck molecular force field by minimization protocol in Discovery Studio 3.5. Various ligand confirmations were generated based on bond energy, CHARM energy, dihedral energy, electrostatic energy, initial potential energy, and initial RMS gradient valves.

Protein preparation

The crystal structure of GSK-3β (PDB ID: 1Q5K) was retrieved from protein data bank. The ligands were designed and the structure was analyzed by Marvin Sketch.

Reference compound

2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo-2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid an inhibitor of GSK-3β kinase was used as a reference compound and is shown in Fig. 2.

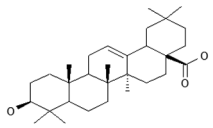
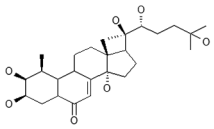
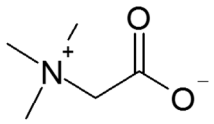
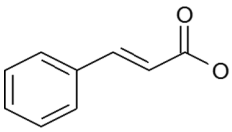
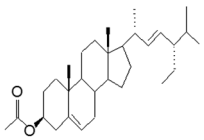
Chemdraw structure	Name of the compound
	Oleanolic acid
	Ecdysterone
	Betaine
	Stigmasterol acetate
	Cinnamic acid

Fig. 1: Represents the structure of the selected phytocompounds (ligand) from Chemsketch

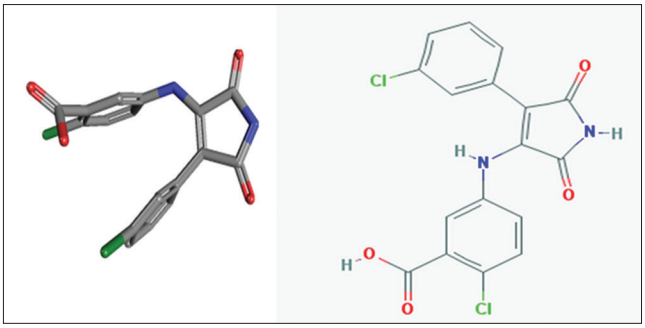


Fig. 2: Represents the structure of 2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo-2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid

Docking using discovery studio

The protocol of docking of ligands with the receptors was performed using DS 3.5 suite. Docking is a virtual screening of a database of compounds and predicting the strongest binders based on various scoring functions. Accelrys Discovery Studio 3.5 was used for docking. In the process, first, a ligand library was generated by placing the ligand PDB files in a single discovery studio file (dsv). The preparation of the library helps in simultaneous docking of multiple ligands against the receptor and in making an easy comparative study between the ligands. Before docking, the ligands were prepared using the "Prepare Ligand" module, which cleans the geometry of the ligands and distributes the uneven charges throughout using CHARMM. Force fields applied in CHARMM are the energies and forces on each particle of the system and also defines the positional relationship between atoms that determine their energy. The ligands were primarily positioned in the binding site using LibDock and then they were docked with both the receptors to understand the mechanism of GSK-3β and GK catalyze enzymatic reactions. A comparative analysis of LibDock scores and the binding energies was also done to examine the role of bioactive compounds interaction with active site residues [8]. The docked ligand–target complexes were analyzed carefully to identify the interactions and binding affinities. The docking scores were recorded, and docking poses were saved for references.

Molecular dynamics simulation study

Molecular dynamics studies performed to investigate further details of the interaction between the protein and the ligand using simulation package in Discovery studio with CHARMm force filed. Top selected inhibitor complexes were subjected to a 100 ps NVT (Constant temperature dynamics using Berendsen weak coupling method) molecular dynamic simulation. Implicit solvation by Distance-dependent dielectric was applied to the system to simulate in solvent environment. The complexes are energy minimized by the steepest descent and conjugate gradient methods until the system reached 0.001 kcal/mol convergence. System was then subjected to 10 ps heating step from 50 to 300 K, followed by 50 ps equilibrium process to thermally equilibrate the molecules of the systems and finally 100 ps full MD production at 300 K with NVT ensemble. All simulation steps were run with a time step of 1 fs. Full MD trajectory was considered for analysis.

Binding energies were calculated for selected four inhibitors in solvent environment which was constructed for each molecule from average Gibbs energy. The relationship between the Gibbs free energy of ligand, receptor and complex was given in the following equation [9].

$\Delta\bar{G}$ binding=$\bar{G}$ complex–($\bar{G}$ ligand+$\bar{G}$ receptor)

The average Gibbs energy which was constructed from each energy component in the above equation is the binding free energy of the complex.

