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Targeting Glycogen Synthase Kinase-3 (Gsk3β) With Naturally Occurring Phytochemicals (Quercetin and its Modelled Analogue): A Pharmacophore Modelling and Molecular Docking Approach

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Abstract *Molecular simulation is a significant tool in drug design and modelling. This work consists of a computational analysis through pharmacophore modelling and molecular docking techniques in order to predict the potential inhibitory activity of quercetin and its analogue. The quercetin analogue QUT1, QUT2, QUT3, QUT4, QUT5, QUT6, QUT7, Quercetin, QUT8, QUT9 and QUT10 were noticed to have minimum energy values of -6.3 kcal/mol, -5.7 kcal/mol, -5.5 kcal/mol, -5.4 kcal/mol, -5.3 kcal/mol, -5.2 kcal/mol, -5.2 kcal/mol, -5.2 kcal/mol, -5.1 kcal/mol,, -5.0 kcal/mol, and +3.0 kcal/mol respectively. Hence, QUT1 (7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) was selected as the lead molecule. The ligand-receptor interaction study of the lead molecule revealed that QUT1 interacted with 15 amino acid residues (CYS 199, ALA 83, LEU 132, ASP 133, TYR 134, VAL 135, PRO 136, THR 138, ARG 141, ILE 62, VAL 61, VAL 110, VAL 70, GLN 72 and LEU 188) within the pocket of glycogen synthase kinase-3β. With favourable ADME prediction of the lead molecule, is possible to conclude that 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one is a probable drug candidate for any disease in which synthase kinase-3β plays a key role in its cell replication.*

Key Words: *Quercetin, molecular docking, ADMET, bioactive compound, pharmacophore modelling*

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1.0 Introduction

The origin of most drugs leads is often traced to natural product which is either obtained as a pure compound or as standardized plant extracts. This is owing to the availability and chemical diversity of natural products (Cos *et al.,* 2006). Consequently, increasing researches on medicinal plants has given birth to the discovery of several novel secondary metabolites that have been isolated from plants. According to Patwardhan *et al*., 2004 over 80% of global medicinal products in the market are associated with plants. However, biological diversity associated with chemical diversity is considered as one of the rich sources of bioprospecting, which leads to the discovery of some of the most significant bioactive compounds (Ramesha *et al*., 2011).

Huberantha Senjiana, a species of *Huberantha* (Annonaceae) was studied in Villupuram District, Tamil Nadu, India the outcome of the study revealed the unique characteristic of this plant ant its close similarity to *Huberantha* jenkinsii

(Ramachandran *et al*., 2015). A further study on the extract of this plant using HPLC with a photodiode array (PDA) detector for the detection and quantification of its phytoconstituents, using quercetin as a marker revealed significant concentration of quercetin in the leaf of *Huberantha senjiana* (Pandiyan & Ilango, 2020). Their findings led to the conclusion that quercetin may have played a significant role in the biological activity of *Huberantha* Senjiana

Quercetin is a flavanol, it is a brilliant citron-yellow needle crystal which is insoluble in cold water, sparingly soluble in hot water and readily soluble in alcohol or lipids (Hollman *et al*., 1999; Ross & Kasum, 2002). Quercetin, a flavonoid is ubiquitous in fruits and vegetables. This secondary metabolite sustains robust biological properties that can enhance mental and physical performances and can also reduce risk of infection (Davis *et al.,* 2009). Flavonoids have also been reported to be significant in enhancing disease resistance capacity such as anti-oxidant(Chan *et al*., 2000; Woodman & Chan, 2004), anti-virus (Lee *et al.,* 1999), anti-diabetogenic activity (Babu *et al*., 2006), anti-inflammatory (Cho *et al*., 2004) and anti-anxiolytic effect (Zanoli *et al*., 2000) amongst others. Hence, it is imperative to design effective clinical trials procedure to evaluate novel dietary supplement further.

GSK-3β is a multifunctional serine/threonine kinase previously designated as the main enzyme responsible for glycogen metabolism *via* phosphorylating (Embi *et al*., 1980). Available literature revealed that GSK controls the functions of various proteins associated with some cardinal biological processes such as glucose metabolism, tumorigenesis, inflammation, gene expression, cell survival, and alzheimer disease (Alonso & Martinez, 2004; Dorronsoro *et al*., 2002; Martinez *et al*., 2002). Studies conducted by Martinez *et* al. (2002) on GSK-3β inhibitors and its potentials as a as therapeutic agents for the treatment of some diseases as diseases like type II diabetes, cancer, Alzheimer's disease, mood disorders, bipolar disorders, stroke, and chronic inflammatory. The results obtained indicated that this molecule is significant in the treatment processes. (Martinez *et al*., 2002). To further assess the benefit of quercetin, we employed computational studies based on pharmacophore modelling and molecular docking simulation to understand the possible inhibitory implication of quercetin against glycogen synthase kinase 3β.

