Potential of the Compounds from Bixa orellana Purified Annatto Oil and Its Granules (Chronic®) against Dyslipidemia and Inflammatory Diseases: In Silico Studies with Geranylgeraniol and Tocotrienols

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Abstract: Some significant compounds present in annatto are geranylgeraniol and tocotrienols. These compounds have beneficial effects against hyperlipidemia and chronic diseases, where oxidative stress and inflammation are present, but the exact mechanism of action of such activities is still a subject of research. This study aimed to evaluate possible mechanisms of action that could be underlying the activities of these molecules. For this, in silico approaches such as ligand topology (PASS and PreTox-II) and molecular docking with the software GOLD were used. Additionally, we screened some pharmacokinetic and toxicological parameters using the servers PreADMET, SwissADME, and SEA servers. The results corroborate the antidyslipidemia and anti-inflammatory activities of geranylgeraniol and tocotrienols. Notably, some new mechanisms of action were predicted to be potentially underlying the activities of these compounds, including inhibition of squalene monooxygenase, lanoster synthase, and phospholipase A2. These results give new insight into new mechanisms of action involved in these molecules from annatto and Chronic®.

Keywords: Bixa orellana; oil; inflammatory process; geranylgeraniol; tocotrienol

1. Introduction

Lipid disorders, such as dyslipidemia, constitute a significant concern among the overall population and researchers due to their role in hyperlipidemia, hypertension, atherosclerosis, and even insulin resistance. Such aggravation is caused by increased levels of total cholesterol and low-density lipoprotein (LDL) and decreased levels of high-density lipoprotein, which together raise the risk of cardiovascular diseases and metabolic abnormalities [1–4].

Bixa orellana is the plant species known as “annatto” and “achiote”. This species is studied for some health issues, including inflammation-related conditions and dyslipidemias [5–7]. Such health benefits can be at least partly due to the presence of tocotrienols and geranylgeraniol from its composition. Tocotrienols are unsaturated forms of vitamin E known for anti-inflammatory, antioxidant, and lipid-lowering activities, which are higher...
than those from tocopherols—their saturated counterparts, also parts of the vitamin E group [8,9]. In turn, geranylgeraniol is an intermediate in the biosynthesis of cholesterol, and it is believed to regulate the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase negatively.

Both tocotrienols and geranylgeraniol are research subjects due to their biological activities, including cardioprotective and neuroprotective effects, hypolipidemic activity, metabolic disorder prevention, and antitumoral activity [10–12]. A fundamental approach in the process of drug discovery is pharmaceutical chemistry. A research can be more efficient through pharmaceutical chemistry by decreasing the necessary time, funds, and number of animals needed.

Some of the parameters often screened in potential new drugs through this approach are biological activity prediction, pharmacokinetic profile, and toxicological potential [13,14]. Hence, by using pharmaceutical chemistry tools, the purpose of this study was to evaluate the pharmaceutical potential of tocotrienols and geranylgeraniol for their main biological activities and possible mechanisms of action. This perspective could hint at safer medications compared with the standard ones.

2. Results and Discussion
2.1. Molecules’ Structure Obtention and Biological Activity Prediction

Tocotrienols and geranylgeraniol are molecules well described and studied in the literature [15,16]. Their structures were obtained from the PubChem database (Figure 1A) and then assessed for possible biological activities and mechanisms of action using the server PASS (prediction of activity spectra for substances) [17–20].

Figure 1. (A) Molecular structure of geranylgeraniol and tocotrienols. (B) Targets used in the docking simulation with their respective PDB ID. 1W6K: lanosterol synthase complexed with lanosterol; 6C6N: squalene monoxygenase complexed with FAD and CPMPD-4; 1HW9: HMG-CoA reductase complexed with simvastatin; 5IKQ: cyclooxygenase-2 complexed with meclofenamic acid; 5G3N: secreted phospholipase A2 complexed with the inhibitor Azd2716.
Geranylgeraniol had a high probability of activity (Pa) values (>0.7) for the following activities: mucous membrane protection (0.953), lipid metabolism regulation (0.885), TNF expression inhibitor (0.840), antilulcerative (0.770), and antineoplastic (0.743). Still notably, the hypolipidemic activity Pa was 0.686, and antihypercholesterolemic Pa was 0.570, both higher than the probability of inactivity (Pi) (0.015 for both).

Tocotrienols also had significant Pa values for lipid peroxidase inhibition (from 0.941 to 0.989), antioxidant activity (from 0.913 to 0.973), anti-inflammatory activity (from 0.813 to 0.866), antihypercholesterolemic activity (from 0.803 to 0.962), cholesterol synthesis inhibition (from 0.663 to 0.702), among other related activities (Table 1). There are some variations among the isomers, but the class consistently shows high Pa’s tendency to improve the blood lipid profile. It is important to notice that in annatto, the most abundant isomer is δ, according to some authors, which can be up to 90% of the isomer composition [21].

Table 1. Biological activity prediction of the compounds according to the PASS server.

| Molecule     | Pa   | Pi   | Activity Prediction                 |
|--------------|------|------|------------------------------------|
| Geranylgeraniol | 0.953 | 0.003 | Mucous membrane protection         |
|              | 0.885 | 0.004 | Lipid metabolism regulation        |
|              | 0.840 | 0.003 | TNF inhibitor                       |
|              | 0.770 | 0.004 | Antiulcerative                     |
|              | 0.743 | 0.049 | Antineoplastic                     |
|              | 0.686 | 0.015 | Hypolipidemic                      |
|              | 0.636 | 0.007 | NF kappa B regulator               |
|              | 0.643 | 0.024 | Anti-inflammatory                   |
|              | 0.570 | 0.015 | Antihypercholesterolemic           |
|              | 0.549 | 0.005 | Antioxidant                        |
|              | 0.538 | 0.03  | Cholesterol antagonist             |
|              | 0.498 | 0.019 | Antineoplastic                     |
|              | 0.437 | 0.007 | Cholesterol synthesis inhibitor    |
|              | 0.989 | 0.001 | Lipid peroxidase inhibitor         |
|              | 0.973 | 0.002 | Antioxidant                        |
|              | 0.962 | 0.002 | Antihypercholesterolemic           |
|              | 0.900 | 0.005 | Treatment of acute neural disorders |
|              | 0.892 | 0.005 | Cerebral anti-ischemic             |
|              | 0.866 | 0.005 | Anti-inflammatory                   |
|              | 0.863 | 0.003 | Peroxidase inhibitor               |
|              | 0.763 | 0.005 | Hepatoprotector                    |
|              | 0.733 | 0.034 | Mucous membrane protection         |
|              | 0.713 | 0.008 | Cholesterol antagonist             |
|              | 0.702 | 0.001 | Cholesterol synthesis inhibition   |
|              | 0.685 | 0.003 | NOS2 expression inhibition         |
|              | 0.621 | 0.009 | Antineoplastic (breast cancer)     |
|              | 0.456 | 0.033 | NF kappa B inhibitor               |
|              | 0.426 | 0.031 | Atherosclerosis treatment          |
| Molecule        | Pa  | Pi  | Activity Prediction                  |
|-----------------|-----|-----|--------------------------------------|
| Molecule        | 0.435 | 0.046 | TNF inhibitor                        |
| 0.397           | 0.044 |     | Antipsoriasis                        |
| 0.255           | 0.017 |     | Phospholipase A₂ inhibition          |
| 0.957           | 0.002 |     | Lipid peroxidase inhibition          |
| 0.951           | 0.002 |     | Antioxidant                          |
| 0.951           | 0.002 |     | Antihypercholesterolemic             |
| 0.881           | 0.004 |     | Hypolipidemic                        |
| 0.835           | 0.005 |     | Anti-inflammatory                    |
| 0.812           | 0.005 |     | Anticarcinogenic                     |
| 0.787           | 0.004 |     | Antiulcerative                       |
| 0.744           | 0.002 |     | NOS₂ expression inhibition           |
| 0.738           | 0.040 |     | Mucous membrane protection           |
| 0.692           | 0.001 |     | Cholesterol synthesis inhibition     |
| 0.714           | 0.026 |     | Cerebral anti-ischemic               |
| 0.685           | 0.008 |     | Hepatoprotector                      |
| 0.648           | 0.035 |     | Antineoplastic                       |
| 0.602           | 0.019 |     | Cholesterol antagonist               |
| 0.475           | 0.027 |     | Antipsoriasis                        |
| 0.481           | 0.034 |     | TNF inhibitor                         |
| 0.355           | 0.010 |     | NF kappa B inhibitor                 |
| 0.271           | 0.026 |     | Lipoprotein disorder treatment       |
| 0.198           | 0.025 |     | Phospholipase A₂ inhibition          |
| 0.977           | 0.002 |     | Lipid peroxidase inhibition          |
| 0.953           | 0.002 |     | Antioxidant                          |
| 0.944           | 0.002 |     | Antihypercholesterolemic             |
| 0.882           | 0.004 |     | Hypolipidemic                        |
| 0.846           | 0.005 |     | Anti-inflammatory                    |
| 0.811           | 0.005 |     | Anticarcinogenic                     |
| 0.776           | 0.017 |     | Cerebral anti-ischemic               |
| 0.762           | 0.004 |     | Antiulcerative                       |
| 0.686           | 0.001 |     | Cholesterol synthesis inhibitor      |
| 0.682           | 0.008 |     | Hepatoprotector                      |
| 0.719           | 0.008 |     | Mucous membrane protection           |
| 0.683           | 0.003 |     | NOS₂ expression inhibition           |
| 0.593           | 0.011 |     | Antineoplastic (breast cancer)       |
| 0.452           | 0.041 |     | TNF inhibitor                         |
| 0.464           | 0.061 |     | Lipid metabolism inhibitor           |
| 0.402           | 0.043 |     | Antipsoriasis                        |
| 0.271           | 0.014 |     | NF kappa B inhibitor                 |
| 0.230           | 0.016 |     | Phospholipase A₂ inhibition          |
| 0.280           | 0.091 |     | Atherosclerosis treatment            |

