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Article

In vitro Photoprotective Evaluation and Development of Novel Nanoemulsion with Chromone Derivative

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Chromone derivatives exhibiting high absorbance values in the UVA/UVB region were synthesized, and their photoprotective properties were evaluated. Chromones were prepared according to known literature procedures and characterized by high resolution mass spectrometry, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The *in vitro* solar protection factor (SPF) was determined by the Mansur method and cytotoxicity was evaluated using the sulforhodamine B assay. Two of the chromones synthesized demonstrated suitable SPF values and displayed no cytotoxic effect towards MRC-5 human fibroblasts at the tested concentrations, indicating great potential for future *in vivo* assays and clinical trials. Finally, the lead compound was incorporated into a nanoemulsion. Nanoemulsions showed high droplet size homogeneity and excellent stability. Chromones bearing methoxy substituents were found to be the most promising compounds with ideal photoprotective properties desirable for utilization and incorporation in sunscreen formulations.

Keywords: Mansur, chromone, organic UV filters, cytotoxicity, nanoemulsion

Introduction

Exposure to sun light and its UV radiation[1,2] is a contributing factor to developing cutaneous malignant melanoma which is one of the fastest growing types of cancer cases.[1] UVB radiation causes the formation of reactive oxygen species (ROS) in the skin and increases oxidative stress, resulting in destructive damage and loss of cellular function. In addition, UVB induces genetic mutation, inflammation, and skin cancer.[3] Sunscreens contain either UV blockers or filters, which have the ability to block or absorb solar radiation. Scientists and cosmetic companies are continually working to develop new UV filters with high stability under ultraviolet (UV) exposure that can be incorporated into sunscreens.[4] UV filter benzophenone-3, also known as oxybenzone, is commonly used in sunscreen products and is suspected to act as a weak estrogen. Studies revealed that oxybenzone caused increased uterine weight in rat researches and increased proliferation of human breast cancer cells *in vitro*.[5] Due to these limitations, studies have been carried out towards the development of new compounds with photoprotective applications. For example, octocrylene analogues, bis(indolyl)methane derivatives, vanillin derivatives, bile acids/azastilbenes conjugates, *N*-acyl hydrazone compounds, benzophenone derivatives and quercetin derivatives have all been

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investigated as potential alternatives.[6-14] *In vitro* methods used for the evaluation and development of new active ingredients are currently accepted and present advantages to *in vivo* methods such as reproducibility, simplicity and no need for human subjects. The photoprotective activity is expressed as its sun protection factor (SPF) and is a convenient way of correlating the dose of sun exposure with the concentration of the photoprotective product applied without the occurrence of erythema.[6] Sunscreens can differ in their pharmaceutical forms and may be purchased as: oils, gels, emulsions, among others.[15] The growth in interest for the development of cosmetic nanoemulsions for application in topical cosmetic products is due to their ability to allow controlled delivery and optimized dispersion of active ingredients into the desired layers of the skin such as the epidermis. The active ingredients in the form of nanoparticles have a high surface-to-volume ratio, which promotes dispersibility in the emulsion, and they are therefore better adapted for multiple functions.[16] This technology has found its way into commercial preparations of cosmetic products. Chromones represent a class of natural products that have displayed a variety of biological activities, including antifungal, anti-microbial, anti-allergenic, antitubulin, antiviral, anti-hypertensive, antitumoral activities[6-9] but up until now, they have not been explored for their photoprotective properties. The aim of this research therefore was to synthetize and evaluate the potential of chromones for innovative sunscreens and to determine their sun protection factor (SPF).

Experimental

Reagents and solvents (Sigma-Aldrich, St. Louis, USA) were used without further purification and were purchased from commercial suppliers. The melting points were valued on the Büchi Melting Point B-540 (Merck KGaA, Darmstadt, Germany). The nuclear magnetic resonance (NMR) spectra were acquired on a 400 MHz NMR, Nuclear Bruker Ascend 400 Instrument (Billerica, Massachusetts, USA). ^{1}H and ^{13}C NMR spectra of these compounds are available in the Supplementary Information (SI) section. Infrared spectra were obtained on a Thermo Scientific Nicolet 380 FT-IR apparatus (600-4000 cm^{-1}, Nicolet Instrument Corp., Madison, WI, USA) using the potassium bromide (KBr, spectroscopic grade) disc method. The *in vitro* solar protection factor (SPF) and photostability were determined by the spectrophotometric method developed by Mansur *et al.*[17] The UV-Vis ultraviolet readings were determined on the Thermo Scientific Genesys 10S spectrophotometer (Waltham, Massachusetts, USA). The average size, polydispersity index and zeta potential of the nanoemulsions were determined by photon correlation spectroscopy and microelectrophoresis associated with laser-Doppler anemometry on the Zetasizer (Malvern, model Zetasizer Nano series-Nano ZS, Malvern, United Kingdom). High resolution mass spectra (HRMS) were acquired using a LCMS-Q-ORBITRAP (Thermo Scientific, Fair Lawn, NJ, USA) mass spectrometer and the samples were solubilized in acetonitrile. The high-performance liquid chromatography (HPLC) was bypassed and the samples were directly injected into the mass spectrometer.

