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Article

A study on 2-(2'-hydroxyphenyl) benzoxazoles derivatives as potential organic UV filters, Part I

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Damage resulting from the incidence of ultraviolet (UV) radiation on the skin is common nowadays, with UVB (290–320 nm) and UVA (320–400 nm) radiation responsible for photoaging, sunburn and carcinogenesis. For this reason, sunscreens represent products of growing interest to prevent such damage. However, there are few organic filters marketed worldwide with photostability and effectiveness at wavelengths greater than 340 nm (long UVA), which justifies the exploration for new compounds. In this work, we determined the photostability and sun protection factor (SPF) of three 2-(2'-hydroxyphenyl)benzoxazole derivative dyes in order to develop new organic UV filters. UV-vis spectrophotometry has high level of reproducibility when compared with *in vivo* human clinical methods. Solubility determinations were performed in different solvents. The compounds absorbed UVA and UVB radiation, with maximum absorption wavelengths ranging from 336 to 374 nm. Photostability was evaluated using a solar simulator (3 $J.m^2.s^{-1}$ UVA radiation) for a maximum of 3 h. The 2-(amino-2'-hydroxyphenyl)benzoxazoles showed higher photostability than the acetylated derivative under the evaluated conditions. The three benzoxazoles presented SPF values of around 40 and preliminary results indicate that they show suitable properties to act as good chemical filters in photoprotective formulations.

Keywords: 2-(2'-hydroxyphenyl)benzoxazole. Photoprotector. Photostability. Sunscreen. UV radiation.

INTRODUCTION

The solar radiation that reaches the earth can be divided into many regions but the ones that are of medical interest are infrared (56%), visible (39%) and ultraviolet (5%) radiation. Ultraviolet (UV) radiation is the one with the greatest biological effect and can be divided into UVA (320–340 nm), UVB (290–320 nm) and UVC (100–290 nm). Ultraviolet light exposure is the most important risk factor for cutaneous melanoma and nonmelanoma skin cancers. Ultraviolet light also causes severe sunburn, photoaging damage to the skin, photoallergies, and melasmas (Stiefel, Schwack, 2015; Herzog, Wehrle, Quass, 2009; Mancebo, Hu, Wang, 2014).

Depending on the wavelength, absorbed UV light interacts with different skin cells at different depths. Energy from UVB radiation is mostly absorbed by the epidermis and affects epidermal cells such as the keratinocytes and also generates reactive oxygen species (ROS), but its main action is the direct induction of DNA damage. Cyclobutane pyrimidine dimers and pyrimidone photoproducts are the main lesions induced by direct excitation of DNA bases by UVA and UVB photons, which may be related to premalignant skin lesions. Ultraviolet radiation also alters RNA and implies the

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formation of dysfunctional proteins (Perdiz *et al*., 2000). The energy from UVA penetrates deeper into the skin affecting both epidermal keratinocytes and the deeper dermal fibroblasts. UVC radiation, due to the high energy associated with its shorter wavelength, is highly harmful to humans with carcinogenic and mutagenic effects. It is mostly absorbed by the ozone layer, so the amount of this radiation that reaches the human population is very small (Montagner, Costa, 2009).

According to the Brazilian Health Surveillance Agency (ANVISA), sunscreen is any cosmetic formulation prepared to contact the skin and lips with the purpose of protecting it against UVB and UVA radiation, absorbing, scattering or reflecting the solar radiation (Brasil, 2016). UV filters are active substances that act by mechanisms of reflection, dispersion or absorption of radiation that affects the skin (Gilbert *et al*., 2013). They can be divided into inorganic (or physical) and organic (or chemical) filters, whose action is based on reflection or absorption of the solar radiation, respectively. Most sunscreens combine organic and inorganic filters in their formulations to achieve the expected level of effectiveness and more uniform coverage of the UVA and UVB ranges (Zaratti *et al*., 2014). The evaluation of protection efficiency is mainly through the induction of erythema in human skin and is expressed as a sun protection factor (SPF; Schuch *et al*., 2012). The *in vivo* method of determination of SPF is officially adopted in several countries (ANVISA, Brazil; FDA, United States; DIN, Germany; COLIPA, European Union; AAN, Australia). UV-vis spectrophotometry is an *in vitro* approach based on spectrophotometric analysis developed by Mansur *et al.* (1986) for the evaluation of approximate SPF values of sunscreen products (Yang *et al*., 2018; Fonseca, Rafaela, 2013; Dutra *et al*., 2004).

A number of organic molecules are employed as UV filters in sunscreen products (Herzog, Wehrle, Quass, 2009; Gilbert *et al*., 2013; Baker, Greenough, Stavros, 2016; Nash, Tanner, 2014; Rastogi, 2002). Among them, compounds that present a photoinduced excited-state intramolecular proton transfer (ESIPT) are strong UV absorbers (Farkas *et al*., 2010; Ignasiak *et al*., 2015). Derivatives of 2-(2'-hydroxyphenyl)benzoxazole are known to emit light by an ESIPT mechanism and are capable of absorbing high-energy UV radiation and dissipate rapidly the harmful UV energy through an intramolecular rearrangement (Rodembusch *et al*., 2007).

