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Analysis of the Oral Bioavailability and Toxicity of Compounds Derived from Caulerpine - A Biologically Active Secondary Metabolite Isolated from The Algae *Caulerpa racemosa* (Forssk.) J. Agardh in An *In Silico* Study

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ABSTRACT

Natural products of marine origin with biological activity have started to be systematically explored recently when compared to natural products of terrestrial origin. The structural variety of these substances is amazing, and they represent an as yet unexplored source of molecular diversity, which should certainly lead to the development of new drugs such as analgesics, antitumor drugs, antibiotics, and anti-inflammatories. Benthic green algae (Chlorophyta), are present in the rocky regions and reefs of the northeast and harbor a rich macroalgal flora of Caulerpa species, which produces several classes of secondary metabolites with pharmacological potential. This genus produces several classes of secondary metabolites with pharmacological potential, particularly bisindolic alkaloids and terpenoids, including sesquiterpenes and diterpenoids with aldehydes and/or acetate alcohols as functional groups. In view of this, the evaluation of theoretical pharmacokinetic parameters, through *in-silico* studies, becomes extremely important for the advancement of pharmacological investigations. Thus, aiming to evaluate the theoretical pharmacokinetic and toxicological properties, the software Molispiration® and AdmetSAR were used to study nine molecules derived from *Caulerpa racemosa*. Of these, only two were not within the "Lipinski's rule of 5" standards. The theoretical toxicity parameters showed that, of the substances analyzed, most showed a good standard of solubility, stability, and did not undergo significant metabolism in the liver and intestine. All showed no mutagenic potential, were weak inhibitors of HERG and not very toxic. Thus, the use of *in sílico* tools is a cheap, fast and of great scientific relevance alternative for the analysis and development of new drugs based on natural products, ensuring the safety for a possible and future clinical stage in humans.

INTRODUCTION:

The oceans are inhabited by a large number of plants, marine invertebrates and microorganisms, sources of natural products with unique structures responsible for different ecological functions. Many of these marine compounds, besides being important for understanding the evolution and maintenance of their communities in different habitats, may serve as structural models for the discovery of new prototype candidates for antitumor, antiviral and anti-inflammatory drugs, arousing interest in the scientific community [1,2,3,4,5].

Thus, in recent years, the study of marine organisms has been valued because they represent a privileged source of prospecting for new drugs due to their structural diversity, since they are responsible for the production of unique and sophisticated chemical structures different from those found in terrestrial organisms [6,7,8], increasing the prospects for the discovery of innovative products with pharmaceutical, cosmetic, veterinary, food, bioenergy and biotechnological applicability [9,10,11,12].

Brazil is among the top 10 countries in the world biodiversity ranking and, by presenting one of the largest coastal areas in the tropical region of the planet, has awakened for research in the area of marine biotechnology having studies performed in pharmacological models proving that the antinociceptive, anti-inflammatory and spasmolytic activities observed in Caulerpa extracts were intensified when performed with the alkaloid substance caulerpine [13,14,15].

The indolic alkaloid caulerpine, the major substance of the genus Caulerpa has been reported in twenty species, including *C. racemosa*[16]**.** Several authors have described the activity of caulerpine, such as: inhibition of some phases of viral replication against Herpes Simplex Virus type 1 being, therefore, candidate for an alternative drug to acyclovir during the treatment of HSV-1 infections [17], inhibition of the activation of the transcription factor HIF-1 (Hypoxia-inducible factor-1), an important molecular target in cancer treatment [18] and recently tuberculostatic [19].

The development of new drugs involves a sequence of experiments that include toxicological and pharmacological screening, having among the toxicological studies, the non-clinical toxicity studies. Thus, in view of the pharmacological potential of the marine products studied, the evaluation of theoretical pharmacokinetic and toxicological parameters (ADMET - Absorption,

Distribution, Metabolization, Excretion and Toxicity) of caulerpine and the extracts obtained from *Caulerpa racemosa*becomes extremely important for the advancement of pharmacological investigations, with these studies being necessary to ensure safety for a possible and future clinical stage in humans, thus fulfilling the steps in the production chain of medicines.

MATERIAL AND METHODS

In silico tests

Test Substances

All chemical information, smiles codes, of the test substances were obtained from the free Pubchem® website (https://pubchem.ncbi.nlm.nih.gov).

