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SYSTEMATIC SELECTION OF LIMONOIDS FROM *CARAPA GUIANESIS* AUBLET WITH ANTI-INFLAMMATORY ACTIVITY, THROUGH IN *SILICO* TECHNIQUES FOR TOPICAL USE

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ABSTRACT

Objective: The aim of this study is to investigate in silico the inhibitory activity of limonoids on the COX-1 enzyme and their liposolubility descriptors for topical actions against inflammation.

Theoretical Framework: Andiroba oil has limonoids with anti-inflammatory potential, highlighting their potential for sustainable bioeconomy due to their classification as non-steroidal. In this context, nine limonoid structures were studied in silico via molecular docking to the COX-1 enzyme.

Method: The 2D chemical structures of the limonoids were prepared using the MarvinSketch program, converted into 3D structures and optimized using classical mechanics methods. The structure of the COX-1 protein complexed with flurbiprofen in its active site was downloaded from the RCSB PDB of Homo sapiens origin and used for molecular docking using the Virtual Molegro Docker program.

Results and Discussion: The results obtained demonstrated that limonoids have the potential to inhibit COX-1. These results showed valuable information on liposolubility and COX-1 inhibitory activity, highlighting the potential for topical use of andirobas oil.

Research Implications: The practical and theoretical implications of this research emphasize that limonoids can inhibit COX-1 by preventing the formation of TXA2 thromboxanes, in addition to being associated with inhibition of platelet aggregation, being an effective measure in the prevention of arterial thrombosis, and which can be extended to vascular diseases, opening up perspectives for possible reduction, not only of microvaricose veins, but also of superficial phlebitis.

Originality/Value: This study contributes to the literature as it is an unprecedented study involving COX-1 and the limonoids present in andirobeira oil.

Keywords: COX-1, Anti-inflammatory Activity, Fat-soluble Descriptors, Andirobeira, Amazonian Plant.

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SELEÇÃO SISTEMÁTICA DE LIMONÓIDES DE CARAPA GUIANESIS AUBLET COM ATIVIDADE ANTI-INFLAMATÓRIA, ATRAVÉS DE TÉCNICAS IN SILICO PARA USO TÓPICO

RESUMO

Objetivo: O objetivo deste estudo é investigar *in silico* a atividade inibitória de limonoides sobre a enzima COX-1 e os seus descritores de lipossolubilidade para ações tópicas contra inflamações.

Referencial Teórico: O óleo de andiroba apresenta limonóides com potenciais anti-inflamatórios, destacando suas vertentes para a bioeconomia sustentável devido serem classificadas como não esteroidais. Neste contexto foram estudadas nove estruturas de limonóides *in sílico* via ancoragem molecular na enzima COX-1.

Método: As estruturas químicas 2D dos limonóides foram preparadas pelo programa MarvinSketch, sendo convertidas em estruturas 3D e otimizadas por métodos de mecânica clássica. A estrutura da proteína COX-1 complexada com flurbiprofeno no seu sítio ativo foi baixada do RCSB PDB de origem Homo sapiens e usada na ancoragem molecular através programa *Virtual Molegro Docker*.

Resultados e Discussão: Os resultados obtidos mostram que os limonóides apresentam potencial de inibição da COX-1. Estes resultados mostraram informações valiosas sobre lipossolubilidade e atividade inibitória da COX-1, ressaltando o potencial em uso tópico do óleo de andirobas.

Implicações da Pesquisa: As implicações práticas e teóricas desta pesquisa ressaltam que os limonóides podem inibir a COX-1 impedindo a formação de tromboxanos TXA2, além de estar associado à inibição da agregação plaquetária, sendo uma medida eficaz na prevenção de trombose arterial, e que pode ser estendida às enfermidades vasculares, podendo abrir perspectivas para possível redução, não só das microvarizes, mas de flebites superficiais.

Originalidade/Valor: Este estudo contribui para a literatura pois é um estudo inédito envolvendo a COX-1 e os limonóides presentes no óleo de andirobeira.

Palavras-chave: COX-1, Atividade Anti-Inflamatória, Descritores Lipossolúveis, Andirobeira, Planta Amazônica.

SELECCIÓN SISTEMÁTICA DE LIMONÓIDOS DE *CARAPA GUIANESIS* AUBLET CON ACTIVIDAD ANTIINFLAMATORIA, MEDIANTE TÉCNICAS *IN SILICO* PARA USO TÓPICO

RESUMEN

Objetivo: El objetivo de este estudio es investigar in silico la actividad inhibitoria de los limonoides sobre la enzima COX-1 y sus descriptores de liposolubilidad para acciones tópicas contra la inflamación.

Marco Teórico: El aceite de Andiroba posee limonoides con potencial antiinflamatorio, destacando su potencial para la bioeconomía sostenible debido a su clasificación como no esteroideos. En este contexto, se estudiaron in silico nueve estructuras de limonoides mediante acoplamiento molecular con la enzima COX-1.

Método: Las estructuras químicas 2D de los limonoides se prepararon mediante el programa MarvinSketch, se convirtieron en estructuras 3D y se optimizaron mediante métodos de mecánica clásica. La estructura de la proteína COX-1 complejada con flurbiprofeno en su sitio activo se descargó del PDB RCSB de origen Homo sapiens y se utilizó para el acoplamiento molecular mediante el programa Virtual Molegro Docker.

Resultados y Discusión: Los resultados obtenidos muestran que los limonoides tienen el potencial de inhibir la COX-1. Estos resultados mostraron información valiosa sobre la liposolubilidad y la actividad inhibidora de la COX-1, destacando el potencial de uso tópico del aceite de andirobas.

Implicaciones de la investigación: Las implicaciones prácticas y teóricas de esta investigación destacan que los limonoides pueden inhibir la COX-1 impidiendo la formación de tromboxanos TXA2, además de estar asociados a la inhibición de la agregación plaquetaria, siendo una medida eficaz en la prevención de la trombosis arterial, y que puede extenderse a las enfermedades vasculares, abriendo perspectivas para una posible reducción, no sólo de las microvarices, sino también de las flebitis superficiales.

