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Original Article

Molecular modeling of plant flavonoids as angiotensin converting enzyme (ACE) inhibitors in hypertension: A docking study

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ABSTRACT

Hypertension is one of the most common worldwide diseases that results in the progression of several cardiovascular disorders and stroke. *Angiotensin converting enzyme* (ACE) has become a major target control for hypertension. Several synthetic ACE inhibitors such as captopril, lisinopril, enalapril etc. are in clinical use but are found to have certain side effects and drug-drug interactions. Therefore, there is a great need to search for more economical, nontoxic and safer inhibitors. Flavonoids, the polyphenolic compounds in plants have been reported for some inhibitory effects against ACE. We conducted a molecular docking study on flavonoid derivatives; *flavones, flavonols, flavanones and flavan-3-ols* with two angiotensin converting enzymes in complex with synthetic drugs, *captopril* and *lisinopri*l respectively by ArgusLab 4.0.1 software. The aim of this study is to investigate the binding modes of flavonoid derivatives with ACE to design more safer natural drugs as alternatives to synthetic drugs. The results showed the best binding affinity of ACE with that of *Apigenin*, a flavone having the lowest binding energy of -8.45kcal/mol in ACE- captopril complex and -8.33 kcal/mol with that of lisinopril complex. This information can be utilized to design potent therapeutic drugs from natural plant flavonoids in the regulation of blood pressure.

Key words: Hypertension, Angiotensin converting enzyme, Flavonoid derivatives, Docking study, Drug design.

INTRODUCTION

Hypertension is a major health issue worldwide and reigns as a leading cause of cardiovascular morbidity and mortality. In spite of its significance, hypertension remains poorly controlled and is estimated to affect approximately two third of the world's adult population. [1] Angiotensin converting enzyme (ACE) is a key component of renin-angiotensin- aldosterone system which plays a significant role in the regulation of arterial blood pressure. ACE catalyzes the formation of potent vasoconstrictor angiotensin II from angiotensin I. [2] It also inactivates the vasodilator bradykinin as well as responsible for the release of aldosterone from adrenal cortex which has a tendency to increase blood pressure by retaining sodium. [3] Therefore, inhibition of ACE is a promising way of controlling blood pressure and its inhibitors are used commonly for the treatment of hypertension, myocardial infarction and other cardio related diseases. [4] Several synthetic ACE inhibitors such as captopril, lisinopril, enalapril and rampiril are widely used clinically for the treatment of hypertension but the long term use of these drugs could cause serious side effects such as dry cough, dizziness, angineurotic oedema, taste disturbances and skin rashes [5,6] They also exhibit interactions with other drugs [7] Many studies have been attempted for the isolation and development of natural ACE inhibitors for example; from food proteins, from marine derived compounds and from microbial resources. [8,9,10] Flavonoids are the polyphenolic compounds that are widely distributed in the human diet, primarily in plant-derived foods and beverages.[11] Flavonoids have

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been studied extensively for their role in the regulation of blood pressure and hypertension. Previous studies demonstrated that flavonoids act as an effective component in the diet not only for the maintenance of coronary heart diseases, neurodegenerative disorders and cancer [12,13] but also for the ACE inhibition properties.[14,15]

The objective of the current study is to evaluate the flavonoids for their ability to inhibit ACE in order to establish the structural basis of their bioactivity. They were also assessed for their potential to serve as more safer, economical and nontoxic natural ACE inhibitors in place of synthetic drugs. For this purpose one of the molecular modeling technique is utilized i.e. the docking. Nowadays, molecular docking study is emerging as an important component in the drug discovery processes and used as a tool for high-throughput screening. Molecular docking techniques are used to predict how a protein interacts with small molecules at molecular level. Therefore the technique was utilized to understand the binding modes of flavonoid compounds with ACE at molecular level which was helpful in identifying the functional groups of flavonoids participating in ACE inhibition. A wide range of molecular modeling (docking) software packages are available nowadays like AutoDock, Glide, FlexX etc. The present work of molecular docking has been done using commercially available software, ArgusLab 4.0.1.[16] It is a molecular modeling and drug designing program based on genetic algorithm. It is implemented with exhaustive search methods; Argus Dock docking engine and AScore scoring function.[17] Computer-based molecular modeling tends to speed up drug discoveries by predicting potential effectiveness of ligand-protein interactions as compare to tedious experiments and costly preclinical trials.