Evaluation of oral bioavailability *in silico*

For the analysis of the theoretical oral bioavailability of the compounds, some parameters should be analyzed, according to Lipinski's "Rule of Five": (a) number of hydrogen bonding acceptor groups (nALH), less than or equal to 10; (b) number of hydrogen bonding donor groups (nDLH), less than or equal to 5; (c) molecular mass (MM), less than or equal to 500 g/mol; (d) octanol-water partition coefficient (milog P), less than or equal to 5; (e) polar surface area (PES), less than or equal to 140 Å. Molecules that do not meet more than one of these parameters may have problems with bioavailability [20]. For determination of this bioavailability, the Molinspiration Cheminformatics program was used, a free software found at: (http://www.molinspiration.com/cgi-bin/properties).

In sílico toxicological test

The test substances were subjected to *in sílico* study for evaluation of ADMET parameters (absorption, distribution, metabolism, excretion and toxicity) and for verification of predictions of potential AMES Toxicity, Carcinogenic Agents, Acute Oral Toxicity and Carcinogenicity using address: (http://lmmd.ecust.edu.cn:8000/).

RESULTS AND DISCUSSION

Evaluation of oral bioavailability *in silico*

Molinspiration

Experimental and computational approaches to estimate solubility and permeability in discovery and development settings are described using the Lipinski method [21]. The research of this researcher and collaborators gave rise to the so-called "rule of 5", which profiled drug molecules within limits of molar mass, lipophilicity which is represented by the partition coefficient, log P, and hydrophilicity, represented by the number of hydrogen bond donors and receptors. The "rule of five" establishes some relevant structural parameters for the theoretical prediction of the oral bioavailability profile [20].

From the methodology employed the oral bioavailability results were observed following the Lipinski rule of five. Nine test substances were obtained through the free site Pubchem®. These results are shown in table 1.

Table No. 1: *In-silico* analysis of oral bioavailability according to the Lipinski 5 rule

Chemical Substance	**MOLECULAR PROPERTIES**								
	miLog	**TPSA**	**Natom**	**MW**	**nON**	**N OHNH**	**nV**	**nrotb**	**vol**
1	4.58	106.18	28	370.36	6	4	0	2	311.40
2	7.35	84.19	36	482.58	6	2	1	8	446.84
3	7.98	84.19	36	482.58	6	2	1	10	447.27
4	6.86	84.19	34	454.53	6	2	1	8	413.66
5	5.86	84.19	32	426.47	6	2	1	6	380.06
6	8.89	84.19	38	510.53	6	2	2	12	480.87
7	8.34	84.49	38	510.63	6	2	2	10	480.44
8	6.39	84.19	34	450.49	6	2	1	8	402.40
9	5.75	96.62	36	482.49	8	0	1	4	418.31

1- (6E,13E)-5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylic acid ;

2-(6E,13E)-diisobutyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

3-(6E,13E)-dibutyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

4-(6E,13E)-dipropyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

5-(6E,13E)-diethyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

6-(6E,13E)-dipentyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

7-(6E,13E)-diisopentyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

8-(6E,13E)-diallyl 5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

9-(6E,13E)-dimethyl 5,12-diacetyl-5,12-dihydrocycloocta[1,2-b:5,6-b']diindole-6,13-dicarboxylate;

milLogp: coeficiente de partição octamol água; TPSA: área de superficie polar total; Natom: número

de átomos; MW: peso molecular; nON: número de aceptores de hidrogênio; nOHNH: número de

doadores de hirogênio; nV: número de violações; nROTB: número de rotações; vol: volume. FONTE:Pubchem® (https://pubchem.ncbi.nlm.nih.gov); Molinspiration Cheminformatics(http://www.molinspiration.com/cgi-bin/properties).

Among the analyzed substances, substance 1 met all the standards required by the "rule of 5" (table 1). The values of its results showed a great advantage for the advancement of studies, because it presented good theoretical bioavailability characteristics, showing itself, therefore, as a promising molecule for future formulation of new drugs.

Evaluating the partition coefficient, which determines the degree of lipophilicity of a drug, all other substances studied showed changes in this value, however, substances 2,3,4,5,8 and 9 violated only this question in Lipinski's "rule of 5", i.e., there was only one violation when it came to molecular properties, therefore, they also fit as promising (table 1).