Originalidad/Valor: Este estudio contribuye a la literatura, ya que es un estudio sin precedentes que involucra la COX-1 y los limonoides presentes en el aceite de andirobeira.

Palabras clave: COX-1, Actividad Antiinflamatoria, Descriptores Liposolubles, Andirobeira, Planta Amazónica.

1 INTRODUCTION

metabolomics and computational chemistry techniques , involving molecular docking, were applied to investigate the metabolic profile of a limonoid concentrate and confirm the COX-1 inhibitory activity at decreasing energy levels, and which of the limonoids present the best potential as chemotherapeutic pre-candidates for inhibition of this enzyme for topical use.

In this sense, inhibition of platelet aggregation in microvaricose veins could be an important solution for body aesthetics, and in general, it is possible that inhibition of COX-1 could be directed towards the reduction, not only of microvaricose veins , but also of phlebitis or superficial thrombophlebitis, in mild cases; sports and accidental injuries, bruises, strains, contusions, tendonitis and tenosynovitis, reducing pain and related inflammation. In these cases, the development of safe anti-inflammatories, produced from vegetable oils, are sources of great interest for topical use, a fact that highlights scientifically investigated natural products, based on their traditional, biocompatible uses and a cost-benefit-effectiveness-safety alternative for the treatment of superficial inflammatory conditions .

2 THEORETICAL REFERENCE

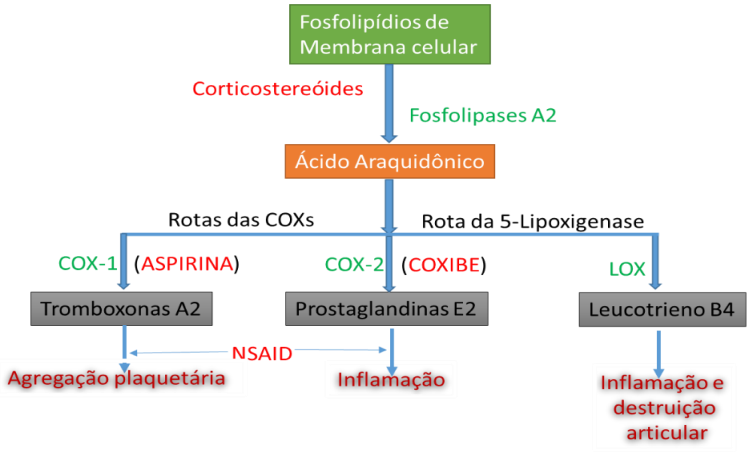
Inflammation is a reactive phenomenon that occurs in the body with biochemical responses against wounds, infections, injuries, hematomas, tendonitis, tenosynovitis, phlebitis or thrombophlebitis and that can also be associated with an autoimmune response or exposure to toxins. This phenomenon is described as a short or long-term process, known as acute or chronic inflammation, respectively; and that presents cyclooxygenases , COX-1 and COX-2, as key enzymes in this process (Chandrasekharan and Simmons 2001) ; (Carter et al., 2014) ; (Orlando and Malkowski, 2016) . The enzyme COX-1 catalyzes the formation reactions of prostaglandins (PGD2 and PGE2), prostacyclins (PGI2) and thromboxanes (TXA2), while COX-2 is responsible for the formation of prostacyclins (PGI2) and prostaglandins (PGE2).

Inhibition of COX-1 prevents the formation of thromboxanes TXA2, and this inhibition is associated with inhibition of platelet aggregation and exerts a vasodilatory action. Thus, in the endothelium, activation of COX-1 allows the production of prostacyclin (PGI2); in platelets, COX-1 is essential for the synthesis of TXA2, which stimulates platelet aggregation and vasoconstriction and has a hemostatic and, consequently, thrombogenic effect. In this sense, inhibition of TXA2 formation also induces inhibition of platelet aggregation, following the same principle of action of aspirin (ASA) in minimizing arterial thrombosis. Thromboxanes are substances that have vasoconstrictor activity in the systemic circulation, but vasodilator activity in the pulmonary circulation. Inhibition of platelet aggregation is an effective measure demonstrated in the prevention of arterial thrombosis, which can be extended to vascular diseases.

In studies performed with rats, results were obtained on the effects of acute COX inhibition on the reactivity of platelet-directed thrombolysis induced by collagen or by thromboxane mimetic TXA2 U46619; it was observed that aspirin, diclofenac and parecoxib , when used to inhibit COX-1 and COX-2, presented different results: Aspirin and diclofenac, as expected, presented thrombus inhibition effects, but there was no thrombus reduction effect with the use of parecoxib , since it is selective for COX-2. COX-1 inhibition reduced collagen-induced thrombus formation (Armstrong et al., 2011) . These results were important to demonstrate that COX-1 inhibition may be useful in minimizing or combating thrombosis and consequently mild thrombophlebitis and/or microvaricose veins . In this study, it can be observed that COX-1 inhibition is important in the selection of molecules with potential for topical use, Figure 1.

Figure 1

Cyclooxygenase inhibition steps and their influences



Arachidonic acid (AA), generated from the cell membrane by the action of phospholipase A2, can be inhibited by the action of steroids and, consequently, inhibit arthritis and pre -thrombotic substances. In this way, AA can be reduced in the body with diets that consume less red meat or through greater consumption of cold water fish, using known COX inhibitors. COX-1 can be inhibited by aspirin in low concentrations; COX-2 can be inhibited by celocoxib , Celebrex , which are selective for this enzyme; and LOX can be inhibited by curcumin or boswellic acid (Figure 1).

