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IN SILICO PREDICTIVE STUDIES OF HISTAMINE H2 RECEPTOR PROTEIN BINDING USING HOMOLOGY MODELLING AND MOLECULAR DOCKING

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ABSTRACT: The present study investigated the 3-D protein model of Histamine H2 Receptor and docking studies with natural flavonoids compounds. The model was generated based on suitable crystal structure as a template. The modelled protein exhibited 96.1% (317 aa) of amino acid residues in the most favored region and molecular docking studies were performed by using natural flavonoids. Almost all the molecules exhibited lower binding energies and showed good interactions with modelled protein. Compound Cratoxyarborenone B showed binding energy of -9.24 Kcal/mol with interacting Gly258, Thr164, Val178 and Arg257. Three molecules exhibited highest affinity towards modelled protein.

KEYWORDS: Histamine H2 Receptor, Homology Modeling, Flavonoids, Docking.

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1. INTRODUCTION

Histamine plays a major role in a variety of pathological conditions and it has a major influence on secretion of gastric acid.[1] It has almost been a hundred years since Sir Henry Dale and his colleagues isolated histamine from mold ergot.[2] Histamine shows a restorative effect on smooth muscles from various organs such as gut, respiratory tract, tonic cardiac contradictory and shock like symptoms when injected into animals.[3] Histamine is a member of G protein-coupled receptors (GPCRs) because it exerts physiologic action by binding to the super family of seven transmembrane GPCRs.[4] Histamine binds to three specific histamine receptors H1, H2 and H3 receptors distinguished by their selective antagonistic drugs.[5] Because of its omnipresent expression and its capacity to activate multiple signaling pathways, H2R controls diverse cellular

functions, inclusive of innate and adaptive immune responses.[6] Histamine stimulates the condition of smooth muscle from various organs, such as the gut and bronchi, and this effect is suppressed by mepyramine, a typical antihistamine drug.[7] Ranitidine is an antagonist of H2receptor-mediated responses to histamine in guinea-pig atrium and rat uterus in vitro and inhibits the gastric acid secretion in rats.[8] H2-receptor opponent is in vitro and can inhibit gastric acid secretion in vivo. This substance is N- {2-[[[5-(dimethylaminomethyl}2-furanyl]methyl]thio] ethyl-N1-methyl-2-nitro-1,1-ethenediamine (AH 19065), with the accepted name ranitidine. Ranitidine has been compared with metiamide and cimetidine to determine its activity as an H2-receptor opponent.[9] Histamine (2-[4-imidazole]-ethylamine) is present in many places with distributed biogenic monoamine. It is produced, stored, and secreted mainly by mast cells and basophils.[10] Drugs like ranitidine, cimetidine, nizatidine, famotidine show great importance for gastric acid regulation. But now-a-days it is clinically used as immune suppressants and also for central nervous system disorders.[11] The main function of H2R is to antagonize block H+ secretion in parental cells of stomach.[12] Among all histamine receptors H2R is the GPCR that can be mostly studied in human cells. Human neutrophils express only H2R but, in mRNA level it express both H2R and H4R. Where as in eosinophils it expresses both H2R and H4R. It is easy to obtain large number in buffy coat and in peripheral blood. [13] In neutrophils and eosinophils, H2R mediates an increase in cAMP formation and in the inhibition of superoxide anion (O_2^-) formation, chemotaxis, and release of cytotoxic enzymes. It was found that histamine H2 receptor was closely related to the development of various cardiovascular disease such as myocardial ischemia [14], hypertension [15], myocardial infarction [16] and congestive heart failure (CHF)[17]. H2RAs are cardio protective for CHF patients and are commonly used in treating peptic ulcers.[18] However, activation of H2RAs may lead to heart failure[19] and when there is a rise in plasma histamine levels at appropriative allergic reactions spontaneously, it leads to the asthmatic attacks in asthma patients[20]. In the present study, in silico studies were performed due to the absence of crystal structure for Histamine H2 receptor. The homology model of the protein was developed using Modeller9.21 and validated by using Procheck. To study the binding affinity of protein-ligand and molecular interactions of Histamine H2 receptor docking studies were performed using autodock4.2.

2. MATERIALS AND METHODS

Sequence alignment and structure prediction

The amino acid sequence of Histamine H2 receptor (Uniprot accession number: P25021) from the species Homo sapiens (Human) was retrieved from the UniProtKB database.[21] A BLAST (Basic Local Alignment Search Tool) search was performed to select the template. The Activated turkey BETA1 Adrenoceptor with bound agonist Isoprenaline and nanobody NB80 (PDB ID: 6H7J_A)[21] was selected. The three dimensional structure was generated using Modeller9.21.

The respective templates were retrieved from protein database like PDB[22]. When choosing the template, it is important to consider the sequence identity and resolution of the template. When both parameters are high the resulting model would be sufficiently good to allow structural and functional research.

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CLUSTAL O(1.2.4) multiple sequence alignment

sp|P25021|HRH2_HUMAN      MAPNGTASSFCLDSTACKITITVVLAVLILITVAGNVVVCLAVGLNRRLRNLTNCFIVSL  60
pdb|6H7J|A                ---------------AAAKVMSLLMALVVLLIVAGNVLVIAAIGSTQRLQTLTNLFITSL  45
                                        *.  .:::::*:::*: *****:*  *:* .:**:.*** **.**

sp|P25021|HRH2_HUMAN      AITDLLLGLLVLPFSAIYQLSCKWSFGKVFCNIYTSLDVMLCTASILNLFMISLDRYCAV  120
pdb|6H7J|A                ACADLVVGLLVVPFGATLVVRGTWLWGSFLCELWTSLDVLCVTASIETLCVIAIDRYLAI  105
                          * :**::****:**.*   :  .* :*..:*:::*****:  **** .* :*::*** *:

sp|P25021|HRH2_HUMAN      MDPLRYPVLVTPVRVAISLVLIWVISITLSFLSIHLGWNSRNETS--KGNHTTSKCKVQV  178
pdb|6H7J|A                TSPFRYQSLMTRARAKVIICTVWAISALVSFLPIMMHWWRDEDPQALKCYQDPGCCDFVT  165
                           .*:**  *:* .*. : :  :*.**  :*** * : *   :: .  *  :  . *.. .