The objective of the current research was to synthesize three derivatives of 2-(2'-hydroxyphenyl) benzoxazole and to evaluate their potential as organic UV filters by application of UV-vis spectrophotometry.

MATERIAL AND METHODS

Chemicals

Reagent grade 2-aminophenol, 4-amino-2-hydroxybenzoic acid and 5-amino-2-hydroxybenzoic acid (Aldrich) were used without purification. Polyphosphoric acid (PPA) was purchased from ACROS Chemicals. All other reagents were from Merck. The silica gel 60 (Merck) was used for chromatographic column separations. All solvents were used as received or were purified using standard procedures. Spectroscopic grade solvents (Merck) were used for the UV–Vis measurements.

Synthesis of 2-(2'-hydroxyphenyl) benzoxazole derivatives (1-3)

The synthesis of 2-(4'-amino-2'-hiydroxyphenyl) benzoxazole (**1**) and 2-(5'-amino-2'-hiydroxyphenyl) benzoxazole (**2**) were prepared according to the procedure described in the literature (Holler *et al*., 2002). The method consists of a condensation reaction of an equimolar amount of 2-aminophenol with aminosalicylic acid in polyphosphoric acid (PPA) at 180 ºC for 4 h (Figure 1). The reactions were accompanied by thin layer chromatography using dichloromethane as eluent. The reaction mixture were poured into ice and the obtained precipitated were filtered, neutralized with sodium carbonate and dried. N-acetylation of compound **2** was performed using catalytic acetic acid and either acetic anhydride or sodium acetate as the acyl source. The acetylation reaction occurs quickly (30 minutes) and leads to the acetylated product **3** without the need of purification (Figure 1).

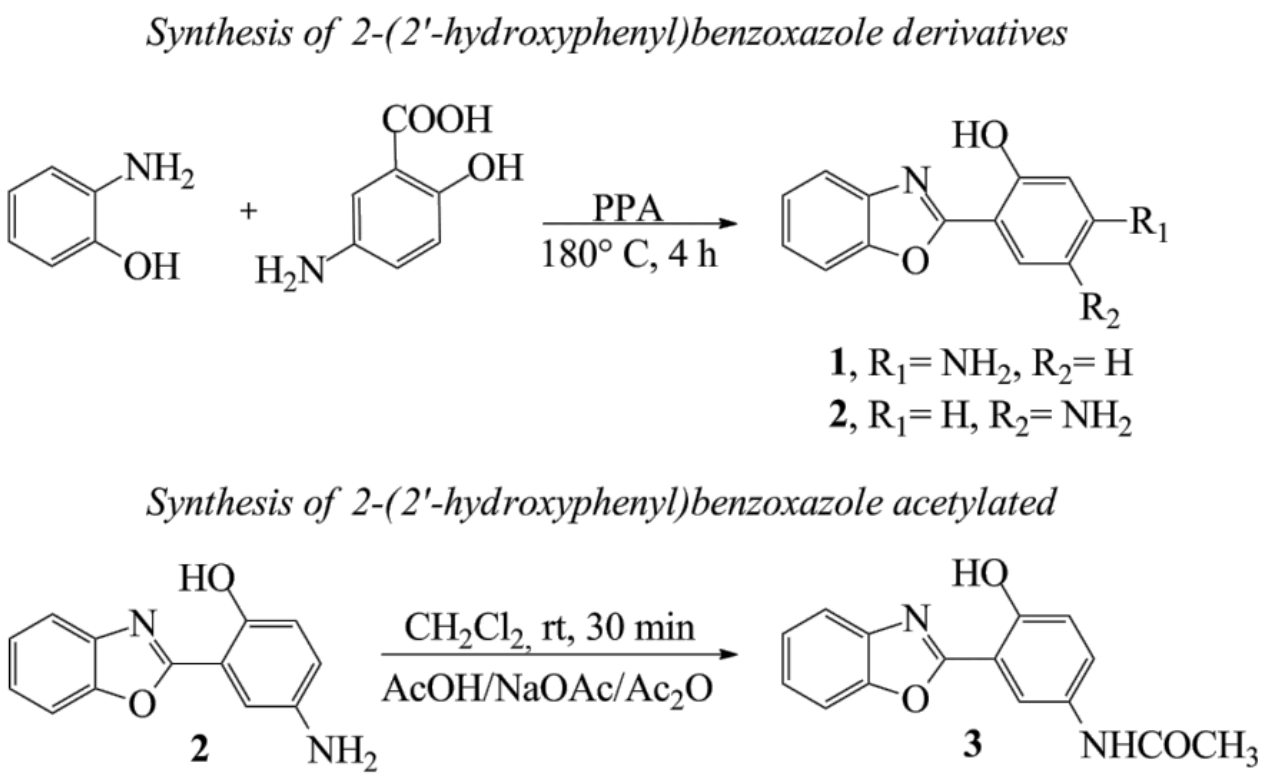

FIGURE 1 - Scheme of synthesis of benzoxazoles compounds 1-3.

