Drug Analytical Research
ISSN: 2527-2616 *Drug Anal. Res., v. 4, n. 2, p. 40-49, 2020*

Novel ultraviolet absorbers derived from cashew nut shell liquid: spectrophotometric, *in silico* and *in vitro* assays

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The use of sunscreens prevents erythema, photodamage and skin cancer. Natural products have been studied as ultraviolet (UV) absorbers due to their structural similarity to organic UV filters and their lower cost. The cashew nut shell liquid (CNSL) constituents were isolated by our group leading to four mixtures and seventeen pure compounds, which had chromophoric groups similar to organic UV filters. In addition, C15 and C8 CNSL-derivatives molecules were rationally planned as UV absorbers. The aim of this work was to evaluate the potential of CNSL's constituents and its derivatives as new UV absorbers using spectrophotometric methods, study their physical-chemical properties and toxicity using *in silico* method, and perform *in vitro* assays. Mixtures and isolated CNSL compounds were demonstrated to be non-phototoxic when evaluated in a phototoxicity assay using the yeast *Saccharomyces cerevisiae*. Considering the absorption values on the UV range, 6 compounds showed appropriate SPF values regarding the spectrophotometric test. Additionally, *in silico* and *in vitro* evaluations were performed, showing non-oral bioavailability, as well as non-mutagenic, non-genotoxic and non-phototoxic properties for the tested compounds. These results contribute favorably to the aimed use of the compounds under analysis as novel organic UV absorbers that have as precursor the phenolic lipid component of CNSL, a waste product obtained as the by-product of cashew nut food processing.

Keywords: ultraviolet absorbers, cashew nut, SPF, spectrophotometry
https://doi.org/10.22456/2527-2616.108405

Introduction

The Cashew tree (*Anacardium occidentale* L.) is a native plant of the Brazilian Northeast [1-3], cultivated in many equatorial and sub-equatorial areas of the world [1, 4]. Only six countries (Brazil, India, Madagascar, Mozambique, Kenya and Tanzania) stand out in a significant way in the production and commercial exploration of the cashew nut [1].

Cashew nut comprises the shell and the kernel. While the kernel is nutritionally valuable, the shell has been considered as a residue of cashew nut production [4]. The byproduct of the chestnut processing, the cashew nut shell liquid (CNSL), was initially used as raw material in the production of antioxidants, thermal insulation and attrition material, plasticizers, surfactants, paints and varnishes [4-7]. Its components also have antioxidant, fungicidal, molluscicidal, anti-tumor, antimicrobial, anti-inflammatory, anti-genotoxic and cytostatic activities [1].

CNSL is a brownish viscous oil composed of phenolic compounds in proportions that vary according to the method of extraction. In general, the initial composition of natural CNSL (solvent-extracted) is a mixture of anacardic acid (70%), cardanol (18%), cardol (10%) and 2-methylcardol (1%) [8]. Our group isolated the CNSL compounds, obtaining seventeen molecules that had similar groups to sunscreen´s active ingredients, the ultraviolet (UV) absorbers.

Additionally, CNSL is considered a versatile raw material for a series of chemical transformations due to the phenolic and lipid constituents' dualistic nature, including the aromatic and acyclic character, associated to the existence of many functional groups in the aromatic ring and presence of multiple unsaturations in the acyclic chain [4,5]. Concerning the chemical nature, ease of obtaining and control of chemical transformations in the structure of some of the CNSL phenolic constituents [1,4,5], the present work was carried out aiming at a potential exploration of CNSL as raw material in the synthesis of new agents for protection against solar radiation. Fifteen CNSL-derived molecules were rationally planned as sunscreens (Patent number INPI Nº PI 0406040-7, WO 2006/042391A2). These derivatives present as main structural characteristics the photoabsorbent chromophoric patterns found in aromatic, cinnamic, sulphonic esters, as well as conjugated arylketones necessary for the photoprotection

activity, along with the natural hydrophobic subunit comprised by the alkylic chain of the CNSL phenolic derivatives.

It is well known that solar ultraviolet radiation is the major etiological cause of skin cancer in humans [9]. Over a million cases are detected each year, whereas 132,000 new cases of cutaneous malignant melanomas occur worldwide each year [10]. Therefore, protection from UV light is a major strategy in the prevention of skin cancer, of which the most popular method is the use of sunscreen [11].