Typical procedure for synthesis of esters (**1a-1c**)

In a round bottom flask (50 mL), 5 mL of pyridine, 2.5 mL (1.77 mmol) of 2-hydroxyacetophenone and 2.0 mL (1.42 mmol) of the requisite benzoyl chloride were added. The reactants were stirred for approximately 15 min at 25 °C. After this time, 1 M HCl (60 mL) was added followed by crushed ice. Next, the solid was filtered and the precipitate was washed with methanol and water (1:1). The powder was recrystallized with methanol to provide white crystals. For esters **1a**-**1c**, the NMR spectra were compared with values reported in the literature.[18,19]

2-Acetylphenyl benzoate (**1a**)

Product yield 71%; mp 86-87 °C (lit 87-88 °C);[20] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (s, 3H), 7.23 (d, 1H, *J* 8.0 Hz), 7.34 (t, 1H, *J* 7.5 Hz), 7.55-7.60 (m, 4H), 7.86 (dd, 1H, *J* 8.0 and 1.5 Hz), 8.21-8.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 123.9, 126.2, 128.7, 129.3, 130.3, 130.4, 131.4, 133.5, 133.9, 149.4, 165.2, 197.6.

2-Acetylphenyl-3,4-dimethoxybenzoate (**1b**)

Product yield 86%; mp 129-131 °C (lit 129-131 °C);[21] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H), 3.98 (s, 6H), 6.98-7.91 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 56.0, 110.5, 112.5, 121.6, 123.9, 130.2, 148.9, 153.8, 164.9, 197.7.

2-Acetylphenyl-4-nitrobenzoate (**1c**)

Product yield 85%; mp 91-92 °C (lit 93-95 °C);[22] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (s, 3H), 7.27 (d, 1H, *J* 9.0 Hz), 7.46 (t, 1H, *J* 7.7 Hz), 7.66 (t, 1H, *J* 7.6 Hz), 7.93 (d, 1H, *J* 7.8 Hz), 8.38-8.42 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 123.8, 126.7, 130.8, 133.9, 148.9, 150.9, 163.6, 197.3.

Typical procedure for synthesis of diketones (**2a-2c**)

In a one necked round bottom flask (50 mL), 0.470 g (1.56 mmol) of ester **1a**-**1c**, 1.10 mL of pyridine and

0.175 g (3.13 mmol) of KOH were added. The mixture was maintained under stirring for approximately 15 min at 50 °C. Upon completion, the reaction was cooled in an ice bath and acetic acid 10% was added resulting in the precipitation of a yellow solid. The solid was filtered and washed with cold ethanol and recrystallized with 70% aqueous ethanol to afford a yellow powder. Diketones **2a-2c** were confirmed by comparison of their melting points to the literature values. NMR spectra was not carried out due to the keto-enol tautomerism that occurs readily in $CDCl_3$.

1-(2-Hydroxyphenyl)-3-phenylpropane-1,3-dione (**2a**)

Product yield 77%; mp 121-122 °C (lit 118-120 °C).[23]

1-(3,4-Dimethoxyphenyl)-3-(2-hydroxyphenyl)propane-1,3-dione (**2b**)

Product yield 89%; mp 130-131 °C (lit 129-130 °C).[24]

1-(4-Nitrophenyl)-3-(2-hydroxyphenyl)propane-1,3-dione (**2c**)

Product yield 42%; mp 197-198 °C (lit 201-203 °C).[25]

Typical procedure for synthesis of chromones (**3a-3c**)

In a one necked round bottom flask (50 mL) equipped with stir bar and reflux condenser was added 2 mmol of diketone **2a-2c**, 2.4 mmol of sodium acetate and 7 mL acetic anhydride. The mixture was stirred for 60 min at 140 °C. Once accomplished, the reaction solution was poured in water and the precipitate formed was filtered and recrystallized with aqueous ethanol to provide 3-benzoylchromone derivatives **3a-3c** as white solids.

