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IN SILICO AND IN VITRO STUDIES OF CANDIDA RUGOSA LIPASE INHIBITION USING AMENTOFLAVONE AND PLANTAGOGUANIDINIC ACID FROM ORIGANUM MARJORANA AND PLANTAGO CILIATA

Benarous Khedidja[a,B*], Salemi Rania[a], Zakhrouf Hiba[a],

a Département de biologie, université Amar Telidji, Laghouat, Algérie

b laboratoire des sciences fondamentales, université Amar Telidji, Laghouat, Algérie

*k.benarous@lagh-univ.dz

Abstract

The inhibitory effect of phenolic compounds extracted from *Matricaria recutita, Saussurea costus*, *Origanum marjorana, Plantago ciliata, Senna Alexandrina* and *Haloxylon scoparium* on *Candida rugosa* lipase was explored. The phenolic extracts of *Origanum marjorana* and *Plantago ciliata* have shown potent lipase competitive inhibition with Ki=0.05 mg/ml for both of them. The enzymatic inhibition produced by these extracts is described here for the first time. Thus, the inhibitor molecules have been proposed and molecular docking has been achieved using AutoDock Vina program to discuss the nature of interactions and the mechanism of inhibition. Therefore, these results could be used for the drugs design to be used for obesity and candidiasis.

Keywords: *Origanum marjorana; Plantago ciliate; Candida rugosa* lipase; molecular docking.

Introduction

Origanum marjorana, a member of the Lamiaceae family, is of great economic and industrial importance. The fresh or dried highly aromatic leaves and flowering tops of marjoram (*O. majorana* L.) are widely used to flavour many foods. The oil is used in perfumery for its spicy herbaceous notes and as fungicides or insecticides in pharmaceutical and industrial products [1].

The genus *Plantago L.* is the largest genus of the family Plantaginaceae with about 265 species. *Plantago ciliata* is uncommon plant, growing on the south of Algeria (Laghouat city). Only few researches have been done for it [2].

Lipases or triacylglycerol acyl hydrolases, (EC 3.1.1.3), catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids [3-7]. Lipases are widely distributed among bacteria, fungi, plants and animals [3-7].

The digestion and absorption of natural lipids begin with hydrolysis by pancreatic lipase [5-6]. The activity of this enzyme greatly affects the metabolism of fat and the concentration of TAG in blood [5-6]. Obesity is caused as a result of an imbalance between energy intake and expenditure. Excess energy is stored in fat cells, which enlarge or increase in number. Human obesity is one of the most serious health problems. Obesity is associated with an increased risk of several serious diseases including hypertension, coronary heart disease, type II diabetes, stroke, osteoarthritis and certain cancers [5-7].

Therefore, there is an increasing interest in finding new lipase inhibitors of natural origin with less secondary effects. Plant secondary metabolites present in herbal drugs and food have shown to be very useful in the prevention and treatment of many diseases [3-4].

Continuing our research for new natural lipase inhibitors [5-6], our purpose through this work is to investigate *in vitro* the inhibitory effect of the phenolic extracts on *Candida rugosa* lipase. Then, to evaluate their binding affinity to this enzyme and predicting the chemical interactions using docking experiments.

Materials and Methods

Plant material

Plantago ciliata was collected in February 2015 in Saharan Atlas of Algeria (at 40 km north of Laghouat), and the other plants (*Origanum majorana, Saussurea costus, Matricaria recutita, senna alexandrine* were purchased from herbalist in the same date. Voucher specimens were deposited in the laboratory of fundamental sciences of Laghouat University.

Drugs and Chemicals

Candida rugosa lipase, *p*-nitrophenyl laurate, p-nitrophenol and all other reagents were purchased from Sigma-Aldrich. All other chemicals and solvents used were of analytical grade.

Preparation of Plant Extracts

After air drying the plants and grounding into fine powder, we have purchased the same protocol extraction published in Benarous et al., 2013 and 2015 [5-6].