2.0 Materials and Methods

2.1 Receptor and ligand preparation

The compound quercetin and it's analogue as ligands were build using ACD/ChemSketch 2018.2.5 Freeware version. The 2D conformation of quercetin-analogue was built by substituting the hydroxyl group at position 19 (shown in Fig. 1) with a different functional group that is thought to enhance the biological activity of the ligand against the target receptor. The chemical structures were converted into their 3D forms and thereafter optimized using molecular mechanics force filed 94 (Merck molecular force field (MMFF94)) in the Avogadro interface (Hanwell *et al*., 2012). The UCSF Chimera dock-prep tools were used to prepare the optimized structures of quercetin and its analogue prior to molecular docking step. The crystallized structure of the glycogen synthase kinase 3 (GSK-3β) in complex AR-A014418 having 1.94 Å resolution was obtained from the Protein Data Bank (https://www.rcsb.org) with ID: 1Q5K. The crystallized structure consists of two similar chains A and B (a dimer) bounded to small chemical residues (TMU). However, due to computational cost and time, the singular chain A of the protein where the AR-A014418 (TMU) is bounded, was prepared for a molecular docking simulation. The preparation of the biological target (1Q5K) was performed on the UCSF Chimera interface(Pettersen et al., 2004).

2.2 Molecular docking

Docking of ligand quercetin and its analogue against target proteins structure was performed by making use of AutoDockVina software (Morris *et al*., 1998). The specific docking of quercetin-analogues to the active site of glycogen synthase kinase 3 (GSK-3β) (ID: 1Q5K) was achieved by generating a grid box coordinate of the ligand to be substituted on the receptor. The grid box that identifies the pocket of 1Q5K receptor was designed by making use of AutoDock Vina functionality on UCSF Chimera interface (Pettersen *et al*., 2004). The grid box size and centre coordinates for the 1Q5K were x(23.9226, 10.7638) Å, y (21.6961, 7.90577) Å and z (9.76081, 7.1836) Å respectively. The quercetin-analogue with the highest binding affinity for the glycogen synthase kinase 3 (GSK-β) (ID: 1Q5K) was selected for further *in silico* ADME assay.

2.3 Validation and ADME analysis of lead molecule

The 2D chemical structure of quercetin and its analogue uploaded to Swiss ADME online web server. Thereafter, the smiles format of the ligands was generated and submitted for ADME (Adsorption, Distribution, Metabolism and Excretion) predictions. Meanwhile, the results retrieved was observed to consists of lipophilicity, water-solubility, physicochemical properties, pharmacokinetics, pharmaco-like and medicinal chemistry

3.0 Results and Discussion

3.1 Molecular docking

The secondary metabolite, quercetin was isolated from *Huberantha senjiana* leaves. Meanwhile, a substantial amount of quercetin was obtained from the *Huberantha senjiana* leaves. Hence, it is imperative to examine the capacity of quercetin and its analogue to inhibit glycogen synthase kinase-3 *via* pharmacophore modelling and specific molecular docking.

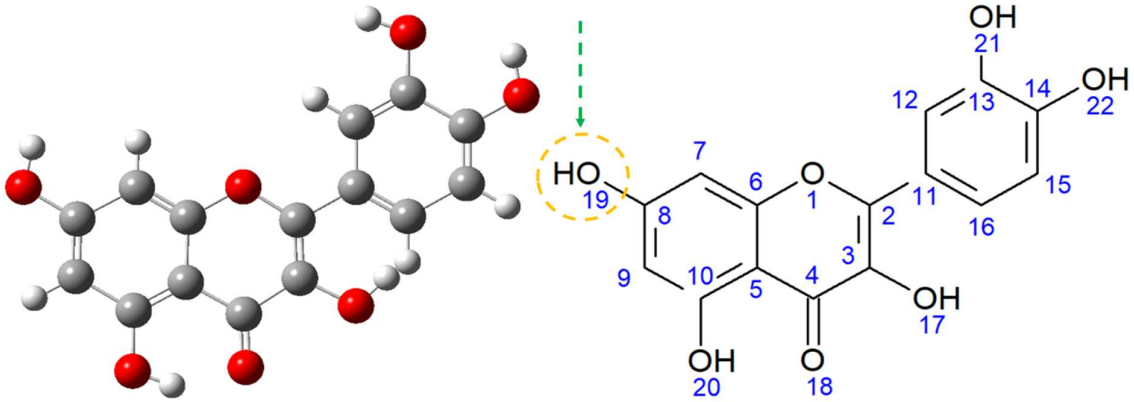


Fig 1: The 2D and 3D chemical structure of quercetin.

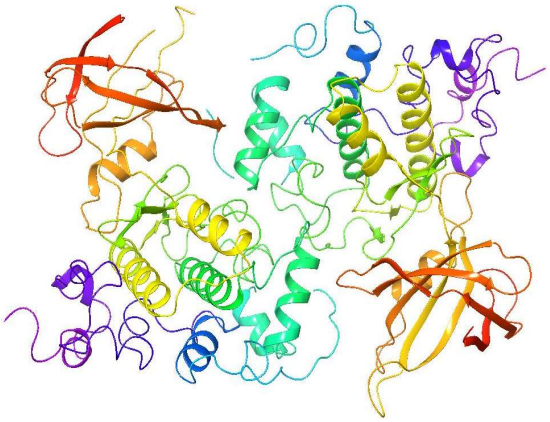
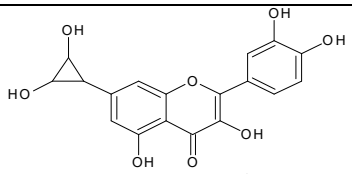
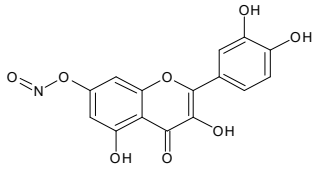
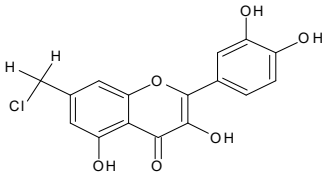
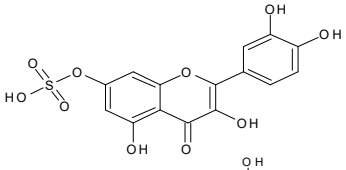
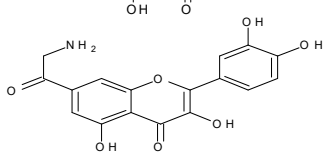
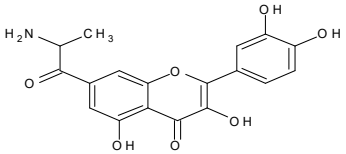
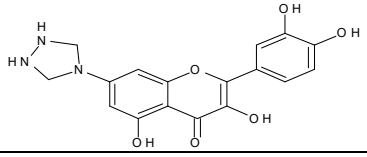