3-Benzoyl-2-methyl-4*H*-chromen-4-one (**3a**)

Product yield 16.6%; mp 118-119 °C; UV-Vis (methanol) λ / nm 365; IR (KBr) ν / cm^{-1} 3000, 1700, 1650, 1500, 1450; ^1H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, H12), 7.39-7.61 (m, 3H), 7.68 (t, 2H, *J* 8.4 Hz), 7.71 (t, 1H, *J* 8.4 Hz), 7.90 (d, 2H, *J* 7.6 Hz), 8.16 (d, 1H, *J* 7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7, 117.9, 123.1, 125.5, 128.7, 130.0, 133.8, 136.9, 155.9, 165.4, 175.9, 193.9, according to Figures S1 and S2 (SI section); HRMS electrospray ionization time-of-flight (ESI-TOF) *m/z*, calcd. for $C_{17}H_{13}O_3$ $[M + H]^+$: 265.0859, found: 265.0851.

3-(3,4-Dimethoxybenzoyl)-2-methyl-4*H*-chromen-4-one (**3b**)

Product yield 77%; mp 182-183 °C; UV-Vis (methanol) λ / nm 365; IR (KBr) ν / cm^{-1} 3000, 1700, 1650, 1500, 1450, 1250, 1000; ^1H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H), 3.99 (s, 6H), 7.01 (d, 2H, *J* 8.5 Hz) 7.25 (d, 1H, *J* 8.7 Hz), 7.37 (t, 1H, *J* 8.4 Hz), 7.61 (m, 2H), 7.70 (d, 1H, *J* 2.0 Hz), 7.9 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 56.1, 110.5, 112.4, 121.6, 123.9, 126.1, 130.2, 131.4, 133.4, 148.9, 149.5, 153.8, 164.9, 197.8, according to Figures S3 and S4 (SI section); HRMS (ESI-TOF) *m/z*, calcd. for $C_{19}H_{17}O_5$ $[M + H]^+$: 325.1071, found: 325.1066.

2-Methyl-3-(4-nitrobenzoyl)-4*H*-chromen-4-one (**3c**)

Product yield 95%; mp 198-199 °C (lit 202-204 °C);[25] UV-Vis (methanol) λ / nm 365; IR (KBr) ν / cm^{-1} 3000, 1700, 1650, 1500, 1450, 1400, 880; ^1H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 7.46 (t, 1H, *J* 8.0 Hz), 7.53 (d, 1H, *J* 9.0 Hz), 7.75 (m, 2H), 8.03 (d, 2H, *J* 9.0 Hz), 8.16 (dd, 1H, *J* 8.0 and *J* 1.5 Hz), 8.32 (d, 2H, *J* 8.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.4, 117.9, 122.0, 123.4, 123.9, 125.9, 126.0, 130.0, 134.6, 142.1, 150.4, 155.9, 167.9, 175.9, 192.8, according to Figures S5 and S6 (SI section); HRMS (ESI-TOF) *m/z*, calcd. for $C_{17}H_{12}NO_5$ $[M + H]^+$: 310.0710, found: 310.0708.

Log P-octanol-water partition coefficient

The log P octanol-water partition coefficient was calculated using the online Molinspiration program Interactive log P calculator (software version v2015.01).[26]

Determination of absorbance maximum of the compounds (**3a-3c**)

For the determination of absorbance in the UVA and UVB region, 10 mg of chromone derivative was diluted in 10 mL methanol, resulting a concentration of 1 mg mL^{-1} that was diluted in methanol again to afford diluted concentrations of 0.025, 0.030, 0.050, 0.070 and 0.1 mg mL^{-1}, according to Figures S7 and S8 (SI section). The scanning was performed for each concentration between the wavelengths of 200 and 600 nm in the UV spectrophotometer, using quartz bucket with an optical way of 1 cm. Methanol was used as white and the experiment was carried out in triplicate.[13,14,17]

In vitro determination of the sun protection factor (SPF) of compounds **3a-3c**

The photoprotection afforded by topical sunscreens against solar ultraviolet radiation exposure can be determined *in vivo* or *in vitro*. *In vitro* methods involve the measurement of absorption or the transmission of UV radiation through sunscreens and are determined based on spectrophotometric analysis of dilute solutions.[13,14,17] Mansur *et al.*[17] developed a very simple mathematical

equation, utilizing UV spectrophotometry and the equation S1 (SI section). Spectrophotometric readings were made in the diluted solutions (0.025, 0.030, 0.050, 0.070 and 0.1 mg mL^{-1}) at wavelengths of 290, 295, 300, 305, 310, 315 and 320 nm. The determined absorbances were then applied to the Mansur method for the respective conversions to SPF using equation S1. The SPF was calculated for each concentration by the spectrophotometric method developed by Mansur *et al.*[17]