In this context, the aim of this study was to evaluate if the CNSL constituents and its derivatives molecules absorb in the ultraviolet, which is one of the characteristics of a substance to be classified as a sunscreen, to determine their Sun Protection Factor (SPF) using spectrophotometric methods, to study their physical-chemical properties and toxicity *in silico*, and perform *in vitro* assays.

Experimental section

Chemicals and reagents

Tetrahydrofuran, chloroform, ethanol and dimethyl sulfoxide were purchased from TEDIA (Brazil), Tween 80 and 4-NQO was from SIGMA-ALDRICH (Brazil). Sodium chloride, potassium dihydrogen phosphate, sodium phosphate dibasic dodecahydrate and glucose were from MERCK (Brazil). Yeast exctract, Bacto peptone and Bacto agar were from DIFCO (Brazil). Octyl p-methoxycinnamate was from PHARMA SPECIAL (Brazil).

Mixtures and isolated compounds from the natural and technical CNSL and their O-acetyl and O-methyl derivatives are shown in Figure 1. Rationally planned C15 and C8 CNSL-derivatives are depicted in Figures 2 and 3. Molecules were synthesized at Laboratório de Desenvolvimento de Estratégias Terapêuticas - LADETER/UCB (MMA/CGEN authorization 167/2014 - Portaria n^{o} 386 de 22/10/2014).

UV absorbance and in vitro SPF determination

For determination of the specific absorbance ($A^{1\%}_{1cm}$) the samples were diluted at 1% (w/v) in diferent solvents considering the solubility of each substance: ethanol (V1-31; V37), chloroform (V32-V35) and DMSO (V36). The absorption values in the ultraviolet range were determined using a Shimadzu UV-1601 spectrophotometer. The molar absorptivity (ε) was calculated for each test solution at the wavelengths of maximum absorbance (λ max) [12].

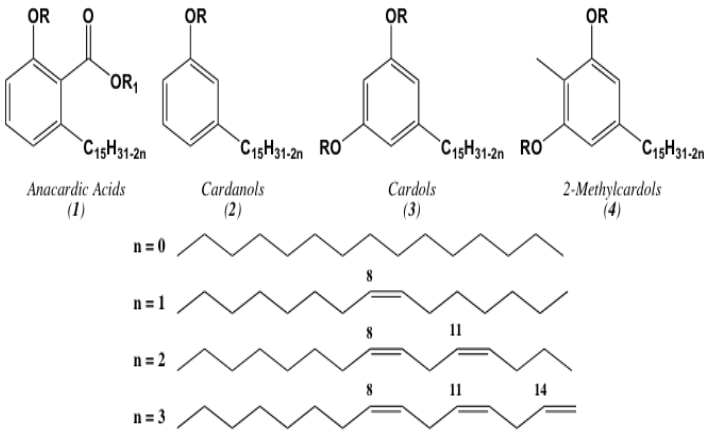
In vitro SPF values were determined according to the method described by Mansur [13]. Absorbance values for each substance at 1 or 5% (w/v), in the same solvents cited above, were determined in triplicate at a final concentration of 0.2 μL/mL and an emission spectrum of 290–320 nm with intervals of 5 nm using 1 cm quartz cuvettes in a Shimadzu UV-1601 spectrophotometer.

The SPF determination, equation (1) and the correlation between the erythemogenic effect (EE) and the radiation intensity at each wavelength (EE × I) were adjusted according to Sayre [14].

$$\text{Spectrophotometric SPF} = CF \sum_{290}^{320} EE(\lambda)\, I(\lambda)\, abs(\lambda) \quad (1)$$

The correction factor (CF) =10, EE (λ) is the erythemogenic effect of radiation on wavelength λ, I (λ) is the intensity of solar light with wavelength λ, and abs (λ) is the spectrophotometric absorbance value of a solution of the preparation at wavelength λ [14].

The *in vitro* SPF were also determined for mixture of V34 and V35 at 5% and 10% (w/v), and two comercial UV filters widely found in sunscreen formulations: octyl-*p*-methoxy-*trans*-cinnamate and octocrylene both at 10% (w/v) in chloroform, this is the maximum concentration alowed by ANVISA for both substances in sunscreen formulations [15].