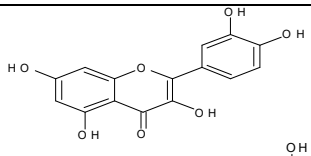
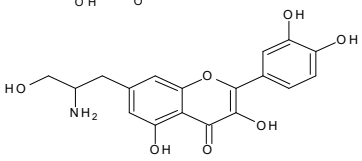
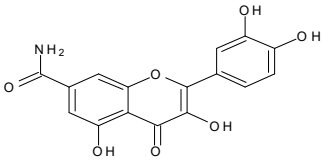
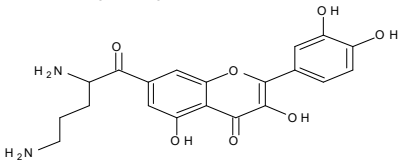
Fig 2: The 3D crystal structure of glycogen synthase kinase 3 (GSK-3β) (ID: 1Q5K).

To select the best candidate among the analogue of quercetin with sufficient inhibition against glycogen synthase kinase-3 (see Fig. 2), a comparison of the minimum energy/full-fitness score and ligand-receptor interaction, nature of H-bonding, hydrophobic potential and electrostatic tendencies of the complexes was employed. As shown in Fig.3, QUT1 (7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) was observed to interact with 15 amino acid residues (CYS 199, ALA 83, LEU 132, ASP 133, TYR 134, VAL 135, PRO 136, THR 138, ARG 141, ILE 62, VAL 61, VAL 110, VAL 70, GLN 72 and LEU 188) within the pocket of glycogen synthase kinase-3β. A full-fitness score of -6.3 kcal/mol was obtained for QUT1-1Q5K complex which was noticed to a display hydrogen bond formation between ASP 133 and a free -OH group on the ligand. The strong electrostatic interaction between ASP 133 and -OH could justify the supreme affinity of this analogue (QUT1). As displayed in Fig 4, about 16 amino acid residues in the pocket of the target interacted with QUT2 (2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4-oxo-4H-1-benzopyran-7-yl nitrite) and a docking score of -5.7 kcal/mol was estimated. QUT3-1Q5K (-5.5 kcal/mol), 7-(chloromethyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one complex was exposed to 17 amino acid residues (see Fig 4). On the contrary, QUT4 (2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4-oxo-4H-1-benzopyran-7-yl hydrogen sulfate) and QUT5 (7-(aminoacetyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) interacted with 14 (-5.4 kcal/mol) and 15 (-5.3 kcal/mol) amino acid residues, respectively. Nevertheless. same minimum energy value of -5.2 kcal/mol was obtained when ~~as~~ QUT 6 (7-(2-aminopropanoyl)-2-(3,4-

dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one), QUT7 (2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(1,2,4-triazolidin-4-yl)-4H-1-benzopyran-4-one) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) were docked against 1Q5K (see Table 1). However, QUT 6 exhibited H-bond interactions with VAL 61 and PRO 136 (see Fig 8). While QUT 7 had H-bond formation with VAL 135 on interaction with 19 amino acid residues. The docked pose of QUT8 (7-(2-amino-3-hydroxypropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) with 1Q5K (-5.1 kcal/mol) exhibited 2H- bond interactions with PRO 136 and GLU 137. QUT8 was also noticed to interact with 18 amino acid residues within the hydrophobic end of the 1Q5K (see Fig 11). The ligands QUT9 (-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4-oxo-4*H*-1-benzopyran-7-carboxamide) and QUT10 (7-(2,5-diaminopentanoyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) had a docking score of -5.0 kcal/mol and +3.0 kcal/mol respectively. However, QUT9 and QUT10 interacted with the same number (16) of amino acids also showed H- bond interaction with PRO 136.

Table 1: 2D representation of quercetin-derivatives and their corresponding Glide score (G-Score) value calculated for the related query.