The photostability test

The photostability was performed using a light chamber with a UVB lamp source at 365 nm. Solutions of 0.07 mg mL^{-1} chromone (**3a-3c**) were prepared in methanol and placed in volumetric flasks without a cap and exposed to radiation for total time of 1 h and 15 min, and evaluated at 15 min intervals upon exposure to UV radiation. The system was open during the irradiation.[27]

Preparation of nanoemulsions

The nanoemulsions were prepared according to the emulsion phase inversion (EPI) method.[28] To obtain the formulations, the distilled water and the oily phase were heated separately at 75 ± 2 °C. The water was slowly poured into the oily phase and the system was maintained under constant stirring at a speed of 500 rpm. Stirring was preformed until a temperature of 25 ± 2 °C was reached. After 24 h, all formulations were macroscopically evaluated. The compositions tested for nanoemulsions are described in Table 1.

Cell viability

Human fibroblasts MRC-5 cells, cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium, were distributed in 96-well microtiter plate using a density of 5×10^4 cell *per* well and after they were incubated at 37 °C with 5% of CO_2 for 24 h. Cells were treated with the sample dissolved in RPMI 2% dimethyl sulfoxide (DMSO), at concentrations ranging from 1000 to 62.5 μg mL^{-1}. The cell viability was evaluated using the sulforhodamine B assay (SRB).[28] After 24 h incubation, the media was removed and cells were fixed with cold 20% trichloroacetic acid for 1 h at 4 °C. The microtiter plate was washed with distilled water and dried. Thereafter, fixed cells were stained for 30 min with 0.1% SRB dissolved in 1% acetic acid. The plate washed again with 1% acetic acid, again allowed to dry and 200 μL of 10 mM TRIS buffer (pH 10.5) were added to stain solubilization at room temperature for about 30 min. Samples absorbance was read in the spectrophotometer (490 nm) and the data were presented as the percentage of viable cells over untreated cells.[28]

Statistical analysis

The results were statistically analyzed by the one-way analysis of variance (ANOVA) test[29] and multiple comparison by Tukey considering a significance level of 0.05 ($p < 0.05$). All statistical analysis was made using Graph Pad Prism 5.0 software.[30]

Results and Discussion

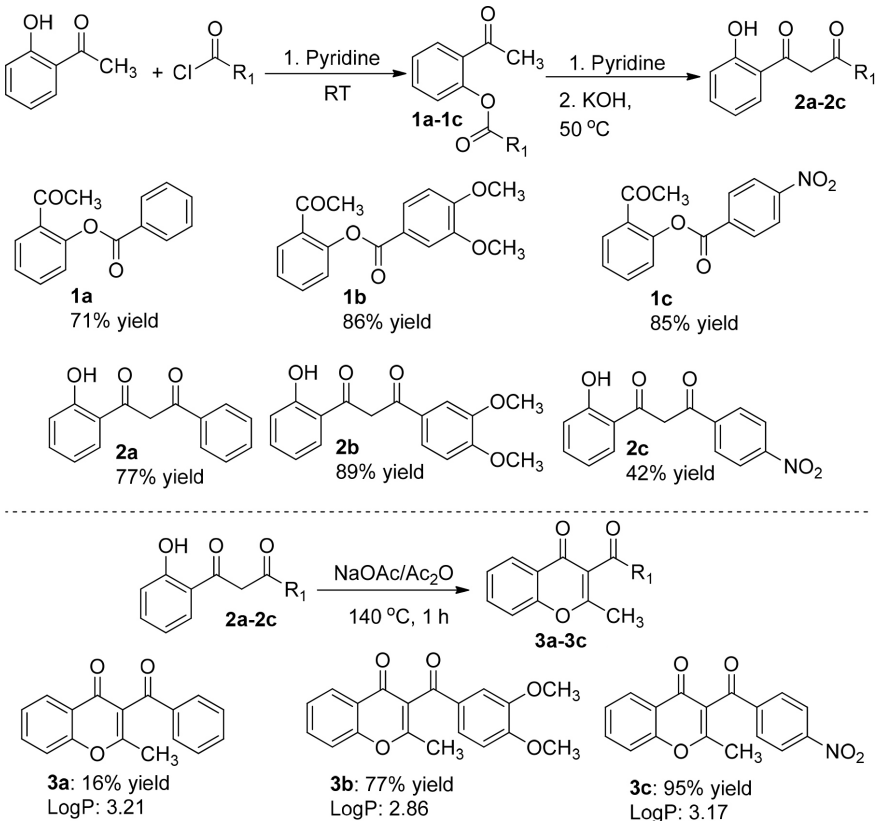
Synthesis of compounds **3a-3c**

Esterification of 2-hydroxyacetophenone with benzoyl chlorides initially yielded intermediates **1a-1c**, which were then subjected to a Baker-Venkataraman rearrangement in pyridine and potassium hydroxide to afford the expected 1,3-diketones **2a-2c** (Scheme 1). Comparison of NMR spectral data and melting point values with literature values (see Experimental section) confirmed the identity of esters **1a-1c** and 1,3-diketones **2a-2c** which are all known compounds. The reaction of 1,3-diketones **2a-2c** with acetic anhydride and sodium acetate provided 3-benzoylchromone derivatives **3a-3c**. Only chromone **3b** is a novel compound. Products **3a-3c** were characterized by high resolution mass spectrometry, Fourier transform infrared (FTIR) and ^1H and ^{13}C nuclear magnetic resonance spectroscopy. In the case of the FTIR analysis, the absorption band at