RESULTS AND DISCUSSION

This study helps us to understand the interaction between the ligand and receptor GSK-3β kinase and also explore their binding mode. Molecular docking continues to hold great drug based design which screens small molecules by orienting and scoring them in binding site of protein. The overall structure of the selected phtyocompounds oleanolic acid, ecdostyrene, cinnamic acid, beatine and stigmasterol acetate was represented in Fig. 2. Ligand was created and prepared for the docking procedure using Chem Sketch. The structures of the ligand obtained from the Chem Sketch are shown in Fig. 1. The secondary structure of the receptor GSK-3β kinase was derived from PDB and used as a target for docking simulation shown in Fig. 3.

GSK-3β had three domains, a crystallographic resolution of 2.00 Å and a molecular mass of 17153.2 Da. The initial and final potential energy calculated by energy minimization was 10801661.13 and −212133.81 kcal/mol, respectively. With the receptor cavity method,

27 amino acids were found in the ligand binding pocket: Ile62, Gly63, Val70, Tyr71, Gln72, Ala73, Leu81, Val82, Ala83, Ile84, Lys85, Val110, Leu132, Asp133, Tyr134, Val135, Pro136, Glu137, Thr138, Arg141, Gln185, Asn186, Leu187, Leu188, Leu189, Asp190 and Lys197. The secondary structure of GSK-3β was shown in Fig. 3.

The lead phytocompounds were docked into the active sites of all the three chains of GSK-3β kinase using ligand Fit Module in Discovery Studio 3.5. The docking score along with binding orientations and hydrogen bonds (H-bonds) was considered for choosing the best pose of the docked compounds. Structure of docked complexes of Lead phytocompounds and GSK-3β receptor was shown in Fig. 4. The details of LibDock score, energy value and H-bond length of the docked lead phtyocompounds against GSK-3β receptor was tabulated in Table 1.

The binding orientations of the hit compounds: (a) Oleanolic acid, (b) ecdysterone, (c) betaine, (d) stigmasterol acetate, and (e) cinnamic acid was represented in Fig. 4. Bioactive compounds such as oleanolic acid, ecdysterone, Betaine, stigmasterol acetate, cinnamic acid were docked against the receptor protein GSK-3β kinase wherein, 2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo-2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid, an inhibitor of GSK-3β Kinase was used as reference compound. Table 1 shows the docking score with respect to LibDock Score, Binding energy, H-bond energies and H-bond distance of the docked lead phytocompounds.

Here through *in silico* approach, it was predicted that ecdysterone has a maximum binding energy of about −63.87 kcal/mol with acceptable affinity for the active pocket also shown to inhibit GSK-3β Kinase receptor as it had good LibDock score of 127.95 with 6 H-bonds which was given in Table 1. Stigmasterolacetate showed a maximum binding energy of about-49.54 kcal/mol with acceptable affinity for the active pocket also shown to inhibit GSK-3β receptor with a LibDock score of 123.83 with 1 H-bond. oleanolic acid, cinnamic acid and betaine showed a LibDock score of about 74.3, 60.8 and 37.4, binding energies of about −38.9, −34.23, −0.89, respectively. 2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo- 2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid was used as a reference compound and it was docked against GSK–3β Kinase. The reference compound shows a LibDock score of about −86.42., binding energy of about 31.5 kcal/mol with 5 H-bonds.

Molecular dynamics simulation has been done to check the stability and interaction of structure during the simulation. The binding free energy is able to determine the ability of enzyme protein to bind its substrate. In this study, binding free energies were calculated in solvent environment. The binding energy of the each complex was listed in Table 1. The compounds which show higher Dock score and H-bonds with crucial amino acids were considered as effective lead compounds for GSK inhibition.

The docking score of reference compound 2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo- 2,5-dihydro-1h-pyrrol-3-ylamino]-benzoic acid was compared with the selected lead phytocompounds. The docking score of the reference compound was −31.5 kcal/mol and the hit compound ecdysterone and stigmasterol acetate from the virtual screening studies show better binding with docking score of about −63.87 kcal/mol and −49.54 kcal/mol.

Ecdysterone had 6 H-bond interactions with LYS85, TYR134, ARG141, GLN185, ASP200, and PRO136. The hit compounds that scored docking score higher than active compound and form interaction with the crucial amino acids were considered as effective leads for designing novel GSK-3β kinase inhibitors. Thus, among the selected 5 phytocompounds ecdysterone scored a good docking score with 6 H-bonds than the other phytocompounds in the order stigmasterol acetate, oleanolic acid, cinnamic acid, and betaine with 1 H-bond interaction when compared with the reference compound which has 5 H-bond interactions.