The use of the Molinspiration software to calculate important physicochemical properties of the molecule and the evaluation of the probability of the compound acting on different pharmacological targets generated an idea of its pharmacodynamic characteristics [22]. The prediction of the bioactivity score by the software was also carried out in the research by [23], showing that *in silico* biological screening indicated that the compounds studied were good drug candidates, having several biological activities.

Like the aforementioned authors, conducted phytochemical screening and *in silico* studies, including the application of Molinspiration software of flavonoids, in view of the recent explosion of interest in the bioactivity of these flavonoids from microalgae and their health benefits [24].

For [25] the "rule of 5" methodology appears to be as useful today in defining medicines as when it was proposed. The authors observed no additional criteria needed nor did they find significant deficiencies in the four "rule of 5" criteria originally proposed by Lipinski *et al*. However, they suggested the BDDCS/rule of five association as a way to broaden the possibilities for theoretical predictions.

Used a series of chalcones and their B-aryl analogues that were prepared and evaluated as inhibitors of myeloperoxidase (MPO) chlorination activity, [26]. They found that the studied substances respected the physicochemical limits outlined by Lipinski, important factors in achieving the pharmacokinetics of the desirable compound indicating, therefore, that they could have no restrictions on oral bioavailability based on these limits.

Recent studies by [27] also used the *in-silico* methodology based on the Lipinski rule of five. Derivatives of α-aryl-α-tetralones and α-fluoro-α-aryl-α-tetralones were synthesized by palladium-catalyzed α-arylation reaction of α-tetralones and α-fluoro-α-tetralones, with bromoarenes in moderate to good yields. These compounds were evaluated for their *in vitro* antiproliferative effects against human breast cancer and leukemia strains with various drug resistance profiles. The in silico results were reflected in the low effects on non-tumor cells. Computational studies found that thiazole derivatives showed zero violation of Lipinski's rule, being, therefore, compounds bioavailable orally and with high gastrointestinal absorption [28].

The molecular mass, which should present values equal to or below 500g/mol, was altered in molecules 6 and 7 (Table 1). These molecules also presented their values of partition coefficient altered, therefore, violated two requirements of the "rule of 5", i.e., they did not present good theoretical availability.

Many contemporary drug candidates exhibit physicochemical properties that are moving towards higher molecular weight and coincidentally also higher lipophilicity in the quest for selectivity and biological specificity generally resulting in lower water solubility. *In silico* studies therefore allow for a formulation strategy for different orally administered drugs [29] [30] emphasized the importance of the interaction between drug properties, formulation, and the gastrointestinal environment in determining oral absorption, parameters that move beyond the "rule of 5." [31] Researched thiazol analogues due to the need for safer and more cost-effective drug discovery for tuberculosis. All synthesized compounds were also evaluated using Molinspiration software, meeting all the required parameters of Lipinski's "rule of five," proving that those analogues showed good antituberculosis potentials. Computational approaches combined with experimental investigations can also be used to design drug manufacturing, improving quality control [32].

Experimental and computational approaches are used to estimate solubility and permeability in discovery and development settings. In the development setting, solubility calculations that focus on predicting the exact value are difficult because of polymorphism. Recent work on linear free energy relationships and Log P approaches are critically reviewed. Useful predictions are possible in closely related analog series when combined with experimental measurements of thermodynamic solubility [21].

AdmetSAR

The nine substances selected via the free Pubchem® website (https://pubchem.ncbi.nlm.nih.gov) were subjected to the *in sílico* study for evaluation of the ADMET (absorption, distribution, metabolism, excretion and toxicity) parameters using: (http://lmmd.ecust.edu.cn:8000/).