**β-tocotrienol**

**γ-tocotrienol**
Table 1. Cont.

| Molecule       | Pa    | Pi   | Activity Prediction                        |
|----------------|-------|------|--------------------------------------------|
| δ-tocotrienol  | 0.941 | 0.002| Lipid peroxidase inhibition                |
|                | 0.913 | 0.003| Antioxidant                                |
|                | 0.813 | 0.006| Anti-inflammatory                           |
|                | 0.803 | 0.005| Antihypercholesterolemic                   |
|                | 0.791 | 0.008| Hypolipidemic                              |
|                | 0.789 | 0.022| Mucous membrane protection                 |
|                | 0.745 | 0.002| NOS2 expression inhibition                 |
|                | 0.683 | 0.005| Antiulcerative                             |
|                | 0.663 | 0.001| Cholesterol synthesis inhibition           |
|                | 0.650 | 0.011| Anticarcinogenic                           |
|                | 0.642 | 0.036| Antineoplastic                             |
|                | 0.589 | 0.013| Hepatoprotector                            |
|                | 0.522 | 0.025| TNF inhibition                             |
|                | 0.512 | 0.027| Antithrombotic                             |
|                | 0.515 | 0.041| Lipid metabolism regulation               |
|                | 0.458 | 0.03  | Antipsoriasis                              |
|                | 0.444 | 0.147| Cerebral anti-ischemic                    |
|                | 0.385 | 0.007| NF kappa B inhibitor                       |
|                | 0.224 | 0.038| Lipoprotein disorder regulator             |
|                | 0.201 | 0.024| Phospholipase A₂ inhibitor                 |

To corroborate the results predicted by PASS, we further assessed these compounds through SEA (similarity ensemble approach) [22,23]. The outputs of this server are shown in Table 2. Geranylgeraniol had significant values ($p$-value < $10^{-10}$ or max Tanimoto coefficient (MaxTC) > 0.6) for squalene monooxygenase ($p$-value = $2.6 \times 10^{-27}$, MaxTC = 0.65) and lanosterol synthase ($p$-value = $4 \times 10^{-19}$, MaxTC = 0.40) interaction probability based on similarity with other compounds. Additionally, the server predicted significant interaction probability with phospholipase A₂ ($p$-value = $7.3 \times 10^{-18}$, MaxTC = 0.3). Tocotrienols had a lower degree of similarity with compounds able to interact with these targets compared with geranylgeraniol; however, the values were still in a considerable range. For squalene monooxygenase interaction, $p$-values ranged from $2.2 \times 10^{-08}$ to $8.6 \times 10^{-09}$, and MaxTC ranged from 0.30 to 0.31; for lanosterol synthase, $p$-values varied from $1.2 \times 10^{-06}$ to $2.0 \times 10^{-08}$, and MaxTC varied from 0.30 to 0.31. Finally, for phospholipase A₂, $p$-values varied from $3 \times 10^{-09}$ to $6.6 \times 10^{-09}$, and MaxTC varied from 0.3 to 0.31.

Table 2. Prediction outputs of the molecules assessed with ligands from the SEA server.

| Molecule         | Target                              | $p$-Value          | Max TC |
|------------------|-------------------------------------|--------------------|--------|
| Geranylgeraniol  | Squalene monooxygenase              | $2.641 \times 10^{-27}$ | 0.65   |
|                  | Lanosterol synthase                 | $4.01 \times 10^{-19}$ | 0.40   |
|                  | Phospholipase A₂                    | $7.305 \times 10^{-18}$ | 0.31   |
|                  | Protein-S-isoprenylcysteine O-methyltransferase | $1.703 \times 10^{-65}$ | 0.53   |
|                  | Geranylgeranyl pyrophosphate synthase | $1.409 \times 10^{-61}$ | 0.50   |
The outputs predicted by PASS and SEA collectively point to these molecules’ tendency to improve the blood lipid profile. However, while in PASS, the most favorable results were achieved by tocotrienols, the highest similarity outputs suggesting that biological action was achieved by geranylgeraniol in SEA. In SEA, the probability of squalene monooxygenase and lanosterol synthase inhibition by tocotrienols was not negligible but was still not high enough. However, it should be kept in mind that these two mechanisms of action are not the only ones that could decrease cholesterol biosynthesis and improve
the blood lipid profile. In fact, tocotrienols have been reported to inhibit the mevalonate pathway of HMG-CoA reductase, a pivotal player in cholesterol biosynthesis [24]. While geranylgeraniol was predicted to inhibit lanosterol synthase and monooxygenase in SEA, this was not predicted by PASS. This divergence between the servers could be a negative indicator of these targets, or it could be due to differences in the servers’ training sets, which could give different outcomes.

Reports support a potential role in improving blood lipid profile by geranylgeraniol. For instance, just like tocotrienols, this molecule was shown to decrease HMG-CoA reductase activity [25,26]. Considering the role of this enzyme in cholesterol biosynthesis, this could be a mechanism in which geranylgeraniol exerts its action. Our group reported that the treatment with geranylgeraniol improved blood lipid parameters; however, the molecule was not administrated alone but with tocotrienols [8]. Altogether, the in silico prediction with its known mechanism of action justifies future studies with this molecule alone in treating blood dyslipidemia in vivo.

As mentioned previously, it is believed that this activity may be at least in part due to HMG-CoA reductase inhibition based on previous studies. However, we sought to assess whether more mechanisms were underlying such activity. Hence, molecular docking was performed with the most promising targets.

2.2. Molecular Docking

Molecular docking is a powerful tool in computation chemistry that allows researchers to assess the molecular interactions’ type and intensity between a ligand and a target biomolecule within an active site [27]. A total of five macromolecular targets acquired from PDB were used in GOLD without the cocrystalized ligands (Figure 1B). Three of them are involved in cholesterol metabolism (OSC, SQLE, and HMGR), and two are directly involved in inflammation (PLA2 and COX-2).

Lanosterol synthase (a.k.a. oxidosqualene cyclase (OSC)) is a membrane-bound protein responsible for synthesizing steroids in mammals. Its cyclization reaction forms lanosterol. Due to its role in the synthesis of steroids, this protein is considered a target to hypolipidemic drugs [28]. When complexed with OSC, lanosterol forms hydrogen bonds with the amino acid residues Trp581 and Asp455 [29].

In the docking performed with OSC, geranylgeraniol and tocotrienol had relevant interactions with the receptors’ active-site amino acid residues. The details of such interactions are shown in Table 3, including the interaction type, distances, and docking scores.

### Table 3. Docking interactions of the molecules with OSC.

| Molecule | Amino Acid | Ligand Atom | Category | Types | Distance (Å) | Score |
|----------|------------|-------------|----------|-------|--------------|-------|
| Geranylgeraniol | A:ASP455 | H25 | Hydrogen bond | Conventional hydrogen bond | 2.08 | 2.65 | 2.81 |
| | A:TRP581 | H28 | Pi-sigma | 2.87 |
| | A:VAL236 | Ligand | 5.36 |
| | A:VAL453 | C14 | Hydrophobic | Alkyl | 3.94 | 87.88 |
| | A:PRO337 | C16 | 4.90 |
| | A:ILE338 | C16 | 5.25 |
| | A:ILE524 | C20 | 3.70 |
| | A:CYS233 | C21 | 4.53 |
| | A:ILE524 | C20 | 4.57 |
| Molecule | Amino Acid | Ligand Atom | Category       | Types     | Distance (Å) | Score |
|----------|------------|-------------|----------------|-----------|--------------|-------|
| A:TRP192 |            |              |                |           | 5.12         |       |
| A:TRP192 |            | C20         |                |           | 5.40         |       |
| A:TRP192 |            | C21         |                |           | 4.63         |       |
| A:HIS232 | Ligand     |             |                | Pi-alkyl  | 4.24         |       |
| A:PHE444 |            | C14         |                |           | 4.92         |       |
| A:TYR503 |            |             |                |           | 4.76         |       |
| A:PHE521 |            | C20         |                |           | 3.93         |       |
| A:PHE696 | Ligand     | C15         |                |           | 3.91         |       |
| A:ASP455 |            | H39         | Hydrogen bond  | Conventional hydrogen bond | 1.86 |       |
| A:TRP581 |            |              |                | Pi-pi stackedPi-pi T-shaped | 4.38 | 4.26 |
| A:TRP387 | Ligand     |             |                |           | 5.60         |       |
| A:VAL236 |            |             |                |           | 5.28         |       |
| A:PRO337 |            |             |                |           | 5.33         |       |
| A:VAL453 | C11        |             |                |           | 4.26         |       |
| A:ILE338 | Ligand     |             |                |           | 5.18         |       |
| A:VAL236 |            |             |                |           | 5.03         |       |
| A:PRO337 | C26        |             |                |           | 4.99         |       |
| A:ILE338 |            |             |                |           | 4.29         |       |
| A:ILE524 | C30        | C31         |                |           | 3.55         |       |
| A:TRP192 |            | C30         |                |           | 5.29         |       |
|          |            | C31         |                |           |              |       |
| ∝-tocotrienol | ligand |             | Hydrophobic |           | 4.84         | 108.40 |
| A:HIS232 |            | C16         |                |           | 5.07         |       |
|          | ligand     |             |                |           | 5.24         |       |
|          |            | C26         |                |           | 5.31         |       |
| A:TRP387 |            | C13         |                |           | 4.69         |       |
| A:PHE444 |            | C16         |                |           | 4.93         |       |
| A:TYR503 | Ligand     |             |                |           | 4.96         |       |
| A:PHE521 |            | C30         |                |           | 4.30         |       |
| A:TRP581 | Ligand     | C14         |                |           | 4.46         |       |
| A:PHE696 | ligand     |             |                |           | 4.55         |       |
|          |            | C31         |                |           | 5.39         |       |
Table 3. Cont.