MATERIALS AND METHODS

Data Set: Flavonoid derivatives from different subgroups were chosen for the study from the literature. These were *Quercetin* and *Kaempferol* (from Flavonols), *Catechin and Gallocatechin* (from Flavan 3 ols), *Apigenin* (from Flavones) and *Naringenin* (from Flavanones). Three dimensional experimentally determined X-ray crystal structures of angiotensin converting enzymes in complex with *lisinopril* and *captopril* were taken from Brookhaven Protein Data Bank (PDB)[18] (http://www.rcsb.org/), having PDB codes 1O86 and 2X8Z respectively.

Preparation and Energy Minimization of Ligands and Proteins: All the structures were drawn manually using ACD Lab Chemsketch software, cleaned in 3D format and energy was minimized using energy convergence function of ArgusLab software carried out by Hartree- Fork calculation method. The minimized structures were then saved in "mdl mol" and "agl" file formats which can then be recognized by ArgusLab for molecular modeling studies. All the X-ray crystal structures of proteins in complex with lisinopril and captopril were prepared by removing all the water molecules from the file as they sometimes interfere with the binding of compounds at the active site of protein in ArgusLab.[19]The resulting receptor protein structures were saved n PDB file format.

Docking Procedure: Once the ligands and the proteins have been minimized and prepared, the files were downloaded in ArgusLab software for docking to assess the interactions. Docking was performed by "Argus Dock" docking function of the software and "Flexible" ligand docking mode with the grid resolution of 0.40 Å was set for each docking run. Flexible docking allows rotations in the form of torsions to create in the group of atoms present in the ligand. At the end of each docking run, interactions are shown in the form of "poses" with the energy values given in kcal cal/mol for each pose. AScore scoring function of the ArgusLab was used to evaluate the stable interactions as indicated by lowest AScore value i.e. the binding energy of the ligand and the protein target. The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations. The best docking conformation or pose was also selected on the basis of hydrogen bonds (in a range of 2-3 Å) made between the ligand and the protein target at the active site.

RESULTS

The cleaned and geometry optimized structures of flavonoid derivatives selected for the study are given in figure 1. All the procedures have been performed using default parameters of ArgusLab 4.0.1 for windows. Flexible docking has been done between angiotensin converting enzymes in complex with lisinopril and captopril obtained from protein data bank (PDB codes 1O86 and 2X8Z) and flavonoid derivatives. Superimposition of flavonoid derivatives with captopril and lisinopril in ACE is represented in Figures 2 and 3 respectively. Out of the 10 poses obtained for each of the flavonoid compounds, only the best conformations, with highest binding affinity have been chosen for the protein-ligand interaction study and are shown in figures 4, 5and 6 for ACE-lisinopril complex and figures 7, 8 and 9 for ACE-captopril complex. The least binding energy obtained from AScore scoring function of ArgusLab for flavonoids with each of the two

enzymes (PDB codes: 1O86 and 2X8Z) is given in Table 1 and 2 respectively. Results representing hydrogen bonds formed between the flavonoid derivatives and ACE with lisinopril and captopril are given in Table 3 and 4 respectively.

DISCUSSION

The docked poses of the flavonoid derivatives were ranked according to the docking scores i.e. the lowest AScore values of the ArgusLab 4.0.1 and only the top ranked and best fitted conformations of the selective flavonoid derivatives were analyzed for the study. The results showed the competitive type of inhibition by flavonoids for ACE. The best binding affinity of ACE (should be ommited) was found with the Apigenin (belongs to the flavone group of flavonoids) in both the complexes of ACE i.e. -8.45 kcal/mol in captopril and -8.33 kcal/mol in lisinopril complex. Other flavonoid derivatives such as *Naringenin, Quercetin and Kaempferol* (from falvanone and flavanol group of flavonoids) also represent good binding affinity with ACE as indicated in Table 1 and 2. Analysis of the receptor - ligand (insert "-" instead of "/") complex models generated after successful docking of the flavonoids was based on the parameters such as hydrogen bond interactions, binding energy and binding orientation of the docked compound within the active site. These parameters play an important role in the biological activity of a compound. Figures 4 and 7 clearly demonstrate the binding of apigenin with the amino acid residues of the active site of ACE i.e. Gln 281, Gln 265, Lys 511 and Tyr 507 with the hydrogen bond distance of 2.01, 2.67, 2.81 and 2.31 Å respectively. While in figures 5 and 8 naringenin also occupied the binding (ommited) site of lisinopril and captopril in the active site cavity of the enzyme and made hydrogen bonds with the active site amino acid residues His 353, His 337, Gln 265 and Tyr 507. In figures 4 and 7 quercetin and kaempferol were also seen to be buried inside the active site of enzymes replacing the synthetic drugs and made H-bonds with the amino acid residues such as Gln 281, His 353, Gln 265 and His 337 respectively which is well correlated with the results in vitro and vivo.[20,21] This may be attributed due to the differences in the position of the functional groups in the compounds. All these results and the superimposition of flavonoids with the synthetic drugs lisinopril and captopril indicate that flavonoids have a potential to inhibit ACE and the inhibitory property varies with the type of flavonoid derivative as well as its content in the natural compounds. The results also revealed that among flavonoids, *flavone* and *flavanone compounds* (apigenin and naringenin in this study) showed best inhibitory effect against ACE although the IC_{50} value for these compounds are much higher than other flavonoids as reported in the literature.[22] As the percentage of Apigenin and Naringenin content is much higher in various natural flavonoids as compared to the other compounds taken for the present study [23], they may act as better inhibitory components. Therefore specific focus on the isolation of *flavones* and *flavanones* and their ACE inhibition activity can generate valuable information about antihypertensive properties of these compounds.