sp|P25021|HRH2_HUMAN      NEVYGLVDGLVTFYLPLLIMCITYYRIFKVARDQAKRINHISSWK---AATIREHKATVT  235
pdb|6H7J|A                NRAYAIASSIISFYIPLLIMIFVYLRVYREAKEQIRKIDRASKRKTSRVMAMKEHKALKT  225
                          *..*.:...:::**:***** :.* *::: *::* ::*:: *. *   . :::****  *

sp|P25021|HRH2_HUMAN      LAAVMGAFIICWFPYFTAFVYRGLRGDDAINEVLEAIVLWLGYANSALNPILYAALNRDF  295
pdb|6H7J|A                LGIIMGVFTLCWLPFFLVNIVNVF-NRDLVPDWLFVAFNWLGYANSAMNPIIYCRS-PDF  283
                          *. :**.* :**:*:* . : . : . * : : * . . ********:***:*.    **

sp|P25021|HRH2_HUMAN      RTGYQQLFCCRLANRNSHKTSLRSNASQLSRTQSREPRQQEEKPLKLQVWSGTEVTAPQG  355
pdb|6H7J|A                RKAFKRLLAFPRKADRRLHHHHHH----------------------------------  307
                          *..:::*:.      .  :   :

sp|P25021|HRH2_HUMAN      ATDR  359
pdb|6H7J|A                ----  307
```

Figure 1: Sequence alingnment of Histamine H2 receptor and template 6H7J

MODELLER 9.21 was then used to generate satisfactory models; an automated approach to homology modelling by satisfaction of spatial restraints. Sequence alignments using the protein and template sequences was then carried out using platforms like ClustalX and ClustalW [23] (Figure1). Homology models for the chosen protein were then constructed using modeler programs like Modeller 9.21 [24]. After manually modifying the alignment input file in MODELLER 9.21 to match the query and template sequence, 20 models were generated. The best model is determined by the lowest value of the Modeller Objective Function. The stereochemical quality of the given models was then evaluated using software like PROCHECK [25] and the model can be