Table No. 2: ADMET classification properties, calculated in admetSAR software

Substances Results and probabilities

Absorption [redacted]	1	2	3	4	5	6	7	8	9
Blood-brain barrier	BBB+ 0.8792	BBB+ 0.8339	BBB+ .8512	BBB+ 0.8402	BBB+ 0.8268	BBB+ 0.8884	BBB+ 0.8168	BBB+ 0.9381	BBB+ 0.9632
Human intestinal absorption	HIA+ 0.9368	HIA+ 0.9894	HIA+ 0.9912	HIA+ 0.9899	HIA+ 0.9913	HIA+ 0.9896	HIA+ 0.9822	HIA+ 0.9659	HIA+ 0.9560
Permeability in Caco-2	Caco2 - 0.7501	Caco2- 0.6051	Caco2 - 0.6179	Caco2 - 0.6448	Caco2 - 0.6399	Caco2 - 0.6302	Caco2 - 0.6003	Caco2 - 0.6230	Caco2 0.6011
P-glycoprotein substrate	No Substr. 0.6942	No Subst.0.5 134	Substr. 0.5898	Substr. 0.5197	No substr. 0.5189	Substr. 0.6549	Substr. 0.5767	No Substr. 0.6008	No Substr. 0.7936
P-glycoprotein inhibitor	No inhib. 0.9691	No inhib. 0.5874	No inhib. 0.7208	No inhib. 0.6981	No inhib. 0.7852	No inhib. 0.7164	No inhib. 0.5721	No inhib. 0.7568	No Inhib. 0.5495
	No inhib. 0.9218	Inhib. 0.5895	No Inhib. 0.8647	No inhib. 0.8320	No Inhib. 0.8263	No Inhib. 0.7946	No Inhib. 0.5729	No Inhib. 0.5665	Inhib. 0.5978
Renal transport of organic cations	No Inhib. 0.9037	No Inhib. 0.8400	No Inhib. 0.7787	No Inhib. 0.7997	No Inhib. 0.8327	No Inhib. 0.7704	No Inhib. 0.7671	No Inhib. 0.7544	No Inhib. 0.8720

ocation cell phone [redacted]	Mitoc. 0.7014	Mitoc 0.8351	Mitoc. 0.7877	Mitoc. 0.8398	Mitoc. 0.8358	Mitoc. 0.7679	Mitoc. 0.8459	Mitoc. 0.7653	Mitoc. 0.7639
Substr. CYP450 2C9	No substr. 0.7882	No substr.0.8269	No substr. 0.8466	No substr. 0.8573	No substr. 0.8547	No substr. 0.8573	No substr. 0.8141	No substr. 0.8596	No substr. 0.7688
Substr. CYP450 2D6	No substr. 0.8476	No substr.0.8376	No substr. 0.8029	No substr. 0.8195	No substr. 0.8465	No substr. 0.8079	No substr. 0.7981	No substr. 0.8366	No substr. 0.8076
Substr. CYP450 3A4	No substr. 0.7890	No substr.0.5727	No substr. 0.6100	No substr. 0.6126	No substr. 0.6841	No substr. 0.5788	No substr. 0.5122	No substr. 0.7265	Substr. 0.5510
Inhibitor CYP450 1A2	Inhib. 0.5918	Inhib. 0.7724	Inhib. 0.8240	Inhib. 0.8357	Inhib. 0.9012	Inhib. 0.7630	Inhib. 0.7071	Inhib. 0.7687	Inhib. 0.5551
Inhibitor CYP450 2C9	No inhib. 0.7604	Inhib. 0.6167	Inhib. 0.7185	Inhib. 0.7419	Inihib. 0.6537	Inhib. 0.6560	Inhib. 0.5827	Inhib. 0.6587	Inhib. 0.5313
Inhibitor CYP450 2D6	No inhib. 0.9234	No inhib. 0.7415	No inhib. 0.8405	No inhib. 0.8231	No inhib. 0.7145	No inhib. 0.8004	No inhib. 0.8499	No inhib. 0.7254	No inibid. 0.8767
Inhibitor CYP450 2C19	No Inhib. 0.8210	Inhib. 0.6327	Inhib. 0.6487	Inhib. 0.6754	Inhib. 0.6533	Inhib. 0.6573	Inhib. 0.5532	Inhib. 0.6923	Inhib. 0.6381
Inhibitor CYP450 3A4	No Inhib. 0.8009	Inhib. 0.5415	Inhib. 0.5827	Inhib. 0.5429	Inhib. 0.6416	Inhib. 0.5335	No Inhib. 0.5480	Inhib. 0.8550	Inhib. 0.6379