CONCLUSION

Most studies have showed that plant extracts rich in phytochemicals found to be effective in ACE inhibition. However, identification of compounds that specifically inhibit ACE is lacking in most of these investigations. In the present molecular modeling study, results clearly demonstrated that flavonoids have a similar binding sites and interactions with ACE as those of the synthetic drugs taken for the study and prove that dietary flavonoids may possess properties of blood pressure regulation. In terms of the mode of action, flavonoids had shown competitive type of inhibition for ACE. Thus *in silico* study is actually an added advantage to screen the ACE inhibition and flavonoids may serve as useful leads for the synthesis of clinically useful antihypertensive drugs. Structural modifications and further studies on flavonoids (specifically *flavones* and *flavanones*) using animal models are required to develop potential chemical entities for the prevention and treatment of hypertension and related disorders.

Table 1: Binding Energies of Flavonoid Derivatives with ACE in Complex with Lisinopril (Pdb code: 1O86) by AScore Scoring Function of ArgusLab

S.No	Flavonoid derivatives	AScore (kcal/mol)
1	Apigenin (Flavone)	-8.33
2	Naringenin (Flavanone)	-8.01
3	Quercetin (Flavonol)	-7.94
4	Kaempferol (Flavonol)	-6.97
5	Catechin (Flavan 3 ol)	-6.95
6	Gallocatechin (Flavan 3 ol)	-6.79

Table 2: Binding energies of Flavonoid derivatives with ACE in complex with Captopril (Pdb code: 2X8Z) by AScore scoring function of ArgusLab

S.No	Flavonoid derivatives	AScore (kcal/mol)
1	Apigenin (Flavone)	-8.45
2	Naringenin (Flavanone)	-8.43
3	Kaempferol (Flavanol)	-8.04
4	Quercetin (Flavanol)	-7.84
5	Chatechin (Flavan 3 ol)	-7.16
6	Gallochatechin (Flavan 3 ol)	-7.06

Table 3: List of H- Bonds formed between Top Ranked Flavonoid derivatives and ACE-Lisinopril complex

S.No	Flavonoid derivatives	Ligand atom	Amino acid residue atom	H-bond distance (Å)	No of hydrogen bonds
1	Apigenin (Flavone)	O4 O4	Gln 281(NH) Lys 511(NH)	2.01 2.81	2
2	Naringenin (Flavanone)	O5 O1	Lys 511(NH) His 353 (NH)	2.82 2.71	2
3	Quercetin (Flavanol)	O5 O4	Gln 281(NH) His 353 (NH)	2.55 2.75	2

Table 4: List of H- Bonds formed between Top Ranked Flavonoid derivatives and ACE-Captopril complex

S.No	Flavonoid derivatives	Ligand atom	Amino acid residue atom	H-bond distance (Å)	No of hydrogen bonds
1	Apigenin (Flavone)	O4 O4	Gln 265 (NH) Tyr 507(OH)	2.76 2.31	2
2	Naringenin (Flavanone)	O4 O5	Tyr 507 (NH) Gln 265 (NH)	2.12 2.03	2
3	Kaempferol (Flavanol)	O4 O1	His 337(NH) Gln 265 (NH)	2.90 2.90	2