Number	Compound or Mixture	N	R	R_1	Code
1	1-4	0-3	H	H	V15
2	1-4	0	H	H	V16
3	2-4	0-3	H	H	V17
4	2-4	0	H	H	V18
5	1	0-3	H	H	V9
6	1	0-3	H	Me	V30
7	1	0-3	Ac	H	V10
8	1	0-3	Me	Me	V11
9	1	0	H	H	V12
10	1	0	Ac	H	V13
11	1	0	Me	Me	V14
12	2	0-3	H	--	V5
13	2	0-3	Ac	--	V6
14	2	0	H	--	V7
15	2	0	Ac	--	V8
16	2	0	Me	--	V19
17	3	0-3	H	--	V1
18	3	0-3	Ac	--	V2
19	3	0	H	--	V3
20	3	0	Ac	--	V4

Figure 1. Constituents of CNSL (1-20)

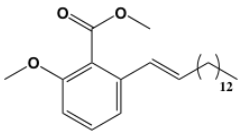
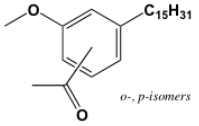
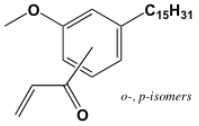
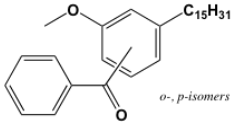
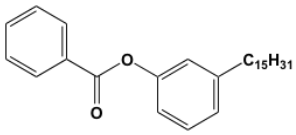
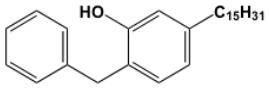
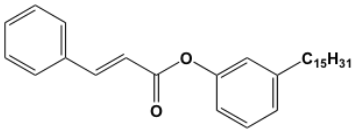
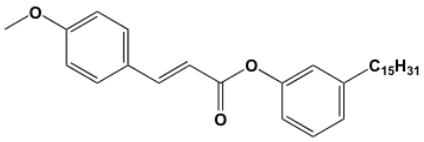
Number	Structure	Code
21		V24
22		V21
23		V22
24		V20
25		V23
26		V29
27		V34
28		V35

Figure 2. Structures 21-28 of CNSL-derived molecules

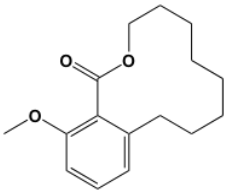
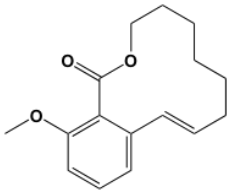
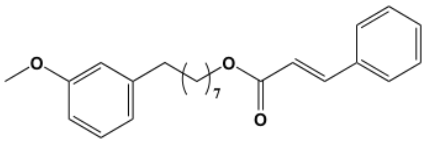
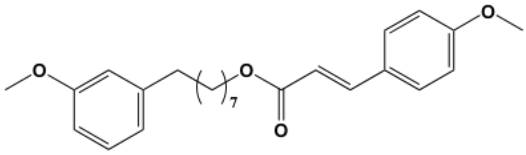
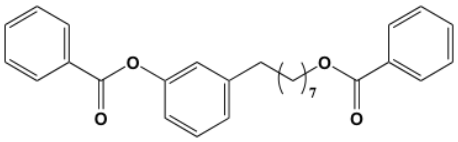
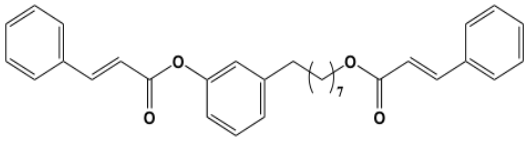
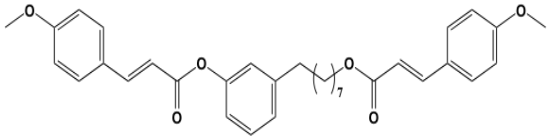
Number	Structure	Code
29		V26
30		V27
31		V33
32		V37
33		V31
34		V32
35		V36

Figure 3. Structures 29-35 of CNSL-derived molecules

In silico studies

The computational tool Osiris® Property Explorer (http://www.organic-chemistry.org/ prog/peo/, Actelion Pharmaceuticals, Ltd.) was used to calculate lipophilicity, expressed as octanol/water partition coefficient (clogP); solubility in water, expressed as the 10-based logarithm of the solubility of a molecule measured in mol/L (logS); molecular weight; drug-likeness indices and drug scores; and toxicological properties such as mutagenic, tumorigenic, irritant and reproductive effects [16].

