 doi:10.20944/preprints201805.0133.v1

Article

In Silico Evaluation of the Antioxidant Potential of Two Caffeine Analogs with Recognized Epithelial Anticancer Potential

Josivan da Silva Costa[1,2,3]*, Cleydson Breno Rodrigues dos Santos[2,3], Karina da silva Lopes Costa[2], Ryan da Silva Ramos[2,3], Davi do Socorro Barros Brasil[5], Rosivaldo dos Santos Borges[1], Joaquín María Campos Rosa[5] and Williams Jorge da Cruz Macêdo[1,3]

[1] Postgraduate Program in Biotechnology and Biodiversity-Network BIONORTE, Federal University of Pará, Rua Augusto Corrêa, 01, Belém, Pará, 66075110, Brazil; josivan.chemistry@gmail.com (J.S.C.)
[2] Laboratory of Modeling and Computational Chemistry, Federal University of Amapá, Department of Biological Sciences. Rod. Juscelino Kubitschek, Km 02, s/n, Macapá, Amapá, 68902-280, Brazil; breno.unifap@gmail.com (C.B.R.S.); karinalopesfarm@gmail.com (K.S.L.C.); ryanquimico@hotmail.com (R.S.R.)
[3] Laboratory of Molecular Modeling and Simulation System, Federal Rural University of Amazônia, Rua João Pessoa, 121, Capanema, Pará, 68700-030, Brazil; williams.macedo@ufra.edu.br (W.J.C.M.)
[4] Institute of Technology, Federal University of Pará, Av. Augusto Corrêa, 01, Belém, Pará, 66075-900, Brazil; davibb@ufpa.br (D.S.B.B.)
[5] Department of Pharmaceutical and Organic Chemistry, University of Granada, Campus of Cartuja, Granada, 18071, Spain; jmcampos@ugr.es (J.M.C.R.)
* Correspondence: josivan.chemistry@gmail.com; Tel.: +55-96-4009-2699

Abstract: The antioxidant activity of molecules constitutes an important factor for the regulation of redox homeostasis and reduction of oxidative stress. Cells affected by oxidative stress can undergo genetic alteration, causing structural changes and promoting the onset of chronic diseases, such as cancer. The *in silico* study performed here was developed to evaluate the antioxidant potential of two molecules, ZINC08706191 (Z91) and ZINC08992920 (Z20), with recognized epithelial anticancer potential. Molecular docking, chemical-quantum calculations and Pearson's correlation were performed. The Z91 and Z20 molecules showed lower binding free energy (ΔG) values for the receptor-ligand interaction than the reference molecules (caffeine – CAF and ascorbic acid – AA), and better results for values of molecular descriptors correlated with ΔG, resulting in a decrease of ΔG. Strong correlations were observed between ΔG values for the five receptors evaluated and ΔG values of the potential epithelial anticancer activity available in literature. These results attest to the significant antioxidant potential of the Z91 and Z20 molecules and their strong relation with the potential epithelial anticancer activity and may be indicated for further analysis in relation to the control of oxidative stress and epithelial anticancer activity.

Keywords: Antioxidant potential; Molecular docking; Molecular descriptors; Binding free energy; Free radicals; Oxidative stress

1. Introduction

The oxidants play a key role in maintaining the redox homeostasis of cells. However in large quantities an imbalance can be triggered. The reactive oxygen species (ROS), resulting from aerobic respiration, are examples of extremely unstable oxidants that can collide with other species (molecules or biomacromolecules), causing their transformation (oxidative damages). Increased oxidative damage causes cell stress, known as oxidative stress [1, 2].

 doi:10.20944/preprints201805.0133.v1

Several chronic diseases such as diabetes, neurodegenerative, cardiovascular and cancer can be caused by increased oxidative stress [3]. This occurs from the activation of various transcription factors, which can express hundreds of different genes, such as growth factor promoters and inflammatory cytokines, for example, which lead to the activation of inflammatory pathways transforming a normal cell into a cancer cell [4].

Thus, maintenance of redox homeostasis and reduction of oxidative stress depend on the efficiency of antioxidant present in the cell, since the first and second defense barriers (antioxidant enzymes and proteolytic and lipolytic enzymes, respectively) have already been overcome [5]. Some enzymes (such as NADPH Oxidase – NO, Citochrome P450 – CP450, Myeloperoxidase – MP, Lypoxygenase – LO, e Xhantine Oxidase – XO) are known to generate ROS during the metabolism of arachidonic acid and its inhibition break the ROS production cycle with consequent reduction of oxidative stress and maintenance of redox homeostasis [6].

The increase of oxidative stress mediated by ROS may lead to the appearance of several diseases, including cancer. Therefore the search for agents that maintains the balance of redox homeostasis (antioxidants) and also possess anticancer potential, has an important role in the discovery of molecules that prevent and halt the growth of cancer cells via reduction of oxidative stress.

In this study, two caffeine analogs with recognized epithelial anticancer potential were tested *in silico* to evaluate the antioxidant potential. For this evaluation were used five receptors (the enzymes NO, CP450, MP, LO, and XO) and two molecules ZINC08706191 (Z91) and ZINC08992920 (Z20) with potential epithelial anticancer proposed by Costa et al. (2018) [7].

2. Results and Discussion

2.1 Evaluation of Molecular docking

Data about validation protocols for molecular docking can be seen in Figure 1. According to literature [8, 9], the RMSD values expressing the relationship between the calculated x crystallographic data of the complexed ligand was less than 2.0 Å. The similarities in the overlapping of experimental (cyan) and calculated (yellow) crystallographic poses (orientation + conformation), which graphically represent the low RMSD value, can also be verified. These results attest that the protocols used can be applied in the molecular docking analyzes between the receptors of the antioxidant activity and the molecules to be evaluated.

The binding free energies (ΔG) for the molecules evaluated at each receptor are shown in Figure 2. In Figure 2, the more external the values in the pentagon, the lower the ΔG and consequently the more significant the interaction between the receptor, and the ligand for the potential antioxidant activity. Thus, the molecule ZINC08706191 (Z91, green line) presented the best results with greater selectivity for the five receptors of the antioxidant activity tested. These inferences can be confirmed from the evaluation of the reference molecules caffeine (CAF, orange) and ascorbic acid (AA, blue), which had higher ΔG values (more internal in the pentagon) and less significant for the potential antioxidant activity, than the tested molecules.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1