Quantification of Total Phenols and Flavonoids

The concentration of total phenols in plant extracts was estimated by the Folin-Denis procedure [11-12], which is the most used procedure for the quantification of phenols in plants. The total phenolic content of the ethyl acetate extracts was expressed as Gallic acid equivalents (GAE). The quantification of flavonoids was performed by a method Lamaison and Carnet (1991) with the use of aluminum trichloride as a reagent, with the standard flavanoid used, is rutin and the total flavonoid content in the ethyl acetate extracts was expressed as rutin equivalents (ER).

Inhibition of Candida rugosa Lipase

The method used for *Candida rugosa* lipase was previously described and performed in Benarous *et al*, 2013 and 2015 [5-6]. Inhibition kinetics was performed using varying concentrations of phenolic extracts in the assay mixture. The concentrations yielding a lipase inhibition of 50% (IC50) were calculated from the inhibition vs. plant extract concentration curves by regression analysis. The Ki values were determined using Dixon plots. All experiments were done at least in triplicate.

Molecular docking

All the used ligands (proposed molecules as amentoflavone and plantagoguanidinic acid) were obtained from ZINC database, they were assembled with Discovery Studio visualizer v4.0. The 3D structure of Candida rugosa lipase (PDB ID: 1CRL) was obtained from protein data bank (PDB). For docking studies, initial protein was prepared by removing all the water molecules, heteroatoms, any co-crystallized solvent and ligands. It is well known that the pdb files have missing hydrogens. Hence, the polar hydrogens and partial charges were added to the structure using Autodock tools (ADT) (version 1.5.4). Docking calculations were performed with AutoDock Vina program [9]. Because it uses rectangular boxes for the binding site, the box center was defined and the docking box was displayed

using ADT. The docking was blind with a grid box of 68x68x68 with grid points separated 1 A° was positioned at the middle of the protein (x = 72.33; y = 55.391; z = -20.425). Default parameters were used except the number of output conformations was set to 40 and the number of runs was 50. The searching seed was random. The preferred conformations were the ones of lowest binding energy within active site. Finally, the docking results generated were directly loaded into Discovery Studio visualizer, v 4.0. The molecular docking was achieved in HP Z820 Workstation with 12 cores [9].

Results and Discussion

Quantification of total phenols and flavonoids

Our extracts have shown a various yields (%) with different aspects and colors. The extraction yields of the five used plants vary from 0.02 to 5.86% (table 1). This result could be interpreted by the polarity and solubility of phenolic compounds and flavonoids in the different extraction solvents [13- 16].

The amount of total phenolic varied in the five plants and ranged from 4,68±0,01 to 143,09±0,07 mg GAE (gallic acid equivalent)/g dw (dry weight) (Table 1). However, the phenolic content of *Origanum majorana* and *Plantago ciliata L* extracts are higher than other plants in other families such as: *Artemisia arboresens* with 3.42±0.50 mg GAE/g dw (dry weight), *Ruta Montana* with 3.13±0.30 mg GAE/g dw, *Artemisia campestris* with 20.38±0.30 mg GAE/g dw and *Globularia alypum* with 21.54±0.81 mg GAE/g dw [17-18]. About the other fractions, we marked that the total phenolic content in the tested plants was lower than those reported for the most of other Asian medicinal and common dietary plants [19-23].

Flavonoids are one of the most diverse and widespread group of natural compounds, they possess a lot of chemical and biological activities including anti-inflammatory activities [18]. In this study, the content of flavonoids (mg/g), in rutin equivalent varied from 4.40±0.00 to 61.00±0.00 mg RE/g dw. Therefore, the studied extracts present important amounts of phenolic and flavonoid compounds and they could be a potent source of antioxidants.

see Table 1.

Inhibitory Effects on Candida rugosa lipase

Candida rugosa lipase has shown normal Michaelis kinetics on p-nitrophenyl laurate (p-NPL) and for each extract the IC50 and Ki values were calculated from the plot of the enzyme activity as a function of phenolics concentration. For comparison, the irreversible and selective pancreatic lipase inhibitor orlistat has used as reference compound. Among the extracts tested, only two *Origanum majorana* and *Plantago ciliata L* extracts have shown a good inhibitory effect on *Candida rugosa* lipase (Table 2 and figures 1 & 2) but less active than orlistat. The different inhibition produced by these extracts is probably related to their substances with different chemical structures and physicochemical properties.