The substances studied were V32, V33, V34, V35, V36, V37, and compared with two commercial UV filters known for their toxic effects: 4-Methylbenzylidene camphor (4-MBC) and Benzophenone-3 (BP-3).

Mutagenicity assays

The Ames method was used to assess mutagenicity [17]. *Salmonella typhimurium* auxotroph mutant strains TA 97 (*his*D6610/ *his*01242 – Δ*uvr*B *rfa* pKM101 (*amp*^{R})), TA 98 (*his*D3052 – Δ*uvr*B *rfa* pKM101(*amp*^{R})), TA100 (*his*G46 – Δ*uvr*B *rfa* pKM101 (*amp*^{R})), and the wild type strain TA102 (*his*G428-wild type *rfa* pKM101(*amp*^{R}) pAQ1 (*tet*^{R})) were grown in Vogel-Bonner E Medium [17]. A 4-nitroquinoline 1-oxyde (4NQO) solution was used as a positive control for genotoxicity.

Samples were diluted in 5% (V32-V37) or 10% (V34-V35) tetrahydrofuran (THF). Aliquots of 10 μL of each sample were applied directly onto the plates without ultraviolet irradiation to assess the mutagenic potential.

Prior to application of the samples onto the plates, two other aliquots were put into glass flasks and irradiated with 20 kJ/m^2 (27 J/m^2/s for 12'34") of UVA and 10 kJ/m^2 (7.8 J/m^2/s for 21'36") of UVB radiation to evaluate the photomutagenic potential.

Genotoxicity assays

SOS Spot test and SOS chromotest, were carried out as previously described [18]. In both tests, *E. coli* PQ35 and PQ37 (*uvr*A *rfa sfi*A*::lac*Z) strains were used. Samples were diluted in 5% THF (V32-V37) and divided into: non-irradiated and irradiated with UV radiation (two aliquots were removed, put into glass flasks, and irradiated with 20 kJ/m^2 (27 J/m^2/s for 12'34") of UVA and 10 kJ/m^2 (7.8 J/m^2/s for 21'36") of UVB radiation. A 4NQO solution was used as a positive control of genotoxicity in both tests [18].

For the SOS chromotest, the induction of β-galactosidase expression and alkaline phosphatase were measured according to Quillardet & Hofnung (1985) [18] and Miller (1972) [19]. The samples were applied directly on the plate containing the culture medium; before applying on the plates, two brackets of the samples were irradiated with UVA and UVB radiation, respectively, to evaluate the photogenotoxic potential of the substances.

The induction factor was calculated as previously described [18, 20]. For the assays, samples V32, V34 and V36 were diluted to 1; 2.5; 4; 5; 10% (w/v). The commercial sunscreen octyl p-methoxycinnamate was tested in the same dilutions.

Irradiation conditions: a lamp with emission in the 290–320 nm range and a peak at 312 nm was used (VL-215 LM, Vilber Lourmat, France). Fluence was measured using an appropriate sensor (Radiometer VLX-312, Vilber Lourmat-France).

Phototoxicity assay using the yeast Saccharomyces cerevisiae

A *Saccharomyces cerevisiae* wild type strain D273-10B was used and the assay was carried out as described previously [21]. Briefly, solutions of 8-methoxypsoralen and of octyl p-methoxycinnamate at 0.1% were employed as positive [22] and negative phototoxic activity controls, respectively. Ethanol was used as solvent. Aliquots of each studied substance (V1-V37 at 5% w/v) were applied onto Whatman n^o 1 sterile filter paper disks, which were fixed on the surface of the culture media plates.

A suspension of *S. cerevisiae* cells was prepared in sterilized water (10 mL). Aliquots of 0.2 mL were applied and spread in the culture plates using a glass loop. Two plates were prepared for each sample. After seeding and applying the samples, one plate was allowed to grow under two UVA lamps (320-390 nm). A control plate was grown in the dark.

For analysis of the results, the following aspects were observed:

- The presence of a clear zone around the test substance in the light and the absence in the dark indicate the sample phototoxicity;
- The absence of a clear zone around the test substance in the light and in the darkness indicates that the sample is not phototoxic [21].

Results and Discussion

UV absorbance and in vitro SPF determination

The ultraviolet absorption spectral properties (λ_{max} and ε values) of the tested substances in different solvents were obtained. The main characteristics of a UV absorption band are its wavelength of maximum absorbance (λ_{max}) and its molar absorptivity (ε) [23]; both values are distinctive features for each substance [12].

