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In Silico Evaluation of Antioxidant Properties of Cinnamaldehyde Phenylhydrazone †

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Abstract: Hydrazone-type Schiff bases derived from natural compounds had demonstrate to be effective and versatile antioxidants. Particularly, the interest of this work is to analyze the antioxidant activity of cinnamaldehyde phenylhydrazone (CPH), an effective antioxidant synthesized by our group, through theoretical calculations. Considering that the enzymes cytochrome P450 (CP450) and NADPH oxidase (NO) are associated with the oxidative stress process, we chose these enzymes to evaluate their interaction with the antioxidant CPH. The interactions were analyzed by coupling studies performed with the SwissDock server. Considering the parameters of the scoring function based on the energy of the system, the best protein-ligand binding geometries were obtained and the interaction distances between the CPH enzymes were studied. Taking into account the information from all the data obtained, it could be concluded that CPH has strong interactions with enzymatic substrates due to electrostatic interactions with amino acid residues present in segments corresponding to hydrophobic domains. These results attest to the significant antioxidant capacity of CPH observed in experimental antioxidant tests.

Keywords: in silico study; antioxidant; hydrazones

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1. Introduction

Hydrazones are well known compounds that have shown potent antioxidant activities with respect to free radical scavenging. Normally, hydrazones are characterized by an imine group (azomethine), which is essential for their antioxidant activities [1].

Cinnamaldehyde phenylhydrazone (Figure 1) was synthesized from cinnamaldehyde and phenylhydrazine and its antioxidant properties have been demonstrated [2].

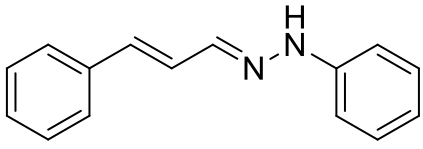

Figure 1. Structure of cinnamaldehyde phenylhdrazone.

Molecular docking techniques are used to predict how a protein interacts with small molecules, such as antioxidants. This ability acts on a significant part of the protein's dynamics which may enhance/inhibit its interaction function in terms of which molecules are targeted [3]. The molecular docking studies can be used to model the interaction between a small molecule and a protein at the atomic level, allowing to characterize the behavior of small molecules at the binding site of target proteins [4]. Thus, the accurate

prediction of the binding modes between the ligand and the protein is of fundamental importance in the design of small transport molecules based on modern structures. Considering that the enzymes cytochrome P450 (CP450) and NADPH oxidase (NO) are associated with the oxidative stress process, in this work we chose these enzymes to evaluate their interaction with the antioxidant CPH. The ability of interaction of CPH with these enzymes will be a measure of the capacity of inhibit the oxidative process.

The aim of the current work was to evaluate the interaction of CP450 and NADPH oxidase NO enzymes, the main fraction of the casein, with cinnamaldehyde phenylhydrazone (CPH) by molecular docking calculations. We used a SwissDock server applying specific scoring functions based on energy terms to obtain the best protein-ligand binding patterns and binding affinity between CPA and CP450-NADPH enzymes.

2. Computational Methodology

2.1. Cinnamaldehyde Phenylhydrazone Structure Preparation

The structure of the antioxidant ligand in this study was optimized employing Gaussian09 program suit [] with the hybrid density functional B3LYP and 6-31+G(d,p) basis sets.

2.2. Proteins Structure Preparation

The amino acid sequence of CP-450 and NO were obtained from the Protein Data Bank of RCSB [5]. The structure of CP-450 corresponding to the PDB: 1OG5 (structure of human cytochrome P450 CYP2C9) with 475 residues of amino acid; and the structure of NO corresponded to the PDB: 2CDU (structure of water-forming NAD(P)H oxidase from *Lactobacillus sanfranciscensis*) with 452 residues. The protonation state of the ionizable residues at pH = 7 was evaluated with the PROPKA program [6]. Final structures were minimized with the USCF Chimera program according to the MM calculation method and were validated by the Mol-Probity server [7].

2.3. Molecular Docking Studies

After the preparation of proteins and ligand structures, molecular docking calculations were performed by SwissDock servers [8]. These docking studies corresponded to a system with flexible ligand and rigid protein. Using specific scoring functions based on energy terms the best protein–ligand binding models were obtained. Interaction types and distances were evaluated with the USCF Chimera program and Discovery Studio Visualizer [9].

3. Results and Discussion

3.1. Molecular Docking Calculation Conducted with CPH/CP-450

The results obtained by molecular docking protocols with SwissDock for the first cluster are shown in Table 1 and Figures 2 and 3. Predicted binding sites were clustered in 57 clusters with populations of 4–16 members. The cluster rank was predicted by the full fitness energy of the members. The best full fitness corresponded to the first member of each cluster which is related to a better affinity of the ligand towards the protein.