Table 1. Compositions tested to obtain nanoemulsions with compound **3a**-**3c** and standards

	Nanoemulsion with **3a** / %	Nanoemulsion with **3b** / %	Nanoemulsion with S1 / %	Nanoemulsion with S2 / %	Phase
Span 120-LQ-(MH)	4	4	4	4	O
Ultramona RH 400	6	6	6	6	O
Licuri oil	10	10	10	10	O
Distilled water	q.s.p	q.s.p	q.s.p	q.s.p	A

q.s.p: amount sufficient to complete 100 mL; O: oily: A: aqueous; S1 (standard 1): Neo Heliopan®: S2 (standard 2): benzophenone-3.



Scheme 1. Synthesis of chromone derivatives: **1a-1c** pyridine, room temperature, 15 min; **2a-2c** pyridine, KOH, 50 °C, 15 min; **3a-3c** NaOAc/Ac_2O, 140 °C, 1 h.

1700 cm^{-1} indicated the presence of a conjugated ketone and IR bands ca. 3000, 1650, 1500 and 1450 cm^{-1}, were attributed to the C–H bond and C=C of the benzenoid ring. The proton NMR spectra displayed characteristic singlet resonance signals corresponding to the methyl group at approximately 2.5 ppm. In the ^{13}C spectrum, the chemical shift for the methyl carbon was recorded at ca. 30 ppm. The lipophilicity of the 3-benzoylchromone derivatives **3a-3c** is important for gauging how well these compounds will be absorbed by the skin. The Log P values for each compound are presented in Scheme 1. The Log P of **3a** is 3.21, **3b** is 2.86 and **3c** is 3.17. A Log P > 1 is indicative of lipophilic character and for a Log P < 1, the molecule is considered to have more hydrophilic character. Interestingly, oxybenzone has been confirmed by *in vivo* studies to be absorbed transdermally[13,14] and the Log P for this compound was calculated to be 3.37 which was very similar to 3-benzoylchromones described in this study. The Log P octanol-water partition coefficient was calculated using Molinspiration Interactive Log P calculator.[26]

UV-Vis absorption spectra of the compounds in solution

The absorption spectra of compounds **3a-3c** in solution are shown in Figure 1. Compounds **3a-3c** exhibited high absorbance in the UVC, UVB and UVA region, with absorption maxima at around 230 nm (UVC) and shoulders at about 300 nm (UVB and UVA), suggesting that the compounds are potential candidates for UV photoprotection products, since a substance to be applied in a sunscreen formulation should present a broad spectrum UVC, UVB and UVA. Using the Beer-Lambert Law, A (λ) = ε (λ)lc, where A is the absorbance, c is the concentration in mol L^{-1} and l is the path length in cm, the maxima molar absorption coefficients (ε) of the compounds **3a**, **3b** and **3c** were found

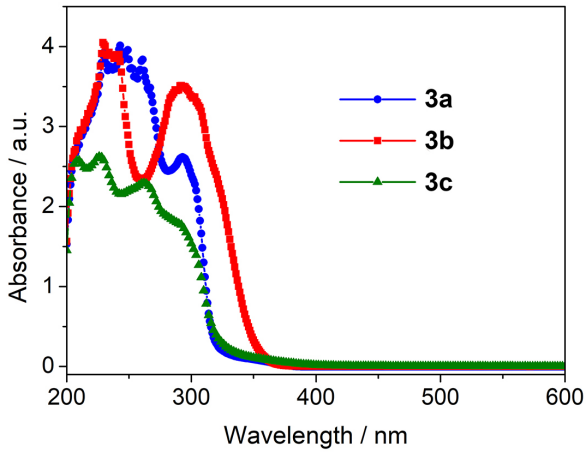


Figure 1. UV-Vis absorption spectra of the compounds **3a-3c** in solution.

to be 1.0×10^4 (ε_1 in 240 nm), 1.3×10^4 (ε_2 in 230 nm) and 0.8×10^4 (ε_3 in 225 nm) L mol^{-1} cm^{-1}, respectively.[13,14] Over the years, the development of ideal photoprotectors to obtain safe and effective formulations included those that provide broad UV protection.[31,32]