The compound **1** was obtained as a white product in yield about 70 %. Purification by column chromatography led to the high purity product showing a single blue fluorescence signal on TLC. Purity was confirmed by melting point determination (227-228 °C). IR (KBr, cm^{-1}): 3485 -3381 (NH_2), 3050 (C-H_{arom}), 1630, 1556, 1498 and 1452 (C-C_{arom}). ^1H NMR (400 MHz, $CDCl_3$): δ = 11.14 (s, 1H, OH); 7.7-7.6 (m, 3H); 7.4–7.3 (m, 2H); 6.28-6.24 (dd,1H); 6.16 (d, 1H); 6.06 (broad, s, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 164 (C2), 160 (C2′), 155 (C8), 148 (C9), 140 (C4′), 128 (C6′), 125.2 (C5 or C6), 124.8 (C5 or C6), 118 (C4 or C7), 110 (C4 or C7), 108 (C1′), 99 (C5′ or C3′), 98 (C5′ or C3′).

The compound **2** was isolated in high purity, showing a single fluorescence signal (TLC) in dichloromethane as eluent, in green color. Melting point (174-175 °C) confirmed the purity of the product (Campo, 2003), which was obtained in a yield about 75 %. IR (KBr, cm^{-1}): 3414 – 3331 (NH_2), 3050 (C-H_{arom}), 1630, 1544, and 1498 (C-C_{arom}). ^1H NMR (400 MHz, DMSO-d^6): δ =10,39 (s, 1H, OH); 7,83 (m, 2H); 7.43 (m, 2H); 7.23 (d, 1H); 6.85-6.78 (m, 2H); 4.91 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d^6): δ 163 (C2), 150 (C8), 149 (C9), 142 (C2′), 140 (C5′), 126 (C3′), 125.6 (C5 or C6), 122 (C5 or C6), 120 (C4 or C7), 118 (C4 or C7), 111.30 (C1′), 110.8 (C6′ or C4′), and 110 (C6′ or C4′).

The compound **3**, (N-[3-(1,3-benzoxazol-2-il)-4-hydroxyphenyl] acetamide, is not described in the literature and was prepared as follows. Compound **2** was acetylated with acetic anhydride in the presence of acetic acid to obtain compound **3**. For this, 2.2 mmol of **2** was dissolved in dichloromethane and a solution containing 3.9 mmol sodium acetate, 35 mmol acetic acid and 4.8 mmol acetic anhydride were added. After 30 minutes, a vacuum filtration was performed and the solid was washed with water and dilute NaOH solution to remove reagents in excess. The product was obtained as a white precipitate in a yield of 87%. The melting point determined was 258-260 °C. IR (KBr, cm^{-1}): 3495-3383 (NH and OH) and 1608 cm^{-1} (C=O). ^1H-RMN (400 MHz, DMSO-d_6): δ 11.21 (s, 1H, OH); 10.20 (s, 1H, NH); 8.25 (s, 1H); 7.9 (d, 1H); 7.76 (s, 1H); 7.49 (s, 1H), 7.4 (m,

2H); 7.2 (d, 1H); 2.07 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 169 (C=O), 163 (C2), 160 (C8), 149 (C9), 144 (C2′), 140 (C5′), 128.3 (C3′), 125.7 (C5 or C6), 125.5 (C5 or C6), 119 (C4 or C7), 117 (C4 or C7), 111(C1′), 106 (C6′ or C4′), 105 (C6′ or C4′) and 25 (CH3). In the mass spectra exhibit the expected ion m/z 268, which represents the molar mass of the compound.

The spectroscopic data and melting point indicated that the synthesis and purifications were successful.

The spectrum can be found in appendix.

Equipments

Melting points were measured with a 498 model Uniscience of Brazil apparatus and were uncorrected. FT-IR spectra were performed on a Shimadzu model IRPrestige-21 spectrophotometer. ^{1}H and ^{13}C NMR spectra were performed on a VARIAN model Avance-400 using tetramethylsilane (TMS) as the internal standard and DMSO-d_6 (Aldrich) or $CDCl_3$ (Merck) as the solvent. UV-Vis absorption spectra were performed on a Varian Cary 100 Bio spectrophotometer.

Solubility test

Solubility of the benzoxazole derivatives **1-3** was performed at 25 ºC with reported results for 1 g of solid evaluated in different solvents. The method adopted for solubility was based on the Brazilian Pharmacopeia, (2010).