Molecular docking of the investigated compounds with *Candida rugosa* lipase

Docking experiments were carried out to predict the ligands binding orientation in order to gain a deeper understanding of the key structural aspects of binding. In this respect AutoDock Vina program [9] for these experiments was used.

Many bibliographic researches have been done to know the identified molecules for each extract of the two plants *Origanum majorana L* and *Plantago ciliata L*. We have found seven molecules and we have download their 3D structures from ZINC database. All of them are docked with autodock Vina. We have found that amentoflavone (from *Origanum majorana L*) and plantagoguianidinic acid (from Plantago genera) are competitive inhibitors for Candida rugosa lipase.

Amentoflavone is a flavonoid with 6 cycles and two are hetercycles (figure 3). The binding mode adopted by this molecule allows the benzyl group to settle within a cavity lined with hydrophobic amino acid residues (figure 4). This hydrophobic pocket includes Gly123, Gly124, Phe133, Phe448, Ile453 and Val454. This group interacts with the different aminoacids with hydrophobic interactions (face to edge and face to alkyl). The benzyl and heterocyclic moiety is involved between two aminoacids (Ser209 & His449) of the triad catalytic (catalytic site) in one hydrogen bond between the NH of Ser209 and oxygen of the benzyl group (the distances here are less than 3 A°) (figure 4). These obtained interactions confirm that amentoflavone is competitive inhibitor.

Plantagoguanidinic acid is composed from carboxylic group and pyridyl group with 3 nitrogen atoms (acceptors of hydrogen). The binding mode of this molecule allows the pyridyl part with the carboxylic groups to go inside the polar cavity consists of Ser209, His 449 (triad catalytic) & Glu208 with many involved hydrogen bonds with the aminoacids of the hydrophobic cavity of lipase consists of Phe296, Glu66, Leu297, Pro74, Asn72, Phe344 & Phe345 (figure 5). Hydrophobic interactions found with the aminoacids residues Phe296 & Phe345 are of type face to alkyl. These obtained interactions confirm that plantagoguanidinic acid is competitive inhibitor.

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References

[1] Sellami, I.H., Maamouri, E., Chahed, T., Aidi Wannes, W., Kchouk, M. I., Marzouk, B. 2009. Effect of growth stage on the content and composition of the essential oïl and phenolic fraction of sweet marjoram (Origanum majorana L). Industrial Crops and Products 30 (2009) 395–402. [2] S. A. Kawashty, E. Gamal-El-Din, M. F. Abdalla and N. A. M. Saleh. Flavonoids of Plantago Species in Egypt. Biochemical Systematics and Ecology, Vol. 22, No. 7. pp. 729-733, 1994.

[3] Benguechoua, M; Benarous, K; Khachebaa, I; Chérif, J, k; Trabelsi-Ayadib, M and Yousfi, M. 2014. Inhibition of Candida rugosa Lipase by Different Extracts of Five Algerian Plants and their Antioxidant Activities. Current Enzyme Inhibition, 2014, 10, 121-128.

[4] Nia, S; Benguechouaa, M; Benarousa, K; Khachebaa, I; Chérif, J, k; Trabelsi-Ayadib, M and Yousfi, M. 2014. Screening of Two Algerian Spontaneous Plants for Anti-lipase and Antioxidant Activities. Current Enzyme Inhibition, 2014, 10, 113-120.

[5] Benarous, K; Djeridane, A; Kameli, A and M. Yousfi.2013. Inhibition of Candida rugosa Lipase by Secondary Metabolites Extracts of Three Algerian Plants and their Antioxydant Activities. Current Enzyme Inhibition, 2013, 9, 75-82.