In vitro determination of the sun protection factor (SPF)

The SPF was evaluated by the Mansur method using a UV spectrophotometer. The values of the acquired absorbance values were placed in equation S1 and used to calculate the SPF values which are presented in Table 2. Neo Heliopan® and benzophenone-3 have been used as positive controls. According to Table 2, compounds **3a** and **3b** showed higher SPF values than benzophenone-3 at the tested concentrations. The SPF of benzophenone-3 (S2) at a concentration of 0.1 mg mL^{-1} was reported to be 19.731.[13,14] By analysis of the obtained data, we verified that compounds **3a** and **3b** presented SPF proportional to the analyzed concentrations. The results are promising and suggest that these are viable candidates to act as a UV filter in sunscreen formulations.

Evaluation of photostability

Compounds **3a-3c** were analyzed separately and in triplicate at room temperature in the photostability assay. The samples were dissolved in methanol, resulting in solutions at concentration of 0.1 mg mL^{-1} before being exposed to UV radiation (at 365 nm) for 1 h and 20 min. The temperature of the experiment was constant over the total period of the study. Figure 2 shows the behavior of the compounds before and after their exposure to UV radiation for different time. The inserts of Figure 2 show the intensity of absorption of the compounds *versus* exposure time to UV radiation. Compounds **3a** and **3b** showed the smallest variation of absorbance during the exposure time to UV radiation. Compound **3c** showed a decrease in absorbance over time and indicating possible degradation under UV exposure.

Cell viability

Compounds **3a** and **3b** displayed no cytotoxic effect towards MRC-5 human fibroblasts at tested concentrations, according to ISO10993-5:2009.[33] On the other hand, compound **3c** exhibited higher toxicity, since at its lowest concentration **3c** was above 70% viability (Figure 3). Therefore, these results indicate safety of compounds **3a** and **3b** at the concentration used to determine its SPF value (100 μg mL^{-1}), but compound **3c** was, in contrast, toxic at this concentration. In view of these cytotoxicity results, along with the SPF values (Table 2), compound **3c** has the least potential for further applications and compounds **3a** and **3b** were therefore selected for the development of nanoemulsions.

Nanoemulsions

Macroscopically, after 24 h of preparation, the nanoemulsions remained stable and with a slightly milky appearance, except the S2 standard, which had a milkier appearance, according to Figure S9 (SI section). As can be seen in Table 3, nanoemulsions containing compounds **3a**, **3b** and standard S1 showed an average droplet size of less than 150 nm and the nanoemulsion containing the standard S2 had a size of 433.70 ± 46.36 nm. The average droplet size values obtained are in accordance with the size range established for nanoemulsions.[34] The polydispersity index (PI) values of nanoemulsions **3a**, **3b** and S1 were lower than 0.3, considering monodispersed,[34] which showed high droplets size homogeneity. The droplet size of an emulsion depends on the emulsification method used.[35] The results demonstrated the efficiency of the phase inversion emulsification method in obtaining nanoemulsions with compounds **3a** and **3b**.

The small size of droplets present in a nanoemulsion stabilizes it against gravitational phase separation and flocculation,[36] besides allowing a more uniform deposition on the skin surface,[36] promoting the formation

Table 2. SPF compounds **3a**-**3c** and standards

Concentration / (mg mL^{-1})	SPF-UVB compounds				
	3a	**3b**	**3c**	S1	S2
0.025	10.728 ± 0.004^a	11.035 ± 0.012^b	5.892 ± 0.010^c	34.912 ± 0.063^d	10.774 ± 0.049^a
0.030	11.787 ± 0.004^a	10.157 ± 0.018^b	8.856 ± 0.003^c	35.264 ± 0.053^d	11.607 ± 0.150^a
0.050	14.053 ± 0.015^a	22.515 ± 0.007^b	11.692 ± 0.006^c	36.027 ± 0.079^d	15.533 ± 0.001^e
0.070	20.312 ± 0.003^a	26.528 ± 0.008^b	14.157 ± 0.011^c	36.492 ± 0.059^d	17.596 ± 0.004^e
0.100	27.436 ± 0.032^a	28.844 ± 0.044^b	16.775 ± 0.002^c	36.817 ± 0.090^d	19.731 ± 0.006^e

$^{a-e}$Indicate $p < 0.05$ at the same line by one-way ANOVA test; SPF: solar protection factor; chromones: **3a**-**3c**; S1 (standard 1): Neo Heliopan®; S2 (standard 2): benzophenone-3; results expressed as mean ± standard deviation.