Code	Score	Structure
QUT1	**-6.3**	
QUT2	**-5.7**	
QUT3	**-5.5**	
QUT4	**-5.4**	
QUT5	**-5.3**	
QUT6	**-5.2**	
QUT7	-5.2	

Quercetin	-5.2	
QUT8	**-5.1**	
QUT9	**-5.0**	
QUT10	**+3.0**	

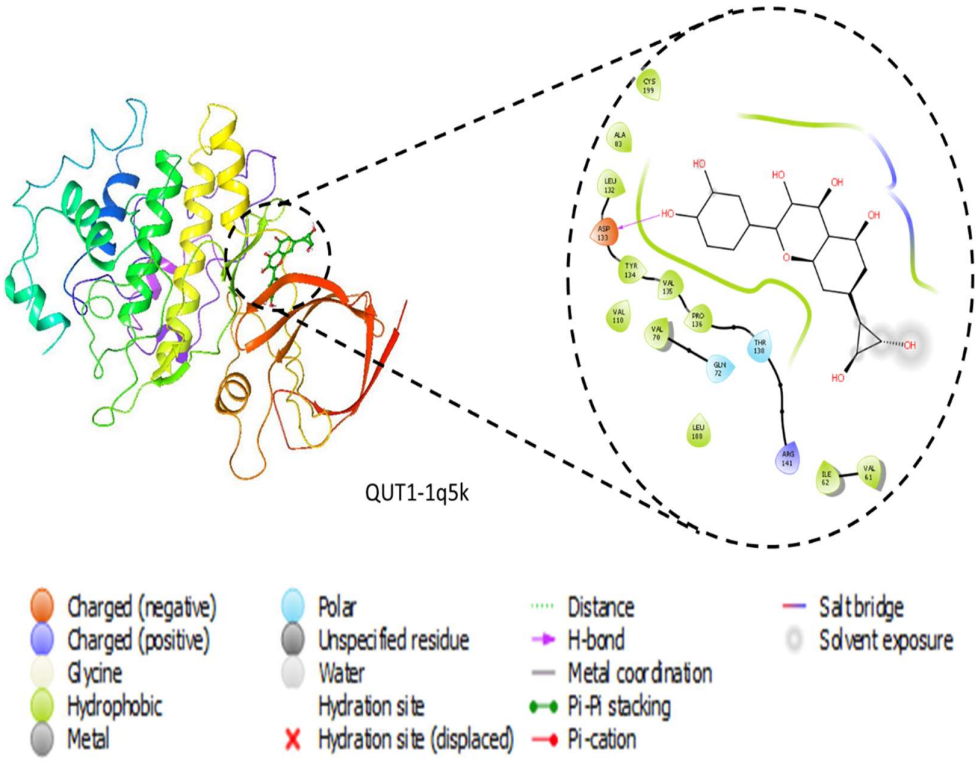


Fig. 3. The 3D X-ray crystal structure of 1q5k complex with QUT1 showing also the binding site region and the residues that constitute this binding site region.

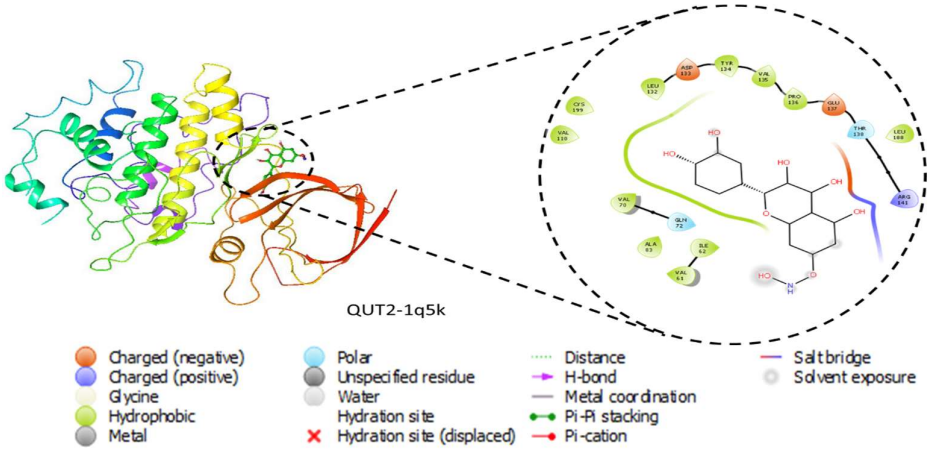


Fig. 4. The 3D X-ray crystal structure of 1q5k complex with QUT2 showing also the binding site region and the residues that constitute this binding site region.

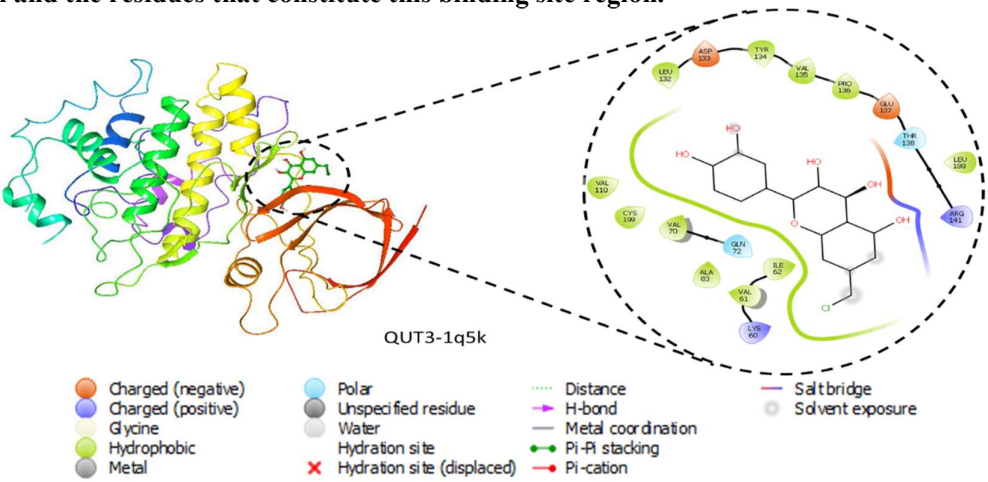


Fig. 5. The 3D X-ray crystal structure of 1q5k complex with QUT3 showing also the binding site region and the residues that constitute this binding site region.