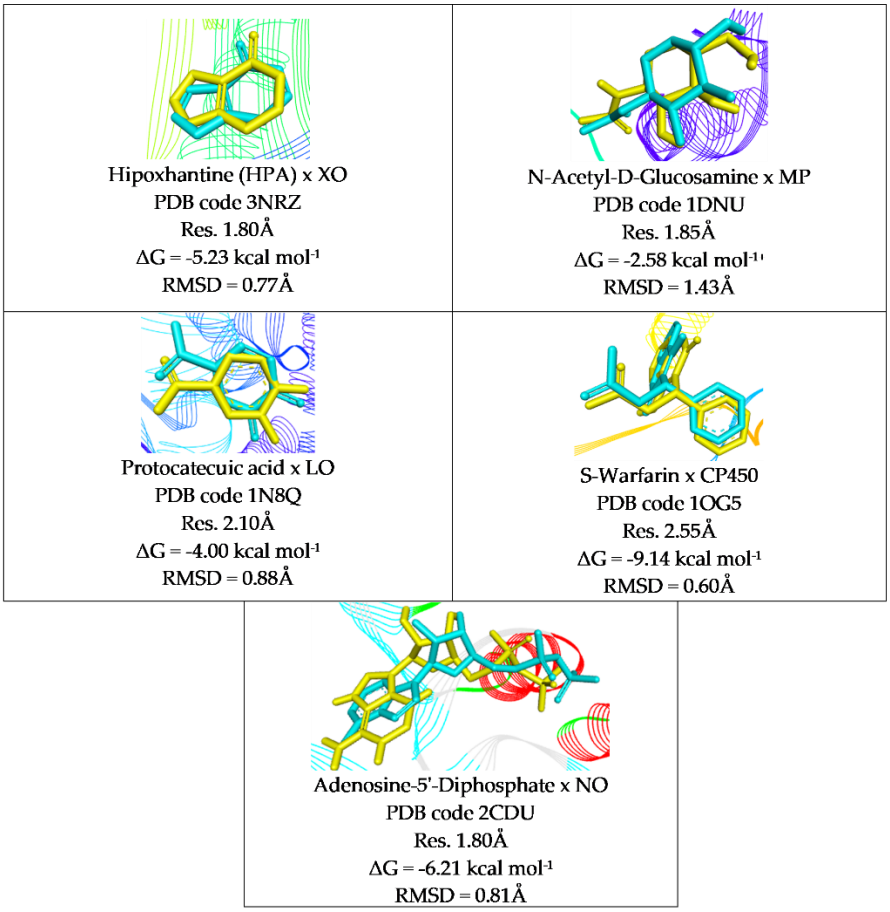


Figure 1. Data obtained in the validation of the molecular docking protocols for the five receptors of the antioxidant activity.

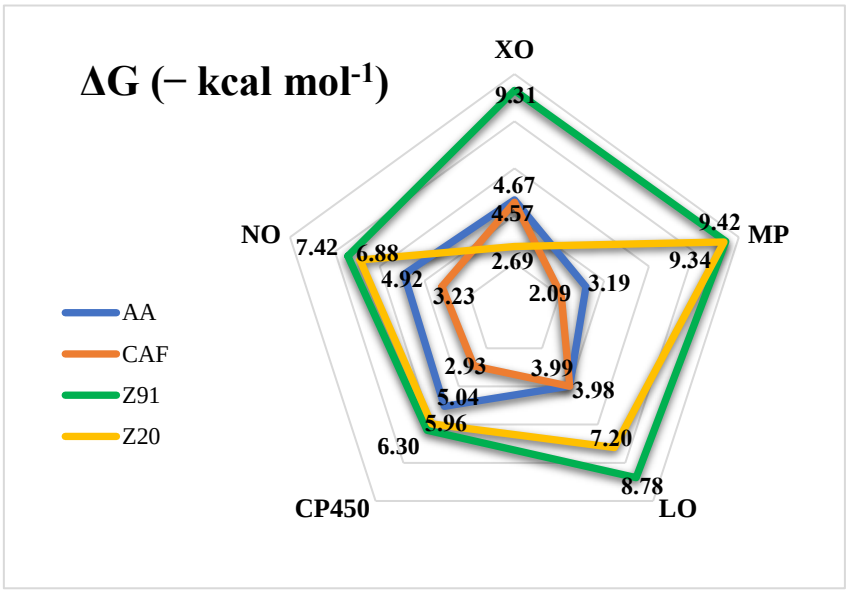
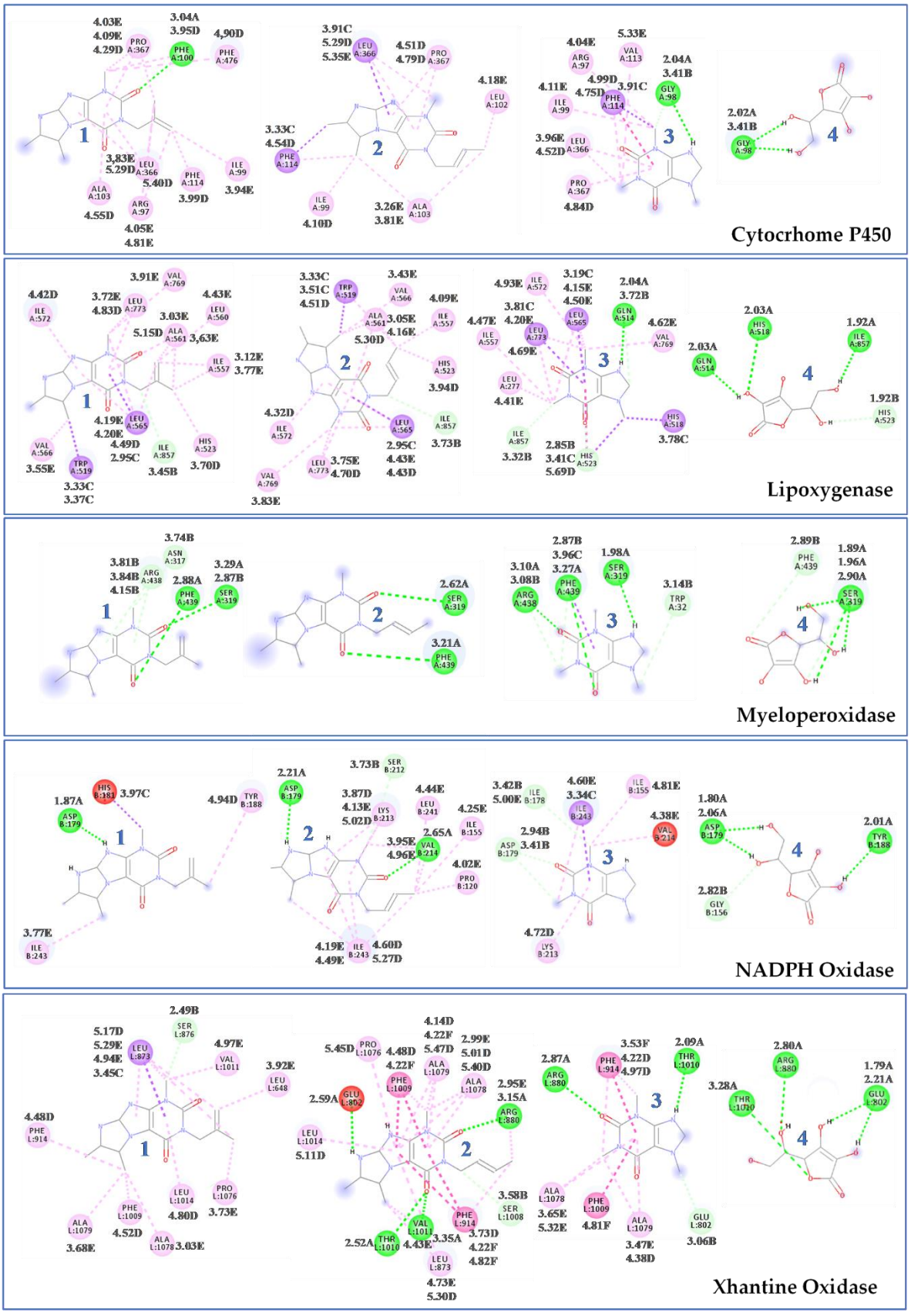


Figure 2. Binding free energy values (– kcal mol-1) resulting from the molecular docking between the molecules and receptors evaluated.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1



Hydrogen bond (A); Carbon-hydrogen bond (B); Pi-sigma (C); Pi-alkil (D); Akyl(E); Pi-pi (F); Z20 (1); Z91 (2); CAF (3); AA (4).

