MOLECULAR DOCKING APPROACH ON CAPSAICIN TO REGULATE BRAIN CHOLESTEROL AN *INSILICO* STUDY

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ABSTRACT:

Red Chilli *(Capsicum annum)* is important as a spice, flavour enhancer, vegetable and component in herbal medicine. The numerous phytochemicals and their medicinally important properties present in diverse germplasm of chilli pepper have been characterized and documented. Capsaicinoids, carotenoids, vitamins, flavonoids such as anthocyanins are present as the major phytochemicals in chilli pepper fruits. Capsaicinoids, pungent analogues of capsinoids, are the most important group of phytochemicals in which capsaicin and dihydrocapsaicin are prominent in providing the basis for pungency and medicinal properties. This study analysed Molecular docking of Pubchem retrieved 2D structures of selected phytocompounds present in *Capsicum annum* with 3D crystal structure of *Cholesterol 24-hydroxylase* protein retrieved from PDB. The human brain accounts for 2% of the whole body mass, yet it contains approximately 25% of the total cholesterol of the body. Cholesterol is a major lipid constituent of the myelin sheath and the membrane lipid rafts in neurons and astrocytes. It has an important role in brain development and neuronal function by regulating cell signaling pathways, gene transcription, and avail- ability of bioactive steroids. Cholesterol synthesis and metabolism in the brain involve complex interactions between astrocytes and neurons. Abnormalities in cholesterol metabolism in the CNS occur in several neurologic disorders, including Niemann-Pick type C and Smith-Lemli-Opitz syndrome. Brain cholesterol has also been implicated in the pathogenesis of Alzheimer disease (AD) and Hun-tington disease. The Docking was done by schrodinger Maestero 12.1 software tool, Capsaicin compound of Capsicum annum having best binding score than the other compounds present in it. Hence it has been concluded Capsaicin can be used in regulation of Brain Cholesterol.

KEYWORDS: *Capsicum annum, Capsaicin, Cholesterol 24-hydroxylase, Brain Cholesterol,* Maestero 12.1, Glide.

INTRODUCTION:

Cholesterol is an integral component of cell membranes, comprising anywhere from 10 to 45% of the lipid bilayer of mammalian cells [1] Cholesterol is a multifaceted molecule. First, it serves as an essential membrane component, as a cofactor for signalling molecules and as a precursor for steroid hormones. Second, its synthesis intercellular transport and intracellular distribution present a logistic force requiring hundreds of cellular components, and third, it plays a crucial role in major human diseases. Despite intense research on this molecule, its metabolism in the central nervous system and its role in neuronal development and function are not well understood. The human brain accounts for 2% of the whole body mass, yet it contains approximately 25% of the total cholesterol of the body. Cholesterol is a major lipid constituent of the myelin sheath and the membrane lipid rafts in neurons and astrocytes. It has an important role in brain development and neuronal function by regulating cell signaling pathways, gene transcription, and availability of bioactive steroids. Although an immense knowledge has accumulated concerning regulation of cholesterol homeostasis in the body, this does not include the brain, where details are just emerging. Approximately 25% of the total amount of the cholesterol present in humans is localized to this organ, most of it present in myelin. Almost all brain cholesterol is a product of local synthesis, with the blood-brain barrier efficiently protecting it from exchange with lipoprotein cholesterol in the circulation[2-6]. Accumulating evidence indicates that neurodegeneration and development of neurological disorders such as Alzheimer's disease (AD) are associated with disturbances in cholesterol homeostasis in the brain [7-12]. Hot peppers, which belong

to the plant genus *Capsicum*, are widely grown for their fruits, which may be eaten fresh (salads, baked dishes, salsa, pizzas, etc.) or cooked, used as a dried powder, or processed into oleoresins. Paprika oleoresin, a viscous, dark red liquid, is prepared industrially by solvent extraction (most commonly employed is hexane) of the dried fruit and the subsequent removal of the solvent[13-15] .The GC/MS analysis revealed the presence of 43 compounds in acetone extract of 'Adorno' cultivar and 33 compounds in acetone extract of 'Etna' cultivar. The active 7 compound from above mentioned compounds were shortlisted for studies to regulate Brain cholesterol inhibiting with crystal structure of cytochrome P450.

MATERIALS AND METHODS:

All the seven phytocompounds were retrived from pubchem data base. Present study deals with the usage of the above compounds in the activity to regulate brain cholesterol. Chemical structures were sketched in 2D sketcher in present software in Structure Data Format (SDF). The docking studies were performed with standard precision (SP) Glide, and extra precision (XP) Glide and MGBSA Prime in Schrodinger software.