used for further structural or functional study. PROCHECK generated a Ramachandran plot which explains residue by residue listing that facilitates the in-depth calculation of Psi/Phi angles and the backbone conformation of the models. The RMSD (root mean square deviation) was calculated by superimposing (6H7J_A) over the generated model to access the accuracy and reliability of the generated model by using SPDBV [26].

Docking methodology

Identification of active site pockets. The active site prediction was carried out using Tripo's Sybyl6.7 [27]. It showed two active site pockets. The amino acids in pocket one were Gly98, Leu109, Trp117, Thr118, Asp200, Phe201, Thr203, Ala208, Ser211, Ser212, Ser215, Trp303, Phe307, Asn310, Asn313, Val314, Phe325, Val326 and Tyr333. Twenty different plant secondary metabolites and four already existing drugs were selected for molecular docking with modelled protein. All the molecules were sketched in Sybyl 6.7 and minimized by adding Gasteiger-Huckel charges and saved in .mol2 format. Molecular docking studies were performed on all the natural compounds separately by using AutoDock4.2 [28]. program, using the Lamarckian Genetic Algorithm (LGA) and empirical free energy function was implemented. Initially, the modelled Histamine H2 receptor protein was loaded and hydrogens were added before saving it in PDBQT format [29]. Later the ligand was loaded and conformations were set and saved in PDBQT format. The grid parameters were selected and calculated using AutoGrid. For all the dockings, a grid-point spacing of 0.375 Å was applied and grid map with 60×60×60 points were used [30]. X, Y, Z (8.141, -44.241, -20.424) coordinates were selected on the basis of the amino acids present in the active site predicted in sybyl6.7 biopolymer module. Default parameters were used to run the Autodock.