Solubility was tested in the following solvents: cyclopentasiloxane, PPG-15 stearyl ether, C12-15 alkyl benzoate, medium chain triglycerides (MCT), ethanol, distilled water and acetone according to the polarity of the molecule and the applicability of the solvents in cosmetic formulations.

In order to evaluate the compound as soluble or slightly soluble, for each solvent, 0.01g the compound to be evaluated was weighed in a glass goblet and 30 parts of the solvent (0.3 mL) were added. In another goblet the same amount of test compound was weighed and 100 parts of the solvent (1 mL) was added. Solvent additions to obtain final volume of 10 mL (1:1000) and 100 mL (1:10,000) were made for classification as poorly soluble and very poorly soluble. Subsequent addition of 10 mL of solvent to the 1:10,000 ratio allowed the classification as insoluble. The preparations that did not contain any solid residues were considered properly solubilized and the formation of the solution was verified.

Preparation of the solutions for optical measurements

The solutions used for UV absorption and photostability analysis were prepared in MCT and ethanol. The dye solutions were prepared weighing 1.4, 6.5 and 3.7 mg of the compounds **1-3** respectively, in 250 mL of the solvent in order to obtain absorbance close to 1. The solutions were kept in an ultrasound bath until a complete dissolution of the dye. Samples were prepared in triplicate and readings were taken in the range of 290 to 450 nm on a UV-Vis spectrophotometer.

Photostability

The photostability tests were conducted with a home-made solar simulator consisting of a white painted carbon steel chamber in accordance to ICH standards. The simulator has the dimensions 25.0 x 47.0 x 13.0 cm (height x width x depth) and contain two Golden Black Light (25W/220V/350 mA) lamps and two Golden Cool Daylight (30W/220V/240 mA) lamps. The chamber was isolated so that there was no interference from external radiation or radiation loss through openings. Lamps were placed in the upper to increase the power of the equipment and thus ensure an efficient heat exchange with the environment so as not to overheat.

Three samples of each compound were prepared and exposed at different time intervals in the solar simulator at a power of 900 W with the lamp emitting 3 $J.m^2.s^{-1}$ UVA radiation. The three samples were irradiated for a period of three hours and the absorbance measured every hour to detect photodegradation of the samples.

Commercial filters 1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione (avobenzone, AVO) **(4)** and hexyl-2-[4-(diethylamino)-2-hydroxybenzoyl] benzoate (Uvinul® A Plus) **(5)** were evaluated under the same conditions for comparison purposes.

Evaluation of the *in vitro* photoprotective potential

The method for the determination of SPF by UV-vis spectrophotometry was based in the application the Mansur mathematical equation (Equation 1) (Mansur, *et al*., 1986).

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda)$$

Equation 1

where EE (λ) - the erythemal effect spectrum; I (λ) - solar intensity spectrum; Abs (λ) - absorbance of sunscreen product; CF- correction factor (=10).

The benzoxazoles **1-3** were diluted to 0.2 mg.mL^{-1} in ethanol and subjected to spectrophotometric scanning from 290 to 320 nm. The absorbance values at 5 nm intervals were multiplied by the normalized weight values as a function of erythema occurrence by the UVB absorption range. The EE x I values (Table I) of are constants and were determined by Sayre *et al.* (1979).

TABLE I - Normalized product function used in the SPF calculation. EE – erythemal effect spectrum; I – solar intensity spectrum.[20]

Wavelength (λ., nm)	EE (λ) x I (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

RESULTS AND DISCUSSION

Absorption properties

The UV-vis spectra (Figure 2) obtained for the benzoxazoles **1**–**3** were compared with reference spectra and showed equivalence in shape and maximum absorption; the spectra were highly similar. In the present study the maximum absorption wavelength (λ_{max}) of sunscreens tested ranged from 336 to 374 nm.