Figure 3. Data from the receptor-ligand interactions obtained from molecular docking.

 doi:10.20944/preprints201805.0133.v1

Figure 3 shows the interactions and their respective lengths for the five receptors evaluated from the molecular docking with the reference molecules (CAF and AA) and with the tested molecules (Z20 and Z91). The numerical values followed by uppercase letters correspond to the bond length values and the type of interaction, respectively.

Regarding the CP450 receptor, three amino acid residues (PRO367, LEU366 and PHE 114) were common. Z20, Z91 and CAF, and two residues (ALA103 and ILE99) were common only to the Z20 and Z91 molecules. The presence of the five mentioned amino acids provides a decrease of ΔG to the Z20 and Z91 molecules (ΔG = -5.96 kcal mol^{-1}) and Z91 molecules (ΔG = -6.30 kcal mol^{-1}) compared to CAF (ΔG = -2.93 kcal mol^{-1}) and AA (ΔG = -5.04 kcal mol^{-1}). Thus, it is possible to verify the tendency of the ΔG value to decrease with the increase of interactions, especially when compared to AA, with only two hydrogen-bonding interactions (GLY98) and a ΔG value higher than Z20 and Z91.

In the LO receptor analysis, except for the interaction with LEU560 present only in Z20, the molecules Z20 (ΔG = -7.20 kcal mol^{-1}) and Z91 (ΔG = -8.78 kcal mol^{-1}) showed all other interactions with the same amino acid residues (ILE572, LEU773, VAL769, ILE557, VAL566, TRP519, LEU565, ILE857 and HIS523). CAF interacted with seven of these amino acids (absentsVAL566, TRP519 and ALA561). Two residues showed interactions with all molecules: ILE857 and HIS523. Two others (GLN514 and HIS518) show interactions with CAF (ΔG = -3.99 kcal mol^{-1}) and AA (ΔG = -3.98 kcal mol^{-1}). The tendency for the ΔG value to decrease with and increasing number of interactions is also observed for this receptor.

In the MP receptor the residues PHE439 and SER319 showed interactions with all molecules, while ARG438 interacted with Z20 and CAF, andTRP32 interacted only with caffeine. For this receptor, Z91 showed stronger interactions (hydrogen bonds), resulting in a lower value of ΔG.

In NO only the ASP179 residue showed interaction with all molecules. ILE243 interacted with Z91 (ΔG = -7.42 kcal mol^{-1}), Z20 (ΔG = -6.88 kcal mol^{-1}) and CAF (ΔG = -3.23 kcal mol^{-1}), whereas TYR188 was common to Z20 and AA (ΔG = -4.92 kcal mol^{-1}). LYS213, VAL214 and ILE155 were common to Z91 and CAF. The tendency of ΔG to decrease with the number of interactions and with the presence of strong interactions (two hydrogen bonds), is verified. Z91 with a higher number of interactions and a lower value of ΔG confirms this motive.

For the XO receptor, the residues PHE914, ALA1078, ALA1079 and PHE1009 were common to Z20 (ΔG = -9.31 kcal mol^{-1}), Z91 (ΔG = -9.31 kcal mol^{-1}) and CAF (ΔG = -4.57 kcal mol^{-1}). GLU802 and ARG880 were common to Z91, CAF and AA (ΔG = -4.67 kcal mol^{-1}), and common to Z20 and Z91 the residues LEU873, VAL1011, PRO1076 and LEU1014. For this receptor also the greater number of interactions resulted in a lower value of ΔG.

Schematic diagrams for each receptor with the interactions of the amino acid residues with one (yellow), two (green), three (red) and four (blue) molecules simultaneously, can be seen in Figure 4.

The blue in LO, MP and NO shows the existence of residues common to all molecules for each of these receptors. The predominance of red already shows that the molecules tested agree well with CAF, in relation to the interaction with the same amino acid residues. This evidence indicates a good antioxidant potential due to interaction with amino acid residues similar to those that interacted with the reference molecules (showed by the blue and red colors).

Green (residues common to two molecules) is more frequent for residues with common interactions between Z20 and Z91. This observation may be related to the decrease of the ΔG value because the additional interaction with these two molecules increases the total amount of interactions with consequent decrease of ΔG.

The antioxidant activities of CAF and AA may be related to the amino acid residues with which the molecules interacted. For this reason, the observation of the common residues between the tested molecules, CAF and AA, allow the inference that the molecules tested may present good antioxidant potential. This relation with the reference molecules favors the molecule Z91, which has lower values of ΔG in all receptors.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1

