

Molecular Docking Studies of Natural Phenolic Compound and Derivates with Phospholipase A2

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Abstract:

The enzyme phospholipase A2 (PLA2) catalyzes the conversion of membrane phospholipids in the inflammatory mediators, such as prostaglandins and leukotrienes. Because of this role, substances with inhibitory activity of PLA2 enzyme, has gained prominence in the scientific community like possible anti-inflammatory. Several studies have shown that phenolic compounds such as flavonoids, phenolic acids and other, has, among various biological activities, anti-inflammatory activity by inhibition of the enzyme PLA2. Based on this context, this study aimed to conduct a molecular docking study of various natural phenolic compounds and some of their derivatives forward to the enzyme PLA2. The crystallographic structure of PLA 2 was obtained from Target Database Protein Data Bank [PDB ID: 1KPM] and the ligands were obtained from PubChem Database. The docking was performed using the AutoDock 4.0 software. It was observed that among the phenolic compounds included in the study, those with better interaction with the enzyme were rosmarinic acid 3'-O-beta-glucoside, 4-nerolidylcatechol, rosmarinic acid methyl ester, quercetin 3-O-malonylglucoside, quercetin pentaacetate and rosmarinic acid, respectively. The present study provides a better understanding of the inhibition of PLA2 by phenolic compounds, which may contribute to the development of new anti-inflammatories.

Keywords: phospholipase A2; phenolic compound; molecular docking; anti-inflammatory

1. Introduction

Phospholipase A2 (PLA2) are enzymes that hydrolyze the bond in the sn-2 position of cell membrane phospholipids and lipoprotein, in a Ca²⁺ dependent reaction [1,2], producing free fatty acids, precursors of various pro-inflammatory lipid mediators such as leukotrienes, prostaglandins and platelet activating factor [3,4]. The mammalian PLA2 are subdivided into two main families: the secretory enzymes with low molecular weight (sPLA2) consisting of four types (I, II, V and X), and cytosolic PLA2 high molecular mass existing as two types (IV or VI or cPLA2 and iPLA2)[5-7].

PLA2 is extremely important in the inflammation cascade by catalyzing the conversion of membrane phospholipids in free

arachidonic acid by hydrolysis [8,9], Then is converted by cyclooxygenases to prostaglandins and leukotrienes, increasing the inflammatory process [10].

Due the role of PLA2 in the inflammatory process, inhibition of this enzyme has won major therapeutic interest¹. Many natural compounds, such as aristolochic acid, indol derivatives, atropine, eugenol and berberine bind to the active site of the enzyme mainly by hydrophobic interactions [11].

The importance of natural products in modern medicine has been well recognized. Natural Compounds isolated from plant sources has an extensive use since the past and is still used until now in the treating of various pathologies. In addition to serving like compounds of interest in its natural form, these compounds can also serve

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as templates for the synthesis of derivatives with higher activity and lower toxicity [12]. Thus, molecular modeling studies PLA2 as the target can be useful in designing new and more potent anti-inflammatory drugs [11].

Among the natural substances, phenolic compounds such as flavonoids, phenolic acids and others have become very prominent due to its diverse biological activities, among them, anti-inflammatory activity by inhibiting the enzyme phospholipase A2 [1,13–15]. These compounds are secondary metabolites of plants that are involved in defense against ultraviolet radiation and pathogens [16]. These compounds have aromatic rings with one or more hydroxyl groups or methoxyl [17].

Currently, the anti-inflammatory non-steroid drugs present on the pharmaceutical market, reduces the conversion of arachidonic acid into prostaglandins by inhibiting the cyclooxygenase enzyme [18–23], however, does not inhibit 5-lipoxygenase enzyme. Thus, the excess of arachidonic acid produced is converted to leukotrienes by the 5-lipoxygenase, which in high levels are directly related to adverse effects on the gastric and renal pathways, as observed in patients who use non-steroidal anti-inflammatory drugs. Then, for an anti-inflammatory drug be effective and with less side effects, the substance may selectively inhibit the PLA2 and avoid not only prostaglandin, but also the formation of leukotrienes [24].

This study objective was to compare interaction's affinity of natural phenolic compounds with the PLA2 enzyme, because the interaction mode and other structural details of the complex formed by the phenolics compounds and PLA2 enzyme may contribute to the new and more potent anti-inflammatory development.

2. Results and Discussion

2.1 Docking Study

Based on the results the compounds that showed better interaction development with the PLA2 enzyme were the rosmarinic acid 3'-O-beta-glucoside, 4-nerolidylcatechol, rosmarinic acid methyl ester, quercetin 3-O-malonylglucoside, quercetin pentaacetate and rosmarinic acid with binding free energy of -9.68, -9.44, -8.87, -8.81, -

8.79 and -8.13, respectively (Table 1).