In this interaction, it is known that aspirin is the only NSAID that binds irreversibly to COX enzymes and therefore molecules similar to these characteristics are sought, as it has been demonstrated that many NSAIDs are not selective in inhibiting COX, acting on both isoforms COX-1 and COX-2. In addition, there are already selective COX-2 inhibitors that confer gastric protection; however, they are associated with an increased risk of major coronary events (Carvalho et al., 2004) , (Sharma et al., 2019) . COX-1 inhibition regulates COX-2 expression, which can neutralize the deleterious influences and subsequent events due to a PG deficiency, and the various studies are focused mainly on the selective inhibition of COX-2 by orally administered substances, due to the adverse effects caused when COX-1 is inhibited. However, the use of topical NSAIDs is more safe and their adverse dermatological effects may include pruritus and dermatitis, which can be stopped by discontinuing the use of the product. In this sense, topical NSAIDs should be considered as the first option before oral NSAIDs, specifically for adult patients with osteoarthritis and for those with comorbid conditions and/or adverse risk factors (Honvo et al., 2019) . In this case, the mechanism of action is the penetration of the NSAID through the skin into the subdermal tissue , including the synovial tissue, to act directly on the site of inflammation (Pradal, 2020) , requiring biocompatible substances that have the potential to penetrate the skin in order to cross the lipid barrier.

One of the strategies used is the search for molecules from natural sources, used by humans throughout existence (Dias et al., 2012) , and specifically in this study, the species *Carapa* stands out. *guianensis* known as Andirobeira , which is a plant with a long vegetative cycle that occurs in the Amazon and that produces a class of metabolites belonging to the tetranortriterpenoids , known as limonoids (Nascimento et al., 2019) , (Reis et al., 2021) , and which are found mainly in the seed oil, which has been widely used by the Amazon population to combat skin inflammation (Chia et al., 2018) (Porfírio-Dias et al., 2020) . However, no one has ever investigated limonoids as COX-1 inhibition ligands, acting in anti-inflammatory action. Thus, nine tetranortriterpene analogues, resulting from the limonoid class , isolated from the seeds of the species *C. guianensis* were selected based on their anti-inflammatory action as

potential COX-1 inhibitors to specifically investigate their action in combating inflammation related to arterial thrombosis, microvaricose hematomas , phlebitis or superficial thrombophlebitis, in mild cases; injuries, strains, contusions, tendonitis and tenosynovitis.

3 METHODOLOGY

3.1 OBTAINING THE SET OF MOLECULES TO BE ANALYZED

The set of limonoid molecules was obtained from the literature (Pereira da Silva et al., 2023) , (Nagatomo et al., 2022) , as well as some structures were obtained from studies carried out in our laboratories (Reis et al., 2021) , from lipidomics studies applied to the oil of Andirobeira seeds (*C guianensis*), under Sigen code AF60078.

3.2 SYSTEMATIC INVESTIGATION AND METABOLIC SCREENING THROUGH MOLECULAR ANCHORING

3.2.1 Limonoid molecules as COX-1 ligands

A dataset of 9 molecules, based on tetranortripernoid structures , known as limonoids , were obtained experimentally from the seeds of *C. guianensis* . The 2D chemical structures of the ligands, limonoids , were prepared by the MarvinSketch program (ChemAction , USA), being converted into 3D structures and optimized by classical mechanics methods. Then, these limonoids were subjected to *in silico tests* via molecular docking, using the 3D structure of COX-1.

3.3 ENZYME PREPARATION

The crystal structure of COX-1 (PDB ID: 1Q4G) was downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) of Homo sapiens origin, X- ray 2.51 Å (Orlando & Malkowski, 2016) . This COX-1 contains the inhibitor flurbiprofen complexed in its binding site, as well as the Fe-containing porphyrin group as a cofactor. Then, the crystallized protein structure was processed by removing the existing ligand molecules (ammonium ion, 2-acetamide-2-deoxy-β-D- glucopyranose , octyl -β-D-glucopyranose , Fe-containing protoporphyrin , (Figure 1) and water). After that, polar

hydrogens and Kollman charges were added to the protein using the *Virtual Molegro Docker* (MVD, v. 6.0) program tools . The *Moldock* scoring function was used to rank the best conformations and their respective affinity energy values (in kcal/mol) for flurbiprofen (self-docking) and limonoids. (Mota et al., 2019) .

3.4 PREPARATION OF BINDERS

The structures of limonoids , from C. guianensis seed oil , were prepared by MarvinSketch (ChemAxon , USA). After that, the structures were converted into 3D structures by the "Clean in 3D" function in MarvinSketch and saved in the structure data file format (sdf). In the last step, all ligands were converted to pdbqt format , using the OpenBabel program . Computer simulations were performed in the Molegro Virtual Docker v. 6.0 (2007) program. The *moldock* scoring function was used to classify the best conformations and their respective affinity energy values (in kcal/mol) for flurbiprofen (self-anchoring) and for limonoids (Mota et al., 2019) .

3.5 APPLICATION OF VIRTUAL SCREENING AND MOLECULAR DOCKING IN LIMONOID MOLECULES

Molegro Virtual Docker 6.0 (MVD 2007) was used for molecular docking analysis. The compounds were coupled to COX-1 using the protein structure and bonding sites, prepared as described above. The water molecules were removed during docking calculations. The binding sites were defined as a sphere of 15 Å with a coordinate center identical to the inhibitor in the corresponding crystallized structure.

During the anchoring experiments, the ligands were treated as flexible, while the protein became rigid. A MolDock-Score (Grid) scoring function was applied in the analysis and the grid resolution was set to 1 Å. The maximum population size and interactions were adjusted to 100 and 1500, respectively. For each ligand 50 independent docking races were held. The lowest power anchor poses have been selected for the analyzes shown below.

To validate the program, a re-docking with the crystallographic inhibitor Flurbiprofen (BFL) was carried out using the original coordinates of the crystallographic complex originating from the PDB. To do so, the BFL ligand was removed from the catalytic cavity leaving it free so that the Moldock algorithm of the MVD could find the conformation closest to the crystallographic experimental coordinates. Next, the nine structures of selected limonoids were

investigated in silica at the binding site, using the anchor-and-grow algorithm (Löwe et al., 2001).