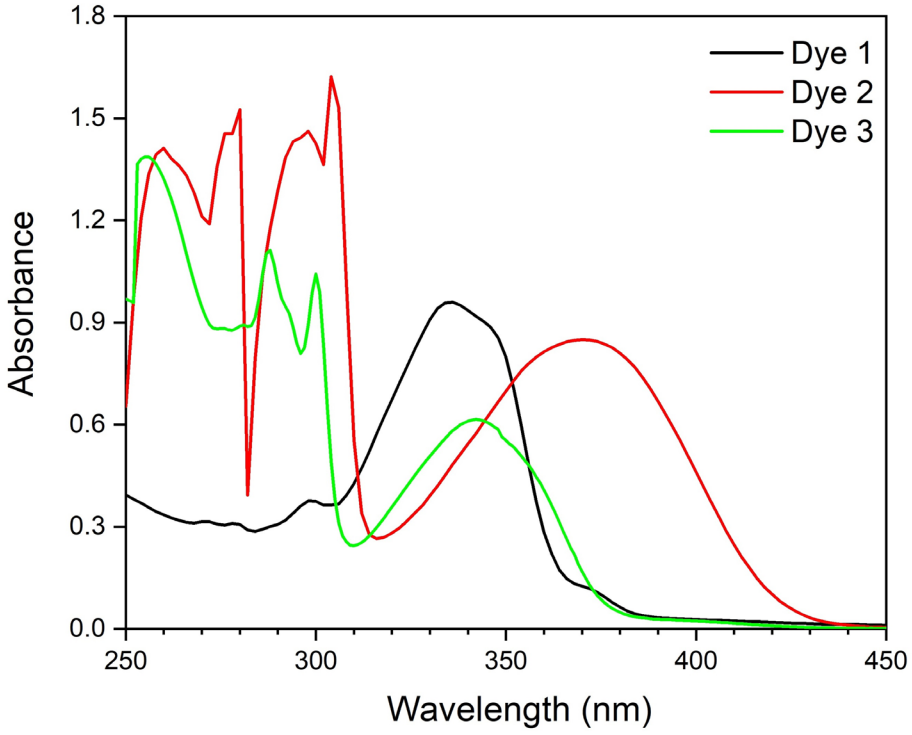

FIGURE 2 - Absorption spectra of benzoxazole dyes 1-3 in ethanol (c= 10^{-4} M).

According to the results obtained in ethanol, compound **1** absorbed UV radiation in the UVA range with λ_{max} at 336 nm, an absorbance value at λ_{max} of 0.96 and $\varepsilon_{max} = 1.83 \times 10^4$ mol^{-1} cm^{-1}. Compound **2** absorbed UV radiation in the UVA range with λ_{max} at 374 nm, an absorbance value at λ_{max} of 0.852 and $\varepsilon_{max} = 5.30 \times 10^4$ mol^{-1} cm^{-1}. Compound **3** absorbed UV radiation in the UVA range with λ_{max} at 339 nm, an absorbance value at λ_{max} of 0.80 and $\varepsilon_{max} = 1.69 \times 10^5$ mol^{-1} cm^{-1}. When MCT was used as the solvent, compound **1** showed a small blue-shift (2 nm) in the absorption maximum while compound **2** showed a red-shift (10 nm); compound **3** showed no change (data not shown). Comparing the three studied 2-(2'-hydroxyphenyl)benzoxazole compounds with respect to absorbance in ethanol solution, compound **3** presented the highest molar absorptivity while **2** resulted in absorption at a longer wavelength (374 nm) in MCT.

All compounds absorbed both UVA and UVB radiation and fulfilled the main requirements for an organic compound to be employed as a photoprotective chemical: a large absorption cross-section in the UVA and UVB spectral regions and the availability of one or more mechanisms by which the absorbed energy can be dissipated without loss of integrity of the chemical filter molecule (Baker *et al.*, 2017). Compounds **1**–**3** showed a large absorption cross-section in the UVA and UVB spectral regions and presented ESIPT mechanisms by which the absorbed energy can be dissipated without loss of chemical integrity. The absorption wavelength maxima were close to those found by Wang *et al.* (2013), who conducted a cross-sectional study of the evolution of sunscreen products in the United States.

Photostability

Photostability is one of the critical requirements for an effective sunscreen. However, most commercially available sunscreens undergo photoreactions that can lead to the formation of harmful products (Abid *et al.*, 2017). Filters that undergo photodegradation after exposure to sunlight or artificial light show a decrease in their UV protection capability and the generation of harmful photolytic products.

The results of the photostability measurements of benzoxazoles **1**–**3** and the two commercial filters over 3

h of exposure are presented in Table II. After exposure, compound **1** showed a slight decrease in the maximum absorption of 6.6% in ethanol and 4.7% in MCT. For compound **2** the decrease in absorbance was 4.2% in ethanol and 3.3% in MCT. The decrease in absorbance of compound **3** was 14.2% in ethanol and 18.6% in MCT. Compound **2** was the most photostable derivative, showing a slight loss in absorption capacity over 3 h exposure in both ethanol and MCT solution. Compound **3** had the greatest loss in absorption intensity compared to the other compounds although the absorption spectrum showed no significant change until 2 h of exposure (Figure 3).