Inhibitor promiscuity CYP [redacted]	Low 0.9102	High 0.7302	High 0.6792	High 0.7491	High 0.8265	High 0.7255	High 0.5261	High 0.8210	High 0.6472
HERG	Weak inhib. 0.9897	Weak inhib. 0.9871	Weak inhib. 0.9707	Weak inhib. 0.9632	Weak inhib. 0.9839	Weak inhib. 0.9626	Weak inhib. 0.9738	Weak inhib. 0.9706	Weak inhib. 0.9953
Inhibition Genetics	No inhib. 0.9014	No inhib. 0.7928	No inhib. 0.7799	No inhib. 0.7619	No inhib. 0.7865	No Inhib. 0.5880	No inhib. 0.7958	No inhib. 0.8484	No inhib. 0.8071
Toxicity In the AMES test	No toxic 0.9322	No toxic 0.7263	No toxic 0.8034	No toxic 0.8204	No toxic 0.7508	No toxic 0.8157	No toxic 0.7751	No toxic 0.7185	No toxic 0.6064
Carcinogen	No carcin. 0.9031	No carcin.0.8501	No carcin. 0.9120	No carcin. 0.9027	No carcin. 0.8276	No carcin. 0.9114	No carcin. 0.9125	No carcin. 0.8883	No carcin. 0.8438
Toxicity to fish	High 0.9563	High 0.9969	High 0.9929	High 0.9806	High 0.9872	High 0.9955	High 0.9966	High 0.9990	High 0.8636
Toxicity in *Tetrahymena Pyriformis*	High 0.5462	High 0.9583	High 0.9918	High 0.9919	High 0.9555	High 0.9940	High 0.9933	High 0.9889	High 0.6862
Honeybee Toxicity	Low 0.6532	Low 0.5410	Low 0.6268	Low 0.6339	Low 0.5751	Low 0.6423	Low 0.5881	Low 0.5466	Low 0.6280

Biodegradation	No pront. biod. 0.9081	No pront. biod. 0.9827	No pront. biod. 0.9648	No pront. biod. 0.9603	No pront. biod. 0.9697	No pront. biod. 0.9798	No pront. biod. 0.9813	No pront. biod. 0.9825	No pront. biod. 0.8259
Acute oral toxicity	III 0.4484	III 0.6679	III 0.7039	III 0.7042	III 0.7071	III 0.7342	III 0.6954	III 0.6675	III 0.5652

Table No. 3: ADMET regression properties, calculated in admetSAR software

Substances **Values and units**

Model [redacted]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Aqueous solubility	-4.1684 Logs	-4.7364 Logs	-4.5081 Logs	-4.2402 Logs	-4.2347 Logs	-4.6402 Logs	-4.6778 Logs	-4.3390 Logs	-3.9410 Logs
Permeability in Caco-2 [redacted]	0.0818 LogPappcm/s	0.7000 LogPappcm/s	0.5702 LogPappcm/s	0.5329 LogPappcm/s	0.4541 LogPappcm/s	0.4466 LogPappcm/s	0.7136 LogPappcm/s	0.4275 LogPappcm/s	1.0611 LogPappcm/s
Toxicity In rats	2.2055 LD50 Mol/kg	2.2607 LD50 mol/kg	2.2956 LD50 mol/kg	2.2302 LD50 mol/kg	2.2353 LD50 mol/kg	2.2808 LD50 mol/kg	2.2849 LD50 mol/kg	2.2494 LD50 mol/kg	2.4938 LD50 mol/kg
Toxicity to fish	1.2264	0.3468	0.4645	0.566	0.7824	0.5957	0.2894	0.0179	0.5409

	pLC50 mg/L	pLC50 mg/L	pLC50 mg/L	1 pLC50 mg/L	pLC50 mg/L	pLC50 mg/L	pLC50 mg/L	pLC50 mg/L	pLC50 mg/L
Toxicity in *Tetrahy-mena Pyriformis*	0.2862 pIgC50 ug/L	0.9583 pIgC50 ug/L	1.0301 pIgC50 ug/L	0.9794 pIgC50 ug/L	0.7810 pIgC50 ug/L	1.1088 pIgC50 ug/L	1.1047 pIgC50 ug/L	0.9856 pIgC50 ug/L	0.5816 pIgC50 Ug/L

Given the results obtained, the theoretical absorption of the substances was evaluated through the parameters of blood-brain barrier penetration (BBB), in which all showed positive values, ranging with probability from 0.8168 (substance 7) to 0.9632 (substance 9), suggesting a possible inactivity in the CNS, since all values were less than 1 (CBrain/Cblood<1) [33]. The human intestinal absorption values, which predicts the absorption of the drug through the intestine, showed high and positive values, thus favoring orally administered drugs, substance 5 stood out, with a probability value of 0.9913. The intestine presents a larger absorptive area due to its microvillus, being more desirable for drugs to be absorbed in this region [34].