Qikprop study:

The minimized ligands (R1-R7) were run in Qikprop tool of Schrodinger glide, which states the similarity between selected ligands and standard drugs and also the ADMET properties[16] of the ligand, on the basis of absorption, distribution, metabolism, elimination and toxicity (ADMET) are important aspects of drug molecules. Which covers the physicochemical properties of drugs, the pH and solubility, and the approaches to improving aqueous solubility as well as the drug metabolism and drug/drug interactions, followed by recent development on databases particularly related to the ADMET profiling and prediction.

***Preparation of Protein*:**

X-ray crystalline Structure of protein 2Q9F (Crystalline structure of cytochrome p450) was imported from Protein Data Bank (PDB) to workspace. Protein was prepared with the Protein Preparation Wizard in Maestro12.1 using default options, bond orders were assigned, hydrogens were added, metals were treated, and water molecules 5 A˚ beyond hetero groups were deleted, which further set to preprocess followed by review and modify to remove unwanted chains and residues, further refined under forcefield of OPLS3e. The results were monitored in job monitor.

Preparation of Ligands:

Structures of ligands sketched and saved in SDF format were imported via selecting file. The imported ligands (R1 – R7) were set to minimize under force field OPLS3e. Minimization calculations can be performed on all structures Nonivamide, Nordihydrocapsaicin, Homocapsaicin, Homodihydrocapsaicin, N-Vanillyldecanamide, capsaicin, Dihydrocapsaicin .

Molecular Docking: As for Glide docking, crystal structures of 2Q9F should be prepared by the protein preparation wizard in Schrodinger suite. Afterwards, receptor grids were generated before docking with the active site determined by the position of co crystal ligand. Crystal structures of 2Q9F were imported into Glide, defined as the receptor structure and the location of active site with a box. The OPLS3e force field was used for grid generation [17-18]. The standard precision (SP) and the extra precision (XP) protocols were set for docking studies with crucial residues, in constrained binding to get accurate results. Binding affinity was retrieved running Prime MM-GBSA. All other parameters were maintained as default. Docking programs have proven relatively successful in accurately reproducing known poses of drug-like molecules from co-crystal structures, with Glide consistently performing among the top of the programs[19].

RESULT AND DISCUSSION:

Validating active group of ligands (R1-R7): Nonivamide, Nordihydrocapsaicin, Homocapsaicin, Homodihydrocapsaicin, N-Vanillyldecanamide, capsaicin, Dihydrocapsaicin .

The 2D structures (R1-R7) of were run in Qikprop tool of Schrodinger Glide software to proceed for further elucidation.

Qikprop Analysis:

All 7 compounds show good affinity towards ADMET properties and falls under the expected range.

Table 1

Title	QPlogPo/w	QPlogHERG	QPPCaco	QPlogBB	QPPMDCK	QPlogKp	QPlogKhsa	PercentHuman OralAbsorption
R1	3.332	-4.045	954	-1.049	723	-1.738	0.020	100
R2	3.248	-4.009	803	-1.074	595	-2.003	0.054	100
R3	3.580	-4.372	668	-1.171	535	-2.023	0.195	100
R4	3.642	-4.138	923	-1.115	647	-1.829	0.165	100
R5	3.694	-4.308	799	-1.245	594	-1.811	0.155	100
R6	3.974	-4.442	788	-1.196	601	-1.867	0.310	100
R7	4.006	-4.347	798	-1.250	597	-1.808	0.287	100

QPlogPo/w Predicted octanol/water partition coefficient. **–2.0 – 6.5**

QPlogHERG Predicted IC50 value for blockage of HERG K+ channels. concern **below –5**

QPPCaco Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport**.<25 Poor, >500 great**

QPlogBB Predicted brain/blood partition coefficient. Note: QikProp predictions are for orally delivered drugs so, for example, dopamine and serotonin are CNS negative because they are too polar to cross the blood-brain barrier **–3.0 – 1.2**

QPPMDCK Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier. QikProp predictions are for non-active transport. **500 great**

QPlogKp Predicted skin permeability, log Kp. **–8.0 – –1.0**

QPlogKhsa Prediction of binding to human serum albumin. **–0.5 – 1.5**

PercentHumanOralAbsorption Predicted human oral absorption on 0 to 100% scale.The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with HumanOralAbsorption, as both measure the same property. **<25% is Low.**