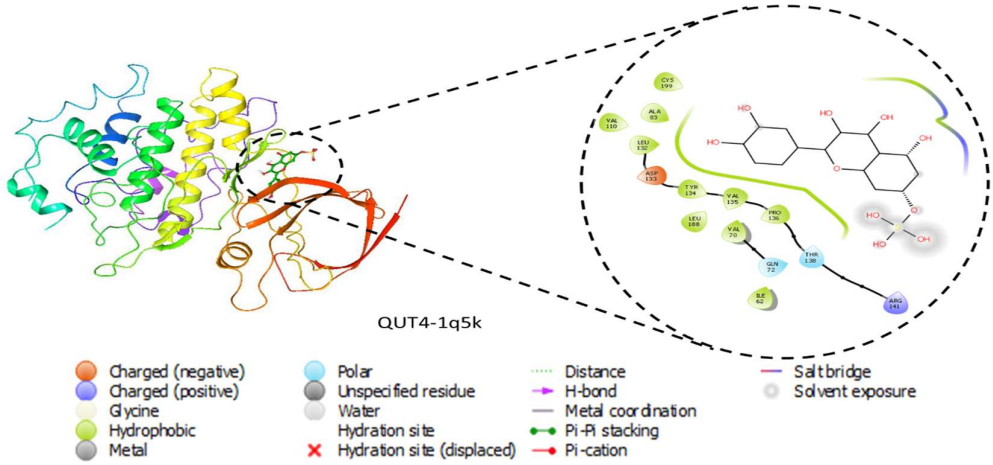


Fig. 6. The 3D X-ray crystal structure of 1q5k complex with QUT4 showing also the binding site region and the residues that constitute this binding site region.

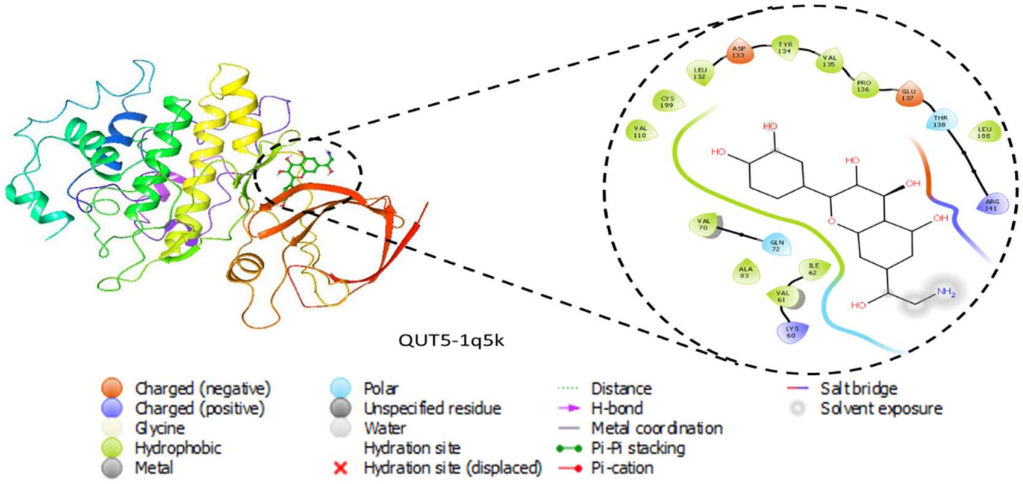


Fig. 7. The 3D X-ray crystal structure of 1q5k complex with QUT5 showing also the binding site region and the residues that constitute this binding site region.

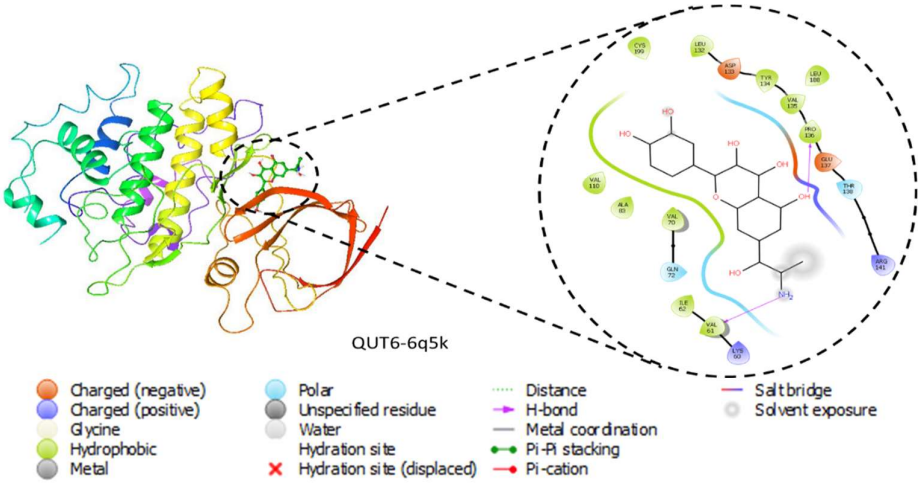


Fig. 8. The 3D X-ray crystal structure of 1q5k complex with QUT6 showing also the binding site region and the residues that constitute this binding site region.