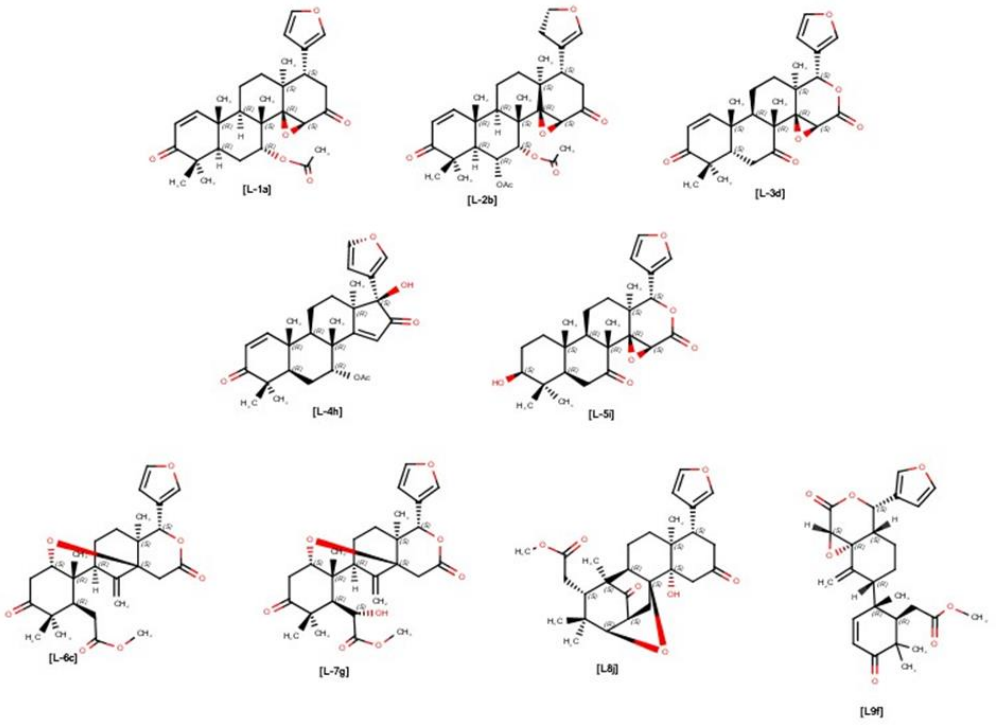
Scoring functions were applied to select the best ligand conformations, van der Waals interactions being evaluated by Lennard-Jones potentials, and electrostatic functions were evaluated by means of time-dependent dielectric functions (Thomsen & Christensen, 2006).

4 RESULTS AND DISCUSSIONS

Andiroba oil is composed of a mixture of triacyl, monoacyl, diacylglycerols, limonoids, steroids, carotenoids and other molecules in minute concentrations. The group of limonoids studied in andiroba species in Brazil, especially in the species *C. guaianensis* , varies considerably according to location, seasonality and oil extraction methods. For this reason, it is practically impossible to detect all limonoids in exclusive samples. The list of molecules presented was analyzed and isolated in different locations and today it is known that nine structures can be identified in *C. guianensis oils in the northern region of Brazil. Thus, nine limonoids were selected and subjected to in silico* investigation of anti-inflammatory activity , aimed at topical use, Figure 2.

Figure 2

Basic structure of natural limonoids obtained from C. guianensis .



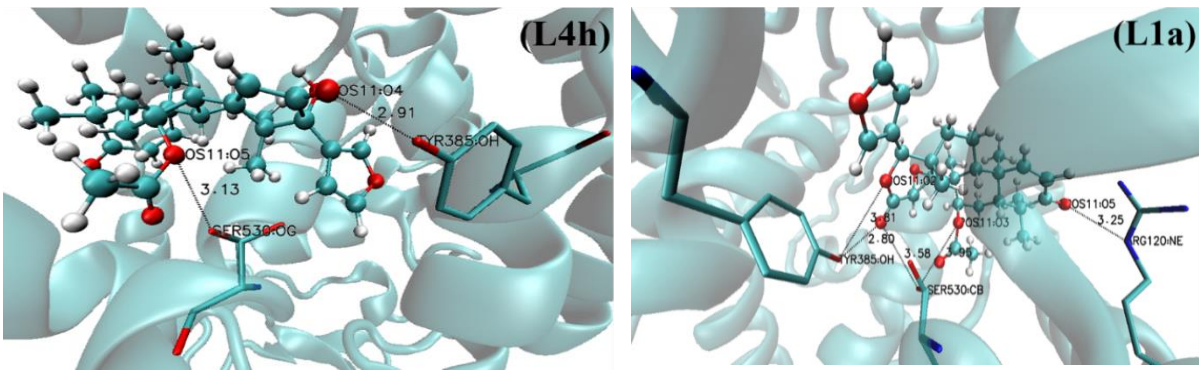
Andiroba oil contains several substances belonging to the tetranortriterpenoid and steroid groups. However, a significant number of these substances are not always found due to several factors, such as seasonality and geological factors that influence the synthesis and production of these metabolites. Several structures belonging to these groups were identified, however, nine of them occur more frequently in the Amazon region. In general, when metabolomics techniques are applied to andiroba oil , 3 to 7 of these substances can be identified, in addition to common fatty acids, and for this reason these nine structures were the target of *in silico investigation* via molecular docking.