[6] Khedidja Benarous, Isabelle Bombarda, Isabel Iriepa, Ignacio Moraleda, Herbette Gaetan, Abderrahmane Linani, Djillali Tahri, Mohamed Sebaa, Mohamed Yousfi, Harmaline and hispidin from Peganum harmala and Inonotus hispidus with binding affinity to Candida rugosa lipase: In silico and in vitro studies, Bioorganic Chemistry, Volume 62, October 2015, Pages 1–7,

[7] M.R. Stolley, M.L. Fitzgibbon, L. Schiffer, L.K. Sharp, V. Singh, L. Van Horn, A. Dyer, Obesity reduction black intervention trial (ORBIT): six-month results, Obesity 17 (2008) 100–106.

[8] Zhou, Q., Lu, W., Niu, Y., Liu, J., Zhang, X., GAO, B., Akoh, C., Shi, H., Liangli, L., 2013. Identification and Quantification of Phytochemical Composition and anti-inflammatory, Cellular Antioxidant, and Radical Scavenging Activities of 12PlantagoSpecies, J. Agric. Food Chem., 61, 6693–6702.

[9] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2010) 455–461.

[10] Ruiz, C., Falcocchio, S., Xoxi, E., Villo, L., Nicolosi, G., Pastor, F.I. J., Diaz, P., Saso, L. 2006. Inhibition of Candida rugosa lipase by saponins, flavonoïde and alkaloids. J. Mol. Catal. B: Enzym40, 138-143.

[11] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin–Ciocalteu reagent, Methods Enzymol. 299 (1999) 153–178.

[12] V.L. Singleton, J.A. Ross, Colorimetry of total phenolics with phosphomolybdic– phosphotungstic acid reagent, Am. J. Enol. Viticult. 16 (1956) 144–158.

[13] J.H. Kwon, J. M.R. Bélanger, J.R. Jocelyn Paré, A. Varoujan Yaylayan, Application of the microwave-assisted process (MAPTM*) to the fast extraction of ginseng saponins, Food Research International, 36 (2003) 491–498.

[14] G. Spingo, L. Tramelli, D. M. De Faveri, Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics, Journal of food engineering. 81 (2006) 200-208.

[15] A.S. Engelberth, E.C. Clausen, D.J. Carrier, Comparing extraction methods to recover ginseng saponins from American ginseng (Panax quinquefolium), followed by purification using fast centrifugal partition chromatography with HPLC verification. Separation and Purification Technology, 72 (2010) 1-6.

[16] A. A. Mujwah, M. A. Mohammed, M. H. Ahmed, First isolation of a flavonoid from Juniperus procera using ethyl acetate extract, Arabian Journal of Chemistry, 3 (2010) 85–88.

[17] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds, Food Chemistry. 97 (2006) 654–660.

[18] A. Djeridane, M. Yousfi, J. Michel Brunel, P. Stocker, Isolation and characterization of a new steroid derivative as a powerful antioxidant from Cleome arabica in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants, Food and Chemical Toxicology. 48 (2010) 2599–2606.

[19] C. Kaur, H. C. Kapoor, Antioxidant activity and total phenolic content of some Asian vegetables.

International Journal of Food Science and Technology, 37 (2002) 153–161.

[20] V. Katalinic, M. Milos, C.T. Kulisi, M. Jukic, Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 94 (2006) 550–557.

[21] K. Tawaha, F. Q. Alali, M. Gharaibeh, M., Mohammad, T., El-Elimat, Antioxidant activity and total phenolic content of selected Jordanian plant species, Food Chemistry. 104 (2007) 1372–1378.

[22] L. Hua-Bin, C. Ka-Wing, W. Chi-Chun, F. King-Wai, C., Feng, J., Yue, Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry 102 (2007) 771–776.

[23] S. Siddharthan, C. Yi-Zhong, C., Harold, C. Mei, Sun, Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry 102 (2007) 938–953.

Table 1. Extraction yield, total phenol and total flavonoid contents for the studied plants mg/g dry weight.

Plants	Extract (mg)	Phe tot (mg/g GAE)	Fv tot (mg/g RE)
Matricaria recutita	22.8	6.64±0.02	4.40±0.00
Saussurea costus	101.2	18.19±0.02	17.41±0.00
Origanum majorana L	589.9	134.17±0.01	61.21±0.00
Plantago ciliata L	160	143.09±0.07	45.88±0.02
Senna alexandrine	31.6	4.68±0.01	7.99±0.01

GAE: Gallic acid equivalent**, RE:** Rutin equivalent, **dw**: dry weight.