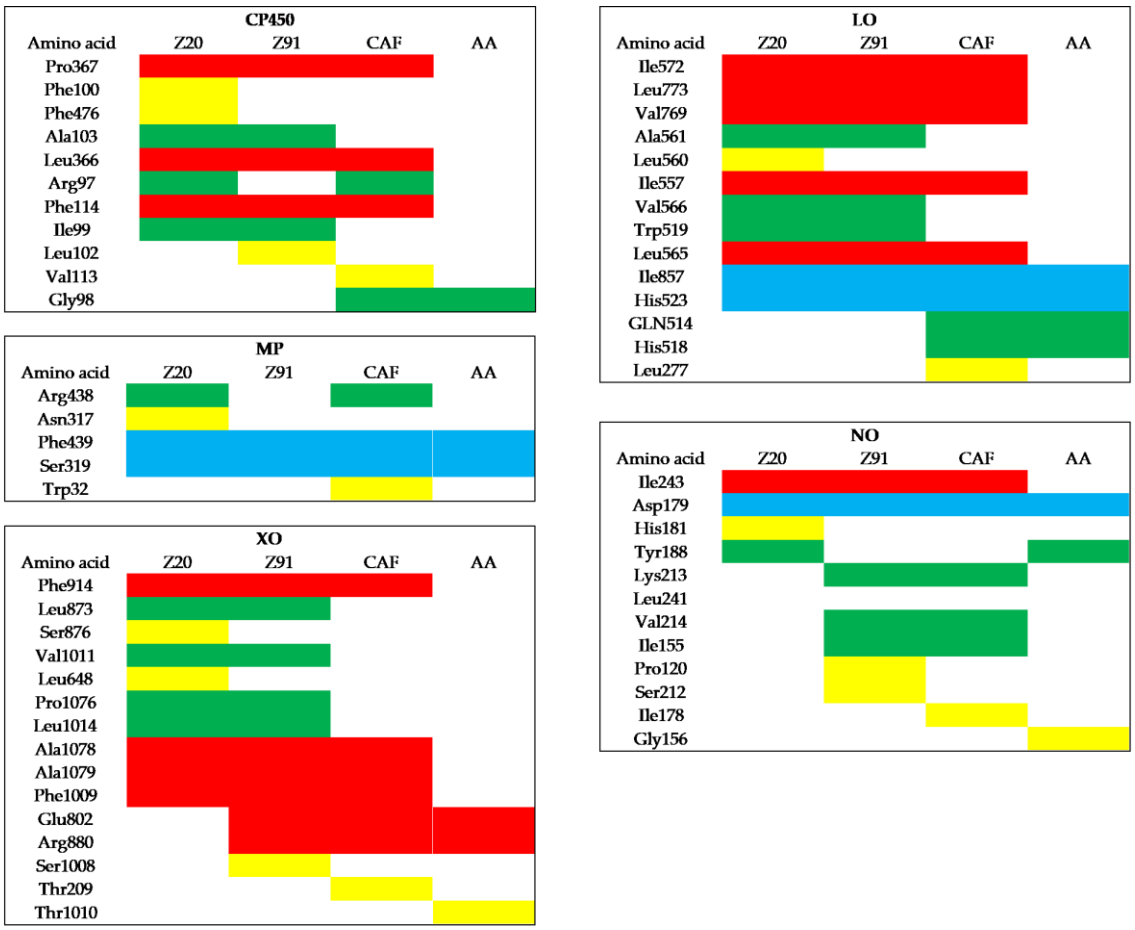


Figure 4. Schematic diagram of the amino acid residues and their interactions with the molecules for the five receptors evaluated.

2.2. Molecular Descriptors and Antioxidant Potential

Table 1 shows data of molecular descriptors and their correlations with ΔG values for the five receptors analyzed. In general, all molecular descriptors showed good correlations with ΔG values (in module most of the values, between 0.5 and 0.99), because in the literature [10, 11], correlation values ≥ 0.3 e ≤ -0.3, are acceptable. This shows that these descriptors have significant relation with the antioxidant potential represented by the values of ΔG.

The Total surface area (TSA) of a molecule is related to its solubility and the molecular interaction that it can performed due to the superficial contact with other molecules [12]. Similar analysis can be done for the molar volume (MV), because the higher the TSA and MV, the higher the level of interaction. From TSA and MV the intermolecular forces operate giving rise to the energy of molecular interaction [13]. In this case, the energy of the molecular interaction is represented by ΔG, which is lower for Z20 and Z91. Its higher values of TSA and MV (457.63 $Å^2$ and 831.4 $Å^3$ for Z91; 432.08 $Å^2$ and 817.96 $Å^3$ for Z20) when compared to AA (272.90 $Å^2$ and 473.75 $Å^3$) and CAF (333.41 $Å^2$and 555.32 $Å^3$), justifies negative correlation values (inversely proportional to ΔG) for these two molecular descriptors.

Molecules formed by different atoms have a polar covalent bond that can be quantified by a dipole moment. This is described with a positive partial charge close to a negative partial charge of the same absolute value, and the measure of the magnitude of these charges is given in debye (D). In the case of polyatomic molecules, a total dipole moment (TDM) is observed. The higher the TDM, the greater the polarity of the molecule and consequently greater a tendency to dissolve and interact in polar environment [14, 15].

 doi:10.20944/preprints201805.0133.v1

Table 1. Molecular descriptors of the tested and reference molecules, and their correlations (CΔG) with ΔG values resulting from molecular docking.

Descriptors	AA	CAF	Z91	Z20
TSA (Å²)	272.9000	333.4100	457.6300	432.0800
MV (Å³)	473.7500	555.3200	831.4000	817.9600
TDM (D)	51.2830	44.3041	5.1008	5.3292
χ (eV)	2.0224	2.6692	2.9553	2.9776
η (eV)	6.6026	5.9236	5.2982	5.3058
1/η (eV)	0.1515	0.1688	0.1887	0.1885
μ (eV)	-2.0224	-2.6692	-2.9553	-2.9776

Descriptors	CΔG-CP450	CΔG-LO	CΔG-MP	CΔG-NO	CΔG-XO
TSA	-0.55	-0.98	-0.91	-0.83	-0.53
MV	-0.61	-0.99	-0.94	-0.86	-0.51
TDM	0.11	0.78	0.63	0.48	0.57
χ	-0.74	-0.04	-0.25	-0.42	-0.30
η	0.43	0.94	0.85	0.74	0.55
1/η	-0.46	-0.95	-0.87	-0.77	-0.55
μ	0.74	0.04	0.25	0.42	0.30

Å = Angstrom; eV = eletron volt. Significant data at $p < 0.05$.

The electronegativity (χ) is described as the capacity that an atom has to attract to itself electrons of a bond that it makes with another atom [16]. It can be used to estimate the tendency of a molecule to attract electrons from another molecule with which it performs interaction [17].

The ΔG values showed positive (directly proportional) correlations with μ e and negative (inversely proportional) correlations with χ. The TDM values for AA (51.283 D), CAF (44.3041 D), Z91 (5.1008 D) and Z20 (5.3292 D), show the tendency for ΔG values to decrease with increase of TDM. An increase in polarity may lead to an increase in repulsive forces (between the molecule and other amino acid residues) in certain regions, making the receptor-ligand interaction difficult. Inverse relation presented between TDM and ΔG was observed between χ and ΔG, where the values of χ for AA (2.0224 eV), CAF (2.6692 eV), Z91 (2.9553 eV) and Z20 (2.9776 eV) increase with the decrease of ΔG.

The molecular hardness (η) and softness (1/η) also are important parameters that describe the reactivity of a molecule. The softness is characterized by having basicity and is related to electron donation with high polarizability and low electronegativity, besides favoring the molecular flexibility with consequent chemical reactivity [18-20]. The hardness is already characterized by high ionization potential and high electronegativity, favoring the molecular stiffness with consequent chemical stability [21, 22].

The 1/η values presented negative correlations with ΔG, while the correlations between η and ΔG were positive. The inversely proportional relationship shown by 1/η can be evidenced by the values presented by AA (0.1515 eV), CAF (0.1688 eV), Z91 (0.1887 eV) and Z20 (0.1885 eV) which are larger for the molecules with lower ΔG values. For η, the relation is directly proportional, because the values for AA (6.6026 eV), CAF (5.9236 eV), Z91 (5.2982 eV) and Z20 (5.3058 eV) decrease with lower values of ΔG.

Chemical Potential (μ) of a species is expressed as a function of thermodynamic quantities. And it can be described as a form of energy absorbed or released from a chemical reaction or change of state. It is related to free energy, being influenced by the number of atoms or molecules that are added or subtracted from the system [23-25]. Positive correlations between μ and ΔG confirm the tendency for increase of ΔG values with higher values of μ, because AA (-2.0224 eV) and CAF (-2.6692 eV) showed higher values of μ and ΔG, when compared to Z91 (-2.9553 eV) and Z20 (-2.9776 eV).