The specific absorbance values are shown in Tables 1 and 2. The higher specific absorbance values found were for V32 (1088) and V36 (1167) (table 2). The ideal for a UV filter is a specific absorbance value higher than 1000, but many commercial UV filters show $A^{1\%}_{1cm}$ lower than 1000 [24], such as 4-MBC (990) [25].

Table 1. UV spectral data of V1-V31 diluted in ethanol

Substance	λ_{max} (nm)[1]	$A^{1\%}_{1cm}$ [2]	ε[3]
V1	276	47	1495.5
V2	275	22	895.9
V3	275	47	1504.9
V4	274	29	1186.7
V5	275	46	1390.1
V6	270	15	516.3
V7	274	57	1733.9
V8	272	16	553.9
V9	302	64	2215.6
V10	301	31	1203.4
V11	280	51	1908.5
V12	305	88	3064.1
V13	304	57	2224.1
V14	280	62	2332.5
V15	299	77	2572.2
V16	302	70	2369.6
V17	275	53	1613.5
V19	275	50	1592.7
V20	282	708	29995.1
V21	285	29	1048.6
V23	266	316	12912.1
V24	221	499	18690.5
V26	280	77	2020.1
V27	292.5	101	2629.3
V29	275	398	16263.1
V30	245	98	3553.0
V31	229	586	25229.6

[1]Wavelength of maximum absorbance, [2]Specific absorbance, [3]Molar absorptivity.

The substances that showed the highest values of molar absorptivity were V20, V31 – V37 (Table 1 and 2). The molar absorptivity is directly proportional to the

chemical´s ability to absorb UV radiation, and it is affected by the nature of the solvent. Therefore, the higher the absorptivity, the more UV radiation the chemical absorbs [12].

Table 2. UV spectral data of V32-V37 diluted in different solvents

Substance	λ_{max} (nm)[1]	$A^{1\%}_{1cm}$[2]	ε[3]	Solvent
V32	294	1088	52509.1	Chloroform
V33	280	528.1	20940.75	Chloroform
V34	284	500.4	21750.39	Chloroform
V35	315	635.5	29531.05	Chloroform
V36	331	1167	63329.6	DMSO
V37	309.6	418	16574.9	Ethanol

[1]Wavelength of maximum absorbance, [2]Specific absorbance, [3]Molar absorptivity.

The molar absorptivity of V20, V29, V31, V33, V34, V35 and V37 were higher than BP-3 (λmax = 287 nm, ε = 14460, in etanol) [26]. While the ε value of V32 and V36 molecules were at least 2 times higher than commertial many UV filters, such as octyl-*p*-methoxy-*trans*-cinnamate (λmax = 310 nm, ε = 24000, in ethanol) [27], butyl methoxy dibenzoyl methane (λmax = 359 nm; ε= 32500, in ethanol) [23], and 4-MBC (λmax = 300 nm, ε = 25183, in metanol) [25].

Cui *et al.* (2012) [28] synthesized two novel N-heterocycle-containing benzotriazole compounds. The UV–Vis spectra of one of the benzotriazole derivatives was measured in chloroform, and the molar absorptivity was 19500 at 339nm (λmax), a lower result than the values found for V31 – V37.

While the CNSL directly derived substances (V1-V20) and others synthesized from it (V20-V31) presented almost insignificant SPF values, substances V32, V33, V34, V35, V36 and V37 presented the best SPF values (table 3): 9.5; 1.5; 2.4; 4.2; 7.7; and 5.2, respectively; and the highest molar absorptivity values (table 2).

Table 3. Results of the sun protection factor (SPF) of substances V32 to V37 at 5% (w/v), with the respective solvents.

Substance	SPF[1]	Solvent
V32	9.5	Chloroform
V33	1.5	Chloroform
V34	2.9	Chloroform
V35	4.2	Chloroform
V36	7.7	DMSO
V37	5.2	Ethanol

[1]Sun protection factor

Nowadays, organic UV absorbers used in sunscreens are aromatic compounds, each containing multiple conjugated π-electron systems [29]. Natural products with polyphenols and flavonoids have been studied as UV absorbers, due to their structural similarity to chemical filters, their lower cost, and some have multiple biological activities [30], such as antimicrobial and antioxidant [31].

Marto *et al.* (2016) [30] studied the green (GCO) and spent coffee oil (SCO), and found that GCO presented an SPF value in ethanol of 5.03 ± 0.23 while SCO presented only 1.57 ± 0.07, but the authors did not specify the concentration used.