| Molecule       | Amino Acid | Ligand Atom | Category         | Types                        | Distance (Å) | Score   |
|----------------|------------|-------------|------------------|------------------------------|--------------|---------|
| β-tocotrienol  | A:ASP455   | H39         | Hydrogen bond    | Conventional hydrogen bond   | 2.17         |         |
|                | A:TRP581   |             |                  | Pi-pi stacked               | 4.80         |         |
|                | A:VAL236   |             |                  |                              | 4.48         |         |
|                | A:PRO337   |             |                  |                              | 4.72         |         |
|                | A:ILE702   | C20         |                  |                              | 4.41         |         |
|                | A:ILE338   |             | Ligand           | Alkyl                        | 5.15         |         |
|                | A:PRO337   | C25         |                  |                              | 4.40         |         |
|                | A:ILE338   |             |                  |                              | 4.09         |         |
|                | A:CYS233   | C29         |                  |                              | 4.57         |         |
|                | A:ILE524   |             |                  |                              | 3.70         |         |
|                | A:TRP192   | C29         | Ligand           |                              | 5.17         |         |
|                |            | C30         |                  |                              | 5.46         |         |
|                |            |             |                  |                              | 5.08         |         |
|                |            |             |                  |                              | 5.26         | 106.85  |
|                | A:TRP230   | C13         | Hydrophobic      |                              | 4.85         |         |
|                |            | C29         |                  |                              | 5.01         |         |
|                |            | C13         |                  |                              | 4.70         |         |
|                | A:HIS232   | Ligand      |                  |                              | 4.15         |         |
|                |            | C25         |                  | Pi-alkyl                     | 5.17         |         |
|                | A:TRP387   | C11         | Ligand           |                              | 5.33         |         |
|                | A:PHE444   | C11         | Ligand           |                              | 4.73         |         |
|                | A:TYR503   | C13         | Ligand           |                              | 4.59         |         |
|                | A:PHE521   | C30         | Ligand           |                              | 3.69         |         |
|                | A:TRP581   | C13         | Ligand           |                              | 4.02         |         |
|                | A:TRP581   | C15         | Ligand           |                              | 4.97         |         |
|                | A:PHE696   | C20         | Ligand           |                              | 5.19         |         |
|                | A:VAL543   | Ligand      |                  |                              | 3.83         |         |
|                | A:ASP455   | H36         | Hydrogen bond    | Conventional hydrogen bond   | 5.32         |         |
| γ-tocotrienol  | A:TRP581   |             |                  | Pi-pi stacked               | 1.83         |         |
|                | A:TRP581   |             |                  |                              | 4.31         |         |
|                | A:TRP581   |             |                  |                              | 4.18         |         |
|                | A:TRP387   |             | Ligand           | Hydrophobic                  | 4.63         |         |
|                | A:VAL236   |             | Ligand           | Alkyl                        | 5.19         |         |
|                | A:PRO337   |             | Ligand           | Alkyl                        | 5.46         |         |
|                | A:ILE338   |             | Ligand           | Alkyl                        | 5.15         |         |
|                | A:VAL236   |             | Ligand           | Alkyl                        | 5.07         |         |
Table 3. Cont.

| Molecule | Amino Acid | Ligand Atom | Category | Types            | Distance (Å) | Score |
|----------|------------|-------------|----------|------------------|--------------|-------|
| A:ILE338 | C24        |             |          |                  | 4.37         |       |
| A:ILE524 | C24        |             |          |                  | 3.63         |       |
| A:TRP192 | C28        |             |          |                  | 4.59         |       |
|          | C29        |             |          |                  | 5.20         |       |
|          |            | Ligand      |          |                  | 4.75         |       |
| A:HIS232 | C14        |             |          |                  | 5.09         |       |
|          | C24        |             |          | Ligand           | 4.95         |       |
|          | C28        |             |          |                  | 4.77         |       |
| A:TRP387 | C30        |             |          |                  | 4.90         |       |
| A:PHE444 | C12        |             |          |                  | 4.17         |       |
|          | C14        |             |          |                  | 5.14         |       |
| A:TYR503 | C12        |             |          | Ligand           | 5.01         |       |
|          | C14        |             |          |                  | 5.30         |       |
| A:PHE521 | C28        |             |          | Ligand           | 4.18         |       |
|          | C29        |             |          |                  | 3.59         |       |
| A:TRP581 | C12        |             |          | Ligand           | 4.61         |       |
|          | C14        |             |          |                  | 5.42         |       |
|          | C29        |             |          | Ligand           | 4.80         |       |
| A:PHE696 | C14        |             |          |                  | 4.34         |       |
|          | C29        |             |          |                  | 5.23         |       |
| A:ASP455 | H36        |             | Hydrogen bond | Conventional hydrogen bond | 1.66         |       |
| A:TRP581 |             |             |          |                  | 4.28         |       |
| A:TRP387 | Ligand     |             |          | Pi-pi stacked    | 4.15         |       |
| A:PRO337 |             |             |          | Pi-pi T-shaped   | 4.80         |       |
| A:ILE338 |             |             |          |                  | 5.00         |       |
| A:VAL236 | C24        |             |          |                  | 5.47         |       |
| A:PRO337 |             |             |          |                  | 5.00         |       |
| A:ILE338 | C28        |             |          |                  | 4.58         |       |
| A:CYS233 | C28        |             |          |                  | 4.29         |       |
|          |            |             |          | Hydrophobic      | 3.72         | 105.88 |
|          |            |             |          |                  | 4.78         |       |
| A:TRP192 | Ligand     |             |          |                  | 5.01         |       |
| A:HIS232 | C12        |             |          |                  | 5.31         |       |
|          | C14        |             |          |                  | 4.85         |       |
| A:PHE444 | Ligand     |             |          |                  | 4.95         |       |
|          | C14        |             |          |                  | 5.03         |       |
|          | C29        |             |          |                  | 4.48         |       |
| A:TYR503 | C29        |             |          |                  | 3.76         |       |
|          | C29        |             |          |                  | 5.22         |       |
|          | C29        |             |          |                  | 5.42         |       |
In Figure 2, it is possible to observe the docking pose in two and three dimensions. It is observed that all molecules could interact with the amino acid residues Asp455 and Trp581 (hydrogen bonds), the same amino acids that can interact with the inhibitor of the enzyme Ro 48-8071, which is considered a structural base for the design of OSC inhibitors. However, the inhibitor performs hydrophobic interactions with Trp581 instead of hydrogen bonds [29].

| Molecule | Amino Acid | Ligand Atom | Category | Types | Distance (Å) | Score |
|----------|------------|-------------|----------|-------|--------------|-------|
| A:PHE521 |            |             |          |       | 3.92         |       |
| A:TRP581 |            | Ligand      |          |       | 5.18         |       |
| A:PHE696 |            |             | H55      |       | 5.18         |       |
|           |            |             |          |       | 4.32         |       |

**Figure 2.** Cont.
Like Ro 48-8071, the molecules could also interact with the residues Trp192 and Phe521, indicating that they can potentially inhibit this enzyme. β-tocotrienol could interact with all the residues mentioned so far plus Trp230, thus performing the same interactions of Ro 48-8071.

Squalene monooxygenase (a.k.a. squalene epoxidase (SQLE)) is the second limiting enzyme in cholesterol biosynthesis accountable to catalyze the conversion of squalene to 2,3(S)-oxidosqualene using flavin adenosine dinucleotide (FAD) as a coenzyme. SQLE inhibition is considered a possible mechanism in treating hypercholesterolemia, fungal infections, and some types of cancer [30]. The docking data with SQLE are shown in Table 4, and the docking poses are depicted in Figure 3.
Inhibition is considered a possible mechanism in treating hypercholesterolemia, fungal infections, and some types of cancer [30]. The docking data with SQLE are shown in Table 4, and the docking poses are depicted in Figure 3.