2.3.Molecular Orbitals and Antioxidant Potential

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1

As in the descriptors AST, VM, χ, 1/η, η, MDT andμ, significant correlation values with ΔG were also obtained for molecular orbital data (Table 2). Most descriptors showed strong correlations with ΔG (majority of the values, in module, above 0.7). These results attest to the strong relationship of the molecular orbitals data with the antioxidant Potential (represented by the ΔG values).

Table 2. Correlations between molecular orbitals data and ΔG's values.

Descriptors	XO	MP	LO	CP450	NO
HOMO-1 (eV)	-0.44	-0.79	-0.82	-0.40	-0.60
HOMO (eV)	-0.52	-0.67	-0.73	-0.25	-0.47
LUMO (eV)	0.36	0.95	0.94	0.68	0.83
LUMO+1 (eV)	0.67	0.99	0.94	0.91	0.98

Significant data at $p < 0.05$.

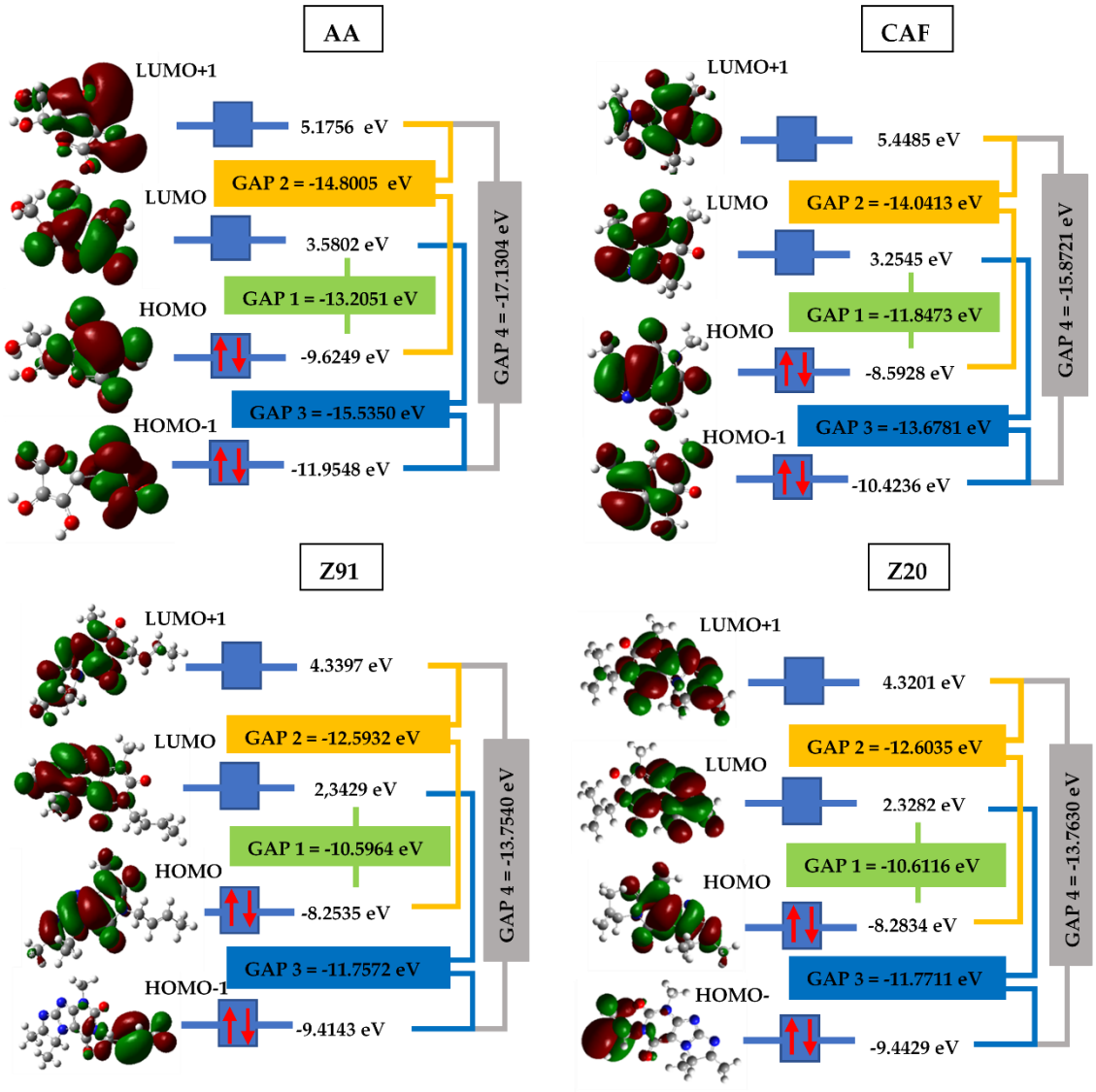


Figure 5. Data of HOMO-1, HOMO, LUMO and LUMO + 1 orbitals.

In Figure 5 data and representations of the molecular orbitals. GAP values (energy variation between frontier orbitals) between all energy states (GAP_1, GAP_2, GAP_3 and GAP_4) are also shown.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1

The energies of the HOMO and HOMO-1 molecular orbitals are strongly related to the ionization potential of a molecule and are indicative of the nucleophilic character of the species [26-28]. Among the species analyzed, the highest values for HOMO and HOMO-1 were presented by Z91 (-8.2535 eV and -9.4143 eV) and Z20 (-8.2834 eV and -9.4429 eV) when compared to AA (-9.6249 eV and -11.9548 eV) and CAF (-8.5928 eV and -9.4429 eV). These data show that Z91 and Z20 have a higher electron donor character and greater ability to perform nucleophilic attacks when compared to the reference molecules AA and CAF. The negative correlations between HOMO and HOMO-1 orbitals and ΔG values attest to the inversely proportional relation of these variables, with a decrease in ΔG values from the increase in HOMO and HOMO-1 values.

The molecular orbitals LUMO and LUMO + 1 have a direct relation with the electron affinity of a molecule, which is related to susceptibility to nucleophilic attacks [29]. When compared to AA (3.5802 eV and 5.1756 eV) and CAF (3.2545 eV and 5.4485 eV), the lowest values of LUMO and LUMO+1 for Z91 (2.3429 eV and 4.3397 eV) and Z20 (2.3282 eV and 4.3201 eV) show that Z91 and Z20 are less susceptible to attack by nucleophiles. The values of LUMO and LUMO + 1 were positively correlated with the values of ΔG, revealing the tendency of the ΔG values to decrease with the reduction of LUMO and LUMO+1 values.

The energy variation between the HOMO and LUMO orbitals is called the GAP. GAP is an important indicator of the chemical reactivity of a molecule. The lower the GAP value, the greater the chemical reactivity of the molecule and the higher the value, the greater the stability [30]. By evaluating the four calculated GAP values (GAP's 1-4) for each molecule, it is possible to observe that low values were obtained. These results show that all molecules have significant chemical reactivity, necessary for interaction with receptors.