Regarding theoretical permeability in Caco-2, only substance 9 (value of 0.6011) showed such a characteristic (table 2). Caco-2 cells are derived from human adenocarcinoma colon cells [35]. The Caco-2 monolayer has been extensively used for high-throughput screening of drug permeability and identification of substrates, inhibitors and inducers of intestinal transporters, especially P-glycoprotein. Traditionally, the Caco-2 monolayer is viewed as a single barrier, rather than a polarized cell monolayer, consisting of metabolic enzymes that are sandwiched between two membrane barriers with distinctly different transporters [36] [37] found that human colon adenocarcinoma cells (Caco-2), when grown on semipermeable filters, spontaneously differentiated in culture to form confluent monolayers that both structurally and functionally resembled the epithelium of the small intestine, thus concluding that the Caco-2 cell system provided useful predictions about the potential for oral absorption of new drug substances.

The absorption analysis also showed that substances 3, 4, 6 and 7 presented as substrates of P-glycoprotein, while 1, 2, 5, 8 and 9 as non-substrates. The intestinal efflux pump, P-glycoprotein (P-gp), located on the apical membranes of intestinal absorptive cells, can reduce the bioavailability of a wide range of drugs that are substrates for this membrane transporter, as well as inhibitory substances increase the bioavailability of the molecule [38,39]. As inhibitors of P-glycoprotein we have 2 and 9. The others were classified as non-inhibitors (table 2). Thus, the substance that presented the best characteristics was 2, classified as non-substrate and inhibitor of P-glycoprotein, showing good theoretical therapeutic results.

With respect to clearance, the proximal tubules of the kidneys play an important role in the excretion of organic drugs and their metabolites. The organic cation transporter is key in the renal excretion of cationic drugs. If there are alterations in these transporters, a renal toxicity can be induced, as well as its inhibition enables the occurrence of drug interactions [40,41]. All nine analyzed substances presented as non-inhibitors of renal transport of organic cations in theory (table 2).

After absorption, the substances evaluated presented as cellular localization of theoretical distribution the mitochondria (table 2). Following on from this point, most drugs are metabolized by enzymes found primarily in the liver. Such metabolization occurs in two phases. Phase I, which consists of the oxidation and reduction reactions, and phase II, which are the conjugation reactions. Generally, metabolism converts lipophilic compounds into hydrophilic derivatives, which can be excreted by the body, usually through urine [42,43].

Regarding metabolization, all substances were non-substrate of the cytochrome P4502C9 and 2D6 complex. Only 9 was a substrate of CYP 450 3A4. All molecules were inhibitors of CYP 1A2 and only substance 1 was shown as a non-inhibitor of cytochrome P4502C9 and 2C19 complex, which favors such molecule because inhibitory substances can develop side effects and therapeutic failures [43]. All were non-inhibitors of CYP4502D6, as well as inhibitors of CYP4503A4, with the exception of substance 1 and 7. Only substance 1 showed low CYP inhibitory promiscuity, i.e., it does not present as a substrate for many enzymes.

The enzymes of cytochrome P450 are essential for the metabolism and bioactivation of many drugs and can be inhibited or induced by drugs, resulting in clinically significant drug

interactions [44,45]. Among the CYP2C19, 2C9, 2D6, 1A2 and 3A4 isoforms, this one interacts with more than half of the drugs used, a fact observed in substances 1 and 7, and CYP2C9 is involved in the metabolism of nonsteroidal anti-inflammatory drugs [33,43,44,46].

Reactions catalyzed by CYPs generally transform xenobiotics into harmless and excretable metabolites, but sometimes a harmless xenobiotic is transformed into a toxic metabolite, thus it is highly desirable to have models that can predict the interaction with specific isoforms. In silico tools are of fundamental importance as they provide a predictability of data on ADME and toxicity properties of compounds [43,44].

For the toxicity parameters, it was theoretically possible to find that all compounds were weak inhibitors of the potassium channel-encoding gene, HERG (human ether-a-go-go related gene), which encodes potassium channels and which are responsible for the normal acting repolarization of cardiac muscle (table 2). Inherited mutations in the HERG gene cause long QT syndrome, a disorder that predisposes individuals to potentially fatal arrhythmias. This side effect is a common reason for drug failure in preclinical safety testing. Computational in silico prediction models provide a rapid and cost-effective way to screen compounds during initial drug discovery [47,48].

