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Photoprotective activity of resveratrol analogues

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ABSTRACT

Resveratrol is a promising agent for protecting human skin from UV radiation and to reduce the occurrence of cutaneous malignancies. We describe the photoprotective activity of six resveratrol analogues using the diffuse transmittance technique to determine the SPF and the protection against UVA radiation. The analogues presented a varied profile of photoprotection, the SPF ranging from 2 to 10 and the UVAPF from 0 to 9. Among the six compounds tested, the protection against UVB sunrays provided by compound **B** was more significant than the protection provided by resveratrol; compounds **C**, **D**, **E** and **F** show photoprotection similar to resveratrol.

1. Introduction

Ultraviolet radiation (UVR) from the sun is known to be the main trigger for numerous pathological problems, notably skin cancer.[1] This fact becomes more relevant when one considers that this is the most common cancer diagnosed worldwide, and it can evolve into its most serious complication, the melanoma, which has a high mortality rate.[2]

UVR can be divided into three distinct regions: ultraviolet A (UVA, 315–400 nm), ultraviolet B (UVB, 280–315 nm) and ultraviolet C (UVC, 100–280 nm). It is currently known that both UVA and UVB are linked to cancer pathogenesis, because of the damage to cellular DNA they can cause, either alone or synergistically.[3,4] In fact, it is estimated that at minimum 10% of all new cancer cases would be prevented if people made proper and continuous use of sunscreens.[1] Yet, 78% of non-melanoma skin cancers are preventable, when one uses these products correctly.[5]

The efficacy of such products is dependent on their capacity to absorb radiant energy, which is proportional to the concentration of active absorbing molecules, and to the absorption range of the wavelength within which their maximum absorption occurs. When using a combination of UVA and UVB filters, broad-spectrum protection to the skin takes place.[6]

It is therefore essential to develop products that protect the human skin against UVR, both UVA and UVB. Resveratrol is an important antioxidant which shows a variable anticancer activity and suppresses, retards or reverses the deleterious effects of UV radiation.[7,8] Within this context, the present work aimed at developing six analogues, which could act as sunscreening agents/UV filters, and at determining their photoprotective activity.

2. Experimental

2.1. Chemicals

Analytical-grade glycerin (Vetec, Brazil) and ultrapure water (18.2 MΩ cm) obtained from an aquaMAX–Ultra 370 Series water purification system (YoungLin, Korea) were used throughout the analysis. Square-shaped (50 × 50 mm) polymethylmethacrylate (PMMA) Helioplate™ HD6 (HelioScreen, France) plates with roughened surface on one side (Sa ≈ 6 μm) were used as the substrate for the determination of photoprotection activity by diffuse transmittance spectrophotometry.

2.2. Equipments

The in vitro photoprotection experiments were conducted in an UV-2000S Ultraviolet Transmittance Analyzer (Labsphere, USA), composed of two photodiode array spectrographs and equipped with an integrating sphere and a xenon flashlamp, which emits a

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continuous spectrum of radiation with no peaks and supplies energy for a 290–450 nm spectral range, with a wavelength increment step of 1 nm, having low irradiance such that the photostability of the product is not unduly challenged.

A long-arc xenon SuntestTM insolator, type CPS+ (Atlas, Germany), filtered with its original UV short cut-off filter combined with a Special UV Glass filter (limiting radiation at approximately 290 nm), providing a VIS + UVA + UVB spectrum, was used as the artificial UV source for irradiation of the sunscreen samples. PMMA plates were supported firmly throughout the irradiation by a Sun-Tray holder which also provided a dark background behind each plate to reduce the risk of any back exposure.

An electronic analytical balance AY-220 (Shimadzu, Japan) and a positive-displacement manual pipette (Mettler Toledo, USA) were used for the preparation of the samples PMMA plates.

2.3. Samples

Six analogs of resveratrol were synthesized in the Laboratory of Chemistry of the Federal University of Juiz de Fora (Juiz de Fora, Minas Gerais, Brazil). As a reference standard, resveratrol (trans-resveratrol 99.0%, Gamma, Brazil) was used. The resveratrol analogues A–F were synthesized by the classical method of imine formation involving condensation between aromatic amine (2-aminophenol) with a variety of aromatic aldehydes in ethanol (Scheme 1). All compounds were characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), infrared (IR) and melting point (mp) (Table 1) and were in accordance with data in the literature.

