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Research article

VIRTUAL SCREENING AND MOLECULAR DOCKING STUDIES OF QUERCETIN AGAINST *BLUETONGUE VIRUS* PROTEINS

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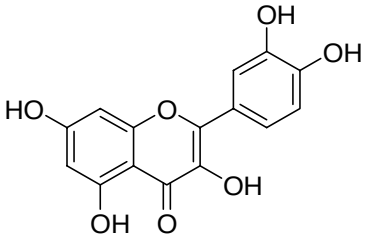
ABSTRACT: Bluetongue is major infectious disease ruminants caused by *Bluetongue virus* (BTV) is an icosahedral, non-enveloped virus belongs to the genus *Orbivirus,* family *Reoviridae* that contains 10 dsRNA genome segments within three concentric protein shells. For this reason, regulatory veterinarians have heightened their interest in this devastating disease. In the present study, we identified the action of Quercetin on 2BTV, 2JH8 and 1BVP proteins obtained from Protein Data Bank using docking studies and quercetin structure retrieved from Pubchem. The active sites were predicted in BTV proteins with CASTP server. Using protein structure, a flexible Docking study was performed between Quercetin and theoretically predicted active sites. The results indicated that amino acids are Arg98, Ser 97, Gln96 and Glu83 present in 2BTV and Thr10, Tyr11, Glu36, Ile23 1BVP proteins are core important for binding studies and these residues are having strong hydrogen bond interactions with Quercetin. We have investigated compound interactions and scoring parameters using GOLD docking package. From the docking studies, we also suggest that Arg98 in 2BTV protein domain and Thr10, Tyr11 were important residues in binding interactions. ADMET server predicted the Pharmalogical studies of quercetin, which shows the acceptable results.

Key words: Quercetin, Bluetongue virus, Molecular docking studies, ADMET studies

INTRODUCTION

Bluetongue is a major infectious disease of ruminants caused by an arbovirus (Bluetongue virus, BTV) transmitted by biting midges (*Culicoides spp.*) [1]–[3]. Historically, bluetongue has been endemic almost exclusively in temperate and tropical areas of the world where the climatic conditions favor both the spread of the susceptible insect vector population and the virus replication cycle within the vector [4]. However, in the last decade BTV has spread extensively in several geographical areas including Southern Europe and also, unexpectedly, in Northern Europe causing a serious burden to both animal health and the economy [5], [6]. From a molecular and structural virology perspective BTV is one of the best understood animal viruses. BTV is a member of the *Orbivirus* genus, within the *Reoviridae* family, and possesses a double-stranded RNA genome formed by 10 segments (Seg-1 to Seg-10) of approximately 19200 base pairs in total [1], [3]. Until now, the BTV genome has been shown to encode for 7 structural and 3 non-structural proteins. The BTV genome is packaged within a triple layered icosahedral protein capsid of approximately 90 nm in diameter [1], [7]–[10]. The outer capsid of the virion is composed by 60 trimers of VP2 and 120 trimers of VP5 [11] and differences within this outer capsid define the 26 BTV serotypes which have been described so far [12], [13]. The outer capsid proteins and VP2 in particular, stimulate virus neutralizing antibodies which in general protect only against the homologous serotype [14]. The internal core is formed by two layers, constituted by VP3 (sub-core) and the immunodominant VP7 (intermediate layer) [7]. Three minor enzymatic proteins, VP1 (RNA dependent RNA polymerase), VP4 (capping enzyme and transmethylase) and VP6 (RNA dependent ATPase and helicase) are contained within the core that is transcriptionally active in infected cells [15]–[21].

Quercetin is the most abundant bioflavonoid and this compound is mainly present in the glycoside form. The bioavailability of quercetin aglycone and its glycosides are different [22] because of different physical and chemical properties. Flavonoids are polyphenolic compounds that usually exist in plants as secondary metabolites. They show strong antioxidative activity as well as other potential effects, including anti-inflammatory, anti-cancer, and anti-viral roles [22]. Flavonols constitute a major group within the flavonoids present in several foodstuffs, such as apples, cherries, and other green vegetables. The most commonly occurring flavonols are those with dihydroxylation in the 3' and 4' positions of the B ring. The bulky glycoside moiety causes a conformational change of the quercetin backbone [23] and modifies the accessibility to active site of the protein. Flavanoids have anti-cancer activity through act as inhibitory agents of P-glycoproteins [24].



In the present study, the interactions between BTV proteins and quercetin were investigated by molecular docking study to predict the binding affinity of quercetin to BTV proteins. Quercetin is effective inhibitor to proteins, and the inhibitory mode belongs to a competitive type.

MATERIALS & METHODS

Docking studies of Quercetin was performed. The structure of the compound was constructed and optimized using chemsketch software. To prepare the bluetongue virus proteins structure, the crystal structure was taken from the Protein Data Bank and PDB_IDs are 2BTV, 2JH8 and 1BVP. From the proteins the chain A was selected for docking studies. Hydrogen atoms were added to the enzyme. The molecular docking method was performed using the Gold version 3.0.1 program to study the binding orientation of compounds into the proteins structure. The docking experiments were performed using the binding site of proteins.