The Figure 1 shows that, with the exception of rosmarinic acid methyl ester and quercetin acetate, compounds that had better binding energy were interacting with the ASP 49 amino acid by H-bond. It can also be observed that the compounds rosmarinic acid methyl ester and quercetin pentaacetate, although it does not interact with ASP 49 amino acid, it interacts with the HIS 48 amino acid by H-bond. These amino acids interactions are important for the compounds pharmacological activity, since the ASP 49 and HIS 48 amino acids are involved in the water molecule activation, and means of basic catalysis, hydrolyze phospholipids at the sn-2 position [24]. Since amino acids TYR 28, GLY 30, GLY 32 and ASP 49, are involved in the coordination of calcium ion are responsible for polarization the correct positioning of the phospholipid ester carbonyl providing a region suitable for water molecule nucleophilic attack [15, 24]. As can be seen, the rosmarinic acid 3'-O-beta-glucoside promotes interaction with the amino acids GLY 30 and ASP 49, the 4-nerolidylcatechol in the ASP 49, quercetin 3-O-malonylglucoside with GLY 32 and ASP 49, quercetin pentaacetate with GLY 30 and the rosmarinic acid with the ASP 49. Only the compound rosmarinic and acid methyl ester does not promote any polar interaction with an amino acid involved in the calcium ions coordination.

The compounds that presented binding energy more favorable were interacted to the amino acids essential for the enzyme catalytic activity, those are responsible for the water molecule activation (HIS 48 and ASP 49) or responsible for coordinating ion Ca^{2+} (TYR 28, GLY 30, GLY 32 and ASP 49) they can be considered as PLA2 inhibitors [11].

Figure 2 shows that all the ligands that are more favorable with docking energy, fits perfectly in active site of the PLA2 enzyme, which also promotes hydrophobic interactions, as well as hydrophilic interactions.

It can be noted that the phenolic hydroxyls have a great importance to the interaction of compounds with the active site of PLA2, however, was not observed any relation in the amount of hydroxyl with the interaction energy. It was also observed that replacement of the phenolic hydroxyls by other polar groups, as in the

quercetin's case pentaacetate, can maintain or improve the interaction efficiency of the compound with the enzyme active site. The quercetin pentaacetate is a semisynthetic quercetin derivative that were obtained by

acetylation [25, 26] and presented a better interaction with the active site of PLA2 than its prototype, quercetin. But unfortunately it was not found in the literature about their inhibitory activity of PLA2.

Table 1. Docking energies of phenolic compounds against the enzyme phospholipase A2.

Compound	ΔG Binding (Kcal/mol) ^a	Ligand Efficiency	ΔG vdW_hb_desolv	ΔG Electrostatic	ΔG Total Internal	ΔG Unbound energy	ΔG Energy torsional
Quercetin	-7.06	-0.32	-8.42	-0.42	-1.12	-1.12	1.79
4-Nerolidylcatechol	-9.44	-0.41	-11.96	-0.46	-1.09	-1.09	2.98
Caffeic Acid	-5.75	-0.44	-6.95	-0.29	-0.47	-0.47	1.49
Ferulic Acid	-5.54	-0.40	-5.82	-1.21	-0.48	-0.48	1.49
Sinapic Acid	-5.95	-0.37	-6.48	-1.26	-0.85	-0.85	1.79
Rosmarinic Acid	-8.13	-0.42	-10.92	-0.79	-1.24	-1.24	3.58
Rosmarinic acid 3'-O-beta-glucoside	-9.68	-0.26	-14.17	-0.58	-4.17	-4.17	5.07
Rosmarinic Acid Methyl Ester	-8.87	-0.23	-12.12	-0.03	-0.92	-0.92	3.28
Rosmarinyl glucoside	-7.62	-0.18	-12.74	-0.25	-1.97	-1.97	5.37
Quercetin 3-Methyl Ether	-7.30	-0.31	-8.6	-0.49	-0.88	-0.88	1.79
Rutin	-2.87	-0.06	-7.21	-0.42	-2.01	-2.01	4.77
Retusin	-7.71	-0.39	-9.08	-0.42	-0.65	-0.65	1.79
Amentoflavone	-5.62	-0.24	-7.9	-0.4	-1.63	-1.63	2.68
Gallic Acid	-4.43	-0.44	-5.18	-0.74	-0.74	-0.74	1.49
Quercimeritrin	-7.76	-0.34	-10.97	-0.37	-0.48	-0.48	3.58
Quercetin pentaacetate	-8.79	-0.31	-11.9	-0.17	-0.24	-0.24	3.28
Isoquercetin	-7.24	-0.24	-10.49	-0.33	-1.14	-1.14	3.58
Quercetin 3-O-malonylglucoside	-8.81	-0.36	-13.23	-0.35	-0.67	-0.67	4.77

^a ΔG binding = ΔG vdW+hb+desolv + ΔG elec + ΔG total + ΔG tor - ΔG unb.