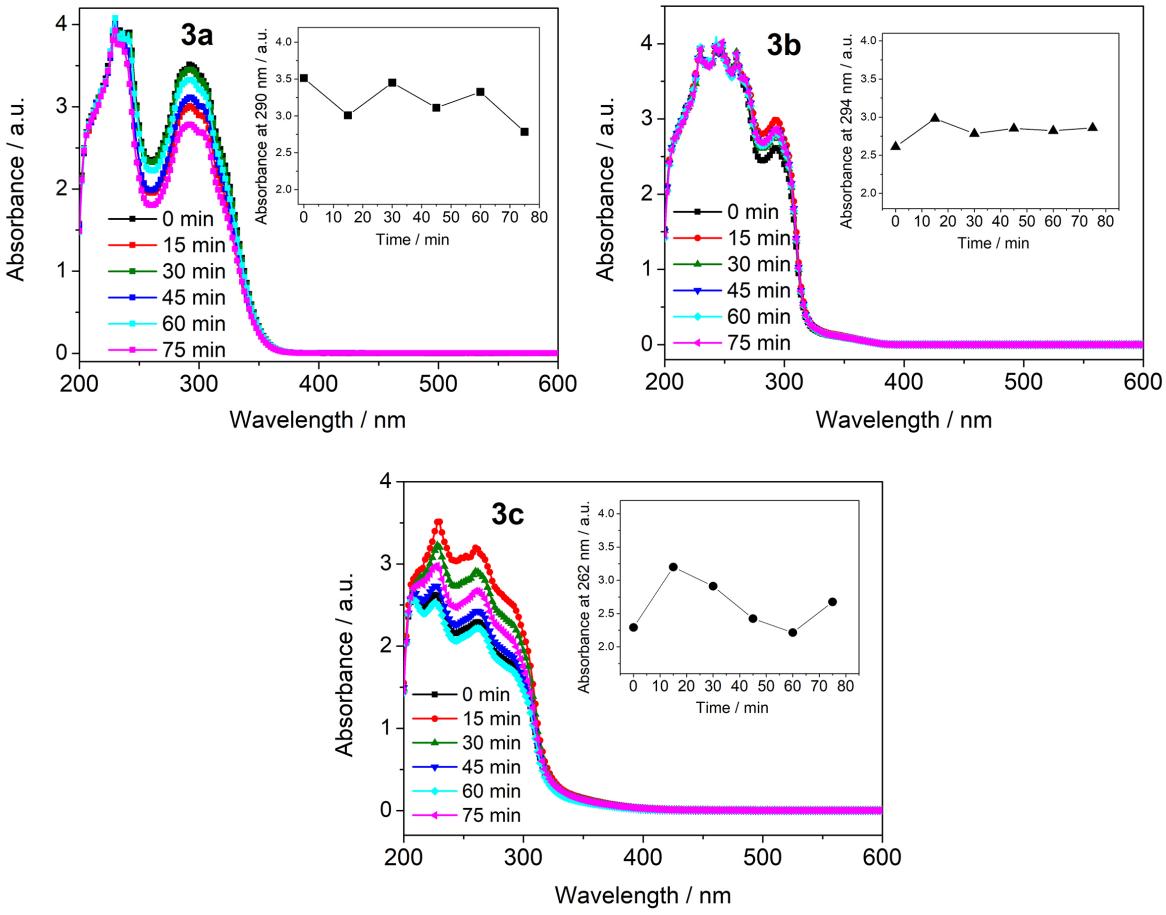


Figure 2. Graphs of photostability of the compounds **3a-3c** after exposure to UV radiation in different times. The inserts show the intensity of absorption *versus* exposure time to UV radiation.

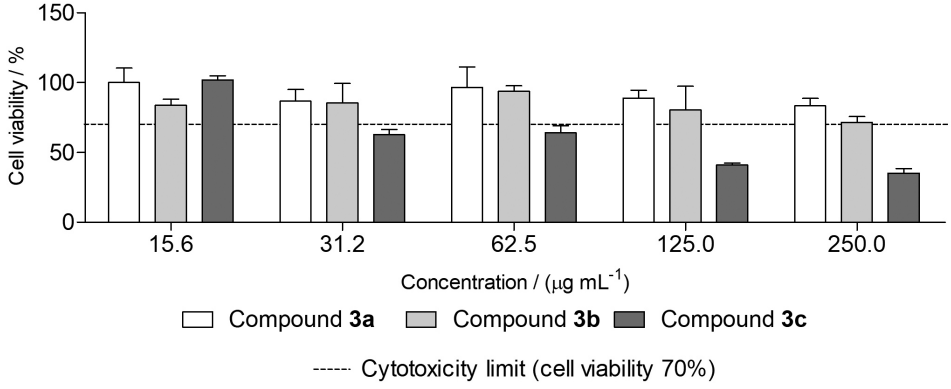


Figure 3. Cytotoxicity of the compounds **3a-3c**.