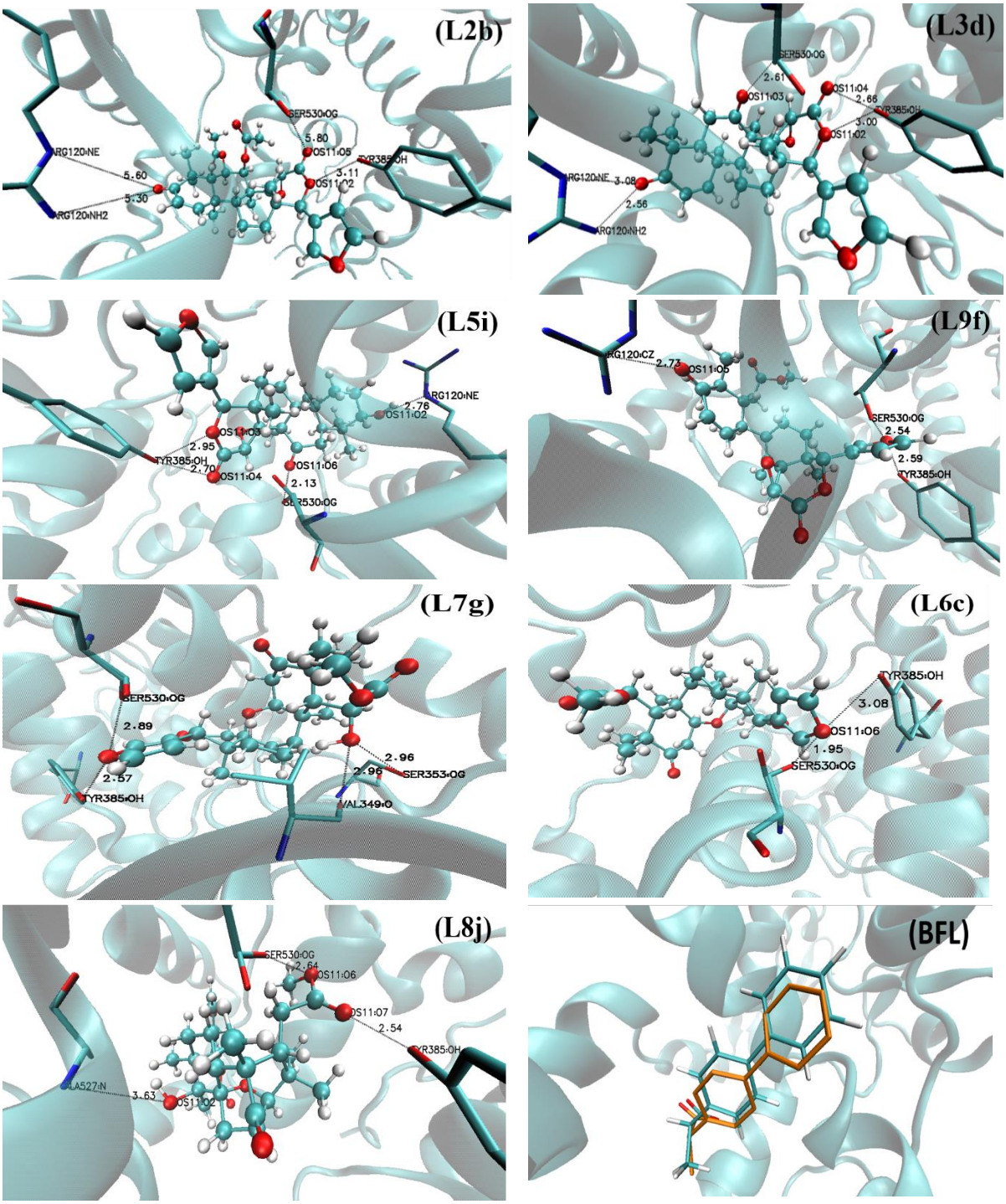
The Molegro Virtual Docker (MVD) program was used in the molecular docking methodology, as it demonstrates better performance when compared to Surflex and Flexx . The evolutionary structural optimization algorithm, Moldock , was used to find the best orientation of the potential ligand in the catalytic cavity. (Thomsen & Christensen, 2006) **.**

The docking (Figure 3) confirms that the Molegro program reproduced the experimental results of the crystallographic ligand BFL, obtaining a similar conformation in the same experimental binding site. The scoring functions applied to the limonoids and BFL, presented in Figure 3, show the conformations selected as potential inhibitors, interacting with the main catalytic residues serine (SER) and tyrosine (TYR) and their respective bond distances , present in their respective binding sites. In addition, it is possible to attribute interactions with the catalytic residues of SER and TYR to all potential inhibitors. The bond distances for SER (530:OG) and all inhibitors ranged from 1.95 Å to 5.80 Å, and in the case of TYR, the bond distances were between 2.70 Å and 3.11 Å .

Figure 3

Conformations interactions of the ligands with the main amino acid residues present in the COX-1 binding site





(L1a) Gedunin , (L2b) 6α- acetoxygedunin , (L6c) methyl- angolensate , (L3d) 7-deacetoxy-7-oxogedunin, (L9f) Andirobin , (L7g) methyl-6-hydroxyangolensate, (L4h) 7-hydroxyazadione, (L5i) 1,2-dihydro-3b-hydroxy-7-deacetoxy-7-oxygedunin, (L8j) Xylocensi k, BFL redocking inhibitor (orange color) and crystallographed BFL inhibitor (blue color).