2.2 Validation of the Methodology

The redocking presented value of RMSD = 1.89 Å, considering the pose most stable of the most populous cluster. This result is considered

satisfactory when the RMSD (which measures the deviation) between the best pose and the ligand complexed crystallographic is less than 2.0 Å [27]. Thus, the value displayed at this step validates the conditions used for the present docking study.

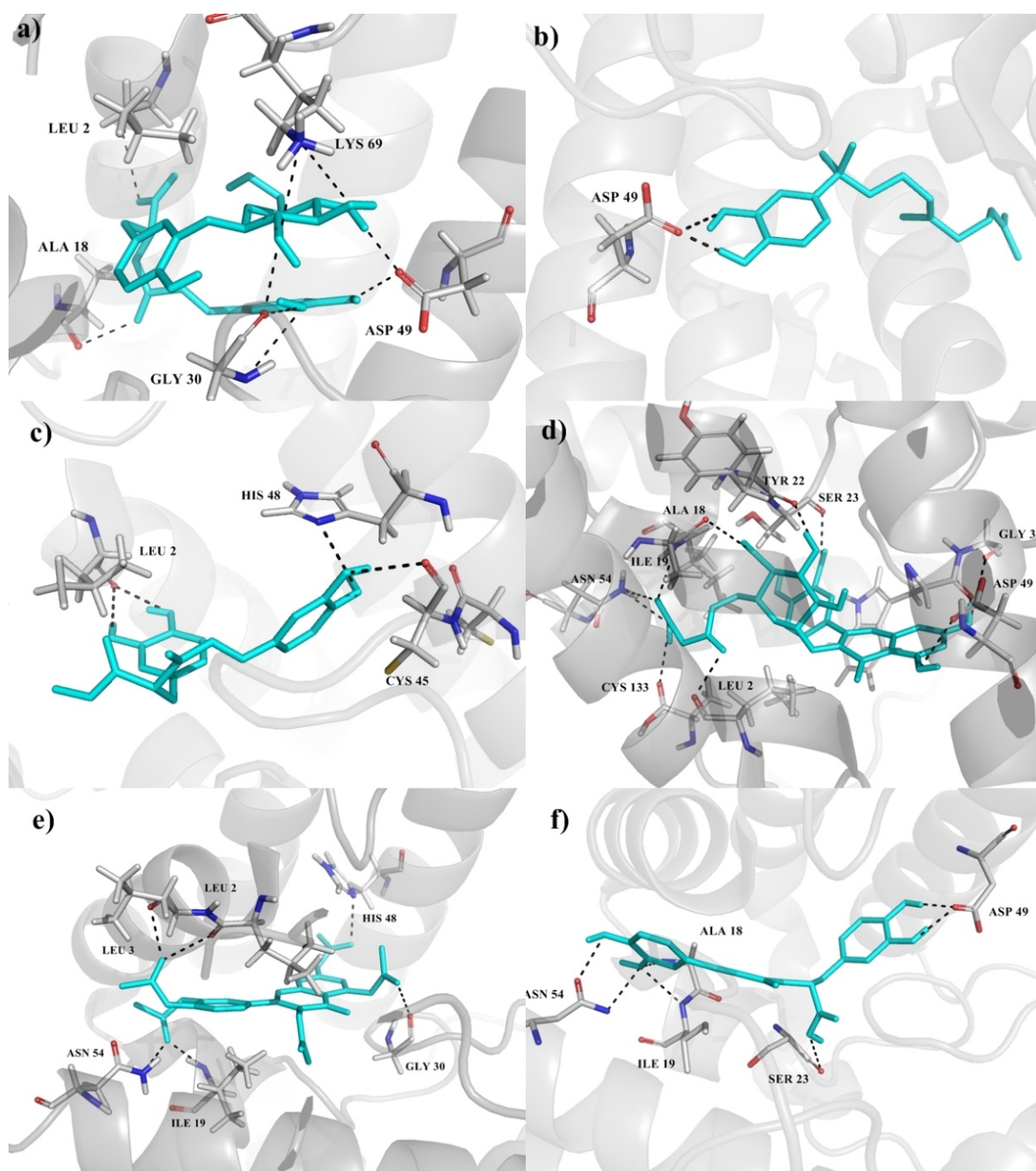


Figure 1. Hydrogen bonds of phenolic compounds **a)** rosmarinic acid 3'-O-beta-glucoside, **b)** 4-nerolidylcatechol, **c)** rosmarinic acid methyl ester, **d)** quercetin 3-O-malonylglucoside, **e)** quercetin pentaacetate **e)** quercetin pentaacetate **f)** rosmarinic acid with amino acids at the active site of PLA2.