2.4. Antioxidant potential x Epithelial anticancer potential

The ΔG values obtained from the interaction of the molecules (CAF, Z91 and Z20) with the five receptors of the antioxidant activity, were submitted to Pearson correlation with the ΔG values obtained in a previous study by Costa et al. (2018) [7], for the interaction with the receptor of epithelial anticancer activity, Chk1 (Table 3).

Table 3. Correlations between ΔG values of potential epithelial anticancer activity and antioxidant potential.

Molecule	ΔG					
	Chk1*	**CP450**	**LO**	**MP**	**NO**	**XO**
CAF	-5.14	-4.57	-2.09	-3.99	-2.93	-3.23
Z20	-7.13	-9.31	-9.42	-8.78	-6.30	-7.42
Z91	-7.41	-2.69	-9.34	-7.20	-5.96	-6.88
CorrChk1	—	0.91	0.98	0.87	0.92	0.69

* Data of ΔG obtained by Costa et al. (2018) [7]. CorrChk1 = correlation with ΔG values of the Chk1 receptor com ΔG values of the antioxidant activity receptors.

The strong positive correlations show that the ΔG values of the antioxidant activity receptors increase with the increase in the ΔG value of the epithelial anticancer activity receptor (Chk1). This corresponds to a directly proportional relationship between the antioxidant potential and the epithelial anticancer potential represented by the ΔG values.

3. Materials and Methods

3.1. Calculation of receptor-ligand interaction for evaluation of antioxidant potential

Initially, five enzymes (receptors) that respond for the production of ROS, Cytochrome P450 (CP450), Lypoxygenase (LO), Myeloperoxidase (MP), NADPH Oxidase (NO) e Xhantine Oxidase (XO) were obtained from PDB (Protein Data Bank) [31]. Receptor-ligand interaction data regarding

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 May 2018 doi:10.20944/preprints201805.0133.v1

the inhibition of these receptors were obtained in the Autodock 4.2.6 program [32] and followed specific protocols for each analyzed receptor. Table 4 shows the protocols used in the validation of molecular docking and subsequent docking analyses of each receptor.

Table 4. Data from protocols used in the molecular docking validation.

Receptor	Ligand	Coordinates of the grid center	Grid size
CP450 (PDB code: 1OG5)	S-warfarin	-20.257x 86.991y 38.581z	22 x-pontos 20 y-pontos 24 z-pontos
LO (PDB code: 1N8Q)	Protocatecuic acid	21.864x 2.184y 18.909z	24 x-pontos 18 y-pontos 12 z-pontos
MP (PDB code: 1DNU)	N-Acetyl-D-Glucosamine	37.859x 8.117y 11.824z	22 x-pontos 30 y-pontos 18 z-pontos
NO (PDB code: 2CDU)	Adenosine-5'-Diphosphate	1.687x 9.885y 54.962z	30 x-pontos 14 y-pontos 32 z-pontos
XO (PDB code: 3NRZ)	Hypoxanthine	89.432x 9.271y 18.011z	16 x-pontos 18 y-pontos 14 z-pontos

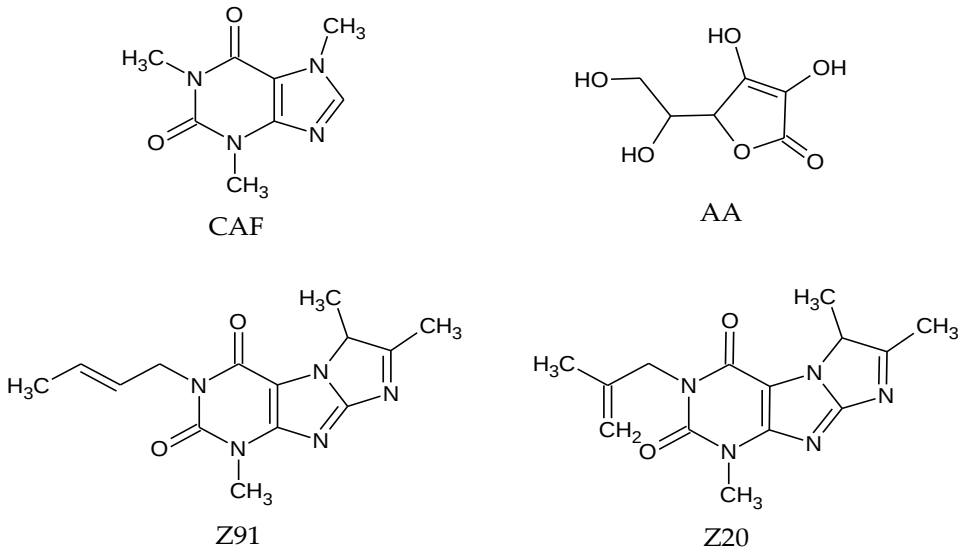


Figure 6. Reference (CAF and AA) and tested (Z20 e Z91) molecules.

Four molecules were docked in the five recipients cited. The molecules are: caffeine (CAF), ascorbic acid (AA), ZINC08992920 (Z20) and ZINC08706191 (Z91), see Figure 6. CAF and AA were used as reference to better evaluate the tested molecules (Z20 and Z91). Test molecules were selected because in the literature [7] they have recognized epithelial anticancer potential. Caffeine was selected because it is analogous to the molecules tested and used as a prototype in the cited literature. And ascorbic acid by having potent antioxidant activity diffused in the scientific society [33, 34].

 doi:10.20944/preprints201805.0133.v1

3.2. Quantum chemical calculations

Calculations to obtain molecular descriptors data were performed in the Gaussian 09 program for the CAF, AA, Z20 and Z91 molecules. The method used was Hartree-Fock in the 6-31G** basis set (HF/6-31G**). The choice of this method was based on the study of Costa et al. (2018) [35], who classified the HF/6-31G** method as the best method for the molecular modeling of caffeine and analogues. The descriptors obtained from calculations were the following: total surface area, molar volume, total dipole moment; molecular hardness (η), molecular softness (1/η), electronegativity (χ), chemical potential (μ), highest occupied molecular orbital energy (HOMO), a level of energy below the highest occupied molecular orbital (HOMO-1), the lowest non-occupied molecular orbital energy (LUMO), and a level of energy above the lowest unoccupied molecular orbital (LUMO+1). GAP values (energy variation between frontier orbitals) were calculated for the four orbitals cited: GAP_1 = HOMO–LUMO, GAP_2 = HOMO–LUMO+1, GAP_3 = HOMO-1–LUMO, and GAP_4 = HOMO-1–LUMO+1.

These data were analyzed and evaluated in relation to their contribution to the antioxidant potential of the tested molecules. Pearson correlation analyzes between the molecular descriptors and ΔG values for the four molecules and the five receptors evaluated were performed by the Statistica 6.2 program [36].

4. Conclusions

The study of the antioxidant activity potential of this article was started from experimental data of biological receptors of the said activity available in the Protein Data Bank. These data were important initially to validate the docking protocols to be used in the molecular docking of the four molecules studied (AA, CAF, Z20 and Z91). The molecular docking protocols for the five receptors (CP450, MP, LO, NO and XO) were all validated for having RMSD (experimental x theoretical) values lower than 2.0 Å. This allows the certainty that the theoretical data have good agreement with the experimental data.