Active site Identification

The binding site identification was carried out using CastP (servers*ts-fw.bioengr.uic.edu/**castp**/calculation.php)* . A new program, CAST, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CAST identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings**.**

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compound was docked to the active site of the VP3, VP4 and VP7 proteins of BTV. The interaction of the compound with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of ligand was observed and their interactions with the protein were studied. The best and most energetically favorable conformation of ligand was selected.

Gold Score fitness function:

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)
Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

ADMET and Drug-likeness studies

The absorption, distribution, metabolism, excretion and toxicity (ADMET) are the most important part of pharmacological studies of the concerned molecule required for drug based discovery. Pre-ADMET is the tool that provides drug-likeliness, ADME profile and toxicity analysis for the ligand. It uses Caco2-cell (heterogeneous human epithelial colorectal adenocarcinoma cell lines) and MDCK (Madin-Darby Canine Kidney) cell models for oral drug absorption prediction and skin permeability, and human intestinal absorption model for oral and trans-dermal drug absorption prediction. Distribution is predicted using BBB (blood brain barrier) penetration and plasma protein binding.

RESULTS AND DISCUSSION

After collecting the crystal model, the possible binding sites of proteins was searched with CASTP server and was shown in Figure 1. From the binding site analysis of proteins we identified that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies. The crystal structures of proteins are similar and we have therefore taken A chains of 2BTV, 2JH8 and 1BVP as representative structure for docking studies. The docking of compounds into the active site of proteins was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function.

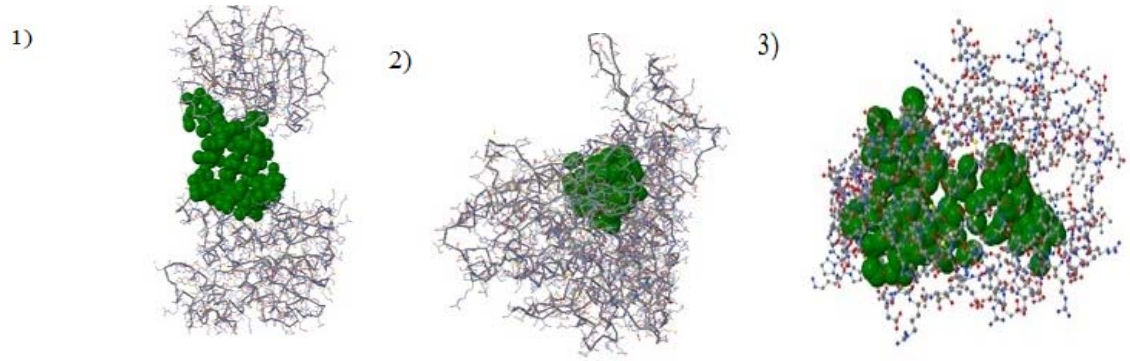


Figure 1, 2 & 3: active sites of 2BTV, 2JH8 & 1BVP respectively

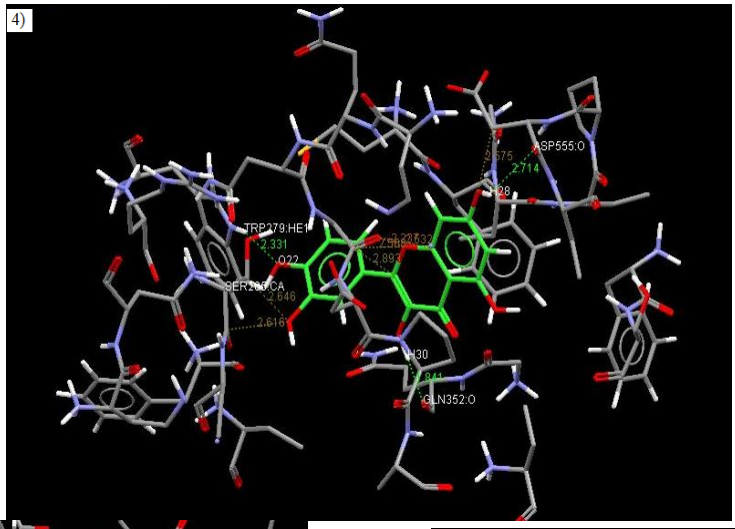
Molecular docking analysis

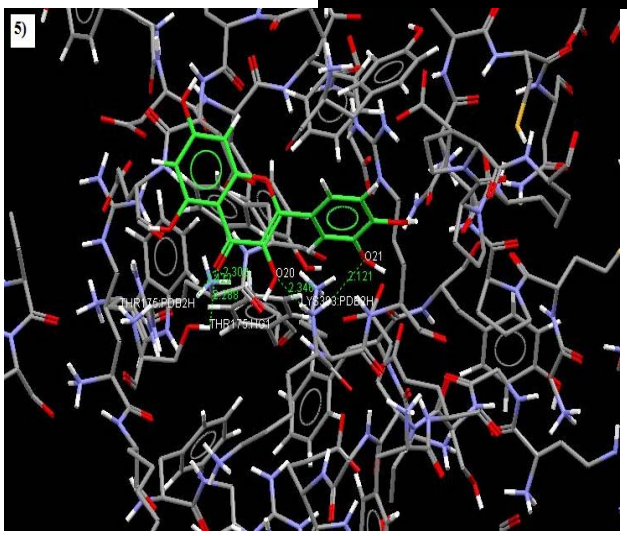
The selected docked conformations of quercetin in the protein binding site showed in figures 4, 5, and 6. The docked conformations revealed that ligand was located in the hydrophobic binding pocket surrounding the binuclear copper active site. In this study, all docked ligand was found to have some interaction between an oxygen and hydrogen atoms of the ligand and proteins. Moreover, these docked conformations also formed an H-bonding interaction with in the active site. The atoms of compounds and proteins involved in bonding and their bond lengths along with docking energies were indicated in table 1.