3. RESULTS AND DISCUSSION

Homology modelling and model evaluation

The present study reports that the template protein (PDB ID: 6H7J_A) having high degree of homology with P25021 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of Histamine H2 receptor (uniprot accession number: P25021_Human) bearing 359 amino acid residues was retrieved from the uniprot protein sequence database with Accession No. P25021. Using BLAST, PDB ID 6H7J_A was identified and selected as template to predict the model. The structure was modelled using Modeller9.21. The generated structure was validated using the protein structure and by PROCHECK. The generated model showed 96.1% of amino acid residues in core region with 317 amino acids, 3.9% of amino acid residues in additionally allowed region having 13 amino acids, there is no amino acid residues in the generously allowed region and disallowed region. The template PDB shows 94.0% (853 aa) of amino acids in core region, 5.7% (52 aa) of the amino acid residues in additionally allowed region, and there is no amino acid residues in the generously allowed region and 2 amino acid residues (0.2%) present in disallowed region. Cartoon model of secondary structure of the modelled protein is shown in figure.2 and Ramachandran plot is shown in (Figure.4). RMSD was calculated for template and generated model by using SPDBV. Both the models were loaded and superimposed using the alpha carbon and RMSD was calculated (Figure 3). It showed RMSD of 1.34Å, which indicates that the generated model shows similarity to the template.