Molecular Docking:

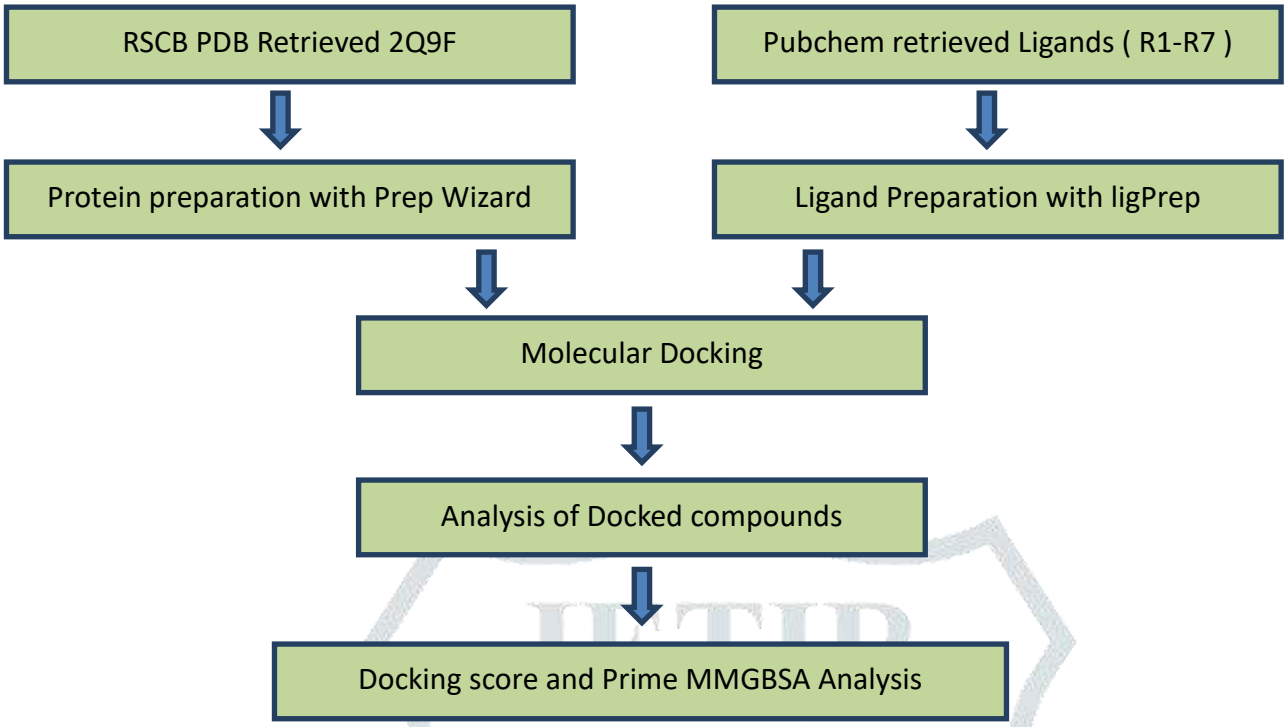


Figure 1(Schematic representation of Molecular docking of R1-R7 ligands with 2Q9F protein

To date, seven structures of ligands have been determined. Meanwhile, these ligands were used for docking to measure the docking conformations. Three different docking programs SP Glide, and XP Glide, Prime MM-GBSA were used for improving the accuracy of prediction. Then, Xscore followed by molecular docking was reliable and accurate for forecasting protein-ligand binding free energies. The docking results were evaluated by comparing values of score energy, SP Glide, XP Glide, and Binding energy. Through analysis of these results of docking simulations, most binding energy scores could accurately forecast the ligand activities. The lowest binding energy and the highest docking score demonstrated that these compounds (ligands) presented well favorable interactions. The docked ligands R1,R2,R3,R4,R5,R6,R7 showed the best range of Docking score, XP Gscore and Binding energy.(Table 1).