### Table 4. Docking interactions of the molecules with SQLE.

| Molecule              | Amino Acid | Ligand Atom | Category     | Types                          | Distance (Å) | Score  |
|-----------------------|------------|-------------|--------------|-------------------------------|--------------|--------|
| Geranylgeraniol       | A:GLY132   | O24         | Hydrogen     | Bond                          | 2.83         | 74.14  |
| (74.14)               | A:GLU153   | H55         |              |                               | 1.70         |        |
|                       | A:VAL133   |             | Hydrophobic  | Alkyl                         | 4.06         |        |
|                       | A:VAL163   |             |              |                               | 5.13         |        |
|                       | A:MET421   |             |              |                               | 5.04         |        |
| α-tocotrienol         | A:GLY146   | O24         | Hydrogen     | Bond                          | 3.03         | 78.60  |
| (80.60)               | A:GLU158   | H55         |              |                               | 1.73         |        |
|                       | A:VAL133   |             | Hydrophobic  | Alkyl                         | 4.05         |        |
|                       | A:VAL163   |             |              |                               | 5.13         |        |
| β-tocotrienol         | A:GLY132   | O24         | Hydrogen     | Bond                          | 2.83         | 88.43  |
| (92.56)               | A:GLU153   | H55         |              |                               | 1.70         |        |
|                       | A:VAL133   |             | Hydrophobic  | Alkyl                         | 4.06         |        |
|                       | A:VAL163   |             |              |                               | 5.13         |        |
| γ-tocotrienol         | A:GLY132   | O24         | Hydrogen     | Bond                          | 2.83         | 87.05  |
| (87.05)               | A:GLU153   | H55         |              |                               | 1.70         |        |
|                       | A:VAL133   |             | Hydrophobic  | Alkyl                         | 4.06         |        |

**Figure 3.** Two-dimensional and three-dimensional representations of the best docking poses calculated by GOLD with SQLE (PDB ID: 6C6N). Pictures produced with Discovery Studio.
Table 4. Docking interactions of the molecules with SQLE.

| Molecule       | Amino Acid | Ligand Atom | Category      | Types                | Distance (Å) | Score  |
|----------------|------------|-------------|---------------|----------------------|--------------|--------|
| Geranylgeraniol| A:GLY132   | O24         | Hydrogen bond | Conventional hydrogen bond | 2.83         |        |
|                | A:GLU153   | H55         |               |                      | 1.70         |        |
|                | A:VAL133   |             |               |                      | 4.06         |        |
|                | A:VAL163   |             |               |                      | 5.13         |        |
|                | A:MET421   |             |               |                      | 5.04         |        |
|                | A:LEU134   | C15         | Hydrophobic   | Alkyl                | 4.41         | 74.14  |
|                | A:VAL163   | C16         |               |                      | 4.56         |        |
|                |             | C20         |               |                      | 4.05         |        |
|                | A:PRO415   | C21         |               |                      | 4.27         |        |
| α-tocotrienol  | A:VAL133   |             |               |                      | 4.33         |        |
|                | A:VAL163   |             |               |                      | 5.27         |        |
|                | A:MET421   | C11         |               |                      | 4.42         |        |
|                | A:PRO415   | C13         |               |                      | 3.96         |        |
|                | A:VAL163   | C14         | Hydrophobic   | Alkyl                | 3.71         | 80.60  |
|                | A:LEU287   | C16         |               |                      | 4.19         |        |
|                | A:VAL133   | C16         |               |                      | 4.70         |        |
|                | A:VAL129   | C30         |               |                      | 4.93         |        |
|                | A:ILE152   | C30         |               |                      | 5.02         |        |
|                | A:VAL250   |             |               |                      | 4.05         |        |
|                | A:ARG154   | C31         |               |                      | 4.14         |        |
|                | A:VAL249   |             |               |                      | 4.20         |        |
|                | A:His226   |             |               |                      | 4.96         |        |
|                | A:VAL163   |             |               |                      | 4.36         |        |
| β-tocotrienol  | A:VAL133   |             |               |                      | 5.10         |        |
|                | A:VAL163   |             |               |                      | 4.65         |        |
|                | A:PRO415   |             |               |                      | 5.00         |        |
|                | A:PRO415   |             |               |                      | 4.55         |        |
|                | A:ALA424   |             |               |                      | 4.92         |        |
|                | A:VAL133   | C13         |               |                      | 4.72         |        |
|                | A:MET421   |             | Hydrophobic   | Alkyl                | 3.84         |        |
|                | A:VAL163   | C15         |               |                      | 4.39         |        |
|                | A:PRO415   | C20         |               |                      | 5.43         | 92.56  |
|                | A:LEU345   |             |               |                      | 4.71         |        |
|                | A:PRO415   |             |               |                      | 4.73         |        |
|                | A:PRO415   |             |               |                      | 4.76         |        |
|                | A:MET388   | C25         |               |                      | 5.16         |        |
|                | A:PRO415   |             |               |                      | 4.75         |        |
|                | A:PRO415   |             |               |                      | 4.32         |        |
|                | A:VAL163   |             |               |                      | 4.76         |        |
|                | A:MET421   | C29         |               |                      | 4.47         |        |
|                | A:PRO415   |             |               |                      | 4.60         |        |
The aromatic groups of the ligand complexed with SQLE (PDB ID: 6C6N) perform nonpolar interactions with the amino acid residues Asp166, Tyr195, Ala322, Leu333, Tyr335, Pro415, Leu416, and Gly418 [30]. Of these residues, only Pro415 could interact with all the molecules tested (hydrophobic interaction) except for δ-tocotrienol. However, other interactions were observed with different amino acid residues. β-tocotrienol was the compound with more interactions with Pro415 (six hydrophobic interactions) and had the highest docking score (92.56).

It is believed that one of the main targets for the hypocholesterolemic activity of tocotrienols is HMG-CoA reductase. This enzyme catalyzes the rate-limiting step in cholesterol biosynthesis [31] and is also targeted by statins, although these molecules inhibit its activity in a different way [8]. As mentioned, there are some reports of HMGR inhibition by geranylgeraniol as well. Here we sought to discover whether the inhibition of these
molecules could involve direct binding to HMGR. The docking interactions are detailed in Table 5 and depicted in Figure 4. The results show that the molecules interacted with the amino acid residues Leu562, Leu853, Ala856, and Leu857 through hydrophobic interactions. It is observed that the highest number of interactions and docking score were obtained by γ-tocotrienol (17 interactions; 57.77 docking score), while geranylgeraniol had the lowest (12 and 51.47, respectively).

**Molecule (docking score)**

| Molecule          | 2D  | 3D  |
|-------------------|-----|-----|
| Geranylgeraniol   | (51.47) |     |
| α-tocotrienol     | (55.05) |     |
| β-tocotrienol     | (56.19) |     |

**Figure 4. Cont.**
γ-tocotrienol (57.77)

δ-tocotrienol (56.59)

Figure 4. Two-dimensional and three-dimensional representations of the best docking poses calculated by GOLD with HMG-CoA reductase. Pictures produced with Discovery Studio.

Table 5. Docking interactions of the molecules with HMG-CoA reductase.

| Molecule   | Amino Acid | Ligand Atom | Category | Types     | Distance (Å) | Score |
|------------|------------|-------------|----------|-----------|--------------|-------|
| Geranylgeraniol | CYS561 | C9          | Hydrophobic | Alkyl     | 4.49         |       |
|              | ALA564     | C17         |          |           | 5.46         |       |
|              | ALA564     | C20         |          |           | 3.30         |       |
|              | ALA564     | C21         |          |           | 3.71         |       |
|              | ALA856     | C17         |          |           | 4.86         |       |
|              | LEU853     | C16         |          |           | 3.49         |       |
|              | LEU562     | C5          |          |           | 4.21         |       |
|              | LEU562     | C15         |          |           | 3.83         |       |
|              | LEU853     | C16         |          |           | 4.26         |       |
|              | CYS561     | C20         |          |           | 3.86         |       |
|              | HIS752     | C5          |          | Pi-alkyl  | 4.36         | 51.47 |
|              | HIS752     | C15         |          |           | 4.97         |       |
| α-tocotrienol | CYS561     | C22         | Hydrophobic | Alkyl     | 4.18         |       |
|              | ALA564     | C31         |          |           | 3.51         |       |
|              | ALA856     | C27         |          |           | 5.19         |       |
|              | ALA856     | C26         |          |           | 3.30         |       |
Table 5. Cont.

| Molecule | Amino Acid | Ligand Atom | Category | Types | Distance (Å) | Score |
|----------|------------|-------------|----------|-------|--------------|-------|
| LEU853   | LEU853     | C9          |          |       | 5.05         |       |
| LEU857   | LEU857     | C13         |          |       | 4.29         |       |
| LEU853   | LEU853     | C14         |          |       | 4.63         |       |
| LEU857   | LEU857     | C17         |          |       | 4.30         |       |
| LEU853   | LEU853     | C21         |          |       | 3.90         |       |
| LEU853   | LEU853     | C26         |          |       | 4.73         |       |
| HIS752   | HIS752     | C9          |          | Pi-alkyl | 5.03         |       |
|          | LEU853     | C13         |          |       | 4.37         |       |
|          | LEU853     | C17         |          |       | 4.95         |       |
|          | LEU853     | C21         |          |       | 5.15         |       |
| β-tocotrienol | CYS561    | C21         |          |       | 4.31         |       |
|          | ALA564     | C26         |          |       | 4.40         |       |
|          | ALA564     | C29         |          |       | 4.37         |       |
|          | ALA856     | C25         |          |       | 4.15         |       |
|          | LEU853     | C9          |          | Alkyl | 5.33         |       |
|          | LEU853     | C13         |          | Hydrophobic | 4.56 | 56.19 |
|          | LEU853     | C16         |          |       | 4.20         |       |
|          | LEU853     | C20         |          |       | 4.19         |       |
|          | LEU562     | C20         |          |       | 3.92         |       |
|          | ARG568     | C30         |          |       | 3.97         |       |
|          | HIS752     | C9          |          |       | 5.32         |       |
|          | HIS752     | C16         |          | Pi-alkyl | 4.53         |       |
|          | LEU853     | C20         |          |       | 4.71         |       |
|          | LEU853     | C20         |          |       | 5.35         |       |
| γ-tocotrienol | CYS561    | C20         |          |       | 4.15         |       |
|          | ALA564     | C25         |          |       | 4.97         |       |
|          | ALA564     | C28         |          |       | 4.87         |       |
|          | ALA856     | C24         |          |       | 3.34         |       |
|          | ALA856     | C29         |          |       | 3.70         |       |
|          | LEU853     | C9          |          | Alkyl | 3.32         |       |
|          | LEU853     | C12         |          | Hydrophobic | 4.68 | 57.77 |
|          | LEU853     | C15         |          |       | 4.41         |       |
|          | LEU853     | C15         |          |       | 4.11         |       |
|          | LEU562     | C19         |          |       | 4.04         |       |
|          | CY561      | C28         |          |       | 3.75         |       |
|          | LEU857     | C30         |          |       | 4.24         |       |
|          | HIS752     | C9          |          | Pi-alkyl | 4.88         |       |
|          | LEU853     | C15         |          |       | 4.40         |       |
|          | LEU853     | C19         |          |       | 5.06         |       |
|          | LEU853     | Anel Ar.    |          |       | 5.01         |       |
Inflammation is tightly associated with lipid and metabolic disturbances [32–34]. According to the results predicted by PASS and SEA, geranylgeraniol and tocotrienols may also decrease inflammation. In accordance with our results, it has been reported that geranylgeraniol suppresses the expression of interleukin-1 receptor-associated kinase-1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6), consequently preventing NF-κB excessive activation in LPS-induced inflammatory response in THP-1 cells. In addition, tocotrienols are thought to exert their effects also in part by decreasing the inflammatory cascade [35–40].