Table 1. Data obtained by molecular docking protocols with the SwissDock server.

Cluster Number	Population	Energy (Kcal/mol)	ΔG (Kcal/mol)	Full Fitness (Kcal/mol)
1	8	15.81	−6.84	−2270.35
2	9	15.43	−6.65	−2269.55
3	16	17.90	−6.99	−2269.50
4	8	14.17	−7.17	−2267.67
5	8	14.65	−7.62	−2267.43

From geometries of predicted binding sites corresponding to clusters 1–5 ranged from −2270.35 and −2267.43 Kcal/mol of full fitness values, the interaction types present in between CPH and the CP-450 chain were identified. The observed interactions between CPH and the amino acid residues consisted of several hydrophobic interactions, especially with the residues corresponding to the sequence from 208–367 residues of amino acid. The cavity of the binding site contains aliphatic and aromatic hydrophobic residues such as Ala447, Phe428, Cys435, Leu366, Pro367, Phe144, Leu208, Ile205, with a distance of interaction between CFH and residues of 3.5–5.1 Å. Similar interactions were observed with binding sites of other clusters not shown in Figures 2 and 3. These results indicate that the CHP interacts with the hydrophobic sites of the central cavity of enzyme.

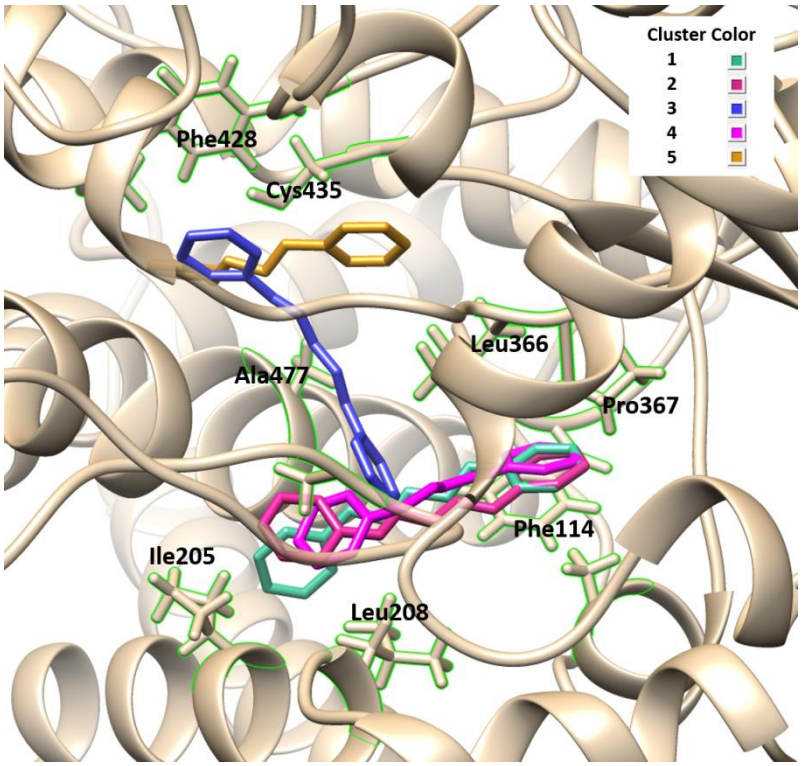

Figure 2. Solutions of the docking simulation of the CP-450 with CPH obtained with the SwissDock server.