Table 2. IC50, Ki values and type inhibition for each extract.

Plant	IC_{50} (mg/ml)	K_i (mg/ml)	Inhibition type
Origanum majorana L	0.26 ± 0.01	0.055 ± 0.00	Competitive
Plantago ciliata L	0.29 ± 0.05	0.05 ± 0.01	Competitive
Orlistat	0.068 ± 0.02	0.01 ± 0.00	Competitive

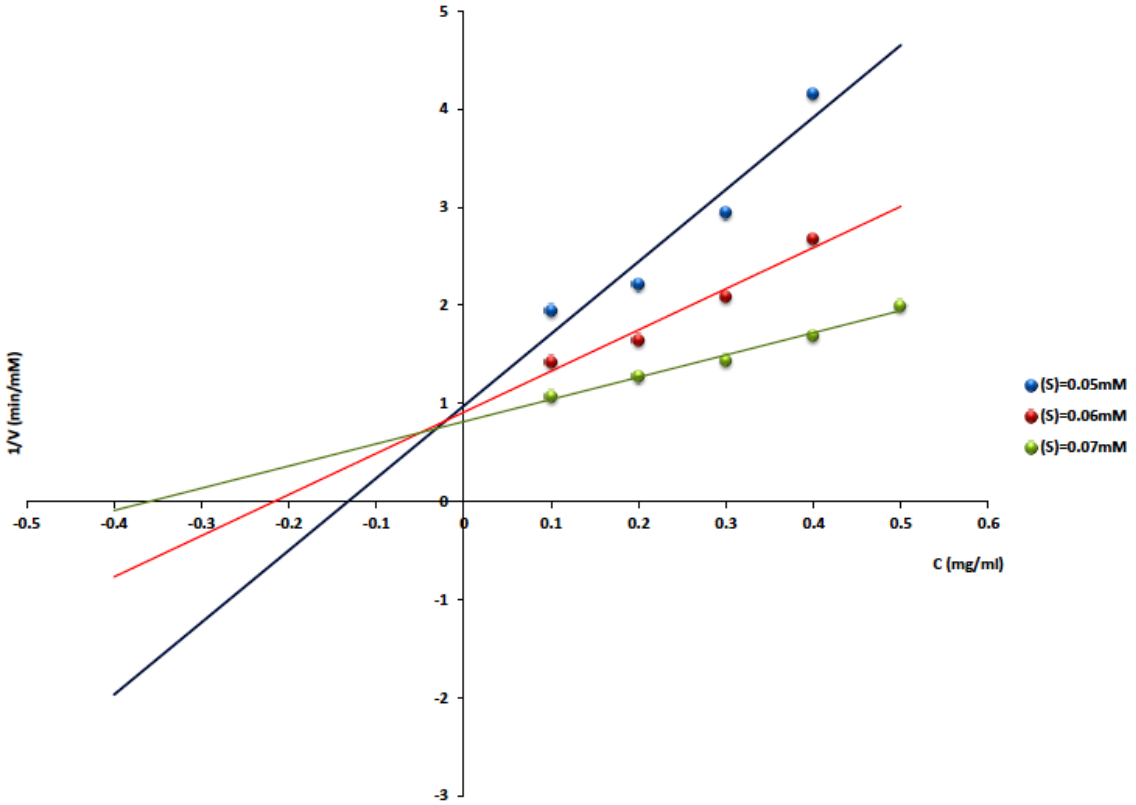


Figure 1. Dixon plots of inhibition of *Candida rugosa* lipase by the extract of *Plantago ciliata (Pc)* expressed in mg/ml. Substrate concentrations: 0.05 mM (S1), 0.06mM (S2) and 0.07 mM (S3). The graph represents the means of three experiments.