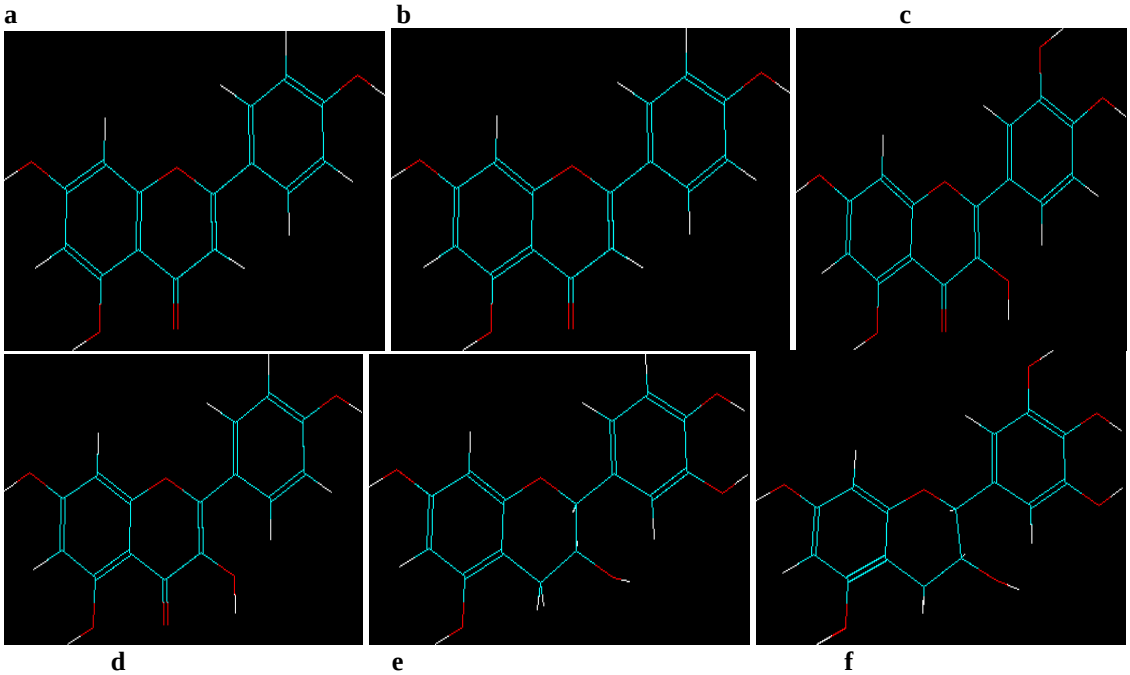

figure 1. 3D cleaned structures of flavonoid derivatives generated by ACD Lab Chemsketch software (a) Apigenin (b) Naringenin (c) Quercetin, (d) Kaempferol (e) Catechin and (f) Gallocatechin

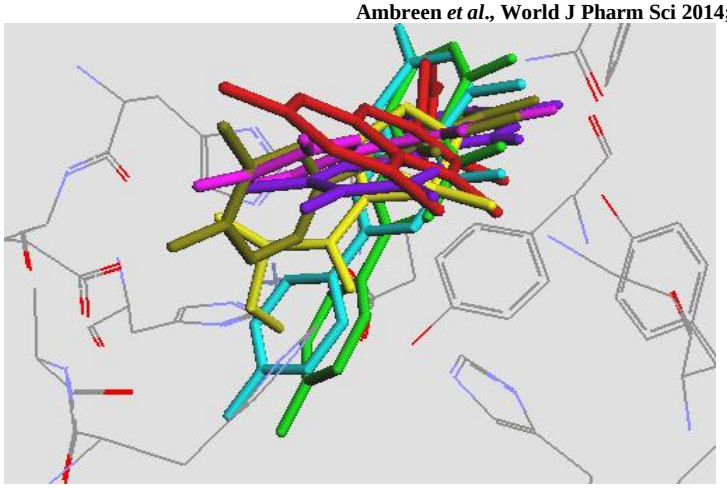

figure 2. Superimposition of flavonoids with *Captopri*l . Captopril is seen in yellow, Naringenin in blue, Apigenin in green, Kaempferol in red, Quercetin in brown, Catechin in pink and Gallocatechin in purple color.