The analogues were incorporated in a cosmetic neutral lotion at 15%, and then subjected to photoprotection assay.

2.4. Photoprotection assay

The samples were accurately and quickly weighed (to reduce product evaporation and dryness) to satisfy the application rate of 1.3 mg cm^{-2} in each PMMA plate (actual quantity applied: 32.5 mg, determined by weighing the plates before and immediately after applying the products). They were directly weighed on the plate surface, applied as a large number of small droplets of approximately equal mass, and distributed in an even manner on the roughened surface of the plate. Then, the products were spread over the whole surface with a fingertip covered with a vinyl glove and pre-saturated with the product, to prevent possible losses of the amount weighed. The spreading was achieved in two steps: (i) quick distribution of the product, without pressure (20–30 s); and (ii) rubbing it into the rough surface using pressure (20–30 s too). For each product, three plates were prepared, which were kept protected from light exposure in a dark chamber at room temperature (≈20 °C) for 15 min, in order to facilitate the formation of a standard stabilized sunscreen film.

After this period, the plates containing the product were placed in the light-path of the transmittance analyzer. The transmission of UV radiation through the sample was measured from 290 to 450 nm at 1 nm intervals on 9 different sites of each plate (total measurement area = 2.0 cm^2). The blank was prepared using the HD6 plates covered with 15-μL of glycerin, because of its non-fluorescence and UV transparency.

Table 1
Spectral data of resveratrol analogues

Compounds	δ *CH*=N	δ *C*=N	*rnu* C=N	Melting point (°C)	Yield (%)
A	8.90	157.18	1625.8	160.0–161.0	78.0
B	8.62	158.46	1622.0	90.1–92.0	66.0
C	8.96	160.77	1631.6	149.0–150.3	90.0
D	8.50	158.84	1614.3	118.0–119.0	50.0
E	8.69	159.73	1625.8	90.8–91.5	83.0
F	8.63	158.87	1623.9	118.7–119.6	72.0

* The NMR experiments were performed at 300 MHz for ^{1}H and 75 MHz for ^{13}C in dimethyl sulfoxide (DMSO-d_6) (ppm) and I.R. experiments were performed at KBr support (cm^{-1}). The ^{1}H NMR of compound **B** is available as Supplementary data, to confirm the purity of the material.

Using the generated data, $SPF_{in\ vitro}$ was calculated using Eq. 1:

$$SPF_{in\ vitro} = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)} \times d\lambda} \quad (1)$$

where $E(\lambda)$ is the erythema action spectrum,[10] $I(\lambda)$ is the spectral irradiance of the UV source, $A_0(\lambda)$ is the mean monochromatic absorbance measurements per plate of the test product layer before UV exposure, and $d\lambda$ is the wavelength step (1 nm).

In order to generate the UVAPF value, the coefficient of adjustment '*C*' was calculated as shown in Eq. 2 and using the SPF label as the value generated by the UV-2000's software.

$$SPF_{in\ vitro,adj} = \text{SPF label} = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)\times C} \times d\lambda} \quad (2)$$

Using the '*C*' value, initial UVAPF was calculated using Eq. 3, and the dose '*D*' of UV irradiation was determined by Eq. 4.

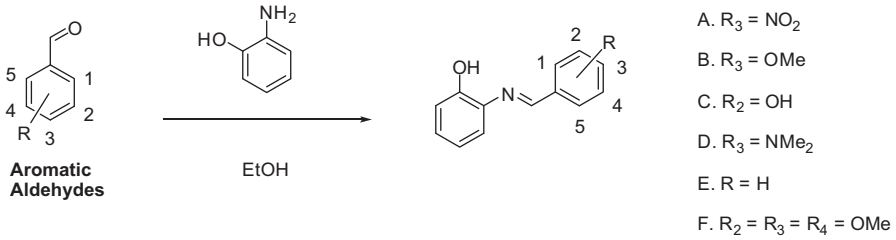
$$UVAPF_0 = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)\times C} \times d\lambda} \quad (3)$$

$$D = UVAPF_0 \times D_0 \quad (4)$$

where $P(\lambda)$ is the PPD action spectrum[10] and D_0 = 1.2 J cm^{-2}.