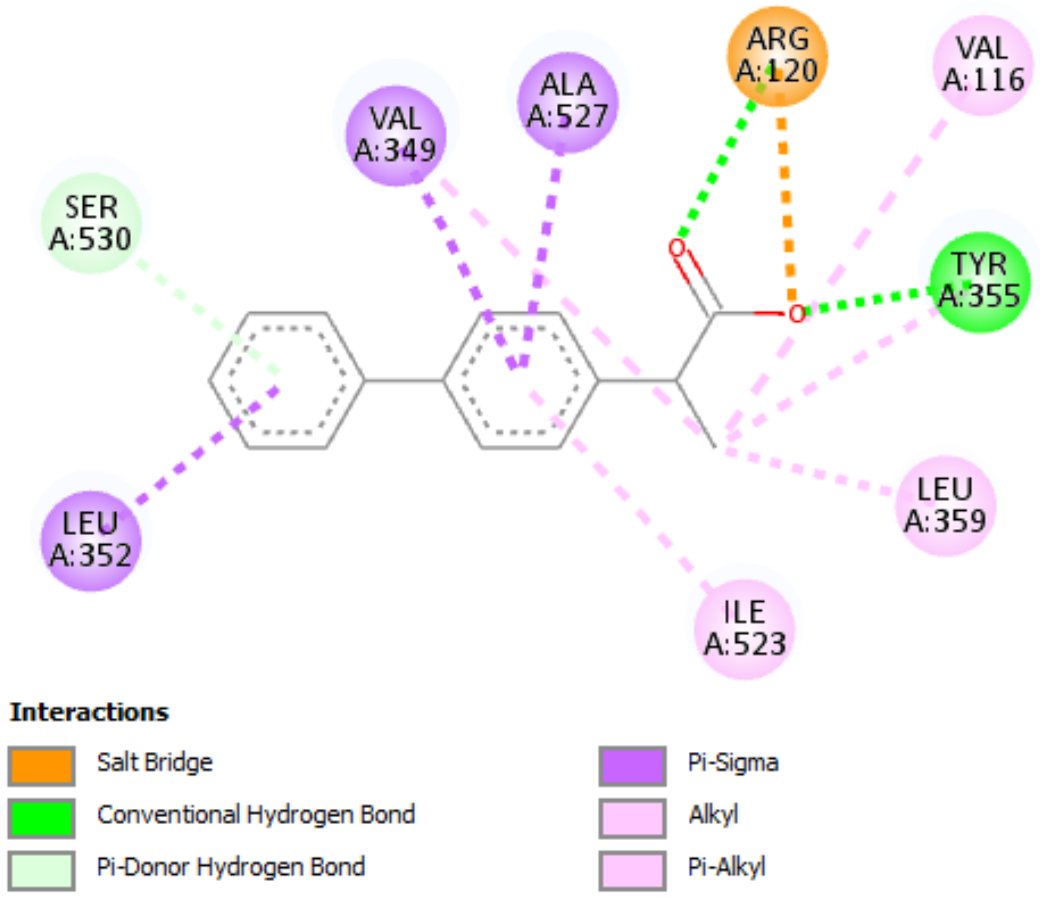
Specific cases (Figure 3) such as inhibitors L1a, L2b, L3d, L5i and L9f presented bond distance values with arginine residue (ARG 120) ranging from 2.56 Å for L3d and the largest distance for inhibitor L2b with a value of 5.60 Å. Exceptions were found for inhibitor L7g which interacted with the valine residue (VAL 349:O) with a bond distance of 2.96 Å and for

inhibitor L8j which presented an interaction of 3.63 Å with the alanine residue (ALA 527:N). Several studies have been carried out using natural products (Levita et al., 2017)

It is noteworthy that at the binding site, interacting with BFL are the following amino acid residues: SER530, VAL349, ALA527, ARG120, VAL116, TYR355, LEU359, ILE523, LEU352, Figure 3. In this case, the conventional hydrogen bond is related Arg120 and TYR355; observing that the greatest contribution to ARG120 is due to the salt binding. These aminoacid residues are relevant in this region and mark the pocket of interaction with ligands that can possibly inhibit this enzyme. Several molecular anchoring studies were carried out, highlighting the active sites of COX-1, which can confirm that the residues present are: VAL116, ARG120, VAL349, LEU352, TYR355, LEU359, TYR385, TRP387, ILE523, ALA527, SER530 (Tziona et al., 2022) .

Figure 4

COX-1 binding site and interaction with BFL



Fonte: BIOVIA Discovery Studio Visualizer. 2022

The binding distance values indicate a probable affinity of the inhibitors in the binding site of the three-dimensional structure of the cyclooxygenase enzyme ; however, the best classified conformations in Table 1, by the Moldock Score, indicate the molecules L1a, L2b, L3d, L4h, L5i and L9f as promising, since their Moldock Score values indicate values lower than -75 kcal/mol . It is observed that the affinity energy values of L9f and L1a present very close values in both binding sites, and in addition, they were equivalent to the value obtained for the crystallographic inhibitor BFL of -106,658 kcal/mol . However, the value of -113,124 kcal/mol of binding affinity energy , calculated by the MVD program, for the inhibitor L5i, proved to be much higher when compared to the other inhibitors and still much higher than the crystallographic inhibitor BFL. However, L3d, L4h and L2b presented values below -77 kcal/mol and in this aspect, the importance of the molecules L1a, L2b and L3d is due to their presence in andiroba oil with greater frequency , presenting good inhibition values which corresponds to a minimum of 72.26% when compared to BFL.

Table 1

Affinity energy values (kcal/mol) obtained in molecular docking.

Binders	**Moldock Score**	**Information**
(L5i)1,2 dihydro-3□-hydroxy-7-deacetoxy-7-oxygedunin	-113.124	Candidate
(L9f) Andirobin	-104,934	Candidate
(L1a) Gedunina	-104,984	Candidate
(L3d) 7-deacetoxy-7- oxogedunin	-96.8876	Candidate
(L4h) 7-hydroxyazadione	-90.1818	Candidate
(L2b) 6α- acetoxygedunin	-77.0742	Candidate
(L6c) methyl- angolensate	-58.4684	Candidate
(L7g) methyl-6-hydroxyangolensate	-53.4826	Candidate
(L8j) Xylocensi k	-45.4288	Candidate
Flurbiprofen (BFL) (re-docking)	-106.658	Reference

In this sense, it can be observed that substance L2b corresponds to 72.26% of the inhibition activity of flurbiprofen , while L1a corresponds to 98.42% of the inhibition.