TABLE II - Absorbance of benzoxazoles 1-3 in EtOH and MCT, Avobenzona 4 and Uvinul® A Plus 5 in EtOH after irradiation

Benzoxazole	Solvent	λ_{max} (nm)	Initial Absorbance	Absorbance after 1h	Absorbance after 2h	Absorbance after 3h
1	EtOH	336	0.960	0.948	0.925	0.897
			% of loss	1.250	3.650	6.560
	MCT	334	0.914	0.913	0.895	0.871
			% of loss	0.100	2.080	4.700
2	EtOH	364	0.852	0.834	0.821	0.816
			% of loss	2.050	3.580	4.220
	MCT	374	0.913	0.910	0.904	0.883
			% of loss	0.270	0.990	3.290
3	EtOH	340	0.649	0.617	0.613	0.557
			% of loss	4.930	5.550	14.18
	MCT	340	0.452	0.399	0.399	0.368
			% of loss	11.72	11.72	18.58
4	EtOH	357	0.909	0.766	0.588	0.411
			% of loss	15.73	35.31	54.79
5	EtOH	354	0.725	0.715	0.713	0.704
			% of loss	1.38	1.66	2.88

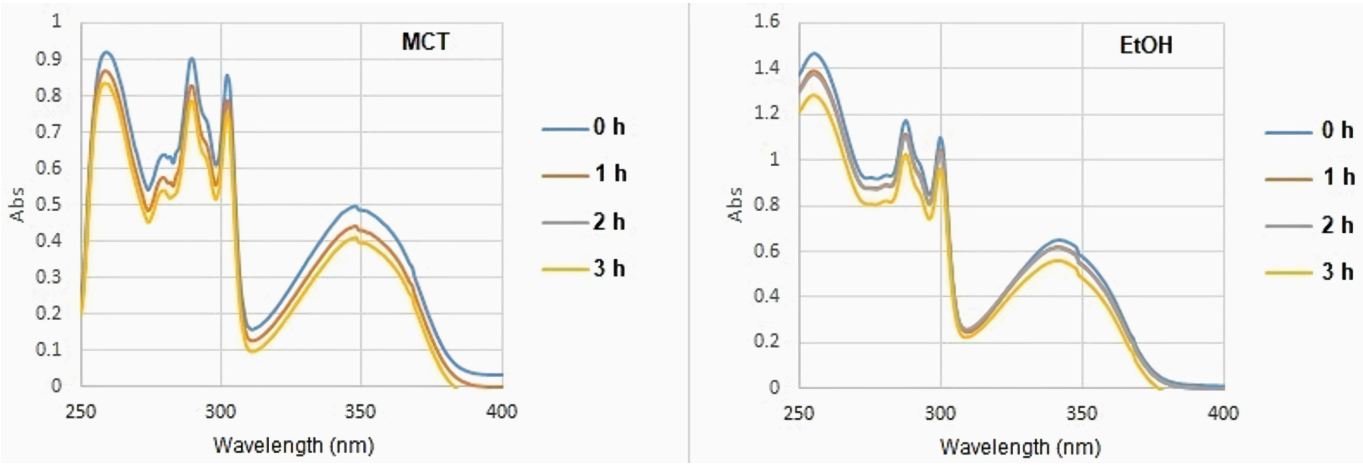

FIGURE 3 - Absorption spectra of benzoxazole dye 3 in MCT and ethanol up to 3 hours of exposition in the solar simulator.

The photostability of the three benzoxazole compounds was compared, under the same conditions, to that of two commercial sunscreens, AVO (**4**) and Uvinul® A Plus (**5**).

Sunscreens containing avobenzone are indicated for providing protection from the sun. AVO is among the most common UV filters; it is included in many commercially available sunscreens, due to its broad absorption spectrum in the UVA region (Gallardo *et al*., 2014). In this study, AVO suffered photodegradation, showing a loss of approximately 55% in photostability at the absorption maximum (357 nm) when irradiated for 3 h under the same conditions, proving it to be much less photostable than the compounds evaluated.

Uvinul® A Plus provides not only reliable filtration of the sun's dangerous UVA rays, but also provides outstanding protection from free radicals and skin damage. It possesses excellent photostability and is toxicologically safe. This filter showed a photostability of 2.88% (354 nm) in a period of 3 h when evaluated under the same irradiation conditions as compounds **1–3**, confirming it to be highly photostable.

The studied compounds were more photostable than the commercial avobenzone filter and the one that came closest to Uvinul® A Plus is the derivative **2** with an amino substituent in position 5' and the least loss in photostability.

The results obtained indicate that the benzoxazole compounds **1–3** are photostable in both solvents used; the presence of the amino group gives a greater photostability to the 2-(2'-hydroxyphenyl)benzoxazole compounds. Therefore, the sunscreen candidates evaluated in this study were proved to be photostable, showing good response to the exposure to solar UV without significant physical or chemical changes.

In preliminary tests the compounds did not induce mutagenic or genotoxic effects, suggesting that these benzoxazoles may not pose genetic risks, although further toxicology assays are necessary. These results will be presented as soon as they are completed.