The plates were inserted into the UV irradiation source (temperature maintained below 40 °C) and then exposed to the calculated UV dose *D*. After that, new transmission measurements of the sunscreen samples were conducted, for acquisition of the second UV spectrum. The final UVAPF was calculated according to Eq. 5. If the coefficient of variation (CV) between the UVAPF's of the individual plates exceeded 20%, then further plates were measured until the CV threshold was achieved.

$$UVAPF = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times 10^{-A(\lambda)\times C} \times d\lambda} \quad (5)$$



Scheme 1. Synthetic pathway for resveratrol analogues.

where $A(\lambda)$ is the mean monochromatic absorbance of the test product layer after UV exposure.

For calculation of the critical wavelength value (λ_c), a series of absorbance values were calculated for each of the three separate plates to which the samples were applied. Absorbance at each wavelength increment $A(\lambda)$ was calculated using Eq. 6, and the λ_c using Eq. 7.

$$A_\lambda = \log(C_\lambda / P_\lambda) \tag{6}$$

where
$C_\lambda = \sqrt[n]{(C_\lambda[1] \times C_\lambda[2] \times \cdots \times C_\lambda[n])}$ and $P_\lambda = \sqrt[n]{(P_\lambda[1] \times P_\lambda[2] \times \cdots \times P_\lambda[n])}$.

$$\int_{290\text{nm}}^{\lambda c} A\lambda \times \mathrm{d}\lambda = 0.9 \int_{290\text{nm}}^{400\text{nm}} A\lambda \times \mathrm{d}\lambda \tag{7}$$

Finally, the UVA/UVB ratio was calculated as the ratio between the final UVAPF and the SPF label.

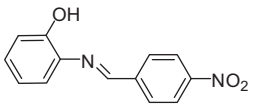
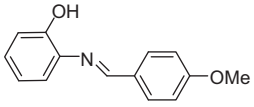
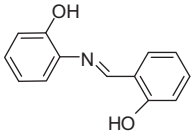
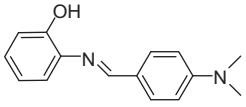
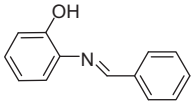
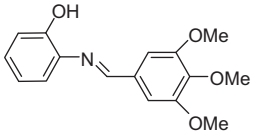
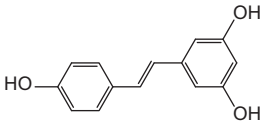
The verification of the validity of the results was obtained using the Cosmetics Europe Reference Sunscreen S2 (determined SPF = 18 ± 1.5, UVAPF = 12 ± 1.1, λ_c = 381 nm, and UVA/UVB ratio = 0.88). All results were expressed as a mean of 27 determinations (3 plates, 9 readings each, at different sites) for lotions containing 15% of each resveratrol analogue in isolation.

3. Results and discussion

Since the discovery of the UVR spectrum by Ritter, in 1801, and of the sunlight role on skin burn by Sir Everard Home, in 1820, many substances had their absorption potential for such radiation analyzed, in the hopes of identifying which ones could be used to minimize its harmful effects on the human skin. The first substance used with that purpose was the acidified quinine sulfate, by Widmark in 1889. The same substance was incorporated in lotions and ointments by Hammer in 1891, determining what today is considered the first chemical sunscreen in history.[11] Since then, several sunscreens were developed and commercially launched, evolving into the products currently available.

The protection level against solar radiations provided by sunscreens are currently represented by the Sun Protection Factor (SPF), which is understood as a measure of the UV solar energy required to produce sunburn on a skin area (generally on the back) protected with a sunscreen product, relative to the amount of solar energy required to produce the same sunburn on an unprotected skin area. Alongside the in vivo methods, several studies were and have been carried out in an attempt to elaborate and standardize an in vitro method for the determination of the SPF. In vitro photoprotection studies are currently considered of utmost importance to elucidate the effective performance of candidates for active sunscreen ingredients or final products against UVR, before the more expensive in vivo tests.[12] They are also a safety issue, as only positive in vitro responses validate the submission of products to in vivo experiments, virtually eliminating any risks of those tests to human volunteers.[13,14] In the present work, we used the diffuse transmittance in vitro technique to determine the photoprotection profile of six resveratrol analogues, because it can determine not only the SPF, but also the protection against UVA radiation (UVAPF), which is important since its role on the skin cancer pathogenesis is currently known.