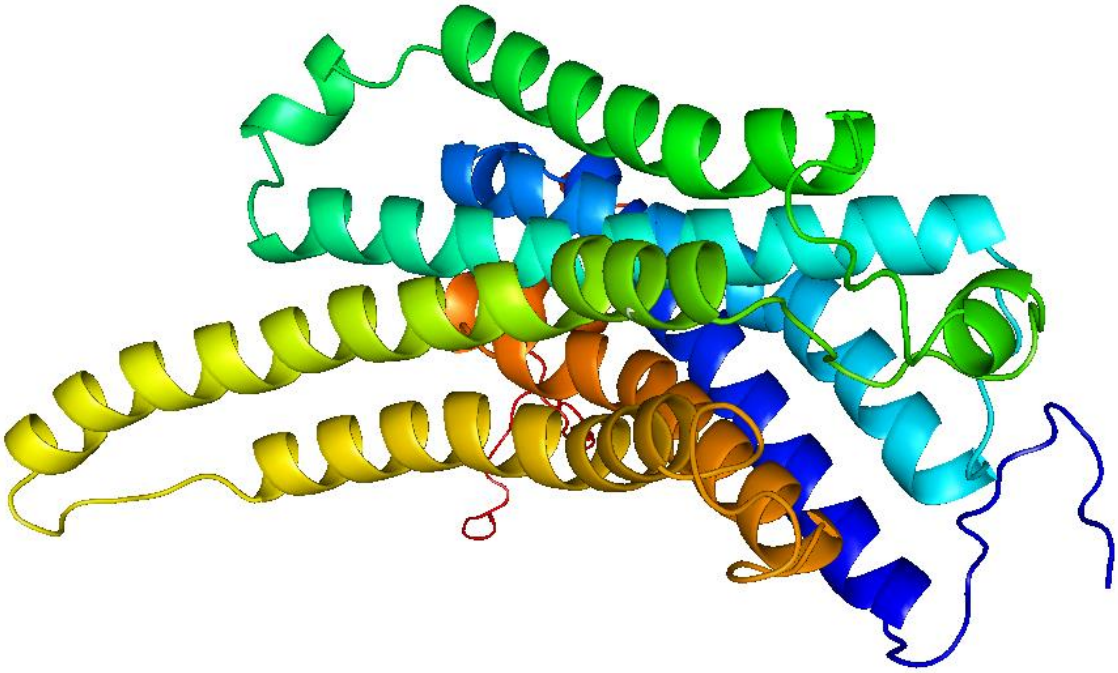


Figure 2: Cartoon model of predicted Histamine H2 Receptor protein.

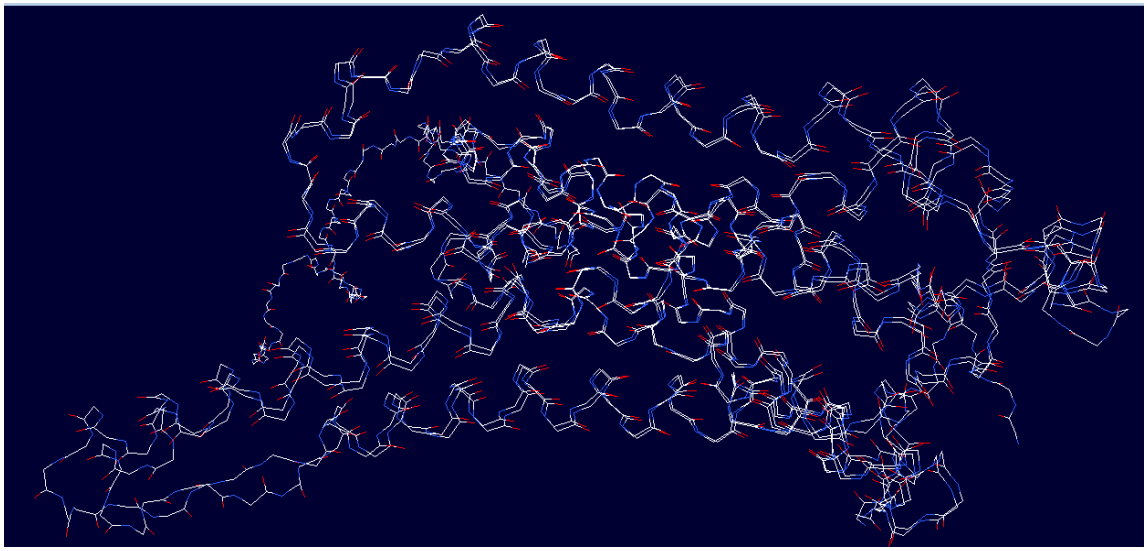


Figure 3: superimposed model of both query and templates

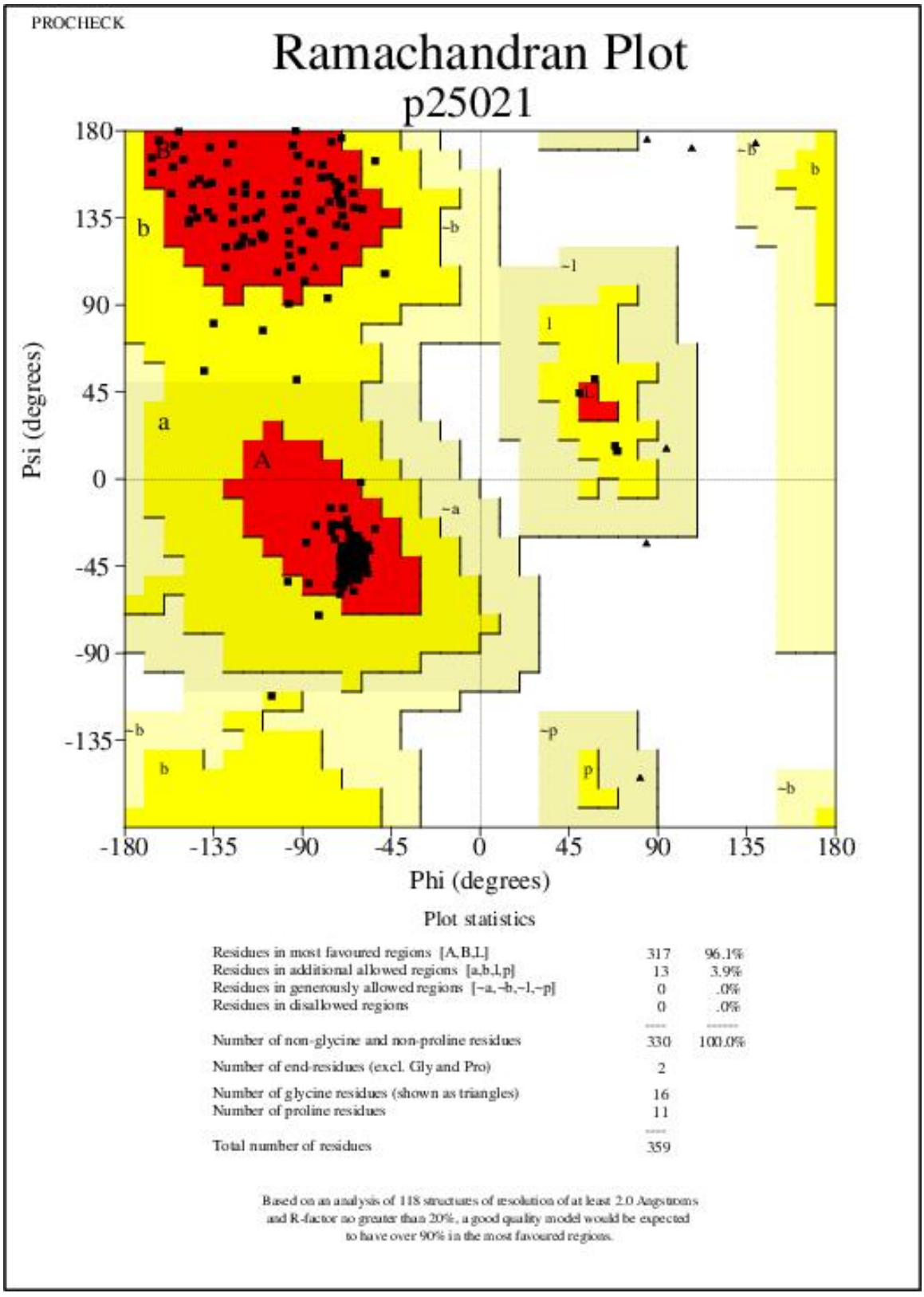


Figure 4: Ramachandran Plot of modelled protein Histamine H2 Receptor showing ~96% residues in most favored region.

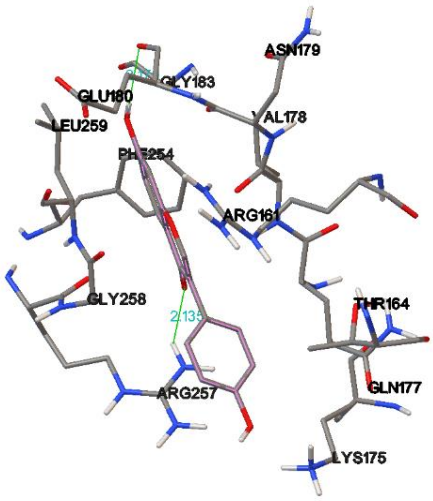
Molecular docking results

Molecular docking is the most extensively used method for the calculation of protein-ligand interactions. It is an efficient method to predict the potential ligand interactions. In the present study, the native plant secondary metabolites have been identified as potent Histamine H2 receptor inhibitors. AutoDock4.2 uses (genetic algorithm) binding free energy assessment to assign the best binding conformation**.** Further, the activity of docked ligand molecules was compared to that of standard drugs which were controls. In total, twenty natural compounds were docked against modelled Histamine H2 receptor protein. However, the compounds Cratoxyarborenone B, Lupinifolin and Khonklonginol-F showed lower binding energies better interactions, indicating more thermodynamically favoured interactions. These three compounds exhibited binding energy of less than -6.26 Kcal/mol and -6.17 Kcal/mol respectively. Specifically, Cratoxyarborenone B, Lupinifolin and Khonklonginol-F exhibited the lower binding energy of value -9.24 Kcal/mol, 9.07 Kcal/mol and 9.00 Kcal/mol with interacting Lys18, Ala2, Ser9; Gly258 and