Since SEA predicted the interaction of all the molecules with phospholipase A2, we performed a docking with this enzyme. We also performed docking with COX-2 because it is a common target for anti-inflammatory compounds (such as the NSAIDs).

COX-2 is an inflammatory enzyme that converts arachidonic acid into prostaglandins, such as prostaglandin H2 [41]. The docking results with COX-2 are shown in Table 6, and the docking poses are depicted in Figure 5. The structure of COX-2 was stored in PDB in a complex with meclofenamic acid, a known inhibitor of this enzyme.

### Table 5. Cont.

| Molecule | Amino Acid | Ligand Atom | Category | Types          | Distance (Å) | Score |
|----------|------------|-------------|----------|----------------|--------------|-------|
| δ-tocotrienol | ALA564 | Anel Ar. | Amide-pi stacked | 4.02 | 56.59 |
|          | CYS561 | C15 | Hydrophobic | C12 | 3.33 |
|          | CYS561 | C12 | Hydrophobic | C12 | 3.32 |
|          | ALA754 | C29 | Hydrophobic | C12 | 3.33 |
|          | ALA856 | C9 | Hydrophobic | Alkyl | 4.26 |
|          | CYS561 | C12 | Hydrophobic | Alkyl | 4.32 |
|          | LEU853 | C20 | Hydrophobic | Alkyl | 4.24 |
|          | LEU562 | C19 | Hydrophobic | Alkyl | 4.24 |
|          | LEU853 | C24 | Hydrophobic | Alkyl | 4.74 |
|          | HIS752 | C20 | Hydrophobic | Pi-alkyl | 4.27 |
|          | CYS561 | C12 | Hydrophobic | Pi-alkyl | 4.56 |
|          | ALA564 | Anel Ar. | Hydrophobic | 4.21 |
|          | ARG568 | | Hydrophobic | 5.40 |

### Table 6. Docking interactions of the molecules with COX-2.

| Molecule | Amino Acid | Ligand Atom | Category | Types          | Distance (Å) | Score |
|----------|------------|-------------|----------|----------------|--------------|-------|
| Geranylgeraniol | B:SER531 | O24 | Hydrogen bond | Conventional hydrogen bond | 2.16 |
|           | B:VAL117 | | | | 3.86 |
|           | B:ARG121 | | Ligand | | 5.14 |
|           | B:VAL524 | | | | 4.13 |
|           | B:ALA528 | C16 | Hydrophobic | Alkyl | 3.99 |
|           | B:LEU353 | | Hydrophobic | Alkyl | 3.99 |
|           | B:LEU532 | | | | 5.04 |
|           | B:LEU385 | C14 | | | 5.05 |
|           | B:LEU353 | C15 | | | 4.18 |
|           | B:VAL524 | | | | 3.88 |
Table 6. Cont.

| Molecule   | Amino Acid | Ligand Atom | Category | Types       | Distance (Å) | Score |
|------------|------------|-------------|----------|-------------|--------------|-------|
| B:VAL350   |            | C16         |          |             | 4.57         |       |
| B:LEU532   |            |             |          |             | 4.40         |       |
| B:VAL89    |            |             |          |             | 5.46         |       |
| B:LEU93    |            | C20         |          |             | 4.92         |       |
| B:VAL117   |            | C21         |          |             | 4.43         |       |
| B:ARG121   |            |             |          |             | 3.45         |       |
| B:TYR356   | Ligand     | C20         | Pi-alkyl |             | 5.30         | 5.09  |
| B:PHE382   |            | C20         | Pi-alkyl |             | 5.48         |       |
| B:TYR386   |            | C14         |          |             | 4.31         |       |
| B:TRP388   |            |             |          |             | 4.91         |       |
| B:VAL524   |            |             |          |             | 3.65         |       |
| B:ALA528   |            |             |          |             | 3.85         |       |
| B:VAL117   |            | C11         |          |             | 5.03         |       |
| B:VAL350   |            | C13         |          |             | 3.68         |       |
| B:LEU353   | Ligand     |             |          |             | 4.79         |       |
| B:VAL350   |            | C16         |          |             | 5.10         |       |
| B:LEU353   |            |             |          |             | 4.14         |       |
| B:LEU385   | C21        |             |          |             | 4.79         |       |
| B:MET523   |            |             |          |             | 4.91         |       |
| B:LEU535   | Ligand     |             |          |             | 4.79         |       |
| B:VAL345   |            | C26         |          |             | 4.76         |       |
| B:VAL350   |            |             |          |             | 5.09         |       |
| B:VAL229   |            | C30         | Hydrophobic |             | 4.50         | 76.42 |
| B:LEU535   | Ligand     |             |          |             | 4.93         |       |
| B:PHE206   |            | C26         |          |             | 4.82         |       |
| B:PHE210   | Ligand     |             |          |             | 4.27         |       |
| B:TYR349   | C26        |             |          |             | 4.24         |       |
| B:TYR356   | C11        |             |          |             | 4.50         |       |
| B:PHE382   | Ligand     | C31         | Pt-alkyl |             | 4.43         |       |
| B:TYR386   | Ligand     | C21         | Pt-alkyl |             | 4.91         |       |
| B:TRP388   |            |             |          |             | 4.99         |       |
| Molecule       | Amino Acid | Ligand Atom | Category      | Types       | Distance (Å) | Score |
|---------------|------------|-------------|---------------|-------------|--------------|-------|
| B:VAL350      |            | Ligand      |               |             | 4.04         |       |
| B:ALA528      |            |             |               |             | 3.86         |       |
| B:VAL524      |            | Ligand      |               |             | 3.84         |       |
| B:ALA528      |            |             |               |             | 4.58         |       |
| B:VAL524      |            | Ligand      |               |             | 4.17         |       |
| B:ALA528      |            |             |               |             | 4.48         |       |
| B:VAL117      |            | C11         | Alkyl         |             | 5.10         |       |
| B:LEU353      | Ligand     |             |               |             | 5.34         |       |
| B:VAL350      |            | C15         | Hydrophobic   |             | 4.49         |       |
| B:LEU353      |             | C20         |               |             | 3.92         |       |
| B:LEU353      | Ligand     |             |               |             | 4.72         |       |
| B:LEU355      |            | C20         |               |             | 4.26         |       |
| B:VAL345      |            | C25         |               |             | 4.39         |       |
| B:VAL350      | Ligand     | C20         |               |             | 4.95         |       |
| B:VAL229      |            | C29         |               |             | 4.89         |       |
| B:PHE206      | Ligand     | C25         |               |             | 4.50         |       |
| B:PHE210      | Ligand     | C29         |               |             | 4.84         |       |
| B:PHE210      |             | C30         |               |             | 3.85         |       |
| B:TYR349      |            | C25         | Pi-alkyl      |             | 4.64         |       |
| B:TYR356      |            | C11         |               |             | 4.77         |       |
| B:PHE382      | Ligand     | C30         |               |             | 4.36         |       |
| B:TYR386      | Ligand     | C20         |               |             | 4.85         |       |
| B:TRP388      |            |             |               |             | 4.53         |       |
| B:VAL350      |            |             |               |             | 4.74         |       |
| B:ALA528      | Ligand     |             |               |             | 3.74         |       |
| B:LEU353      |            |             |               |             | 4.74         |       |
| B:VAL524      |            | Ligand      |               |             | 3.62         |       |
| B:ALA528      |            | C12         | Hydrophobic   | Alkyl       | 4.85         | 85.12 |
| B:VAL350      | Ligand     | C12         |               |             | 4.50         |       |
| B:LEU353      | Ligand     | C14         |               |             | 3.69         |       |
| B:VAL350      | Ligand     |             |               |             | 4.81         |       |
| B:LEU353      |             |             |               |             | 4.19         |       |
| B:VAL350      | Ligand     |             |               |             | 4.95         |       |
| B:LEU353      |             |             |               |             | 4.83         |       |
| B:LEU353      |             |             |               |             | 3.85         |       |
Table 6. Cont.