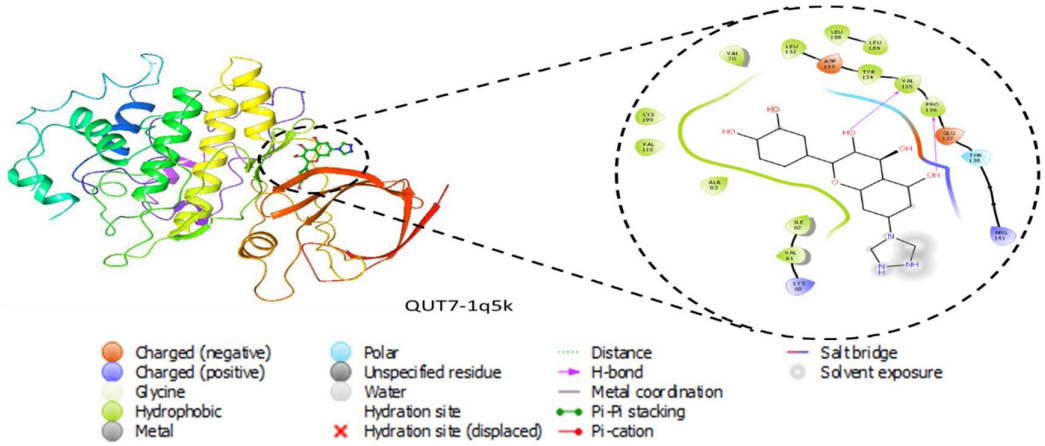


Fig. 9. The 3D X-ray crystal structure of 1q5k complex with QUT7 showing also the binding site region and the residues that constitute this binding site region.

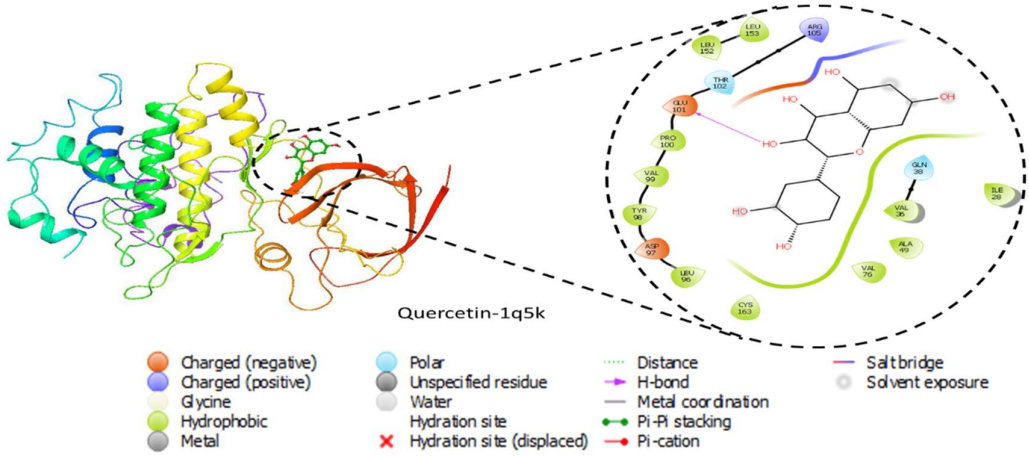


Fig. 10. The 3D X-ray crystal structure of 1q5k complex with quercetin showing also the binding site region and the residues that constitute this binding site region.

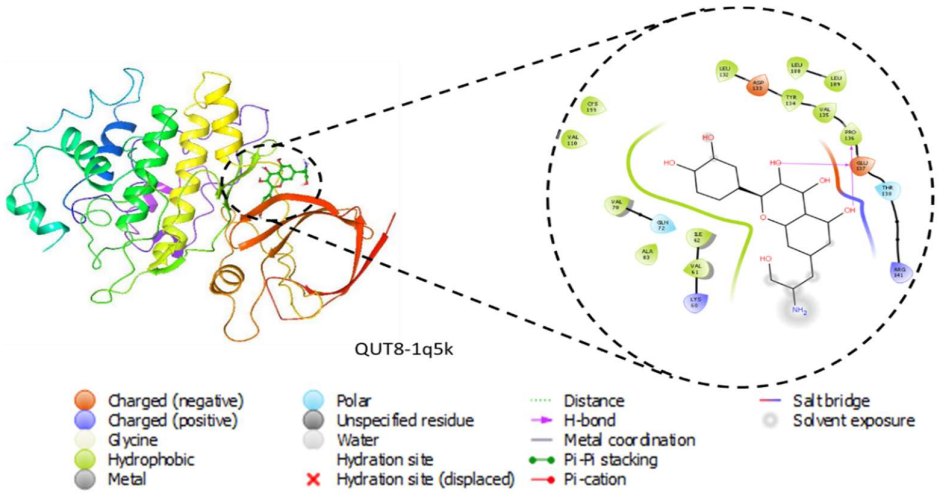


Fig. 11. The 3D X-ray crystal structure of 1q5k complex with QUT8 showing also the binding site region and the residues that constitute this binding site region.