The limonoid molecules , L1a and L2b, were evaluated *in silico* for their anti-inflammatory activity, observing whether both substances present evidence of inhibiting COX-1. The molecular docking simulations were validated using as reference the inhibitor BFL already complexed to the COX-1 enzyme. The same standards were applied to the limonoids L1a and L2b. The energy values obtained suggest that the compound L1a (-104.984 kcal/mol) has the potential to inhibit the COX-1 enzyme close to the inhibitor BFL (-106.658 kcal/mol), while the compound L2b, although it has affinity with the COX-1 enzyme, has a lower inhibition potential in relation to both L1a and BFL.

Studies of the activities and physicochemical parametric descriptors are presented in Table 2. It can be observed that all molecules, including the reference, interact with plasma proteins, which highlights that the L2b molecule presents a greater amount of penetrability when compared to L1a and the reference. Thus, the liposolubility , expressed by log P, presents a numerical value lower than 5 and, consequently, they present a high liposolubility potential , which allows them to cross the cell membrane.

Table 2

Parametric descriptors of physicochemical interactions of ligands

LIGTE	PPB cm/H	Skin permeability Log KP, cm/H	SPW mg/L	SKLogP Mol/L	SKlogS Mol/L	HA*	HD*
L1a	92,25	-1,8082	0,2272	3,7317	-6,3272	6	0
L2b	88,91	-2,0469	0,2535	3,1546	-6,3288	8	0
L3d	92,95	-1,8655	0,4085	3,4416	-6,0307	6	0
L4h	93,28	-1,1987	0.5559	3.9871	-5,9239	6	1
L5i	90.94	-2,5577	0.3931	3,3975	-6,0514	6	1
L6c	91.03	-1,7564	1,1948	3,4524	-5,5953	7	0
L7g	87.99	-2,2778	2,3697	2,6636	-5,3124	8	1
L8j	82.74	-2.7659	3,4871	2,1941	-5.1447	7	1
L9f	92.44	-2.4209	1,3566	3,3677	-5.5251	7	0
BFL	78.89	-2.5262	2,7085	2,4853	-5.3009	2	1

PPB=plasma protein binding: Strong >90%, Weak <90%; LogKP : high permeability <0.1 and Low permeability > 0.1 ; SPW=solubility in pure water: high >1, low<1; HA=H acceptor, HD=H donor, SKlogP = lipid solubility : medium-high lipid solubility >3, low-moderate lipid solubility <3. LIGTE=ligands. * SwissAdme (http://www.swissadme.ch/ index.php)

It is observed that the solubility of the substances in pure water highlights BFL as being the most soluble, followed by L2b, which has a lower skin permeability value (Log KP) when compared to L1a and L3d. However, all of them present high permeability. The liposolubility values are acceptable and correlate with the levels of high acceptance (HA) and no donation (HD) of hydrogen, thus characterizing their skin penetrability potential.

In both cases, the limonoids present highly favorable descriptors for topical use; however, sample processing is necessary to generate a process for obtaining a limonoid concentrate . In this case, the separation of the saponifiable fraction from the unsaponifiable fraction could be a concrete alternative.

Thus, Figure 5 presents the radar graphs of the physical-chemical descriptors of the studied limonoid structures .