Solubility test

Sunscreen formulations include the main sunscreen agents and excipients specific to the type of formulation, including an appropriate solvent or vehicle system. The selection of the contents is determined by the intended use and the physical-chemical nature of the ingredients. The solubility of UV absorbers for sunscreens is essential for the creation of formulations. Regardless of the type of formulation (gel, cream, lotion) containing a sunscreen, those compounds need to be dissolved to ensure a homogeneous distribution in the formulation and also afterwards on the skin. Thus, the solubility aspects of solid UV filters such as compounds **1–3** should not be overlooked during the formulation process.

Compound **1** was slightly soluble in acetone and ethyl acetate, both in the proportion 1:50 but insoluble in water, cyclopentasiloxane, ethanol, octylmethoxycinnamate, octocrylene, octyl palmitate, PPG-15 stearyl ether, propylene glycol and MCT when tested in the proportions according to the Brazilian Pharmacopeia.

Compound **2** was properly solubilized in acetone in a ratio of 1:100, which classifies it as slightly soluble. However, insolubility was found in various solvents and emollients tested (cyclopentasiloxane, PPG-15 stearyl ether, C12–15 alkyl benzoate, ethanol and distilled water).

Compound **3** compound was insoluble in all solvents tested in the proportions described in the Brazilian Pharmacopeia. Suspended solid dispersions were obtained in the oils employed, allowing a possible use in cosmetic formulations. It should be noted that acetone allowed better apparent result with fewer insoluble particles, especially in solvent ratios above 1:500.

The best solubility results among the tested solvents were obtained in acetone and ethyl acetate. Although the descriptive results were the same for all solvents, acetone allowed a better apparent result with fewer insoluble particles. The solubility of benzoxazoles **1–3** was facilitated by the use of an ultrasonic bath, achieving complete solubilization in the solvents tested, thus allowing for their possible use in cosmetic formulations. It should be noted that the concentration required for effective formulations of the three evaluated benzoxazoles compounds is in the 10^{-5} molar range, so a very low amount of filter is employed in the formulation to achieve the desired photoprotection. The benzoxazole compounds **1**–**3** evaluated in this study were poorly soluble in lipophilic solvents without the use of an ultrasonic bath.

Evaluation of *in vitro* photoprotective potential

To determine the photoprotective potential according to ANVISA (Brasil, 2016) it is recommended to use an *in vivo* method employing healthy volunteers with different skin types Alternatively, there are *in vitro* methods that are based on the absorptive or reflective properties of the UV filter and can be used to evaluate the SPF. This method has been shown to be effective, fast and simple and shows good correlation with *in vivo* results (Yang *et al*., 2018; Fonseca, Rafaela, 2013; Dutra *et al*., 2004).

In the test using the Mansur method at the concentration of 10^{-4} M, benzoxazole **3** had an SPF of 39 while **1** and **2** showed SPF values of 38 for absorption between 290 and 320 nm in ethanol. The photoprotective potential of the studied compounds showed values much higher than that found for many filters according to published studies (Polonini *et al*., 2013; Sohn *et al*., 2016). The level of sun protection achievable when using commercial products is indicated by the SPF value, which is the parameter used worldwide. This value reflects the protection level mainly in the UVB range. However, UVA radiation also has deleterious effects on the skin and it is therefore essential that products offer a broad spectrum of protection.

CONCLUSIONS

The three 2-(2**'**-hydroxyphenyl)benzoxazole compounds were obtained in good yields and presented appropriate photophysical characteristics to act as sunscreens, meeting the requirements of the Brazilian and international regulatory agencies.

The evaluated compounds showed both UVA and UVB absorbance, which represents a great advantage over many sunscreens currently marketed, since few of them have absorption in both regions and a very high extinction coefficient that allows the highest possible protection in low concentration with the minimum possible number of UV filters.

They also showed high photostability and solubilities that are satisfactory for applications in sunscreen formulation in the solvents tested using ultrasound. As for photoprotective potential, the compounds showed SPF values comparable to good organic UV filters. The results indicated that the benzoxazoles evaluated show suitable properties to act as good chemical filters in photoprotective formulations.

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REFERENCES

Abid AR, Marciniak B, Pedzinski T, Shahid M. Photostability and photosensitizing characterization of selected sunscreens. J Photochem Photobiol A. 2017;332:241-50.

Baker LA, Greenough SE, Stavros VG. A Perspective on the ultrafast photochemistry of solution-phase sunscreen molecules. J Phys Chem Lett. 2016;7(22):4655-65.

Baker LA, Marchetti B, Karsili TNV, Stavros VG, Ashfold MNR. Photoprotection: extending lessons learned from studying natural sunscreens to the design of artificial sunscreen constituents. Chem Soc Rev. 2017;46(12):3770-91.

Brazil, ANVISA, RDC 126/2016.

Brazil, Brazilian Pharmacopeia. 2010;1: 548p.

Campo LF. Sistemas de materiais fotossensíveis baseados em corantes fluorescentes como meio ativo para dispositivos ópticos. **[**Thesis]. Porto Alegre: Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Química; 2003.