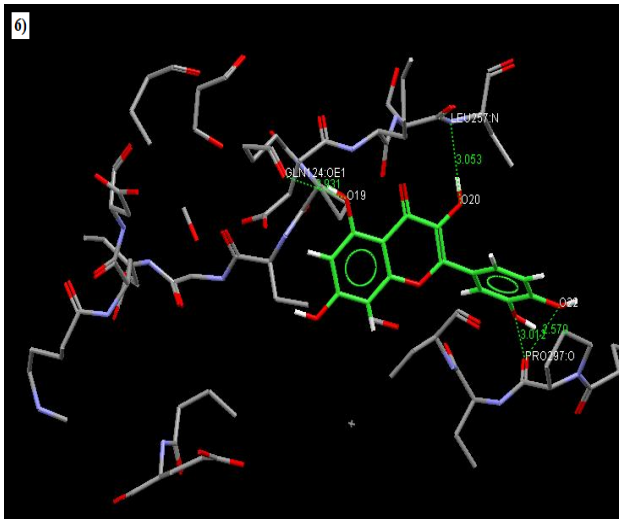
Table 1: Molecular Docking studies of Quercetin

Compound	Proteins	No.of.H_2 bonds	Atoms of compound	Bonding length (A^0)	Amino acids of proteins	Docking score (Kcal)
Quercetin	VP3	1	19(OH)	4.6	Arg98,Thr95,Ser97 Glu71,Leu175	32.703
	VP4	2	19(OH) 21(OH)	2.25 1.43	Ile17,Asp26,Glu10 3,Met91,His16	35.238
	VP7	2	19(OH) 22(OH)	1.04 2.5	Thr10(HG1),Tyr11 (O), Glu36, Ile23	41.064

In binding pocket of VP3 protein, common H-bond interactions were formed between quercetin and THR95, SER97, GLU71, ARG98, and LEU17. The specific H-bonding interaction with ARG98 only was found in the docked conformation of quercetin. In Figure 4, strong H-bonding interactions in the hydroxyl group (OH19) of quercetin and an oxygen atom of ARG98 active site of VP3. In the case of VP4 docked with quercetin, H-bonds with ASP10 and MET91 were formed. Hydrogen bonding interactions between hydroxyl group (OH19) of quercetin and oxygen atom of enzyme residue ASP20 was observed and another H-bond formed between hydroxyl group (OH21) and hydroxyl group of protein residueLEU23 and molecular interactions shown in figure 5. From the docking of quercetin into the active site of VP7, we observed hydrogen bond between the Hydroxyl group (OH22) of quercetin and oxygen atom of TYR16 and another hydrogen bond between hydrogen atom of THR10 and hydroxyl group (OH19) of quercetin.







Figures : 4) molecular interactions of quercetin and VP3 protein, 5)molecular interactions of quercetin and VP4 protein, 6) molecular interactions of VP7 protein

ADME and Druglikenes studies

The three dimensional coordinates of Quercetin was retrieved from PubChem. The drug likeness results were obtained using with the PreADMET tool results were shown in table 2.

Table 2: The drug likeness and General properties of Quercetin using PreADMET tool.

S.No	Properties	Quercetin
1	Liponski rule of five	Suitable
2	Lead like Rule	Suitable
3	CMC like Rule	Qualified
4	MDDR like Rule	Midstructure
5	WDI like Rule	In 90% cutoff
6	Solubility	-1.95
7	Molecular weight	304.0
8	Drug likeness	2.03
9	nON	7
10	nOHNH	5

The PreADMET tool provided information about the absorption, distribution, metabolism, and excretion details about the quercetin and was found to be in acceptable limits, Table 3.

Table 3: ADME studies of Quercetin using the PreADMET tool

S.No	ADME studies	Quercetin
1	Human Intestinal Absorption	Moderate
2	Caco2 Cell Permeability	Middle
3	MDCK Cell Permeability	Low
4	Skin permeability	-4.43341
5	Blood Brain Barrier Penetration	Middle

CONCLUSION

The anti-viral activity of the quercetin was agreed with the molecular docking results, PreADMET, Drug-likeness and Bioactivity studies. The docking study revealed the binding orientation of compounds in the VP3, VP4 and VP7 binding pocket surrounding the active site, which resulted in inhibition of proteins activity. From the results we can conclude that quercetin is the one of the inhibitory compound of bluetongue virus proteins.

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