Table 2
Photoprotection results of resveratrol analogues

Substance		SPF	UVAPF	λ_c (nm)	UVA/UVB ratio
Analogue A		2 ± 0.5	–	–	–
Analogue B		10 ± 0.2	9 ± 0.7	383	0.74
Analogue C		6 ± 0.7	6 ± 1.0	389	1.00
Analogue D		5 ± 0.3	8 ± 1.8	388	1.21
Analogue E		6 ± 1.1	6 ± 0.6	387	0.89
Analogue F		6 ± 1.1	6 ± 0.3	387	0.90
Resveratrol		7 ± 1.7	2 ± 0.2	362	0.46

SPF: Sun Protection Factor. UVAPF: UVA Protection Factor.

Table 3
Boots Star Rating criteria for classification of UVA protection in sunscreens

		Initial mean UVA/UVB ratio			
		0.0–0.59	0.6–0.79	0.8–0.89	0.89 and over
Post exposure mean UVA/UVB ratio	0.0–0.56	No rating	No rating	No rating	No rating
	0.57–0.75	No rating	* * *	* * *	* * *
	0.76–0.85	No rating	* * *	* * * *	* * * *
	0.86 and over	No rating	* * *	* * * *	* * * * *

Resveratrol was chosen as a model molecule as it is an important antioxidant that shows a variable anticancer activity, including on the skin cancer spectrum. Additionally, resveratrol suppresses, retards or reverses the deleterious effects of UV radiation.[7,8] Unfortunately, this substance shows problems such as instability as it converts to the cis-form (a less active form), particularly after exposure to UV light.[15–17] According to Walle and colleagues, methylation of the polyphenols effectively blocks metabolic conjugation reactions, thereby dramatically increasing stability.[17]

Based on: (i) the concept of bioisosterism,[19] the basic skeleton of trans-stilbene was modified by replacing the central C=C linkage with a C=N double bond; (ii) the knowledge that the products presented significant antioxidant activity in a previous work;[20] and (iii) the fact that resveratrol has activity as a skin anticancer agent, in this work we proposed six resveratrol analogues using hydroxyl at position 2 of the aromatic ring and several group substituents.

The six analogues presented a varied profile of photoprotection, the SPF ranging from 2 to 10 and the UVAPF from 0 to 9, as shown in Table 2.

As one can see from Table 2, all the molecules, resveratrol and its analogues, presented a significant photoprotection for a single UV-filter substance, except for analogue A. Their SPF, that is, protection against UVB sunrays, was similar, but the activity in analogue B was more significant than in resveratrol itself. This demonstrates that the presence of a 4-methoxy group (electron donating group) at the para position is relevant. On the other hand, the presence of a nitro group (electron withdrawing) at the para position of the aromatic ring of compound **A** considerately diminishes the value of photoprotection. One can also consider that the compounds **B** ($R = -OCH_3$) and **D** [$R = -N(CH_3)_2$] show similar chemical structure, which would lead to similar SPF profiles, but we must consider that there are differences between oxygen-based compounds and nitrogen-based ones. For instance, amines have a very 'active' lone pair, that is, they are much more basic, and this basicity can influence the activity of hydrogen bonds [O–H···:N (29 kJ/mol or 6.9 kcal/mol) and O–H···:O (21 kJ/mol or 5.0 kcal/mol)], therefore possibly explaining the difference in the final UVB absorption by these molecules.

Analogues from B to F also presented very similar protection against UVA rays (although compound **B** is little more effective than **C**–**F**), and it is important to highlight that the structural modifications led to a very significant improvement in activity in this solar spectrum range, as the UVAPF increased from 2 in resveratrol to 6–8 in the referred analogues (three-fold greater than the original compound). This is of sheer relevance, as UVA rays are known to play an important role on skin cancers, and the analogues were more active in the protection from this type of radiation.

Critical Wavelength is another parameter determined to measure the UVA protection, being defined as the wavelength at which the integral of the area under the absorption spectrum of the sample reaches 90% of the total absorption, from 290 to 400 nm,[10] and thus the protection spectrum is measured (sunscreens with λ_c values near 400 nm are considered broad spectrum). The classification of the spectrum, however, can be different according to the reference adopted. For instance, the US Food and Drug Administration (FDA)[21] classifies products on a scale consisting of five numerical categories: 0 ($\lambda_c < 325$ nm), 1 ($325 \leqslant \lambda_c < 335$), 2 ($335 \leqslant \lambda_c < 350$), 3 ($350 \leqslant \lambda_c < 370$), and 4 ($370 \leqslant \lambda_c$). Under such classification, all the analogues (except A) were rated '4', the highest category. Resveratrol, in turn, is category '3', and this ratifies the improvement in the protection produced by the modifications in the chemical structure. Another possible classification is the one created by Springsteen et al.,[22] who classifies broad-spectrum sunscreen products as those which have a λ_c value greater than 370 nm. Thus, the same, previously mentioned analogues may be considered broad spectrum products by this classification, offering protection against UVA and UVB.