The ethanolic extract of *Marcetia taxifolia* was dissolved in ethanol, and extracts with different concentrations (25% and 12.5%) had satisfactory sunscreen activity (SPF 15.52 and 8.35, respectively), the SPF values of the tested extracts were concentration-dependent. This activity was attributed to the flavonoids found in species of the Marcetia family [31]. We tested our compounds at 5%, a lower concentration, and found higher SPF values, for V32 (SPF 9.2).

According to Wolf (2009) [32] each sunscreen active´s concentration must be sufficient to contribute a minimum SPF of not less than 2 to the finished product. The V32-V37 molecules could be combined to get higher SPF values for the sunscreen product. For example, V34 and V35 do not have high SPF values, however a mixture V34 and V35 at 5% and 10% (w/v) in chloroform was tested by the Mansur method (table 4), resulting in an acceptable SPF value of 5.2 (5%) and SPF 8,5 (10%), which also indicates a compatibility between the tested molecules. While the mixture of these substances at 10% (w/v) in resulted in SPF 8,5. The SPF value of a mixture containing octyl-*p*-methoxy-*trans*-cinnamate (OMC) and octocrylene both at 10% (w/v) was also tested in chloroform and resulted in SPF 12.

Table 4. Results of SPF *in vitro* for mixtures of V34, V35 and commercial UV filters in chloroform.

Mixture	Concentration (w/v)	SPF[1]
V34 + V35	5%	5.2
V34 + V35	10%	8,5
OMC[2] + OC[3]	10%	12

[1]Sun protection fator, [2]octyl-*p*-methoxy-*trans*-cinnamate, [3]octocrylene

In silico studies

The results of cLogP for V32-V37 were between 6.37-10.42 (table 5). For UV filters a cLogP > 5 are desirable because indicates that the substance will show low cutaneous permeability [24]. These values also show low oral bioavailability [33], suggesting a low toxicity if the substance is accidentally ingested. According to drug-likeness and drug-scores results, V32-V37 do not qualify as new potential medicinal substances, also corroborating with the proposed topical and cosmetic use. The commercial filters BP-3 and 4-MBC showed cLogP < 5 (table 5), indicating good oral absorption and cutaneous permeability. This indicates that the substances under study (V32-V37) could have lower toxicity than these commercial filters.

Experimental studies confirm substantial absorption and distribution of commercial filters. Organic UV filters, including BP-3 and 4-MBC, are easily absorbed by the skin and reach the systemic circulation, and accumulate in various tissues, such as adipose tissue, liver, brain and placenta. These filters seem to be associated with altered estrogen, androgen and progesterone activity,

reproductive and developmental toxicity and impaired functioning of the thyroid, liver or kidneys [34].

None of the substances (V32-V37) showed mutagenic or tumorogenic risks (table 5). *In silico* approaches are widely used to study important parameters that may guide a medicinal chemist in the evaluation of chemical and physicochemical properties of a compound, and to avoid unnecessary expenses associated with biological assays of compounds with a high probability of presenting future toxicity risks, and thus save time and investments [35]. The ultraviolet filter BP-3, a substance approved by FDA and widely used showed toxicity risks (mutagenic, tumorogenic and reproductive) (table 5). There are studies that corroborate these results, which indicated estrogenic [36] and antiandrogenic activity of this compound [37].

Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified. Only V35 did not show fragments with potential irritant risk, and V37 showed a potential reproductive effect risk. However, risk alerts are by no means a fully reliable toxicity prediction, nor the absence of risk alerts should lead to the conclusion that a particular substance is completely free of any toxic effect [38]. The underlying principle of the program used, OSIRIS®, is to take advantage of existing information, to focus on non-animal tests and on non-test information as much as possible, to group information about similar substances and to integrate exposure considerations. Ideally, with regard to the 3R principle of Reduction, Refinement and Replacement of animal testing, non-testing (*in silico*) and experimental non-animal (*in vitro*) methods are preferred for this purpose [39].

In fact, *in silico* predictions do not replace or disqualify experimental tests, and both should work in partnership with each other. Experimental *in vitro* and *in vivo* tests are uniquely important for the evaluation of a new compound and should not be replaced by *in silico* studies [40].

Mutagenicity

The samples that presented the best SPF values, V32, V33, V34, V35, V36, V37 were selected for the mutagenic and genotoxic tests because these molecules are the most promising to be considered as new UV filters.