Thr164, Arg257. When compared to the standard drugs i.e., (Ranitidine, Nizatidine, Cimetidine and Famotidine). Ranitidine exhibited binding energy of -6.73 Kcal/mol while interacting with Val178, Glu267 and Glu270. All the compounds showed good binding energy with modelled protein. The natural compounds with their corresponding interactions and binding energies are shown in Table 1 and figure 5. Standard drug interactions and binding energies are shown in table 2 and fiture6.

Table 1: binding energy and interacting amino acids of flavonoids against modelled protein

S.No	Compound Name	Interacting amino acids	Binding energy (KCal/mol)	Dissociation constant (ΔG)
1	Eriodictyol	Leu259	-8.45	637.59 nM
2	Glepidotin A	Asp262, Val178, Arg257, Arg161	-7.72	2.20 µM
3	Lupinifolin	Gly258	-9.07	222.09 nM
4	Blumeatin	Arg257, Glu267	-7.76	2.05 µM
5	Formononetin	Arg257, Gly183	-8.25	893.91nM
6	Cirsimaritin	Arg257	-6.65	13.27 µM
7	Flemichin-D	Thr164	-5.08	190.12 µM
8	Macakurzin B	Arg257, Val177, Glu163	-8.12	1.124 µM
9	Corymbosin	Glu163	-7.51	2.37 µM
10	Sophoraflavanone L	Gly258	-7.01	7.25 µM
11	Dihydroquercetin-7,4’-dimethyl ether	Arg257, Arg161	-6.60	14.55 µM

12	Khonklonginol-F	Thr164, Arg257	-9.00	252.31nM
13	Cratoxyarborenone A	Lys18, Ala2, Ser9	-5.27	136.52 µM
14	Cratoxyarborenone B	Val178, Arg257, Gly258, Thr164	-9.24	51.79 nM
15	Cudraflavone C	Gly258	-7.35	4.08 µM
16	Vitexicarpin	Val178	-6.45	18.82 µM
17	Khonklonginol-H	Arg161, Glu180	-4.64	396.1 µM
18	Cratoxyarborenone E	Arg257, Glu180	-4.48	517.57 µM
19	Luteolin-7-methyl ether	Glu163	-7.13	5.98 µM
20	Chaplashin	Lys175, Arg257, Glu163	-6.74	11.47 µM
21	Quercetin	Arg257, Val176, Gly183	-7.58	2.76 µM