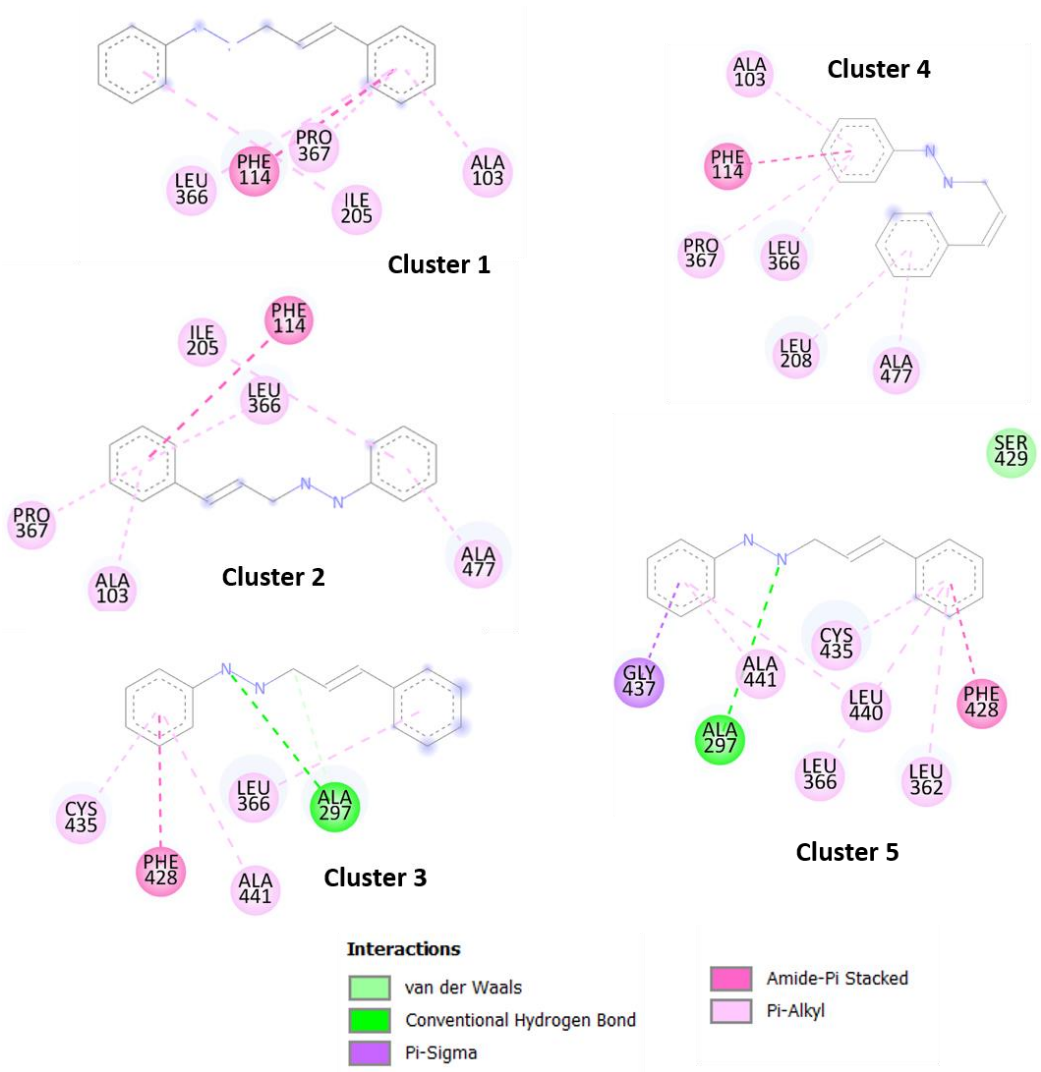

Figure 3. 2D representation of interaction types of CPH with CP-450 enzyme for the first five clusters.

3.2. Molecular Docking Calculation Conducted with CPH/NO

The results obtained by molecular docking protocols with SwissDock for the first cluster are shown in Table 2 and Figures 4 and 5. Predicted binding sites were clustered in 52 clusters with populations of 1–12 members. The cluster rank was predicted by the full fitness energy of the members. The best full fitness corresponded to the first member of each cluster.

Table 2. Data obtained by molecular docking protocols with the SwissDock server.

Cluster Number	Population	Energy (Kcal/mol)	ΔG (Kcal/mol)	Full Fitness (Kcal/mol)
1	6	10.38	−7.15	−2379.45
2	8	14.76	−6.72	−2375.41
3	7	15.87	−6.91	−2375.05
4	1	15.92	−6.61	−2374.25
5	8	19.00	−6.68	−2374.23