Title	***Compounds***	***Docking score***	***Glide gscore***	***XP GScore***	***MMGBSA dG Bind***
R1	***Nonivamide***	***-6.119***	***-6.119***	***-6.119***	***-48.62***
R2	***Nordihydrocapsaicin***	***-6.384***	***-6.384***	***-6.384***	***-40.31***
R3	***Homocapsaicin***	***-7.821***	***-7.821***	***-7.821***	***-47.89***
R4	***Homodihydrocapsaicin***	***-6.909***	***-6.909***	***-6.909***	***-45.63***
R5	***N-Vanillyldecanamide***	***-7.406***	***-7.406***	***-7.406***	***-53.84***
R6	***Capsaicin***	***-7.892***	***-7.892***	***-7.892***	***-59.59***
R7	***Dihydrocapsaicin***	***-7.387***	***-7.387***	***-7387***	***-37.14***

Table 2

Diagrammatic representation of Binding energy and Docking score

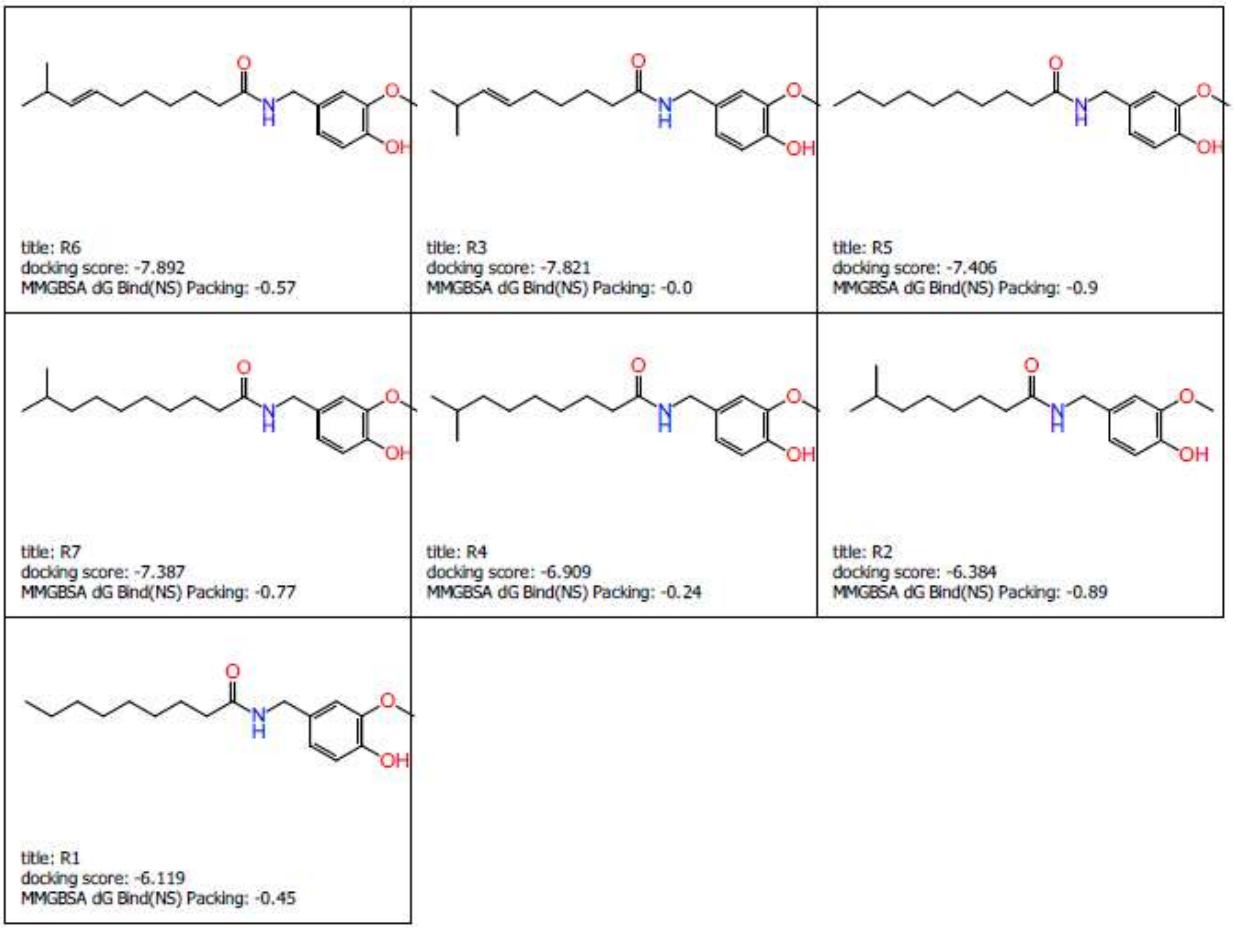


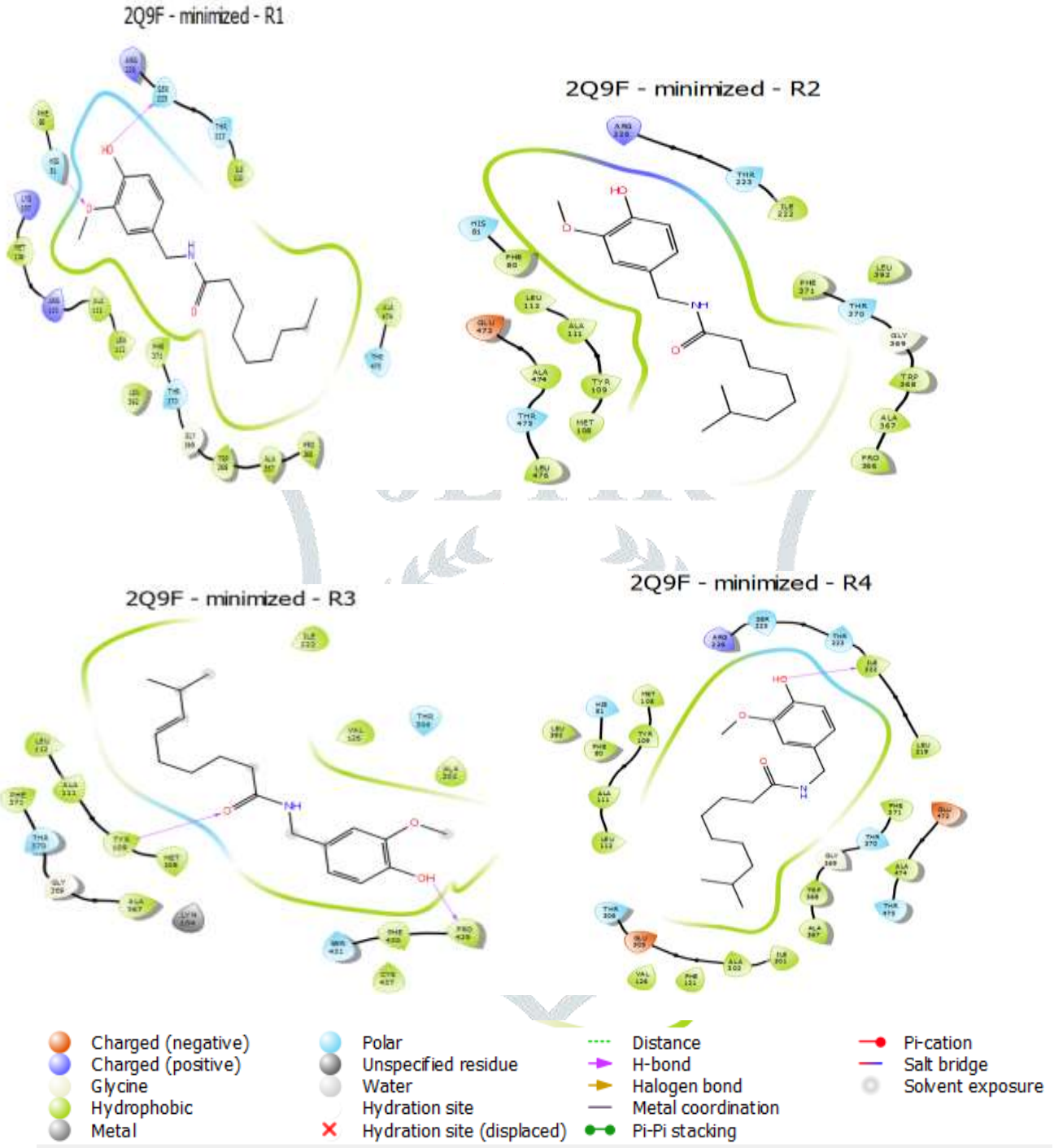
Figure 2