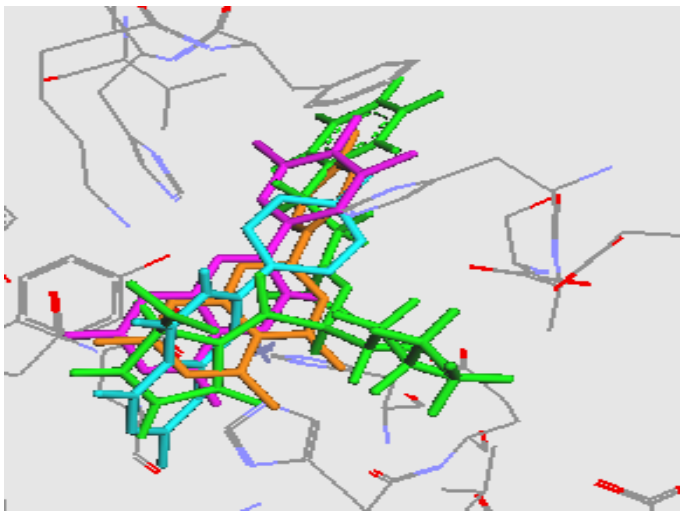

figure 3. Superimposition of flavonoids with *lisinopril*. lisinopril is seen in green, Apigenin in blue Quercetin in pink and Naringenin in orange color.

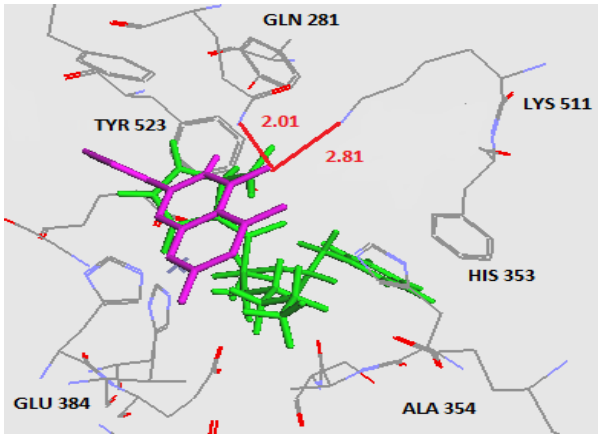

figure 4. Docking of *Apigenin* into ACE complexed with co-crystallized ligand lisinopril. Lisinopril is seen in green and apigenin in pink color. Hydrogen bond interactions are represented in red lines with the distance given in red color.

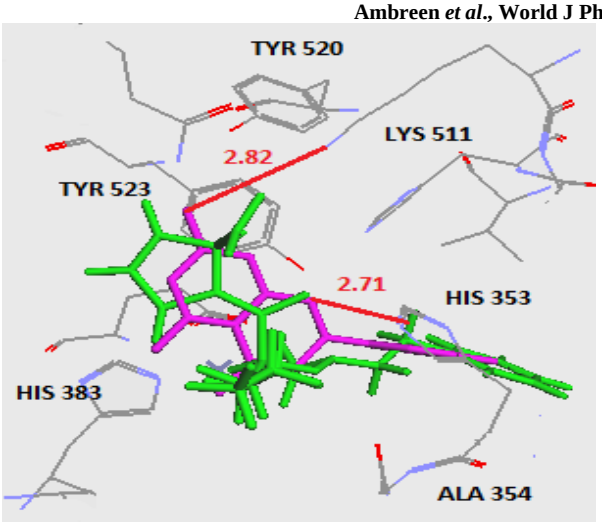

figure 5. Docking of *Naringenin* into ACE complexed with co-crystallized ligand lisinopril. Lisinopril is seen in green and naringenin in pink color. Hydrogen bond interactions are represented in red lines with the distance given in red color

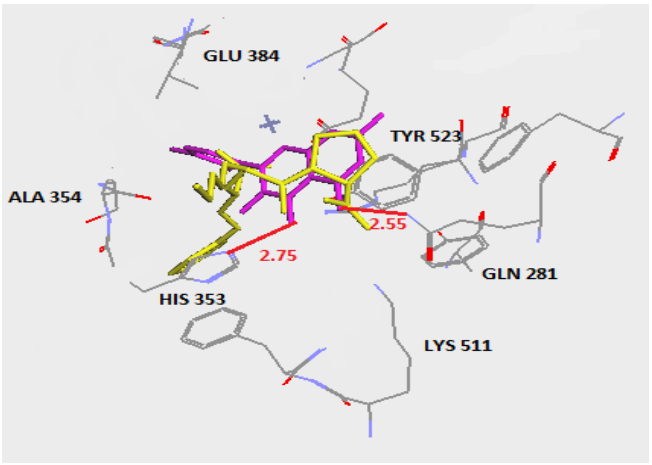

figure 6. Docking of *Quercetin* into ACE complexed with co-crystallized ligand lisinopril. Lisinopril is seen in yellow and quercetin in pink color. Hydrogen bond interactions are represented in red lines with the distance given in red color