of a continuous film, and consequently, improving the effectiveness of the nanoemulsion in protecting against UVA and UVB rays.[14]

The zeta potential (surface charge) of a nanoformulation can interfere with its stability. If the value of the zeta potential is relatively high (> 30 mV in modulus), the repulsive forces exceed the attractive forces of van der Waals, culminating in obtaining an electrostatically stable system. Thus, a greater electrostatic repulsion between the nanoparticles reduces the probability of formation of aggregates, preventing the flocculation of the nanoemulsion from surface.[37] All nanoemulsions produced had zeta potential values greater than –30 mV, which contributes to maintaining the stability of these systems. After 24 h, the two nanoemulsions were read to analyze the SPF using the Mansur formula, as shown in Table 4.

The data in Table 4 leads us to conclude that among the chromone derivatives investigated, the most promising

Table 3. Average size, polydispersity index and zeta potential of nanoemulsions with compound **3a**, compound **3b** and standards

Nanoemulsion	Average size ± SD / nm	PI ± SD	Zeta potential ± SD / mV
3a	144.40 ± 0.31	0.28 ± 0.03	−36.00 ± 1.16
3b	148.00 ± 0.70	0.26 ± 0.02	−36.70 ± 1.50
S1	135.60 ± 1.57	0.20 ± 0.01	−30.90 ± 1.94
S2	433.70 ± 46.36	0.57 ± 0.04	−35.70 ± 1.85

SD: standard deviation; PI: polydispersity index; chromones: **3a** and **3b**; S1 (standard 1): Neo Heliopan®; S2 (standard 2): benzophenone-3.

Table 4. SPF of the nanoemulsions of compounds and standards

Concentration / (mg mL^{-1})	Nanoemulsion			
	S1	S2	**3a**	**3b**
0.025	36.348 ± 0.001^{a}	11.130 ± 0.008^{b}	12.894 ± 0.001^{c}	13.889 ± 0.001^{d}
0.030	36.917 ± 0.001^{a}	13.118 ± 0.001^{b}	19.561 ± 0.001^{c}	23.745 ± 0.051^{d}
0.050	38.508 ± 0.002^{a}	13.343 ± 0.001^{b}	24.560 ± 0.001^{c}	27.882 ± 0.037^{d}
0.070	43.492 ± 0.001^{a}	20.769 ± 0.001^{b}	35.439 ± 0.006^{c}	37.938 ± 0.003^{d}
0.100	45.115 ± 0.002^{a}	22.954 ± 0.004^{b}	37.98 ± 0.001^{c}	38.555 ± 0.002^{d}

Chromones: **3a** and **3b**; S1 (standard 1): Neo Heliopan®; S2 (standard 2): benzophenone-3. Results expressed as mean ± standard deviation; different letters indicate $p < 0.05$ at the same line by one-way ANOVA test.

was the nanoemulsion containing **3b**. At the highest concentration of 0.1 mg mL^{-1}, **3a** has an SPF of 37.98 and the **3b** has an SPF of 38.55.

Conclusions

The photoprotection study performed according to the Mansur methodology showed promising applications for compound **3b**, which presented SPF values similar to benzophenone-3. In addition, the photostability test suggests that compound **3b** is stable with only a small drop in its absorption in the first 15 min of exposure to UV light. 3-Benzoylchromone derivatives **3a** and **3b** are promising compounds that can be incorporated into formulations of sunscreens. Chromone **3b**, as well as compound **3a**, displayed no cytotoxic effect to MRC-5 human fibroblasts at tested concentrations, indicating a great potential for clinical trials. Finally, compounds **3a** and **3b** were incorporated into nanoemulsions that were stable, with a small average droplet size and high SPF value, being strong candidates for the development of sunscreens formulations.

Supplementary Information

NMR spectra used in the characterization of the compounds are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Amanda S. Antunes, Ricardo G. Penido, Ana Paula Gouveia and Gabriela M. Diogo were responsible for data curation, investigation, synthesis and characterization of organic compounds; Jason G. Taylor for conceptualization, data curation, investigation, validation; Orlando D. H. dos Santos and Fernanda B. Perasoli for formal analysis funding acquisition and validation; Paula M. A. Vieira, Lucas R. D. Sousa, Tatiane R. Amparo for data curation and resources; Thiago Cazati for data curation, formal analysis funding acquisition, investigation, resources, validation and writing original draft; Viviane M. R. dos Santos for investigation, project administration resources, writing original draft and writing-review and editing.

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