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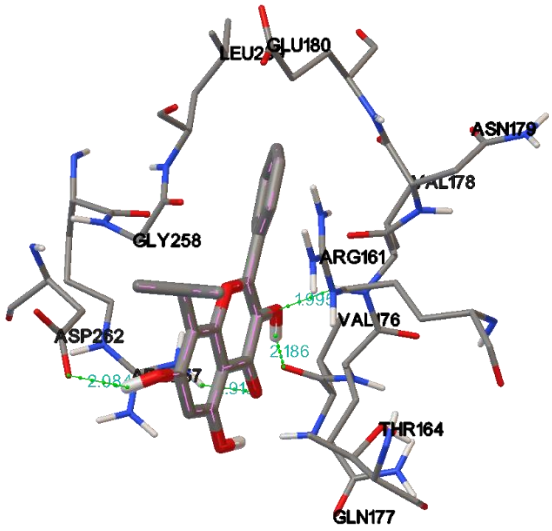

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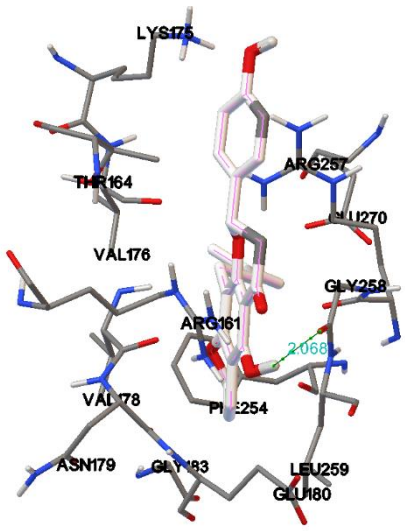

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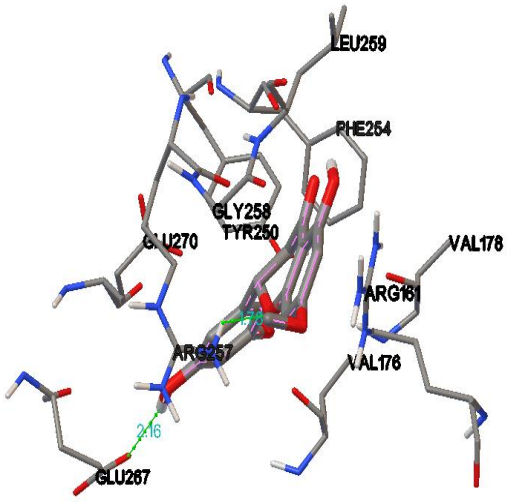

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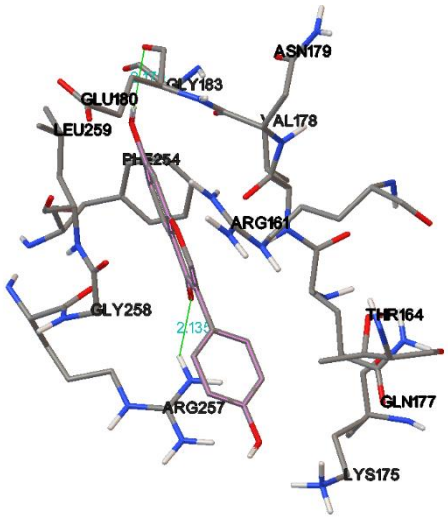

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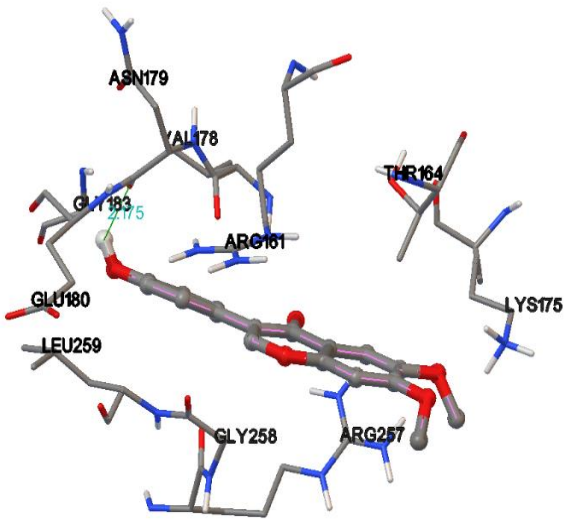

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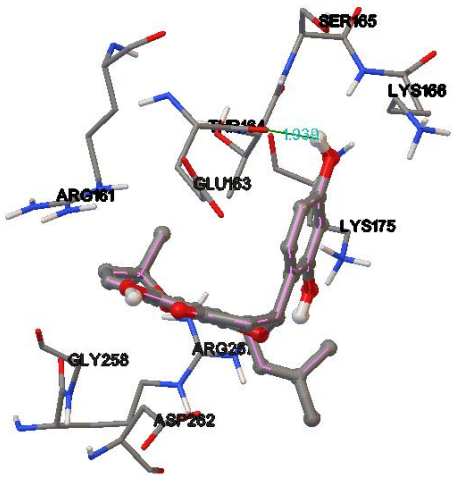

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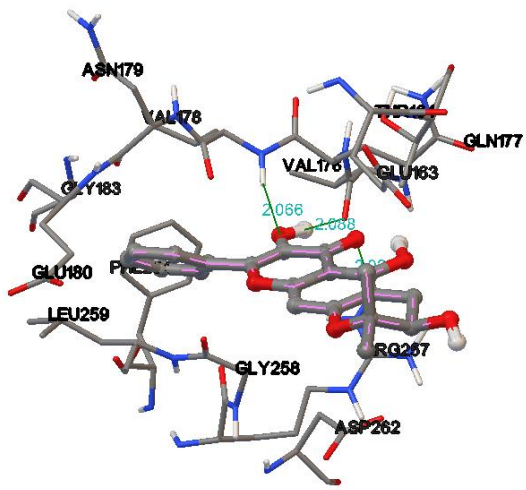

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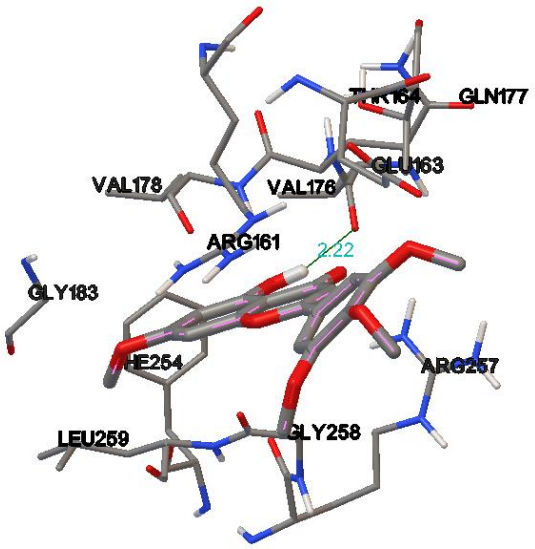

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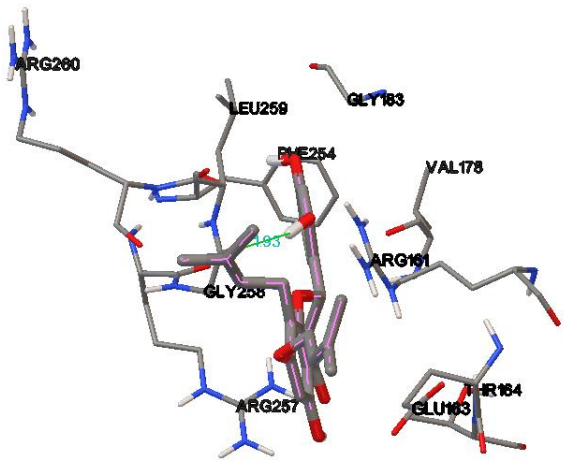

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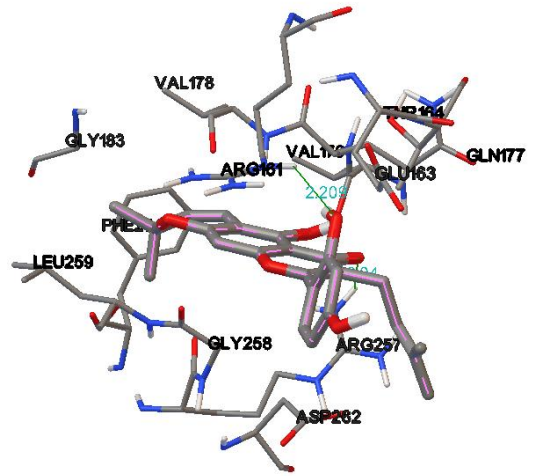

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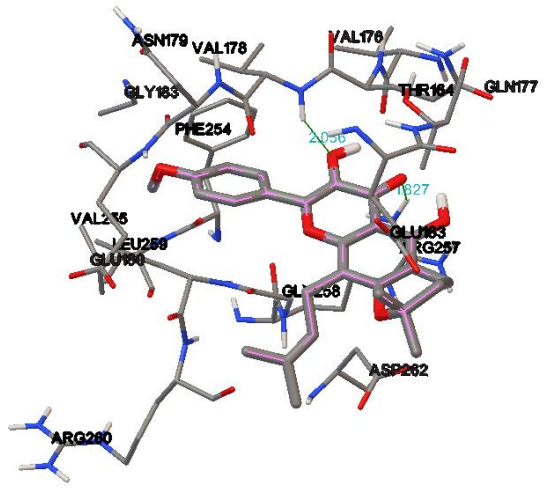

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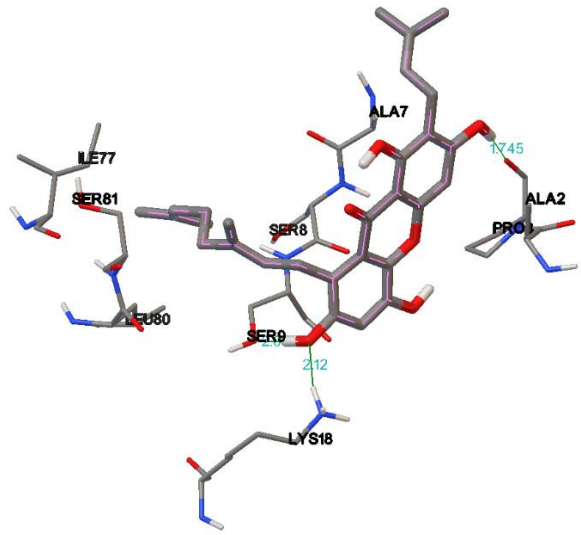

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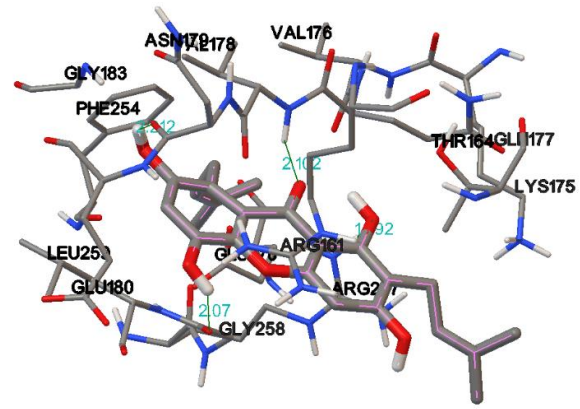

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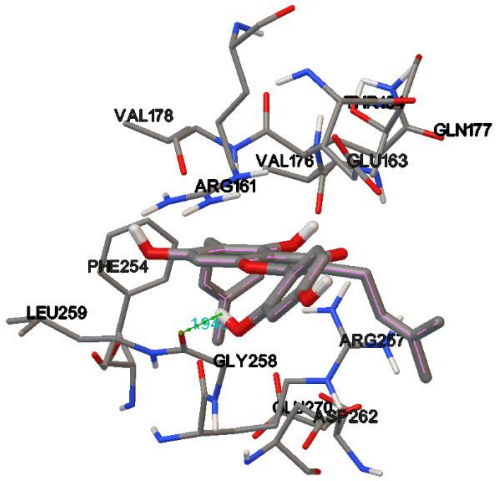

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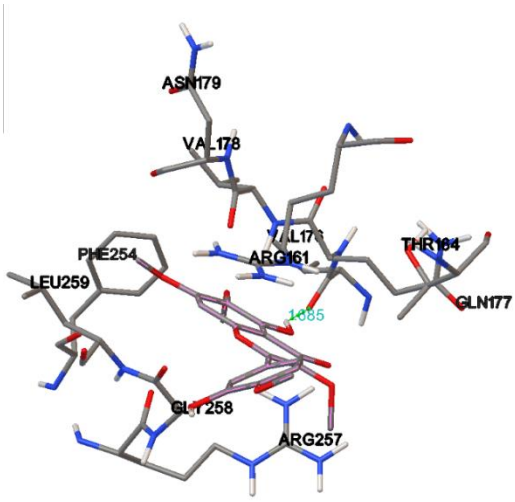

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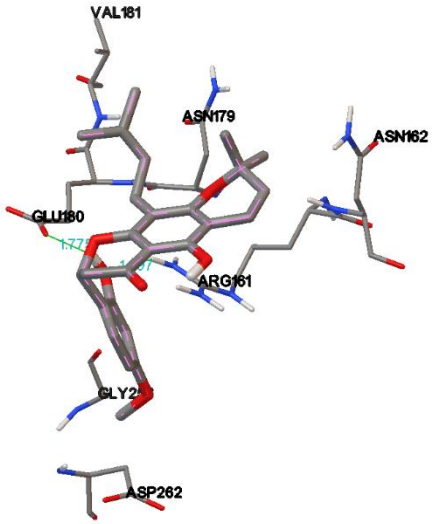

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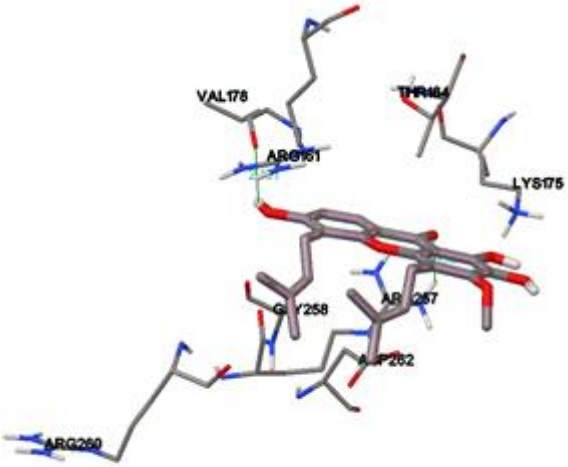