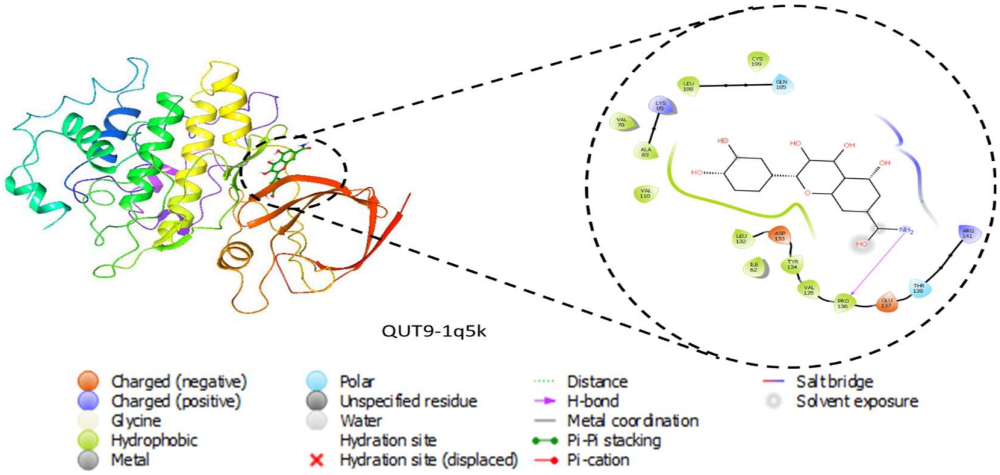


Fig. 12. The 3D X-ray crystal structure of 1q5k complex with QUT9 showing also the binding site region and the residues that constitute this binding site region.

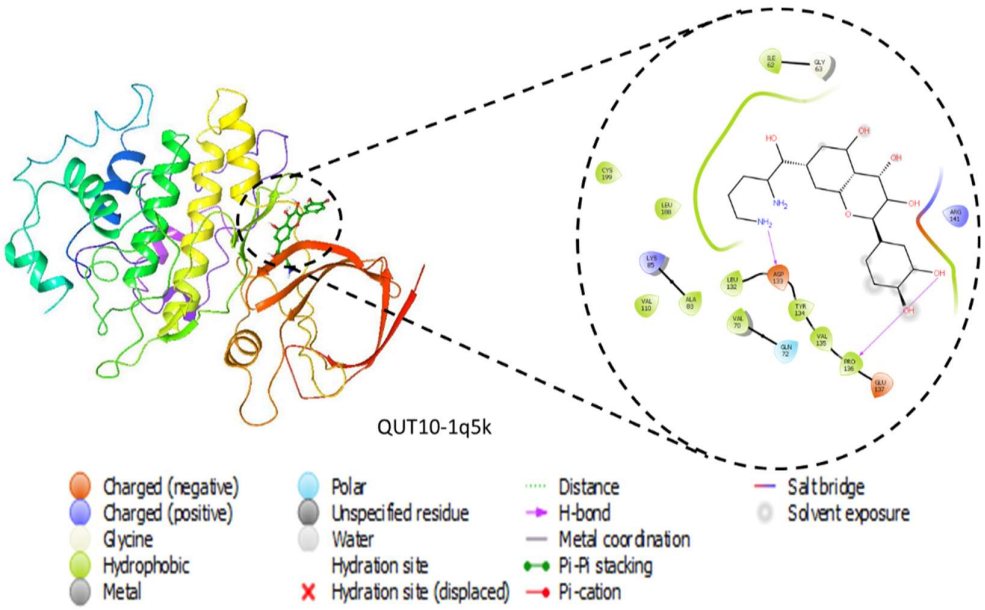


Fig. 13. The 3D X-ray crystal structure of 1q5k complex with QUT10 showing also the binding site region and the residues that constitute this binding site region.

3.2 ADME assessment of potential glycogen synthase kinase-3 inhibitors

The bioavailability radar of 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one displayed the physicochemical space (coloured zone) of QUT1 needed to predict its pharmacokinetics and drug-likeness characteristics of the lead molecule (Fig 14).

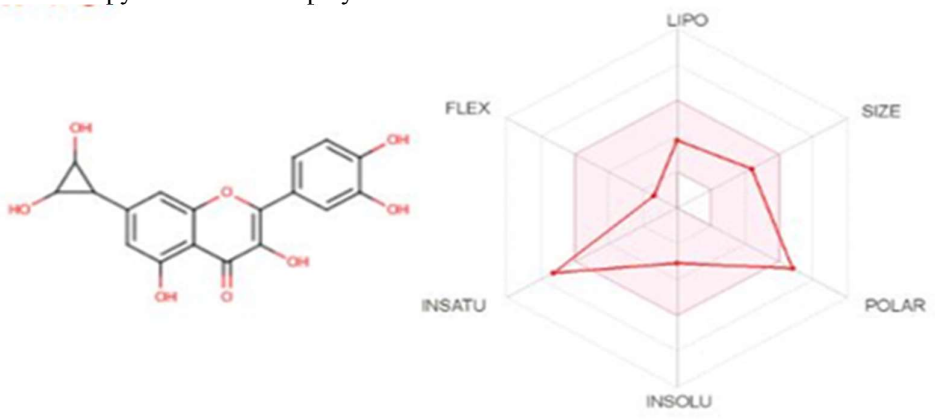


Fig. 14. The bioavailability radar of QUT1 using Swiss ADME predictor.