| Molecule   | Amino Acid | Ligand Atom | Category | Types     | Distance (Å) | Score |
|------------|------------|-------------|----------|-----------|--------------|-------|
| B:LEU385   | C19        |             |          |           | 4.93         |       |
| B:LEU353   | Ligand     |             |          |           | 4.36         |       |
| B:VAL345   | C24        |             |          |           | 4.59         |       |
| B:VAL229   | C28        |             |          |           | 4.90         |       |
| B:VAL350   | C30        |             |          |           | 4.34         |       |
| B:PHE206   | Ligand     |             |          |           | 4.67         |       |
| B:LEU353   |           | C24         |          |           | 4.89         |       |
| B:PHE210   | Ligand     |             |          |           | 4.82         |       |
| B:VAL345   | C24        |             |          |           | 4.63         |       |
| B:PHE206   |            | C29         |          |           | 4.55         |       |
| B:PHE210   |            | C28         |          |           | 3.84         |       |
| B:PHE382   | Ligand     | C29         |          |           | 4.45         |       |
| B:LEU353   |           | C28         |          |           | 4.55         |       |
| B:TYR386   | Ligand     | C19         |          |           | 4.54         |       |
| B:TRP388   | C19        |             |          |           | 4.90         |       |
| B:VAL350   | Ligand     |             |          |           | 4.42         |       |
| B:ALA528   |            |             |          |           | 4.37         |       |
|            |            |             |          |           |              |       |
| B:VAL524   | Ligand     |             |          |           | 3.81         |       |
| B:ALA528   | C12        |             |          |           | 3.63         |       |
| B:LEU353   | Ligand     |             |          |           | 5.45         |       |
| B:VAL524   | C12        |             |          |           | 4.34         |       |
| B:LEU532   |            |             |          |           | 4.66         |       |
| B:LEU353   | Ligand     |             |          |           | 4.84         |       |
| B:VAL350   | C14        |             |          |           | 4.32         |       |
| B:LEU353   | C14        |             |          |           | 4.32         |       |
| B:LEU385   | C19        |             |          |           | 3.75         |       |
| B:MET523   |            |             |          |           | 3.75         |       |
| B:LEU535   | Ligand     |             |          |           | 4.74         |       |
| B:LEU385   | C19        |             |          |           | 4.74         |       |
| B:VAL345   | C24        |             |          |           | 4.65         |       |
| B:VAL350   | C28        |             |          |           | 4.89         |       |
| B:VAL350   | C24        |             |          |           | 5.08         |       |
| B:VAL229   | C28        |             |          |           | 4.84         |       |
| B:LEU535   | C28        |             |          |           | 4.71         |       |
| B:PHE206   | Ligand     |             |          |           | 4.84         | 89.07 |
| B:LEU353   | Ligand     | C28         |          |           | 5.37         |       |

δ-tocotrienol: 89.07
Inflammation is tightly associated with lipid and metabolic disturbances \[32 – 34\]. According to the results predicted by PASS and SEA, geranylgeraniol and tocotrienols may also decrease inflammation. In accordance with our results, it has been reported that geranylgeraniol suppresses the expression of interleukin-receptor-associated kinase-1 (IRAK1) and tumor necrosis factor-receptor-associated factor 6 (TRAF6), consequently preventing NF-κB excessive activation in LPS-induced inflammatory response in THP-1 cells. In addition, tocotrienols are thought to exert their effects also in part by decreasing the inflammatory cascade \[35 – 40\]. Since SEA predicted the interaction of all the molecules with phospholipase A2, we performed a docking with this enzyme. We also performed docking with COX-2 because it is a common target for anti-inflammatory compounds (such as the NSAIDs). COX-2 is an inflammatory enzyme that converts arachidonic acid into prostaglandins, such as prostaglandin H2 \[41\]. The docking results with COX-2 are shown in Table 6, and the docking poses are depicted in Figure 5. The structure of COX-2 was stored in PDB in a complex with meclofenamic acid, a known inhibitor of this enzyme.

### Table 6. Cont.

| Molecule   | Amino Acid | Ligand Atom | Category | Types | Distance (Å) | Score |
|------------|------------|-------------|----------|-------|--------------|-------|
| B:PHE210   |            | C28         | Ligand   |       | 5.04         |       |
|            |            | C29         |          |       | 4.26         |       |
| B:TYR349   |            | C24         |          |       | 4.51         |       |
| B:PHE382   |            | C29         | Ligand   |       | 5.13         |       |
| B:TYR386   |            | C24         |          |       | 4.81         |       |
| B:TRP388   |            | C19         |          |       | 4.65         |       |
| B:VAL350   |            | C24         |          |       | 4.52         |       |
| B:ALA528   |            | C19         |          |       | 5.15         |       |
| B:LEU532   |            | C24         |          |       | 4.84         |       |
| B:LEU532   |            | C20         |          |       | 3.55         |       |

![Figure 5. Cont.](Image)
The hydrogen bonds between the inhibitor’s carboxylate and the phenolic oxygen of Tyr385 and Ser530 are considered important interactions for the inhibition of this enzyme [42]. It was observed that all the structures could interact with COX-2, but none of them could interact with the amino acid residues Tyr385 and Ser530. The highest docking score was achieved by δ-tocotrienol (89.07), and the other molecules had good scores as well (>70).

Phospholipase A₂ is another enzyme involved in the inflammatory response that catalyzes the hydrolysis of two glycerophospholipids and releases two fatty acids and
lysophospholipids. The secreted PLA$_2$ is involved in the rate-limiting step of eicosanoid biosynthesis by releasing unesterified arachidonic acid from membrane phospholipids [43].

Table 7 shows all the interactions of this enzyme with geranylgeraniol and tocotrienols, and the best docking poses are depicted in Figure 6. The results show that all molecules interacted with the amino acid residue His47; except for α-tocotrienol, all molecules could interact with Cys28 as well. Most of the molecules assessed could interact with PLA$_2$’s hydrophobic pocket (Leu2, Phe5, His5, Ile9, Ala17, Ala8, Gly22), suggesting this enzyme’s potential inhibition. The highest docking score was achieved by α-tocotrienol (90.64).

| Molecule          | (docking score) | 2D                  | 3D                  |
|-------------------|-----------------|---------------------|---------------------|
| Geranylgeraniol   | (80.76)         | ![Geranylgeraniol](#) | ![Geranylgeraniol](#) |
| α-tocotrienol     | (90.64)         | ![α-tocotrienol](#) | ![α-tocotrienol](#)  |
| β-tocotrienol     | (86.47)         | ![β-tocotrienol](#) | ![β-tocotrienol](#)  |

*Figure 6. Cont.*
Figure 6. Two-dimensional and three-dimensional representations of the best docking poses calculated by GOLD with PLA$_2$ (PDB ID: 5G3N). Pictures produced with Discovery Studio.

Table 7. Docking interactions of the molecules with PLA$_2$.

| Molecule     | Amino Acid | Ligand Atom | Category     | Types                     | Distance (Å) | Score |
|--------------|------------|-------------|--------------|---------------------------|--------------|-------|
| Geranylgeraniol | HIS47      | O24         | Hydrogen bond| Conventional hydrogen bond| 1.61         |       |
|               | ASP48      | H55         |              |                           | 1.97         |       |
|               | ALA1       | C21         |              |                           | 3.79         |       |
|               | VAL3       | Ligand      |              |                           | 4.87         |       |
|               | ALA17      | C15         |              |                           | 3.70         |       |
|               | LEU2       | Ligand      |              |                           | 5.21         |       |
|               |            |             |              |                           | 4.34         |       |
|               |            |             |              |                           | 4.89         |       |
|               | CYS28      | C14         | Hydrophobic  | Alkyl                      | 4.24         | 80.76 |
|               | CYS44      |             |              |                           | 4.33         |       |
|               | ILE9       | C15         |              |                           | 4.98         |       |
|               |            | C16         |              |                           | 3.94         |       |
|               | LEU2       | C20         |              |                           | 4.71         |       |
|               |            | C21         |              |                           | 5.30         |       |
|               | VAL3       | Ligand      |              |                           | 4.51         |       |
|               | PHE5       | C14         |              | Pi-alkyl                  | 4.84         |       |
|               |            | C15         |              |                           | 5.20         |       |
|               |            |             |              |                           | 4.31         |       |
| Molecule | Amino Acid | Ligand Atom | Category | Types   | Distance (Å) | Score |
|----------|------------|-------------|----------|---------|--------------|-------|
| HIS6     | Ligand     | C15         |          |         | 4.98         |       |
|          |            |             |          |         | 5.02         |       |
| PHE63    | Ligand     | C20         |          |         | 4.26         |       |
| PHE98    | Ligand     | C14         |          |         | 4.85         |       |
| ALA17    | Ligand     | C13         |          |         | 3.99         |       |
| LEU2     | Ligand     | C11         |          |         | 5.28         |       |
| ILE9     | Ligand     | C21         | Hydrophobic | Alkyl   | 4.98         |       |
| LEU2     | Ligand     | C21         |          |         | 5.49         |       |
|          |            | C26         |          |         | 3.95         |       |
|          |            |             |          |         | 4.39         |       |
| LY56     | Ligand     | C21         |          |         | 4.68         |       |
|          |            | C30         | α-tocotrienol | Hydrophobic | 3.99         | 90.64 |
| PHE5     | Ligand     | C13         |          |         | 4.58         |       |
| PHE5     | Ligand     | C21         |          |         | 4.92         |       |
| HIS6     | Ligand     | C21         |          |         | 4.59         |       |
| HIS47    | Ligand     | C21         |          |         | 5.40         |       |
|          |            | C26         | β-tocotrienol | Pi-alkyl | 4.84         |       |
| TYR51    | Ligand     | C30         |          |         | 4.71         |       |
| PHE98    | Ligand     | C21         |          |         | 4.78         |       |
|         |            |             |          |         | 4.82         |       |
| ALA18    | Ligand     |             |          |         | 4.51         |       |
|         |            |             |          |         | 4.67         |       |
| HIS6     |            |             |          |         |              | Pi-sigma 2.89 |
| ALA17    | Ligand     |             |          |         | 4.15         |       |
|          |            |             |          |         | 5.17         |       |
| LEU2     | Ligand     | C11         |          |         | 4.76         |       |
|          |            |             |          |         | 5.17         |       |
| CYS28    | Ligand     | C20         |          |         | 4.68         |       |
| CYS44    | Ligand     | C20         |          |         | 4.80         |       |
| LEU2     | Ligand     | C25         |          |         | 4.90         |       |
|          |            |             |          |         | 4.90         |       |
| VAL30    | Ligand     |             |          |         |              | β-tocotrienol | Hydrophobic | 5.02 | 86.47 |
| PHE5     | Ligand     | C20         |          |         |              | Pi-alkyl 4.80 |
|          |            |             |          |         | 4.96         |       |
| HIS6     | Ligand     | C11         |          |         | 5.03         |       |
| HIS47    | Ligand     | C20         |          |         | 5.03         |       |
| TYR51    | Ligand     | C30         |          |         | 5.06         |       |
| PHE98    | Ligand     | C20         |          |         | 5.11         |       |
| ALA18    | Ligand     |             |          |         | 4.50         |       |
Table 7. Cont.