In relation to the ΔG values resulting from the interaction between the molecules studied and the antioxidant activity receptors, the Z20 and Z91 molecules presented lower values than the reference molecules (AA and CAF), evidencing the best interaction profile of Z20 and Z91. Due to these results, Z20 and Z91 presented remarkable antioxidant potential, with Z91 being better classified as having lower values of ΔG in all the receptors evaluated.

Concerning the interactions obtained for each receptor, was observed the tendency of ΔG values to decrease with the increase in the number of interactions. CAF and AA that present higher ΔG values performed a smaller amount of interactions on the contrary of Z20 and Z91, which presented lower values of ΔG and greater number of interactions. Thus, an increase in the number of interactions results in a decrease of the total energy (binding molecule + receptor), being released in the ΔG form.

The negative correlations presented between ΔG and the molecular descriptors AST, VM, χ and 1/η provide an increase in the antioxidant potential represented by the decrease in the ΔG values. While the positive correlations confirm that the antioxidant potential decreases (ΔG increases) when the values of MDT, η and μ increase. In general, Z91 and Z20 presented better results for molecular descriptors than the AA and CAF molecules.

The highest values of AST, VM, χ and 1/η for Z91 and Z20, show that larger surface areas and volumes result in a greater number of interactions. Higher electronegativities allow these molecules greater capacity to attract electrons from the interactions with the amino acid residues and greater flexibility to twist and adapt to the receptor active site, with a consequent decrease in the ΔG values. The lowest values of MDT, η and μ, suggest that Z91 and Z20 have a good charge distribution resulting in medium polarity, low stiffness and relationship with thermodynamic properties relative to the quantities of atoms involved in the interactions, and as a consequence also the ΔG decrease.

The values of the energies obtained for the molecular orbitals HOMO-1, HOMO, LUMO and LUMO+1 and GAP values, allow the assignment of a strong nucleophilic character (resulting in low ΔG values) and high reactivity) to the analyzed molecules (mainly, Z91 and Z20).

The results obtained in this study reveal that Z91 and Z20 have potential antioxidant activity in at least five via of ROS generation (five receptors analyzed), and this potential is strongly related (correlation values between 0.69 and 0.98) to the potential epithelial anticancer activity presented in the study by Costa et al. (2018) [7]. Thus, Z91 and Z20 may be indicated for more analysis in order to further evaluate their efficiency in the reduction of oxidative stress and treatment of epithelial cancer.

Author Contributions: Conceptualization, R.S.B.; Data curation, J.S.C.; Formal analysis, K.S.L.C. and R.S.R.; Investigation, J.S.C; Methodology, J.S.C.; Project administration, J.S.C. and W.J.C.M.; Resources, D.S.B.B.; Software, C.B.R.S.; Supervision, J.S.C. and W.J.C.M.; Validation, C.B.R.S.; Visualization, K.S.L.C. and R.S.R.; Writing – original draft, J.S.C.; Writing – review & editing, J.S.C., C.B.R.S and J.M.C.R.

Acknowledgments: We acknowledge the Postgraduate Program in Biodiversity and Biotechnology of Amazon (PPG-Bionorte), Paraense Museum Emilio Goeldi (MPEG). Laboratory of Molecular Modeling and Simulation System of Federal Rural University of Amazônia (UFRA-Brazil) are acknowledged for computational and methodological support. Finally, financial support provided by the PROPESP/UFPA is greatly acknowledged.

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

References

1. Silva, A.A.; Gonçalves, R.C. Reactive oxygen species and the respiratory tract diseases of large animals. *Ciência Rural* **2010**, *40*, 994-1002. http://dx.doi.org/10.1590/S0103-84782010005000037.
2. Yun, J.; Mullarky, E.; Lu, C.; Bosch, K.N.;Kavalier, A.; Rivera, K.; Roper, J.;Chio, I.I., Giannopoulou, E.G.; Rago, C.; et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* **2015**, *350*, 1391–1396. https://doi.org/10.1126/science.aaa5004.
3. White, P.A.; Oliveira, R.C.; Oliveira, A.P.; Serafini, M.R.; Araújo, A.A.; Gelain, D.P.; Moreira, J.C.; Almeida J.R.; Quintans, J.S.; Quintans-Junior, L.J.; et al. Antioxidant Activity and Mechanisms of Action of Natural Compounds Isolated from Lichens: A Systematic Review. *Molecules* **2014**, *19*, 14496-14527. https://doi.org/10.3390/molecules190914496.
4. Reuter, S.; Gupta, S.C.; Chaturvedi, M.M.; Aggarwal, B.B.; et al. Oxidative stress, inflammation, and cancer: How are they linked?. *Free Radic Biol Med* **2010**, *49*, 1603–1616. https://linkinghub.elsevier.com/retrieve/pii/S0891584910005381.
5. Cadenas, E. Basic mechanisms of antioxidant activity. *BioFactors* **1997**, *6*, 391-397. https://doi.org/10.1002/biof.5520060404.
6. Dharmaraja, A. T. Role of Reactive Oxygen Species (ROS) in Therapeutics and Drug Resistance in Cancer and Bacteria. *J Med Chem* **2017**, *60*, 3221–3240. https://pubs.acs.org/doi/10.1021/acs.jmedchem.6b01243.
7. Costa, J.S.; Costa, K.S.L.; Cruz, J.V.; Ramos, R.S.; Silva, L.B.; Brasil, D.S.B.; Silva, C.H.T.P.; Santos, C.B.R.; Macêdo, W.J.C. Virtual screening and statistical analysis in the design of new caffeine analogues molecules with potential epithelial anticancer activity. *Curr Pharm Desing* **2018**, *24*, 576-594. http://www.eurekaselect.com/154114.
8. Hevener, K.E.; Zhao, W. Ball, D.M.;Babaoglu, K.; Qi, J.; White, S.W.; Lee R.E.; et al. Validation of Molecular Docking Programs for Virtual Screening against Dihydropteroate Synthase. *J Chem Inf Model* **2009**, *49*,444-460. https://doi.org/10.1021/ci800293n.
9. Gowthaman, U.; Jayakanthan, M.; Sundar, D. Molecular docking studies of dithionitrobenzoic acid and its related compounds to protein disulfide isomerase: computational screening of inhibitors to HIV-1 entry. *BMC Bioinformatics* **2008**, *9*, 1-10. https://doi.org/10.1186/1471-2105-9-S12-S14.
10. Vieira, J.B.; Braga, F.S.; Lobato, C.C.; Santos, C.F.; Costa, J.S.; Bittencourt, J.A.; Brasil, D.S.; Silva, J.O.; Hage-Melim, L.I.; Macêdo, W.J.; et al. A QSAR, Pharmacokinetic and Toxicological Study of New Artemisinin Compounds with Anticancer Activity. *Molecules* **2014**, *19*, 10670-10697. https://doi.org/10.3390/molecules190810670.
11. Santos, C.B.R.; Vieira J.B.; Lobato, C.C.; Hage-Melim, L.I.S.; Souto, R.N.P.; Lima, C.S.; Costa, E.V.M.; Brasil, D.S.B.;Macêdo, W.J.C.; Carvalho, J.C.T.A SAR and QSAR Study of New Artemisinin Compounds with Antimalarial Activity. *Molecules* **2014**, *19*, 367-399. https://doi.org/10.3390/molecules19010367.