The photoreactivity of the UV filter Butyl methoxy dibenzoylmethane was investigated in different solvents, including tetrahydrofuran (THF) and it was stable after irradiation in a solar simulator at a complete dose of 60 kJ/m2 (4 min interval at 250 W/m2) [41]. Therefore, this solvent was used, besides it has no mutagenic or genotoxic potential risk [42], and was tested alone and did not show a mutagenic response.

Table 5. Toxicity risks and physicochemical properties of compounds V32-V37 in comparison with 4-Methylbenzylidene camphor and Benzophenone-3, predicted by OSIRIS Property Explorer.

Substance	**Toxicity risks**				**Physicochemical properties**				
	Mutagenic	Tumorogenic	Irritant	Reproductive effect	MW[1]	clogP[2]	Solubility	Drug Likeness	Drug Score
V32	(-)	(-)	(+)	(-)	482	8.11	-7.02	-13.00	0.07
V33	(-)	(-)	(+)	(-)	366	6.41	-5.2	-12.98	0.12
V34	(-)	(-)	(±)	(-)	434	10.22	-7.47	-22.00	0.09
V35	(-)	(-)	(-)	(-)	464	10.12	-7.49	-20.20	0.11
V36	(-)	(-)	(+)	(-)	542	7.9	-7.06	-11.2	0.06
V37	(-)	(-)	(+)	(+)	396	6.37	-5.22	-11.39	0.07
BP-3[3]	(+)	(+)	(-)	(+)	228	2.85	-3.44	0.08	0.14
4-MBC[4]	(±)	(-)	(-)	(-)	254	4.29	-4.19	-6.64	0.28

[1]Molecular weight (g/mol), [2]Lipophilicity, [3]Benzophenone-3, [4]4-Methylbenzylidene camphor.

The samples tested through the Ames method, at 5% (V32, V33, V34, V35, V36, V37), and 10% (V34, V35) in THF, did not demonstrate mutagenic or photomutagenic activity (n=3). The non-irradiated samples did not demonstrate mutagenicity when compared to the positive control for this test, 4NQO. When the samples were irradiated with UVA and UVB radiation, they did not show a photomutagenic response either (n=3). These results corroborate the predicted from the *in silico* results.

Carvalho et al (2011) [43] investigated the mutagenic, acute and subacute toxicity of anacardic acids isolated from CNSL, performing *in vivo* assays via BALB/c mice, and they did not produce any mutagenic effects, or biochemical and hematological alterations using doses under 300 mg/kg.

Genotoxicity

The non-irradiated and samples that were irradiated with UVA and UVB radiation, V33, V35, and V37 (at 5% in THF) did not present blue halos for PQ35 and PQ37, indicating they were non-genotoxic in the tested concentration (n=3). The THF solvent was tested alone and did not demonstrate genotoxic activity.

Samples V32, V34 and V36 (at 5% in THF) presented a light blue halo just for strain PQ37, when irradiated with UVA and UVB radiation, they demonstrated a light genotoxicity and cytotoxicity for both strains (n=3). Therefore, the SOS Chromotest was used for quantification of this supposed genotoxicity (table 6).

Table 6. Genotoxic activity of the V32, V34, V36, octyl p-methoxycinnamate substances in culture of *E. coli* (PQ37) (n=3).

Substance	Concentration (g%)	Unit AF[1]	Units β-gal[2]	IF[3]
V32	0	0.067	0.551	0.424
	1	0.121	0.376	0.375
	2.5	0.151	0.612	0.492
	4	0.076	0.464	0.739
	5	0.092	0.372	0.489
	10	0.096	0.235	0.296
V34	0	0.067	0.551	0.424
	1	0.124	0.784	0.765
	2.5	0.077	0.725	1.136
	4	0.229	0.772	0.407
	5	0.201	0.797	0.479
	10	0.155	0.861	0.674
V36	0	0.075	1.599	1.097
	1	0.079	0.972	0.577
	2.5	0.053	0.773	0.677
	5	0.059	0.154	0.123
OMC[4]	0	0.067	0.551	0.424
	1	0.078	0.753	1.16
	2.5	0.241	0.647	0.32
	4	0.0493	0.488	1.19
	5	0.086	0.614	0.85
	10	0.080	0.639	0.966