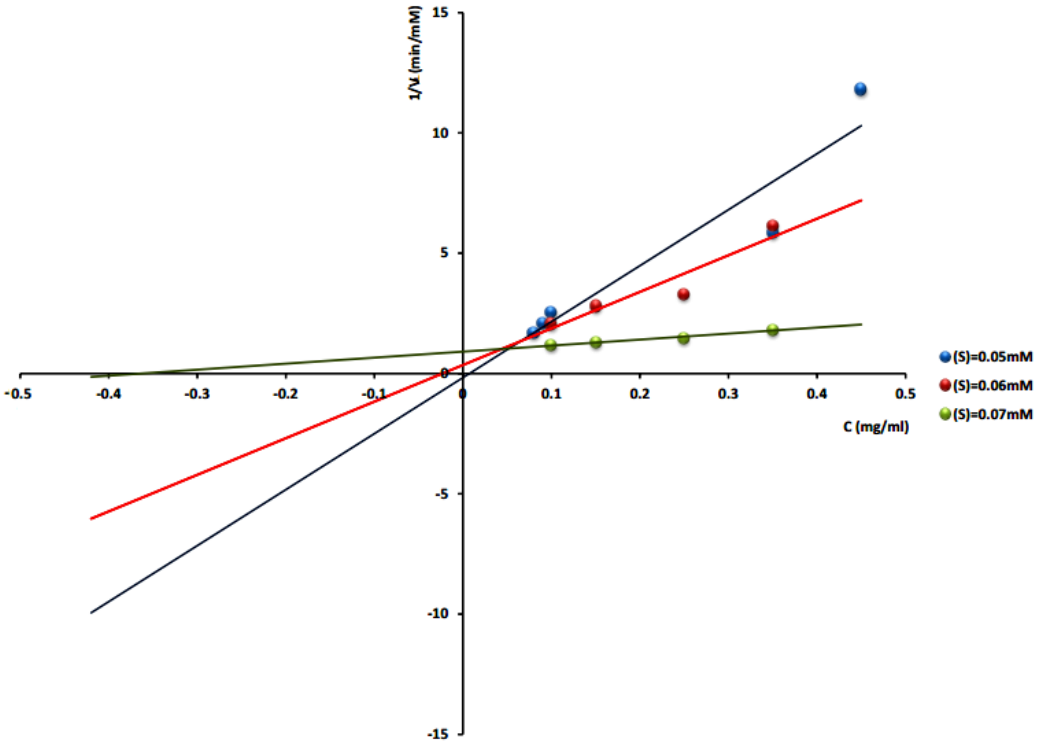
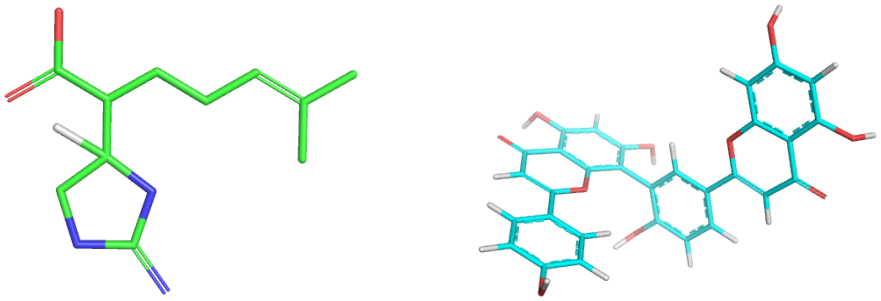


Figure 2. Dixon plots of inhibition of *Candida rugosa* lipase by the extract of *Origanum majorana (Om)* expressed in mg/ml. Substrate concentrations: 0.05 mM (S1), 0.06mM (S2) and 0.07 mM (S3). The graph represents the means of three experiments.