Figure 5

Comparison of physicochemical descriptors and their structures

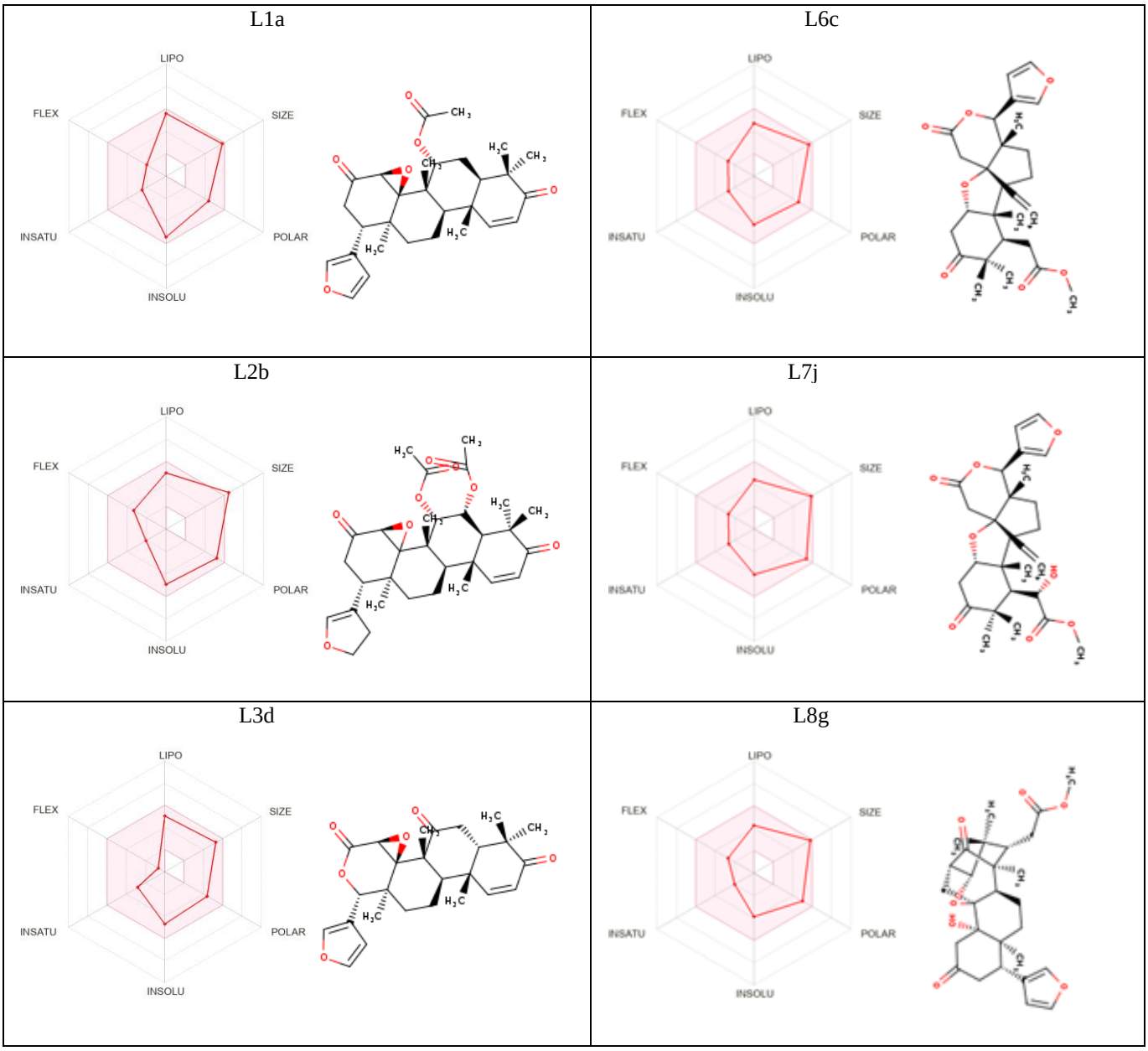
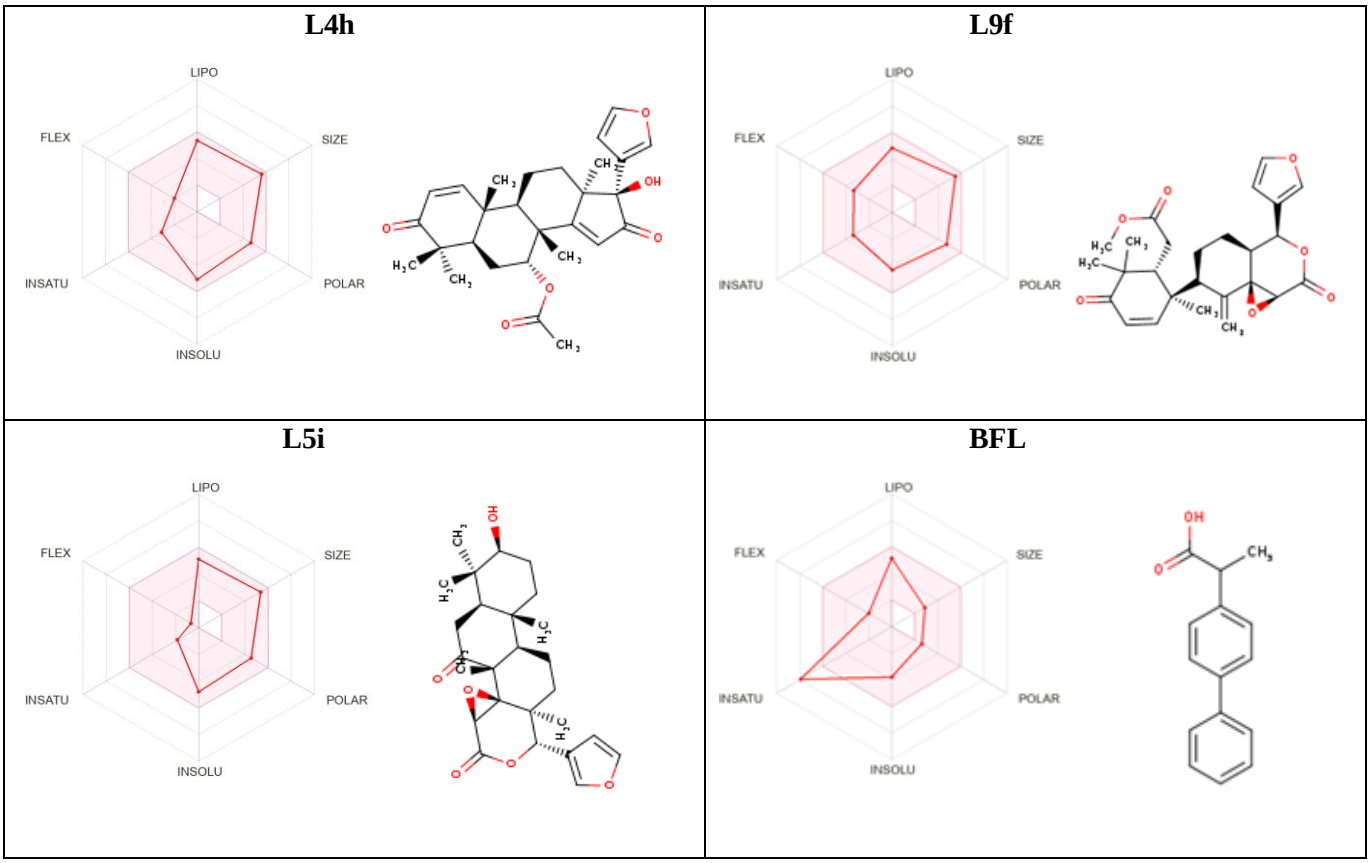


Figure 4

Continuation



It is observed that L3d and L5i present similar tendencies regarding liposolubility (LIPO), molecular size (SIZE), polarity (POLAR), insolubility (INSOLU), degree of unsaturation (INSATU) and flexibility (FLEX). L1a presents a slight tendency of similarity with L4h. In the same sense, the molecules L6c, L7j and L9f present similarities. However, structures 1, 3, 4 and 5 present the same level of liposolubility , followed by all structures presenting similarities in the level of molecular size. This information is consistent with the importance of using these molecules in topical actions.

5 CONCLUSION

Molecular docking simulations highlighted the greater anti-inflammatory effects of the L1a and L2b molecules, with the L1a molecule showing better interaction affinity than the L2b molecule when compared to the BFL reference. The penetrability results demonstrated that the candidates have liposolubility potential and cross the skin barrier. The physicochemical descriptors added the necessary evidence for all molecules to present liposolubility , with L11a

and L3d standing out in particular. The information from the physicochemical descriptors describes the solubility behavior of all molecules; however, it is important to emphasize that they will not be used in oral administration, but that these studies are focused on topical use.

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