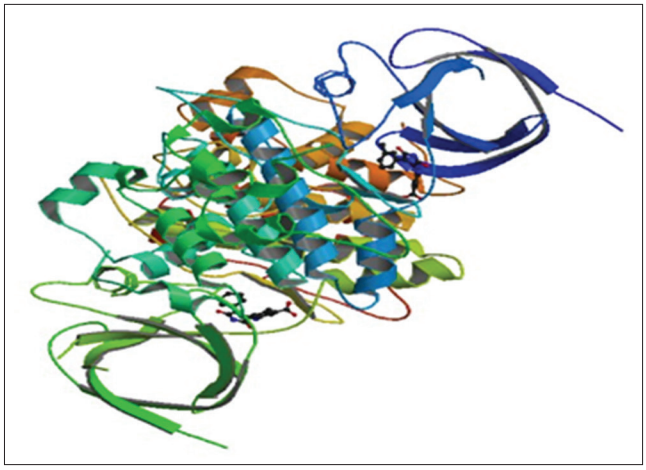


Fig. 3: Representation of secondary structure of glycogen synthase kinase-3 beta

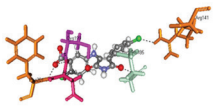
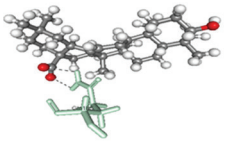
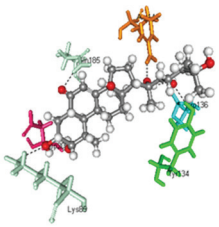
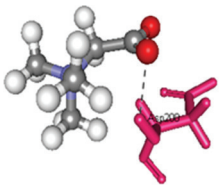
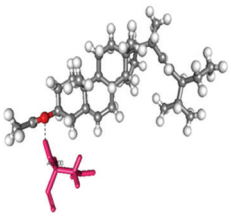
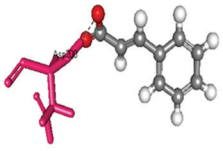
Docking images	Name of the compound
	2-chloro-5-[4-(3-chloro-phenyl)-2, 5-dioxo- 2,5-dihydro-1h-pyrrol-3-ylamino]- benzoic acid
	Oleanolic acid
	Ecdysterone
	Betaine
	Stigmasterol acetate
	Cinnamic acid

Fig. 4: The docking images of the selected phytocompounds with ligand binding pockets against glycogen synthase kinase-3 beta kinase

Table 1: Docking binding energy (kcal/mol) and LibDock score and no of H-bond and H-bond monitoring from docking compounds

Compound name	LibDock Score	Binding energy (kcal/mol)	Number of H-bonds	H-bonds	H-bond monitoring	H-Bond distance
2-chloro-5-[4-(3-chloro-phenyl)-2,5-dioxo-2,5-dihydro-1h-pyrrole-3-ylamino]-benzoic acid	86.42	−31.5	5	ARG141, GLN185, CYS199, ASP200, PHE201	A: ARG141:HH22 - Compound1:Cl25 A: GLN185:HE22 - Compound1:O20 A: CYS199:HG - Compound1:O19 A: ASP200:HN - Compound1:O24 A: PHE201:HN - Compound1:O24	2.41, 2.41, 2.25, 2.30, 2.38
Oleanolic acid	74.3	−38.9	1	GLN185	A: GLN185:HE22 - Compound17:O28 A: GLN185:HE21 - Compound17:O29	2.31, 2.48
Ecdysterone	127.95	−63.87	6	LYS85, TYR134, ARG141, GLN185, ASP200, PRO136	A: LYS85:HZ2 - Compound33:O28 A: TYR134:HH - Compound33:O24 A: ARG141:HH22 - Compound33:O26 A: GLN185:HE21 - Compound33:O18 A: ASP200:HN - Compound33:O27 Compound33:H58 - A: PRO136:O Compound33:H62 - A: PRO136:O	2.1466, 2.15423, 1.82036, 2.20173, 2.2795, 1.73541, 2.23386
Betaine	37.43	0.89	1	GLN185	A: GLN185:HE21 - Compound49:O3	2.07
Stigmasterol acetate	123.83	−49.54	1	TYR134	A: TYR134:HH - Compound50:O24	1.79
Cinnamic acid	60.81	−34.23	2	VAL135, ASP200	A: VAL135:HN - Compound66:O11 A: ASP200:HN - Compound66:O10	2.05, 1.98

CONCLUSION

In molecular docking studies, the important interactions with inhibitors and active site residues were determined. Among the selected lead phytocompounds that docked into the active site of GSK-3β, ecdysterone showed acceptable 6 H-bond interactions with residues LYS85, TYR134, ARG141, GLN185, ASP200, PRO136 when compared to the reference compound with 5 H-bond interactions. Based on the docking score Ecdysterone could be considered as a novel compounds that can be used for experimental studies for the inhibition of GSK-3β kinase. These results can be helpful for further design of novel GSK-3β inhibitors.

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