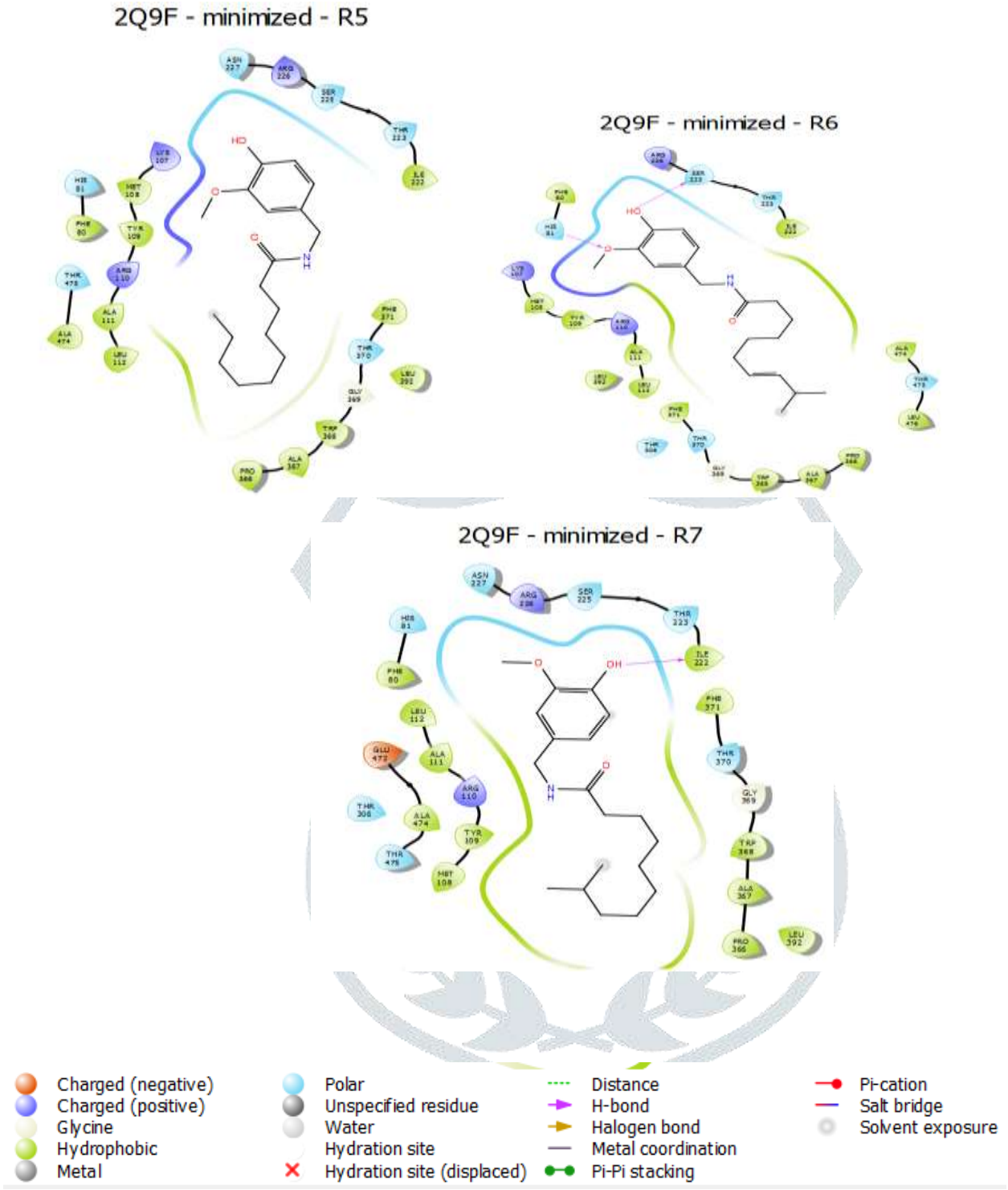
Inhibitor Binding Analysis:

The least binding energy and the most rational binding pattern between the inhibitors and 2Q9F were selected by the three docking protocols. As expected, Capsaicin (compound R6) bound in the active site validating the prediction by molecular docking with 2Q9F. Among the set, top compounds were selected, which represented good interactions with the target protein (Figure 2). From the docking results, Capsaicin (R6) shown interaction with SER 225,HIS 81 which had two Hydrogen bond interactions. Viz rest six compound show only one Hydrogen bond.

2D representation of Docking Analysis

Figure 3





CONCLUSION:

As a result of this computational experimental study of seven competitive ligands of *Capsicum annum* and 2Q9F. To identify the docking accuracy about this target, docking simulation were evaluated. Interestingly, these docking results showed good interactions for all seven inhibitors. Docking results were merged, which allowed us to weigh different binding patterns in the active sites. In a word, we identified that seven hydrogen bond acceptors and heterocyclic rings with Methoxy and -OH, were essential anchoring points in Capsaicin (R6) played a pivotal role in binding affinity. This provides lowest energy ligands, docked into the target pocket with best possible pose. The compound Capsaicin are quantified using the docking score to act regulating Brain cholesterol, The study is conducive for designing an accurate drug for treating Brain related disorder and in maintaining the Brain cholesterol.

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