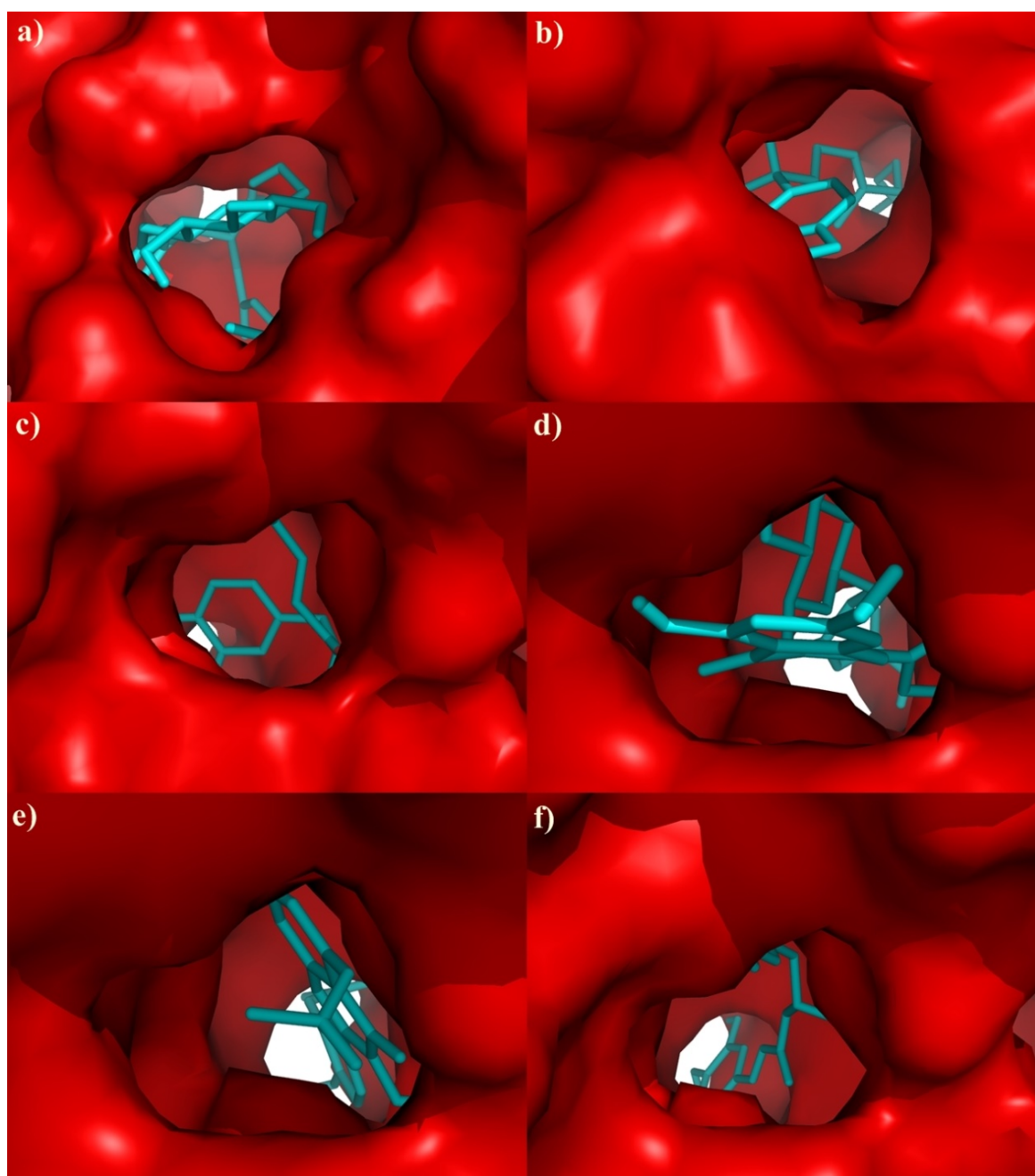


Figure 2. Compounds **a)** rosmarinic acid 3'-O-beta-glucoside, **b)** 4-nerolidylcatechol, **c)** rosmarinic acid methyl ester, **d)** quercetin 3-O-malonylglucoside, **e)** quercetin pentaacetate **e f)** rosmarinic acid in active site of PLA2.