| Molecule | Amino Acid | Ligand Atom | Category   | Types              | Distance (Å) | Score |
|----------|------------|-------------|------------|--------------------|--------------|-------|
| γ-tocotrienol | GLY29 | Ligand | Amide-pi stacked | 4.93 | 88.94 |
|           | ALA17 | C24 | | 4.79 | |
|           | VAL30 | Ligand | | 5.15 | |
|           | LEU2 | C14 | | 5.21 | |
|           | CYS28 | C19 | Alkyl | 4.35 | |
|           | CYS44 | | | 4.29 | |
|           | LEU2 | Ligand | | 3.82 | |
|           | ILE9 | C24 | | 4.82 | |
|           | LEU2 | C28 | | 5.24 | |
|           | VAL3 | C29 | Hydrophobic | 4.61 | |
|           | PHE5 | C19 | Ligand | 5.18 | |
|           | C24 | | | 5.39 | |
|           | HIS6 | C24 | Pi-alkyl | 4.96 | |
|           | C29 | | | 4.33 | |
|           | HIS47 | C14 | Ligand | 4.72 | |
|           | TYR51 | | | 4.12 | |
|           | PHE98 | C19 | | 5.09 | |
|           | VAL30 | Ligand | | 4.31 | |
|           | LYS62 | | | 4.8 | |
| δ-tocotrienol | ASP48 | H36 | Hydrogen bond | Conventional hydrogen bond | 1.71 | |
|           | GLY29 | Ligand | Pi-donor hydrogen bond | 2.93 | |
|           | CYS28 | | Other | 5.93 | |
|           | CYS44 | | Pi-sulfur | 4.86 | |
|           | HIS47 | C29 | Pi-pi T-shaped | 4.78 | |
|           | ALA17 | Ligand | | 4.90 | |
|           | C12 | | | 4.89 | |
|           | LEU2 | Ligand | | 4.11 | |
|           | VAL3 | C24 | Hydrophobic | 4.23 | |
|           | LEU2 | C29 | Alkyl | 4.79 | |
|           | VAL3 | C29 | | 4.36 | |


Table 7. Cont.

| Molecule | Amino Acid | Ligand Atom | Category | Types | Distance (Å) | Score |
|----------|------------|-------------|----------|-------|--------------|-------|
| PHE5     | Ligand     |             |          |       | 4.52         |       |
| HIS6     | C19        |             |          |       | 4.64         |       |
| HIS47    | C12        | Pi-alkyl    |          |       | 5.20         |       |
| PHE63    | Ligand     |             |          |       | 4.95         |       |
|          | C28        |             |          |       | 4.69         |       |

In the docking studies, it was observed that geranylgeraniol could interact with all the targets assessed. For OSC, SQLE, and PLA₂, these interactions were similar to their corresponding crystalized inhibitors, corroborating the predictions by SEA and suggesting a potential hypocholesterolemic and anti-inflammatory activity. Tocotrienols also could interact with the assessed enzymes; notably, β-tocotrienol had an interesting interaction profile with OSC, similar to Ro 48-8071. As regards SQLE, δ-tocotrienol could not interact with the target’s active site amino acid residues, while all others could interact with Pro415, specially β-tocotrienol.

Although all molecules could interact with COX-2, none of these interactions are reported in the literature to inhibit this enzyme activity. For PLA₂, an important interaction that inhibits this enzyme is with the amino acid residues His47 and Cys28. All tocotrienols could interact with His47, and all but α-tocotrienol could interact with Cys28 as well (even though this molecule had the highest docking score).

Collectively, the docking supports the biological activity prediction. The results support the hypocholesterolemic and anti-inflammatory potential for geranylgeraniol and tocotrienols, following previous reports in the literature. Although these activities are not new for these molecules, our results suggest some potential new action mechanism that has not been reported, such as lanosterol synthase inhibition, which is different from HMG-CoA reductase inhibition.

2.3. Pharmacokinetic Property Prediction

Despite having a desired biological activity, a compound must effectively reach its therapeutic targets, and for this, the molecule must have a favorable pharmacokinetic profile (absorption, distribution, metabolism, excretion (ADME)). Nowadays, several approaches are available to predict ADME data from compounds [44]. The servers PreADMET and SwissADME were used to indicate such activities based on the compounds’ structures. The data are shown in Table 8.

Table 8. ADME prediction by PreADMET and SwissADME.

| Molecule            | PreADMET |            |            | SwissADME |            |            |
|---------------------|----------|------------|------------|-----------|------------|------------|
|                     | %HIA     | Caco-2 (nm/sec) | MDCK (nm/sec) | BBP% | BBB (Cbrb/Cbbm) | GI absorption | BBB | P-gp |
| Geranylgeraniol     | 100      | 37.1       | 62.05      | 100       | 17.58      | High        | No  | No  |
| α-tocotrienol       | 97.91    | 29.13      | 21.78      | 100       | 19.21      | Low         | No  | Yes |
| β-tocotrienol       | 97.9     | 27.94      | 24.31      | 100       | 19.01      | Low         | No  | Yes |
| γ-tocotrienol       | 97.9     | 27.94      | 24.31      | 100       | 18.99      | Low         | No  | Yes |
| δ-tocotrienol       | 97.89    | 26.83      | 27.42      | 100       | 18.83      | Low         | No  | Yes |

In PreADMET outputs, %HIA represents the human intestinal absorption, which, as the name suggests, refers to the amount of the molecule that is absorbed. HIA is important because most drugs are administered orally and hence need to be absorbed in satisfactory amounts in the gastrointestinal tract [45]. The server PreADMET considers that good drug candidates should have a %HIA of at least 70%. Hence, all the molecules had a great degree of intestinal absorption with %HIA > 97%, and geranylgeraniol had 100%.
SwissADME bases the gastrointestinal absorption and blood–brain barrier permeation on a different model called BOILED-Egg (brain or intestinal estimated permeation method) [46,47]. In this distinct model, geranylgeraniol but not tocotrienols were predicted to be highly permeant to the GI tract due to their high $\text{Lop } P$.

A popular model to assess drug absorption in drug discovery is using Caco-2 or MDCK cells as test systems. PreADMET can predict the molecular permeation in these cells by comparing the molecules from those of its database. According to the server, $<4 \text{ nm/s}$ represents low permeation, values from 4 to 70 nm/s have intermediate permeation, and values above that represent high permeation. For MDCK, values below 25 represent low permeability, values from 25 to 500 represent intermediate permeation, and values above 500 represent high permeation [48,49].

All molecules assessed had intermediate absorption values in Caco-2 cells, while in MDCK, only geranylgeraniol and $\delta$-tocotrienol had intermediate absorption values, and the others had low values. Overall, geranylgeraniol had superior results to tocotrienols. Among tocotrienols, $\alpha$-tocotrienol had the highest absorption values (Table 8).

For PreADMET, good drug candidates must have $<90\%$ of blood protein binding (BPB) because the molecules should be free to be able to interact with their biological targets [50]. In our prediction, the molecules had an unfavorable BPB profile (higher than 90%). Another distribution parameter assessed was the interaction with P-glycoprotein (P-gp) calculated by SwissADME. This macromolecule is responsible for hampering the intracellular accumulation of potentially toxic compounds and removing them from the CNS through the blood–brain barrier as well [51]. The server predicted that tocotrienols could interact with these targets while geranylgeraniol could not.