The absorption, distribution, metabolism and excretion (ADME) prediction of 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one was achieved using SWISSADME online webserver. The physiochemical properties of the lead molecule (7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) include: 26 heavy atoms, 8 hydrogen bond acceptors, 6 hydrogen bond donors, molar refractivity of 90.80and topological polar surface area (TPSA) of the molecule is found to be 151.59 Å^2. However, the lipophilicity of the lead molecule includes: iLOGP is 1.99, XLOGP3 is 1.11, WLOGP is 1.10, MLOGP is -0.88, SILICOS-IT is 1.19 and Consensus P0/W is 0.90. The egg's yolk molecule falling model was used to describe pharmacokinetic data obtained (see Fig 15). There was no noticeable blood-brain barrier permeant and the gastrointestinal absorption (GI) was low. Table 2, showed the solubility nature of 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one in different solvent fractions. From Table 3, it is evident that CYP 1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isoenzyme were not inhibited by 7-(2,3-

dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one. This shows that the lead molecule may not cause drug-drug interactions. Hence, the possibility of metabolites accumulation within the biosystem is eliminated.

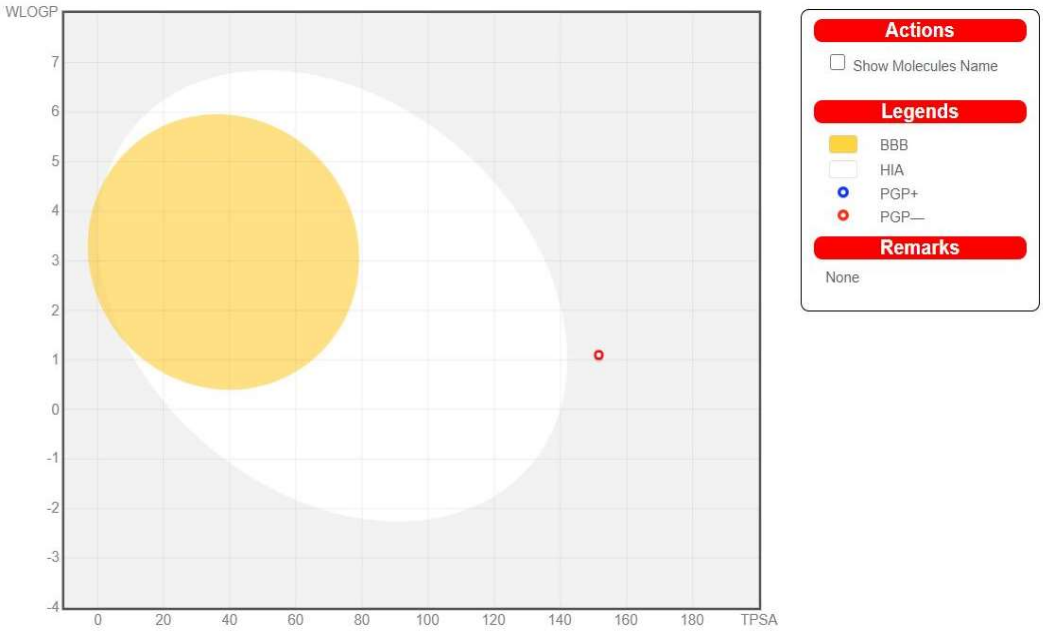


Fig. 15. Molecule falling in egg's yolk prediction QUT1.

Table 2: Water solubility of QUT1.

Log *S* (ESOL)		-3.08
	Solubility	$2.95e^{-01}$ mg ml^{-1}; $8.24e^{-04}$ mol ml^{-1}
	Class	soluble
Log *S* (Ali)		-3.89
	Solubility	$4.65e^{-02}$ mg ml^{-1}; $1.30e^{-4}$ mol ml^{-1}
	Class	soluble
Log *S* (SILICOS-IT)		-2.77
	Solubility	$6.06e^{-01}$ $mgml^{-1}$; $1.69e^{-03}$ mol ml^{-1}
	Class	soluble

Table 3. Pharmacokinetics of QUT1.

GI adsorption	Low
BBB permeant	No
P-gp substrate	No
CYP 1A2	No
CYP2C19	No
CYP2C9	No
CYP2D6	No
CYP3A4	No
Log Kp (skin permeation)	-7.70 cm s^{-1}

The lead molecule (7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one) obeys Lipinski's rules Ghose score with no violation. On the contrary, Egan Veber and Muegge score rule was not obeyed with a violation. But, a bioavailability score of 0.55 was obtained.

Table 4. Druglikeness of QUT1.

Lipinski	Yes, 0 violation: NH or OH >5
Ghose	Yes
Veber	No; 1 violation TPSA >140
Egan	No; 1 violation TPSA >131.6
Muegge	No; 1 violation TPSA >150. H-don>5
Bioavailability score	0.55

4.0 Conclusion

The inhibition of glycogen synthase kinase-3β protein with quercetin and its analogue was successfully studied using pharmacophore modelling and molecular docking techniques. 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one was observed to have the highest minimum energy and was selected as the lead molecule. Further *in silico* ADME assay of the lead molecule, presented 7-(2,3-dihydroxycyclopropyl)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4H-1-benzopyran-4-one as a probable drug candidate for any disease in which synthase kinase-3β plays a key role in its cell replication.

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6.0 References

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