In silico prediction of genotoxicity, which has evolved greatly in recent years, provides an established and accepted method that defines the first step in the evaluation of reactive DNA impurities. This has been possible due to the increasing amount of reliable Ames screening data, attempts to understand the pathways of activity, and the subsequent development of computer-based prediction systems [49].

The Ames test is performed by using *Salmonella thiphimurium* bacterial strains to measure the mutagenicity of molecules, i.e., it is used to analyze the mutagenic activity of chemical compounds [34]. Thus, the AdmetSAR program predicted that all the molecules analyzed presented themselves as non-toxic by the Ames test, as well as non-carcinogenic and not readily biodegradable, presenting acute toxicity category III (Table 2).

According to the evaluation of the parameters of acute toxicity in rats in the regression properties (table 3), the theoretical results of the AdmetSAR tool showed that all substances studied presented values between the range of 2205555 (substance 1) and 24938 (substance 9), toxicity

grade III, which includes compounds with LD50 values higher than 500 mg/kg and lower than 5000 mg/k, highlighting, therefore, substance 1 as the one with the lowest theoretical toxicity. They also showed low solubility in aqueous media, with substances 1 (LogS of -4.1684) and 9 (LogS of -3.9410) standing out, therefore possessing values that represent a higher absorption power [50].

The absorption, distribution, metabolism, excretion and toxicity (ADMET) properties assessed in the in silico study play important roles in new drug discovery/development, and this information is quite useful when conducting environmental and human risk assessment [51].

The current pharmaceutical industry is seeking to develop drug candidates with less potential for environmental impact, although such risk has been shown to be very low. Through modern analytical techniques, residues of many pharmaceuticals can still be detected in the aquatic environment. Thus, a better understanding of drug metabolism and pharmacokinetics can result in the administration of lower doses to achieve the same therapeutic effect, shorter duration of therapy, better targeting and better drug delivery, combined with greater specificity, directly leading to lower emissions from the patient to the environment and therefore lower environmental waste [52].

According [53], use of *in sílico* tools in the drug development process predicts a wide range of properties, including absorption, distribution, metabolism, elimination, and toxicity, becoming increasingly important due to changes in legislation and the ethical and economic factors for reducing animal testing. Although such tools have been used for decades, there remains a reluctance to accept predictions based on these methods, particularly in regulatory settings. This apprehension arises in part due to a lack of confidence in the reliability, robustness, and applicability of the models. To address this problem, the authors proposed a scheme for in-silico model verification that allowed the scientific validity of the models to be assessed according to the principles of good computational modeling practices. They reported on the implementation of the scheme within the "eTOX" (electronic toxicity) project and its application to *in sílico* models.

Drug discovery is a complex and expensive process in which several areas of knowledge converge. Computational methods have contributed, among other applications, to the efficient

analysis of data, the filtering of compound collections to select molecules for experimental evaluation, the generation of hypotheses to help understand the mechanism of action of drugs, and the design of new chemical structures. In addition, computational methods have made significant contributions to the development of drugs that are in clinical use [54].

In-silico processes require not only a good understanding of fluid mechanics, but also investment in hardware, software, and know-how. On the hardware front, reaping the full potential of CFD requires high-performance computing (HPC) resources and powerful compute clusters. Due to the advancement of cloud-based computing, there are numerous low-cost options for computing power worldwide, but the dilemma of edge versus cloud computing must be resolved with respect to development needs and corresponding process time scales [55].

CONCLUSION

It can be concluded that, according to the results obtained, among the substances derived from Caulerpa racemosa, a total of nine substances, seven showed promising pharmacokinetic activities from the results obtained by the Molinspiration tool, and only two violated the principles that govern the rules of theoretical bioavailability in silico. The theoretical toxicity parameters showed that, of the analyzed substances, most showed a good pattern of solubility, stability, and did not undergo significant metabolism in the liver and intestine. All showed no mutagenic potential, were weak inhibitors of HERG and not very toxic. Thus, the use of *in sílico* tools to search for drug candidates is a cheap, fast and scientifically relevant alternative for the analysis and development of new drugs based on natural products.

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