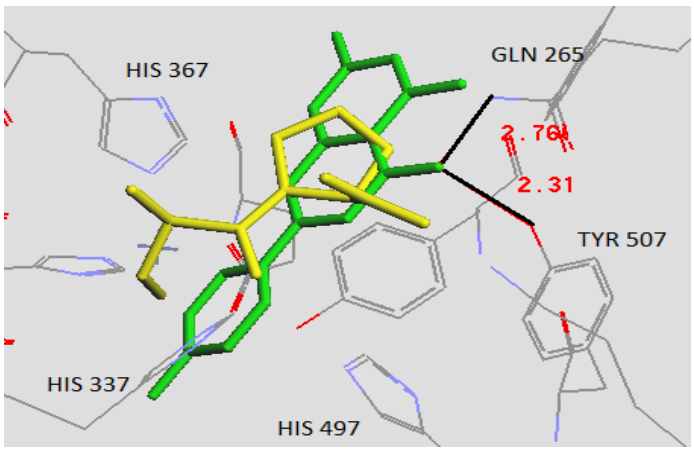

figure 7. Docking of *Apigenin* into ACE complexed with co-crystallized ligand captopril. Captopril is seen in yellow and apigenin in green. Hydrogen bond interactions are represented in black lines with the distance given in red color

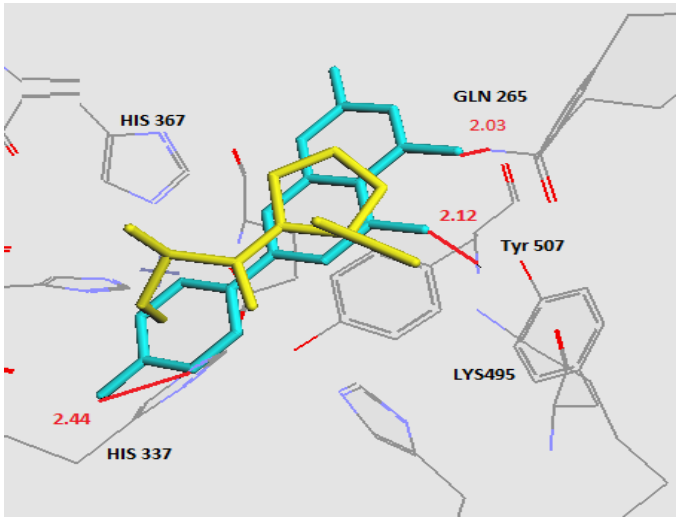

figure 8. Docking of *Naringenin* into ACE complexed with co-crystallized ligand captopril. Captopril is seen in yellow and naringenin in blue color. Hydrogen bond interactions are represented in red lines with the distance given in red color

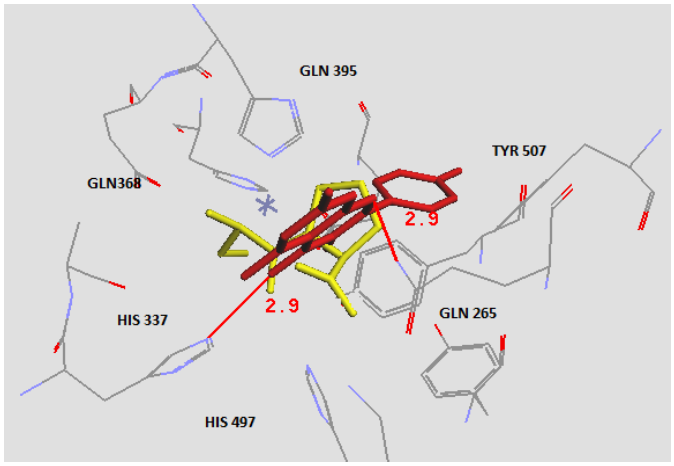

figure 9. Docking of *Kaempferol* into ACE complexed with co-crystallized ligand captopril. Captopril is seen in yellow and kaempferol in red color. Hydrogen bond interactions are represented in red lines with the distance given in red color

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