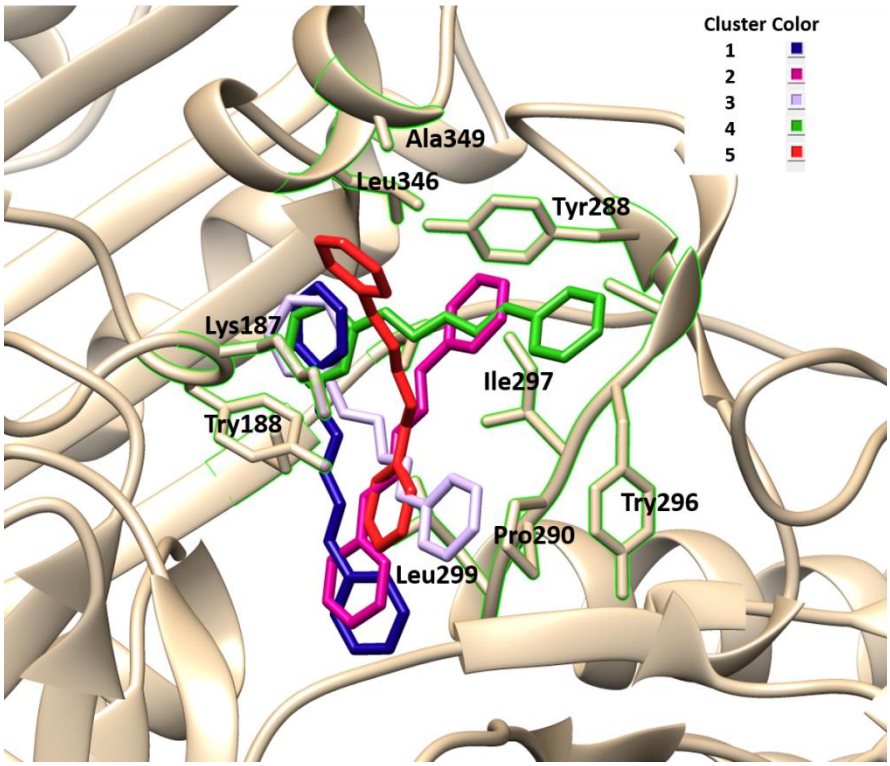

Figure 4. Solutions of the docking simulation of the NO with CPH obtained with the SwissDock server.

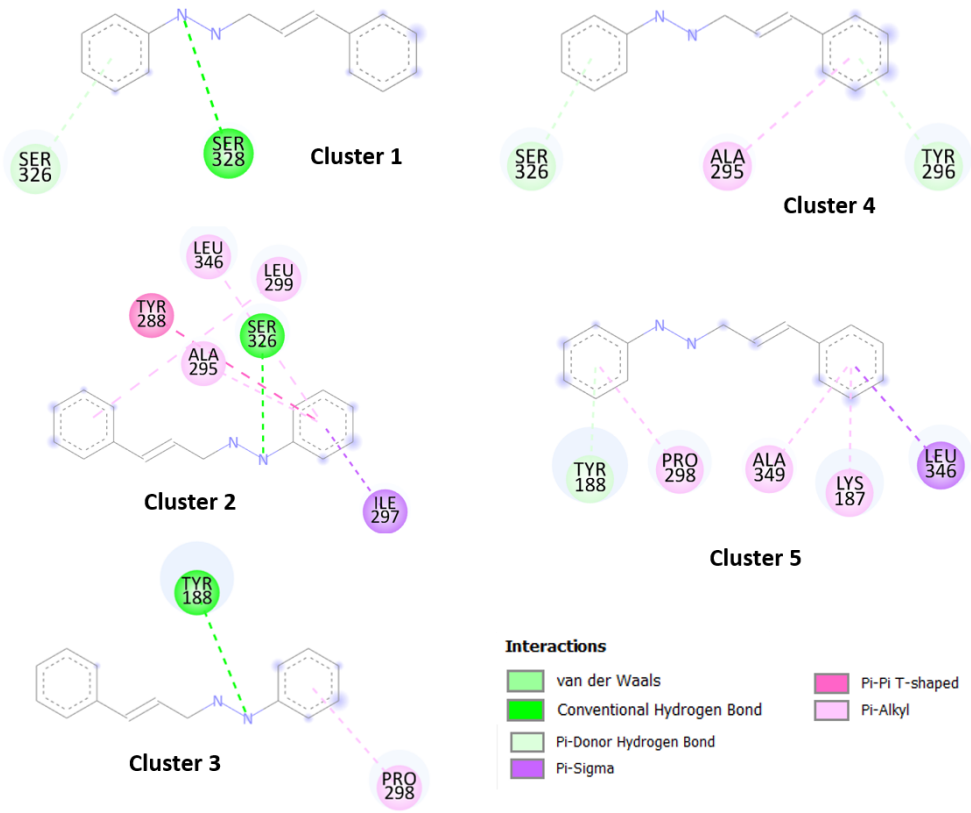

Figure 5. 2D representation of interaction types of CPH with NO enzyme for the first five clusters.

From geometries of predicted binding sites corresponding to clusters 1–5 ranged from −2379.45 and −2374.23 Kcal/mol of full fitness values, the interaction types present

in between CPH and the NO chain were identified. The observed interactions between CPH and the amino acid residues consisted of several hydrophobic interactions, especially with the residues corresponding to the sequence from 208–367 residues of amino acid. The cavity of the binding site contains aliphatic and aromatic hydrophobic residues such as Lys187. Try188, Try288, Ala295, Try296, Pro298, Ser326, Leu346, Ala349, with a distance of interaction between CFH and residues of 3.0–4.7 Å. Similar interactions were observed with binding sites of other clusters not shown in Figures 3 and 4. These results indicate that the CHP interacts with the hydrophobic sites of the enzyme.

4. Conclusions

In silico calculation is an useful tool to study the binding modes of CFH with CP-450 and NO enzymes employing SwissDock server.

If we compare the total energies, it is observed that in both cases the values are of the same order, so that the affinity of the binding would be equivalent in both cases, although a little higher in the case of the NO enzyme. These values are high, implying high affinity for the enzymes.

The observed interactions between CFH and the residues consisted of hydrophobic interactions of an electrostatic nature.

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Conflicts of Interest: The authors declare no conflict of interest.

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