Both servers give outputs about blood–brain barrier (BBB) permeation and, hence, have potential to reach the CNS. However, the results are in disagreement. According to PreADMET, compounds with $\text{Cbrain/Cblood values higher than 2.0 can cross the BBB, and all the molecules had high values, while in Swiss ADME, which uses the BOILED-Egg model, the molecules were predicted not to cross the BBB. However, these molecules probably cross the BBB according to in vivo data of tocotrienols and other vitamins E in SNC disorders [52,53]. The pharmacokinetics of tocotrienols have been reported in patients with favorable results and safety profiles [54,55].}

### 2.4. Toxicological Property Prediction

The toxicological prediction from geranylgeraniol and tocotrienols were assessed with PreADMET and ProTox-II. This online server is accessible and can help screen possible toxicities from compounds [56]. The prediction outputs are shown in Table 9.

**Table 9.** Toxicity prediction in ProTox-II.

| Molecule         | Toxicity Class | Predicted DL$_{50}$ | Toxicity Type   | Prediction | Probability |
|------------------|----------------|--------------------|----------------|------------|-------------|
| Geranylgeraniol  | 5              | 5000 mg/kg         | Hepatotoxicity | Inactive   | 0.79        |
|                  |                |                    | Carcinogenicity| Inactive   | 0.76        |
|                  |                |                    | Immunotoxicity | Inactive   | 0.99        |
|                  |                |                    | Mutagenicity   | Inactive   | 0.97        |
|                  |                |                    | Cytotoxicity   | Inactive   | 0.85        |
| $\alpha$-tocotrienol | 4             | 500 mg/kg          | Hepatotoxicity | Inactive   | 0.93        |
|                  |                |                    | Carcinogenicity| Inactive   | 0.77        |
|                  |                |                    | Immunotoxicity | Inactive   | 0.89        |
|                  |                |                    | Mutagenicity   | Inactive   | 0.92        |
|                  |                |                    | Cytotoxicity   | Inactive   | 0.87        |
Table 9. Cont.

| Molecule     | Toxicity Class | Predicted DL$_{50}$ | Toxicity Type   | Prediction | Probability |
|--------------|----------------|---------------------|----------------|------------|-------------|
| β-tocotrienol| 4              | 500 mg/kg           | Hepatotoxicity | Inactive   | 0.93        |
|              |                |                     | Carcinogenicity| Inactive   | 0.77        |
|              |                |                     | Immunotoxicity | Inactive   | 0.79        |
|              |                |                     | Mutagenicity   | Inactive   | 0.92        |
|              |                |                     | Cytotoxicity   | Inactive   | 0.87        |
| γ-tocotrienol| 4              | 500 mg/kg           | Hepatotoxicity | Inactive   | 0.93        |
|              |                |                     | Carcinogenicity| Inactive   | 0.77        |
|              |                |                     | Immunotoxicity | Inactive   | 0.61        |
|              |                |                     | Mutagenicity   | Inactive   | 0.92        |
|              |                |                     | Cytotoxicity   | Inactive   | 0.87        |
| δ-tocotrienol| 4              | 500 mg/kg           | Hepatotoxicity | Inactive   | 0.94        |
|              |                |                     | Carcinogenicity| Inactive   | 0.79        |
|              |                |                     | Immunotoxicity | Inactive   | 0.93        |
|              |                |                     | Mutagenicity   | Inactive   | 0.91        |
|              |                |                     | Cytotoxicity   | Inactive   | 0.86        |

All the molecules were predicted to be nonmutagenic in bacteria and nonhepatotoxic, cardiotoxic, immunotoxic, or cytotoxic. The predicted median lethal doses were high, especially for geranylgeraniol. ProTox-II classifies the molecules according to the predicted toxicity from 1 to 6, in which higher values represent less toxic compounds. The highest value was achieved for geranylgeraniol (5), while tocotrienols were classified as 4.

3. Materials and Methods

3.1. Molecules Studied

This study used the major molecules found in the purified annatto oil (PAO) and its granules (Chronic®). The samples were kindly provided by Ages Bioactive Compounds Co. (São Paulo-SP, Brazil). The batch analysis certificate is described as URU200401 (12 March 2020, expiration date: 22 March 2022), composition: bixin (1.7%), tocotrienols (9.59%), and geranylgeraniol (28.32%), as described by Matias Pereira et al. [8].

All structures used were confirmed in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 1 October 2021) (Figure 1A). The molecules were drawn using ChemDraw [56] and optimized using HyperChem through the semiempirical method RM1 [57].

3.2. Biological Activities Prediction

The prediction of biological activity was based on analysis of the structure–activity relationship of a training set using the PASS server (prediction of activity spectra for substances; http://www.pharmaexpert.ru/passonline, accessed on 1 October 2021), which can predict 4,130 biological activities in the compounds with an average accuracy of 95%. PASS is based on the naïve Bayes classifier approach and multilevel neighborhoods of atoms descriptors. The predicted activities are given as Pa (probability of being active) or Pi (probability to be inactive). Molecules with a Pa superior to 0.7 are considered promising candidates for the given activity; however, molecules with Pa > 0.4 and Pa > Pi could still be good candidates [17–20].

In addition, the SEA server (similarity ensemble approach; http://sea.bkslab.org/, accessed on 1 November 2021) was used to assess potential targets of the studied molecules. This server predicts small-molecule activity based on the macromolecular targets they...
interact with, which is inferred according to topology similarity with other molecules’ fingerprints from its database [22,23]. The server gives the \( p \)-value as similarity output representing the expected value (E-value) and the max Tanimoto coefficient (MaxTC). In a prediction, the lower the \( p \)-value, the more significant it is, evidencing that the prediction is less likely to be by chance; ideally, a prediction should be \(< 10^{-10}\) to be highly significant, while a \( p \)-value > 1 is considered insignificant. A MaxTC is considered highly significant when the value is >0.6, and insubstantial when <0.3 [22,58].

3.3. Molecular Docking

The docking was performed using the software GOLD (Genetic Optimization for Ligand Docking [59]) using biological targets acquired from Protein Data Bank [60]. A total of five targets were selected: the human lanosterol synthase (an oxidosqualene cyclase (OSC)) complexed with lanosterol, human squalene epoxidase (a.k.a. squalene monooxygenase (SQLE)) complexed with FAD and CPMPD-4, human HMG-CoA reductase (HMGR) complexed with simvastatin, secreted phospholipase A\(_2\) (sPLA\(_2\)) complexed with the inhibitor Azd2716, and human cyclooxygenase-2 (COX-2) complexed with meclofenamic acid (Figure 1B). All the cocrystallized ligands were removed to perform the docking.

Before the dockings, validation was performed for each target by calculating the root mean square deviation (RMSD), which is the root mean square distance of nonhydrogen atoms of the ligand from the crystal structure and their corresponding docked pose. All the crystallized targets had RMSD < 2 Å and considered the upper limit of satisfactory docking [61]. Other parameters assessed were the docking sphere radius and x, y, and z coordinates (Table 10).

| Molecule                               | PDB ID  | Resolution (Å) | RMSD (Å) | Docking Radius (Å) | x, y, z Coordinates |
|----------------------------------------|---------|----------------|----------|---------------------|--------------------|
| Lanosterol synthase (OSC)              | 1W6K    | 2.1            | 0.622    | 11.49               | 28.79, 69.02, 8.45 |
| Squalene epoxidase (SQLE)              | 6C6N    | 2.3            | 1.038    | 15.08               | −23.75, 92.76, 63.37 |
| HMG-CoA reductase (HMGR)               | 1HW9    | 2.3            | 1.482    | 8.41                | 2.31, −8.29, −9.21 |
| Cyclooxygenase-2 (COX-2)               | 5IKQ    | 2.4            | 0.507    | 8.867               | 16.06, 43.11, 60.99 |
| Phospholipase A\(_2\) (sPLA\(_2\))    | 5G3N    | 1.8            | 0.507    | 9.132               | 7.48, 3.44, −0.16  |

Cocrystallized ligands, ions, and water molecules were removed from the crystallographic structures to perform the docking. Additionally, hydrogens were added to the ligands, and their atomic charge was calculated using HyperChem, as described in [62].

3.4. Pharmacokinetic Prediction

An in silico ADME (absorption, distribution, metabolism, excretion) prediction was performed using the servers PreADMET (https://preadmet.bmdrc.kr/, accessed on 1 November 2021) and SwissADME (http://www.swissadme.ch, accessed on 1 November 2021). These servers can calculate the physicochemical and pharmacokinetic properties of molecules, including human intestinal absorption, Caco-2 cell and MDCK permeability, percentage of plasma protein binding, blood–brain barrier penetration, glycoprotein P interaction, metabolism by P450 cytochromes, among others [46,48,63].

3.5. Toxicological Prediction

The toxicological prediction was performed using ProTox-II. This server can predict different toxicity parameters, such as acute toxicity, organ-specific toxicity, cytotoxicity, carcinogenicity, and immunotoxicity [64].

4. Conclusions

The biological activity results follow what is reported in the literature, mainly for the antioxidant, anti-inflammatory, and antidyslipidemia potential of geranylgeraniol and tocotrienols. The molecular docking corroborated the predicted activities of the servers.
Notably, the in silico data presented another mechanism of action that could be involved in the activity of this molecule, which is inhibition of squalene monoxygenase and lanosterol synthase, which will need to be confirmed in vitro.

These in silico data corroborate the use of these molecules against lipid disorders, coronary disease due to cholesterol accumulation, and several chronic diseases in which oxidative stress and inflammatory cascade have a role. Geranylgeraniol and tocotrienols are major molecules from *Bixa orellana* and Chronic®. The results also point to a good pharmacokinetic profile for these molecules and a good safety profile, according to previously reported experimental data.

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