12. Yalkowsky, S.H.; Valvani, S. C. Solubilities and partitioning. 2. Relationships between aqueous solubilities, partition coefficients, and molecular surface areas of rigid aromatic hydrocarbons. *J Chem Eng Data* **1979**, *24*, 127–129. https://pubs.acs.org/doi/abs/10.1021/je60081a021.
13. Panayiotou, C. Redefining solubility parameters: Bulk and surface properties from unified molecular descriptors.*J Chem Thermodyn* **2017**, *111*, 207-220. https://doi.org/10.1016/j.jct.2017.03.035.
14. Gubskaya, A.V.; Kusalik, P.G. The total molecular dipole moment for liquid water. *J Chem Phys* **2002**, *117*, 5290-5302. https://doi.org/10.1063/1.1501122.
15. Farasat, M.; Shojaei,S.H.R.;Golzan M.M.; Farhadi, K. Theoretical study of the potential energy surface and electric dipole moment of aniline. *J Mol Struct* **2016**, *1108*, 341-346. https://doi.org/10.1016/j.molstruc.2015.12.044.
16. Tognetti, V.; Morell, C.; Joubert, L. Atomic electronegativities in molecules. *Chem Phys Lett* **2015**, *635*, 111-115. https://doi.org/10.1016/j.cplett.2015.05.057.
17. Cunha, E.L.; Santos, C.F.; Braga, F.S.; Costa, J.S.; Silva, R.C.; Favacho, H.A.S.; Hage-Melim, L. I. S.; Carvalho, J.C.T.; Silva, C.H. T. P.; Santos, C.B. R. Computational Investigation of Antifungal Compounds Using Molecular Modeling and Prediction of ADME/Tox Properties. *J ComputTheorNanosci* **2015**, *12*, 3682-3691. https://doi.org/10.1166/jctn.2015.4260.
18. Yang, W.; Lee, C.; Ghosh, S. K. Molecular Softness as the Average of Atomic Softnesses: Companion Principle to the Geometric Mean Principle for Electronegativity Equalization. *J Phys Chem* **1985**, 89, 5412-5414https://pubs.acs.org/doi/abs/10.1021/j100271a019.
19. Nalewajski, R.F.; Korchowiec, J.; Zhou, Z. Molecular hardness and softness parameters and their use in chemistry. *Int J Quantum Chem* **1988**, *34*, 349-366. https://doi.org/10.1002/qua.560340840.
20. Liu, P.H.; Hunt, K.L.C. Molecular softness, hypersoftness, infrared absorption, and vibrational Raman scattering: New relations derived from nonlocal polarizability densities. *J Chem Phys* **1995**, *103*, 10597-10604.https://doi.org/10.1063/1.469844.
21. Ghanty, T.K.; Ghosh, S.K. Molecular Hardness, Polarizability and Valency Variation of Formamide and Thioformamide on Internal Rotation: A Density Functional Study. *J Phys Chem A***2000**, *104*, 2975–2979. https://pubs.acs.org/doi/abs/10.1021/jp993830b.
22. Kaya, S.; Kaya, C. A new method for calculation of molecular hardness: A theoretical study**.** *ComputTheor Chem***2015**, *1060*, 66–70.https://doi.org/10.1016/j.comptc.2015.03.004.
23. Tarazona, M.P.; Sáiz, E. Understanding Chemical Potential.*J Chem Educ* **1995**, *72*,882-883. https://pubs.acs.org/doi/abs/10.1021/ed072p882.
24. Cook, G.; Dickerson, R.H. Understanding the chemical potential. *Am J Phys* **1995**, *63*, 737-742. https://doi.org/10.1119/1.17844.
25. Job, G.; Herrmann, F. Chemical potential—a quantity in search of recognition. *Eur J Phys* **2006**, *27*, 353-371. http://iopscience.iop.org/article/10.1088/0143-0807/27/2/018/meta.
26. Anh, N.T. Regio- and stereo-selectivities in some nucleophilic reactions. In*Organic Chemistry Syntheses and Reactivity*. Topics in Current Chemistry. Springer: Berlin, Heidelberg, Germany, 1980. *Volume 88*, pp. 145-162, 3-540-09817-8.
27. Bassindale, A.R.; Glynn, S.J.; Taylor, P.G. Reaction Mechanisms of Nucleophilic Attack at Silicon. In *The Chemistry ofOrganic Silicon Compounds*, Patai, S.; Rappoport, Z., Eds.; Wiley Online Library: New York, USA, 1898,*Volume 2*, pp. 495-511,9780470857250.
28. Mihçiokur, O.; Özpozan, T. Molecular structure, vibrational spectroscopic analysis (IR & Raman), HOMO-LUMO and NBO analysis of anti-cancer drug sunitinib using DFT method. *J Mol Struct* **2017**, *1149*, 27-41. https://doi.org/10.1016/j.molstruc.2017.07.064.
29. Grant, G.H.; Richards, W.G. *Computational Chemistry*. Oxford Science Publications: Oxford, USA, 1996; pp. 1-96, 9780198557401.
30. Zhang, G.; Musgrave, C. B. Comparison of DFT Methods for Molecular Orbital Eigenvalue Calculations. *J Phys Chem A* **2007**, *111*, 1554-1561. https://pubs.acs.org/doi/abs/10.1021/jp061633o.
31. PDB – Protein Data Bank. Available in: <http://www.ncbi.nlm.nih.gov/protein/>. Access: 20 de jan. 2018. https://www.rcsb.org/.
32. Morris, G.M.;Goodsell, D.S.; Halliday, R.S.; Huey, R.; Hart, W.E.;Belew, R.K.; Olson, A.J.; et al. Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. *J Comput Chem* **1998**, *19*, 1639-1662. https://doi.org/10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B.

33. Seib, P.A.; Tolbert, B.M. *Ascorbic Acid: Chemistry, Metabolism, and Uses,* 1st ed.; American Chemical Society: Washington, DC, USA, 1982; pp. 395-497, 13: 9780841206328.
34. Uluata, S.; McClements, D.J.; Decker, E.A. How the Multiple Antioxidant Properties of Ascorbic Acid Affect Lipid Oxidation in Oil-in-Water Emulsions. *J Agric Food Chem* **2015**, *63*, 1819-1824. https://pubs.acs.org/doi/abs/10.1021/jf5053942.
35. Costa, J.S.; Santos, C.B.R.; Costa, K.S.L.; Ramos, R.S.; Silva, C.H.T.P.; Macêdo, W.J.C. Validation of computational methods applied in molecular modeling of caffeine with epithelial anticancer activity: Theoretical study of geometric, thermochemical and spectrometric data. *Quím Nova,* p. 1-24, 2018. (accepted)
36. STATISTICA (Data Analysis Software System); Version 6.1, StatSoft, Inc., 2004.