[1]Alkaline phosphatase, [2]β-gal - β-galactosidase, [3]Induction factor, [4]Octyl p-methoxycinnamate

A compound is classified as "non-genotoxic" if the induction factor remains <1.5; as "marginal" if the induction factor is between 1.5 and 2.0; and as "genotoxic" if the induction factor exceeds 2.0 [44].
Substances V32, V34 and V36, at concentrations varying from 1% to 10%, presented induction factors lower than 1.5 (table 6), being considered non-genotoxic. Additionally, their induction factors were lower compared to octyl p-methoxycinnamate, a very used and non-genotoxic sunscreen, approved for use by FDA and by Brazil´s regulatory organ (ANVISA).
Phenolic lipids, like anacardic acid, cardanol and cardol present in the CNSL can be incorporate by erythrocytes and liposomal membranes, exerting antigenotoxic activity [1]. The novel molecules under study derived from these phenolic lipids also showed no genotoxic effect.

Phototoxicity in vitro

None of the 35 tested substances at 5% (w/v) led to the appearance of growth inhibition halos in both plates (irradiated and in the absence of light) demonstrating no phototoxic result (figure 4).
The positive control for phototoxicity, 8-methoxypsoralen resulted in the appearance of growth inhibition halo in the irradiated plates (figure 5); and octyl *p*-methoxycinnamate did not promote the appearance of growth inhibition halo.

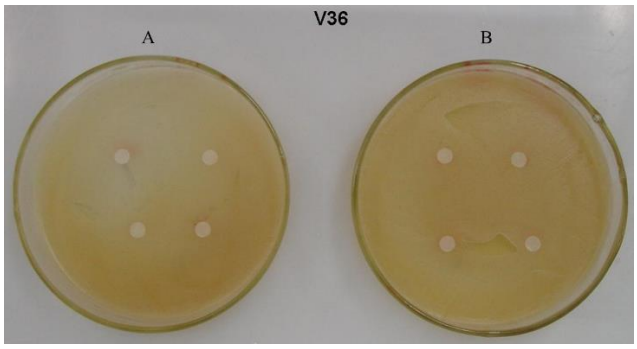


Figure 4. Negative result of phototoxicity V36: absence of growth inhibition halos in the plate irradiated with UV light (A) and in the absence of light plate (B).

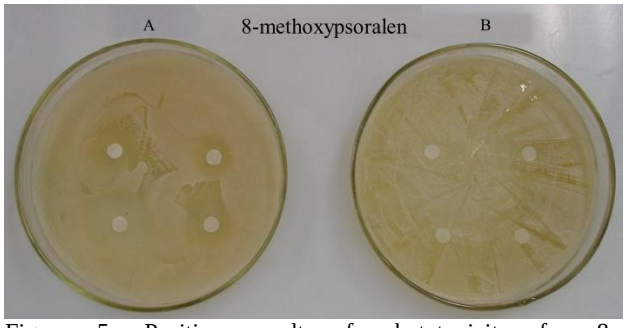


Figure 5. Positive result of phototoxicity for 8-methoxypsoralen: presence of growth inhibition halo in the plate irradiated with UV light (A).

It is particularly important to test the phototoxic potential of pharmaceuticals and cosmetic products because the photoexcited forms of certain chemical compounds are known to produce phototoxic insult to cellular biomolecules [45].

Conclusions

Six of the 37 molecules studied showed potential to be used as new UV filters, showed appropriate SPF values, and demonstred to be non-phototoxic, non-genotoxic and non-mutagenic and the *in silico* results also indicated low cutaneous permeability and low oral bioavaibility. Moreover, these substances may have a lower cost because they are derived from the cashewnut that has an abundant industrial production in Brazil.
The use of this structural pattern for sunscreens has not been previously reported, and, therefore, the compounds tested in this study and their synthetic methodology represent a novelty among the organic photoprotective agents. Additionally, these compounds conjugate, in a single structure, different photoabsorbent chromophores, providing relevant synthesis cost reduction in relation to the isolated molecules found in the literature and in the market.

Acknowledgments

Recognition is due to CNPq (process number 310509/2011-4) for the fellowship to L.A.S. Romeiro.

The authors also gratefully acknowledge the help from the colleagues from UFRJ – Rio de Janeiro/Brazil: Zaida Freitas, Célia Santos da Silva, Elisabete P. Santos, Luiz Mauricio T.R. Lima and Ana Luisa P. Miranda.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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