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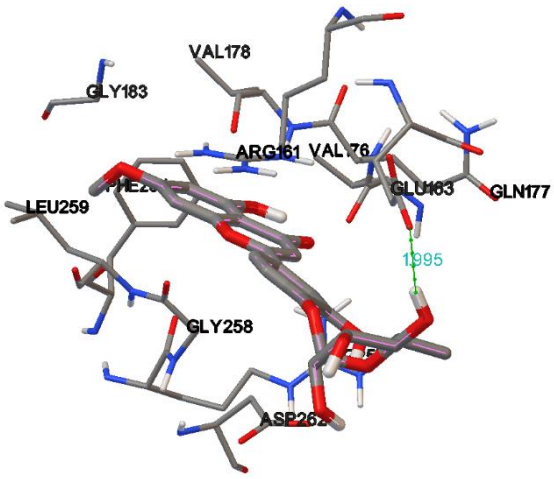

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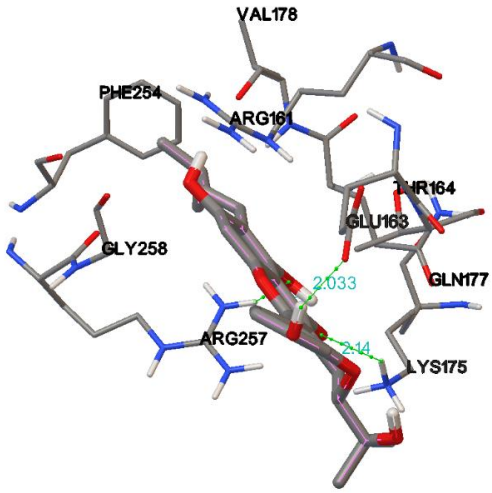

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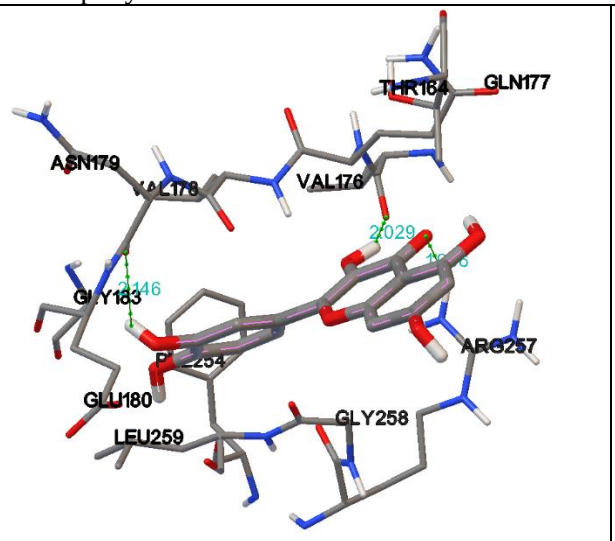

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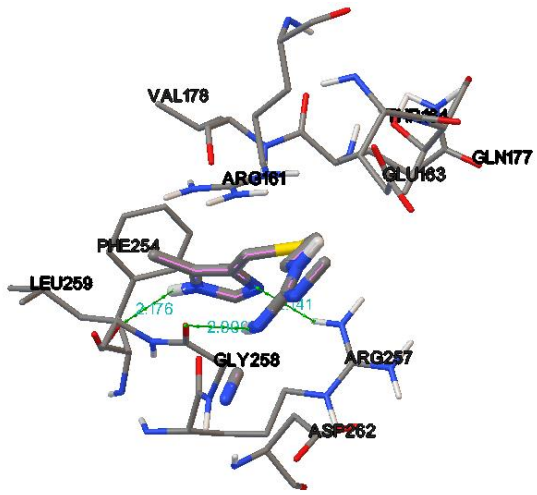
Figure 5: Molecular docking interactions of the 21 flavonoids against modelled protein

Table 2: binding energy and interacting amino acids of standard drugs against modelled protein

Standard Drugs:

S.No	Compound Name	Interacting amino acids	Binding energy	Dissociation constant (µM)
1	Cimetidine	Arg257,Gly258,Phe254	-5.87	49.93µM
2	Nizatidine	Glu180	-6.59	14.65 µM
3	Famotidine	Val178,Glu178,Ile265,Phe254	-5.85	51.47 µM
4	Ranitidine	Val178,Glu267,Glu270	-6.73	11.63 µM



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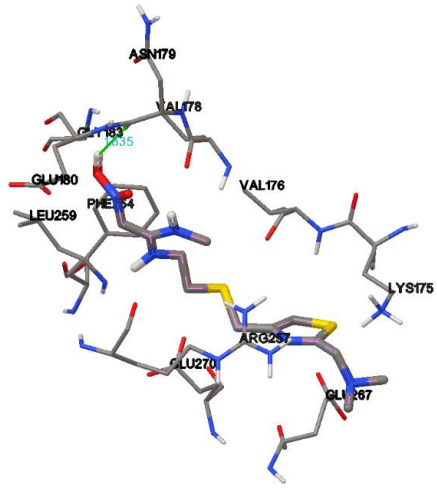

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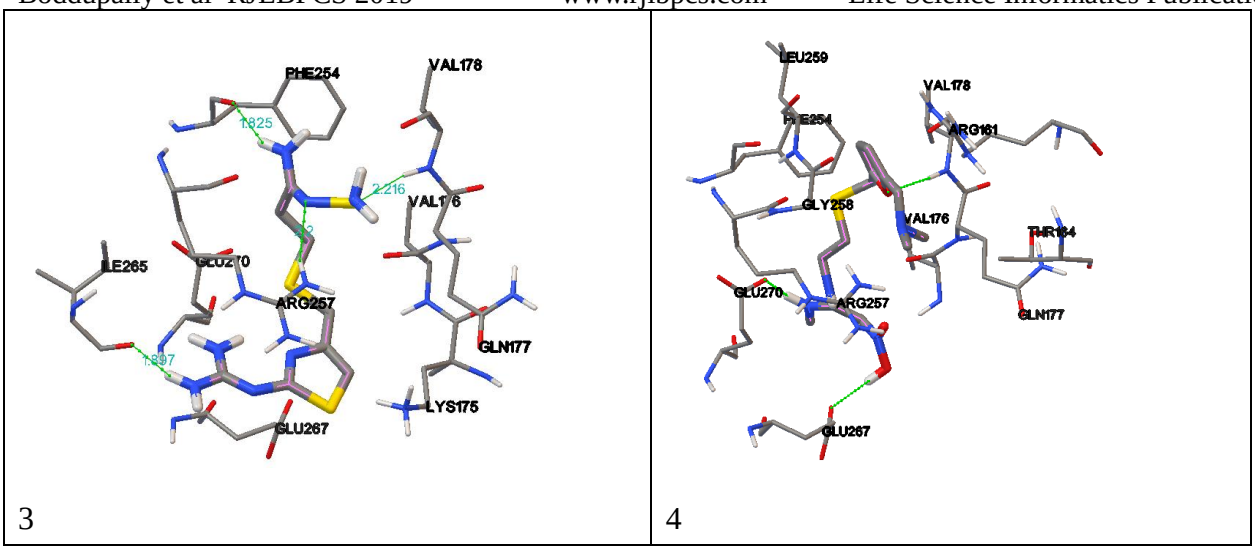

Figure 6: molecular docking interactions of the standard drugs against modelled protein

4. CONCLUSION

Molecular modelling of Histamine H2 Receptor was performed. It showed 96.1% of amino acid residues in core region and Molecular docking studies were also performed to the modelled Histamine H2 Receptor with phytochemicals has revealed lower binding energies and good interactions. Three compounds Cratoxyarborenone B, Lupinifolin and Khonklonginol-F showed lower binding energies of -9.24 Kcal/mol. These predictions will essentially lead to effective treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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