Dutra EA, Oliveira DAGC, Kedor-Hackmann ERM, Santoro MIRM. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Braz J Pharm Sci. 2004;40(3):381-85.

Farkas R, Lhiaubet-Vallet V, Corbera J, Törincsi M, Gorchs O, Trullas C, et al. Synthesis of new 2-(2′-hydroxyaryl) benzotriazoles and evaluation of their photochemical behavior as potential UV-filters. Molecules. 2010;15(9):6205-16.

Fonseca AP, Rafaela N. Determination of sun protection factor by UV-Vis spectrophotometry. Health Care Curr Rev 2013;1:1.

Gallardo C, Pinillos MJF, Pazmiño AJD, Munera EA. Characterization of Encapsulation Process of Avobenzone in Solid Lipid Microparticle Using a Factorial Design and its Effect on Photostability. J App Pharm Sci. 2014;4(12):35–43

Gilbert E, Pirot F, Bertholle V, Roussel L, Falson F, Padois K. Commonly used UV filter toxicity on biological functions: review of last decade studies. Int J Cosmet Sci. 2013;35(3):208-19.

Herzog BH, Wehrle M, Quass K. Photostability of UV absorber systems in sunscreens. Photochem Photobiol. 2009;85(4):869-78.

Holler MG, Campo LF, Brandelli A, Stefani V. Synthesis and spectroscopic characterization of 2-(2'-hydroxyphenyl) benzazole isothiocyanates as new fluorescent probes for proteins. J Photochem Photobiol A. 2002;149(1-3):217-25

Ignasiak MT, Houée-Levin C, Kciuk G, Marciniak B, Pedzinski T. A Reevaluation of the photolytic properties of 2-hydroxybenzophenone-based UV sunscreens: Are chemical sunscreens inoffensive? Chem Phys Chem. 2015;16(3):628-33.

Mancebo SE, Hu JY, Wang SQ. Sunscreens: a review of health benefits, regulations, and controversies. Dermatol Clin. 2014;32(3):427-38.

Montagner S, Costa A. Bases biomoleculares do fotoenvelhecimento. An Bras Dermatol. 2009;84(3):263-9.

Mansur JS, Breder MN, Mansur MC, Azulay RD. Determination of sun protection factor by spectrophotometry. An Bras Dermatol. 1986;61:121-24.

Nash JF, Tanner PR. Relevance of UV filter/sunscreen product photostability to human safety. Photodermatol Photoimmunol Photomed. 2014;30(2-3):88-95.

Perdiz D, Gróf P, Mezzina M, Nikaido O, Moustacchi E, Sage E. Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. Possible role of Dewar photoproducts in solar mutagenesis. J Biol Chem. 2000;275(35):26732-42.

Polonini HC, Dias RM, Souza IO, Gonçalves KM, Gomes TB, Raposo NR, et al. Quinolines derivatives as novel sunscreening agents. Bioorg Med Chem Lett. 2013;23(16):4506-10.

Rastogi SC. UV filters in sunscreen products - a survey. Contact Dermatitis. 2002;46(6):348-51.

Rodembusch FS, Leusin FP, Campo LF, Stefani V. Excited state intramolecular proton transfer in amino 2-(2'-hydroxyphenyl) benzazole derivatives: Effects of the solvent and the amino group position. J Lumin. 2007;126(2):728-34.

Sayre RM, Agin PP, LeVee GJ, Marlowe E. A comparison of *in vivo* and *in vitro* testing of sunscreening formulas. Photochem Photobiol. 1979;29(3):559-66.

Schuch AP, Lago JC, Yagural T, Menckl CFM. DNA Dosimetry assessment for sunscreen genotoxic photoprotection. PLoS ONE. 2012;7(6):1-8.

Sohn M, Herzog B, Osterwalder U, Imanidis G. Calculation of the sun protection factor of sunscreens with different vehicles using measured film thickness distribution - Comparison with the SPF *in vitro*. J Photochem Photobiol B. 2016;159:74-81.

Stiefel C, Schwack W. Photoprotection in changing times – UV filter efficacy and safety, sensitization processes and regulatory aspects. Int J Cosmet Sci. 2015;37(1):2-30.

Wang SQ, Tanner PR, Lim HW, Nash JF. The evolution of sunscreen products in the United States - a 12-year cross sectional study. Photochem Photobiol Sci. 2013;12(1):197-202.

Yang SI, Liu S, Brooks GJ, Lanctot Y, Gruber JV. Reliable and simple spectrophotometric determination of sun protection factor: A case study using organic UV filter-based sunscreen products. J Cosmet Dermatol. 2018;17(3):518-22.

Zaratti F, Piacentini RD, Guillén HA, Cabrera SH, Lileye JB, McKenzie RL. Proposal for a modification of the UVI risk scale. Photochem Photobiol Sci. 2014;13(7):980-85.

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