3. Material and Methods

3.1 Preparation of target protein

The crystallographic structure of the target enzymatic PLA2 was obtained from the Protein Data Bank database [PDB ID: 1KPM] [28]. This structure was chosen because it has been used in other molecular docking studies [9, 29,]. The enzyme was elucidated by X-ray crystallography, with a resolution of 1.80 Å. Gasteiger charge and

polar hydrogens required for the potential calculations were added considering the target structure, and the water molecules removed.

3.2 Preparation of ligand molecules

All the structures of ligands were obtained by PubChem Database [30] (<https://pubchem.ncbi.nlm.nih.gov/>). They were included in the study 18 structures of natural

phenolic compounds and some of its derivatives (**Table 2**). The molecules of the binders were optimized by 32quantum mechanical, semi-empirical method PM6 [31], with software

MOPAC7 [32]. The ligands non-polar hydrogens were suppressed and torsional links of each binder were set automatically.

Table 2. Natural phenolic compounds included in the study.

Compound	IUPAC Name	PubChem CID
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	5280343
4-Nerolidylcatechol	4-[(6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-yl]benzene-1,2-diol	5352089
Caffeic Acid	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	689043
Ferulic Acid	(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	445858
Sinapic Acid	(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid	637775
Rosmarinic Acid	(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid	5281792
rosmarinic acid 3'-O-beta-glucoside	(2R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-[4-hydroxy-3-[(2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]propanoate	25245848
Rosmarinic Acid Methyl Ester	methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[3-(3,4-dihydroxyphenyl)prop-2-enoyloxy]propanoate	3012090
Rosmarinyl glucoside	(2R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-[3-hydroxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]propanoic acid	11606086
Quercetin 3-Methyl Ether	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	5280681
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	5280805
Retusin	2-(3,4-dimethoxyphenyl)-5-hydroxy-3,7-dimethoxychromen-4-one	5352005
Amentoflavone	8-[5-(5,7-dihydroxy-4-oxochromen-2-yl)-2-hydroxyphenyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	5281600
Gallic Acid	3,4,5-trihydroxybenzoic acid	370
Quercimeritrin	2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	5282160
Quercetin pentaacetate	[2-acetyloxy-4-(3,5,7-triacetyloxy-4-oxochromen-2-yl)phenyl] acetate	14005
Isoquercetin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	5280804
Quercetin 3-O-malonylglucoside	3-[[[(2R,3S,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxochromen-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid	44259188

3.3 Docking Study

The *AutoDock 4.0* [33] software was used as the choice for conducting studies on PLA2 target. The AutoDock Tools module was used to prepare

and analyze the computer simulations. The AutoDock require three-dimensional maps pre-calculated, arranged in a housing composed of three-dimensional grid points (grid maps) in a

defined region in the macromolecule (target site). The AutoGrid 4.0 program was used to generate the maps for the ligands. The box was placed in the catalytic region of the enzyme. The dimensions of the box in the X-, Y- and Z-axis were respectively 52 Å 60 Å and 48 Å spacing of 0.375 Å. The Lamarckian Genetic algorithm (GA-LS) was chosen to search for the best conformations 100 runs for each ligand (genetic algorithm with local search). During the search process, the enzyme was kept rigid, while the ligands were kept flexible. The initial population was defined as 150 and the search process occurred through random initial conformations. The maximum power ratings chosen, was 25,000,000. The maximum number of generations was 27,000. The number of elitism chosen was 1. The gene mutation and crossover rates were defined respectively as 0.02 and 0.80. At the end of the calculations, 100 different poses were obtained and grouped into different clusters defined by proximity and energy RMSD values ("root mean square deviation") in accordance with the default of AutoDock.

3.4 Validation of the Methodology

The validation of the method was performed by the technique of redocking, using the same target protein and its complexed ligand ("native"): vitamin E.

4. Conclusions

This study aimed to investigate, through molecular docking, affinity interaction of various natural phenolic compounds and some of their derivatives with phospholipase A2, and see how this interaction occurs in order to contribute to the development of new drugs with anti-inflammatory potential.

It was observed that compounds which showed the best energy interaction with the active site of the enzyme interacted with the amino acids essential for the enzyme catalytic activity, and even if the phenolic hydroxyl groups are essential for interaction of compounds, there was no relationship between the amount hydroxyls with the energy of interaction. Another important observation was regarding the phenolic hydroxyls replacement of the flavonoid quercetin with other

polar groups, such as the quercetin pentaacetate case, which showed better interaction energy when compared with quercetin.

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