(a) **(b)**

Figure 3. The 3D structures of proposed molecules for docking

(a) : plantagoguinidinic acid, (b) : Amentoflavone

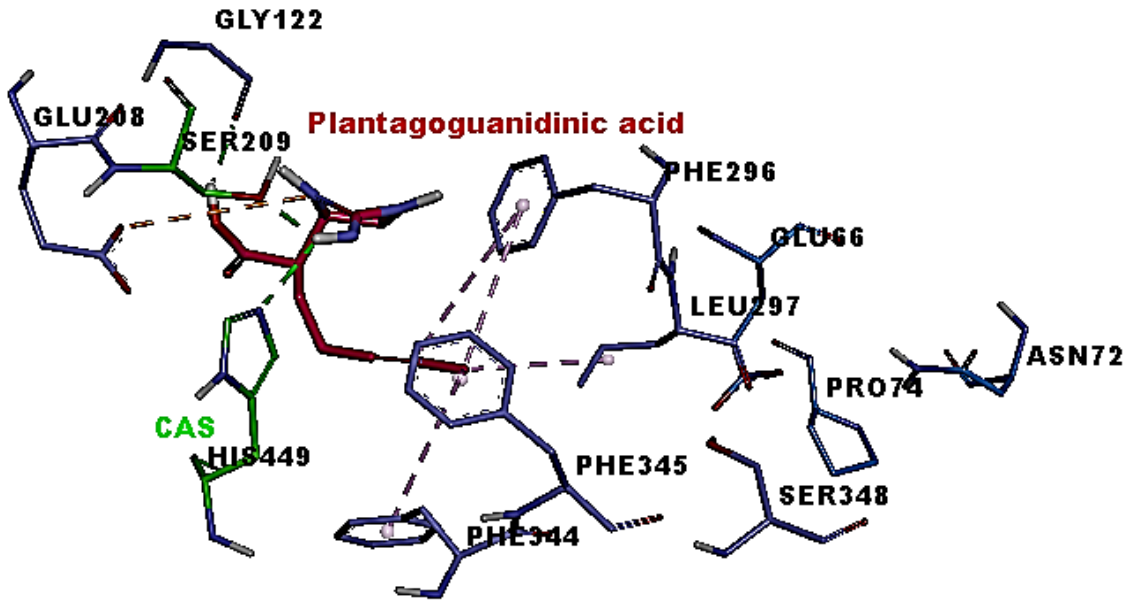


Figure 4. Docking pose of Plantagoguanidinic acid inhibitor (red sticks) into CRL. Amino acid residues of the binding site are color-coded.

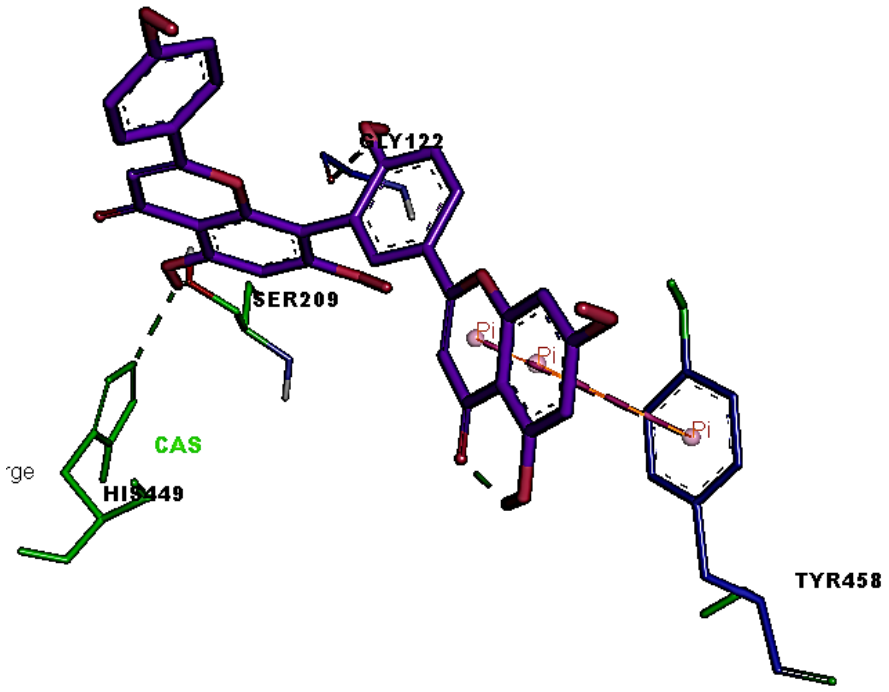


Figure 5. Docking pose of amentoflavone inhibitor (Purple sticks) into CRL. Amino acid residues of the binding site are color-coded.