Finally, we have determined the UVA/UVB ratio, which provides a good idea of which UV region is better blocked by the substances. Again, the analogues had a greater value than the original compound. The UVA/UVB ratio can also be used to provide the so-called Boots Star Rating,[23] which classifies the products into categories from 0 to 5 stars. Such classification should be done according to Table 3.

The Boots Star Rating is important to determine the stability of the generated photoprotection values, since the components of the sunscreens may degrade. Analogues C–F are, therefore, 5 stars; B is 3 stars; and resveratrol receives no stars, because its protection is mainly focused on the UVB region.

As a final consideration, it is important to highlight that the photoprotective activity achieved by those molecules is due to the fact that they were capable of absorbing the short wavelength, high-energized UV rays, and converting them into less energetic radiations of the infrared region.[24,25] This occurs because the UV photon absorbs enough energy to cause the transfer of electrons to a more energetic orbital in the molecule which contains this chromophore group,[25] that is, the absorption of UV leads to the excitation of electrons found in orbital π HOMO and their subsequent transference to the π* LUMO orbital.[24]

4. Conclusions

In conclusion, one can infer that the structural modifications among the analogues B–F were of great value, as they played a role in increasing the photoprotection of the original compound, resveratrol, mainly in the UVA region, one of the primary causes for the emergence of skin cancers.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.11.052.

References and notes

1. Brasil, Ministério da Saúde. Secretaria de Atenção à Saúde. Instituto Nacional de Câncer. Estimativa 2010: incidência de câncer no Brasil: Rio de Janeiro, 2010.
2. Polonini, H. C.; Raposo, N. R. B.; Brandão, M. A. F. *Rev. APS.* **2011**, *30*, 604.
3. Delgado, J. A.; Quesada, I.; Montaño, L. M.; Anasagasti, L. *Mexic. J. Phys.* **2006**, *52*, 78.
4. Saladi, R. N.; Persaud, A. N. *Drugs Today* **2005**, *41*, 37.
5. Polonini, H. C.; Gomes, T. B. B.; Gonçalves, K. M.; Brandão, M. A. F.; Raposo, N. R. B. *Lat. Am. J. Pharm.* **2012**, *31*, 353.
6. Ghissassi, F.; Baan, R.; Straif, K.; Grosse, Y.; Secretan, B.; Bouvard, V., et al *Lancet Oncol.* **2009**, *10*, 751.
7. Signoreli, P.; Ghidoni, R. *J. Nutr. Biochem.* **2005**, *16*, 449.
8. Caddeo, C.; Teskac, K.; Sinico, C.; Kristl, J. *Int. J. Pharm.* **2008**, *363*, 183.
10. Cosmetics Europe, Method for in vitro determination of UVA protection, 2011.
11. Urbach, F. *J. Photochem. Photobiol., B* **2001**, *64*, 99.
12. Heinrich, U.; Tronnier, H.; Kockott, D.; Kuckuk, R.; Heise, H. M. *Int. J. Cosmet. Sci.* **2004**, *26*, 79.
13. Maia Campos, P. M. B. G.; Gianeti, M. D.; Kanashiro, A.; Lucisano-Valim, Y. M.; Gaspar, L. R. *Photochem. Photobiol.* **2006**, *82*, 683.
14. Velasco, M. V. R.; Sarruf, F. D.; Salgado-Santos, I. M. N.; Haroutiounian-Filho, C. A.; Kaneko, T. M.; Baby, A. R. *Int. J. Pharm.* **2008**, *363*, 50.
15. Fremont, L. *Life Sci.* **2000**, *66*, 663.
16. Aggarwal, B. B.; Bhardwaj, A.; Aggarwal, R. S.; Seeram, N. P.; Shishodia, S.; Takada, Y. *Anticancer Res.* **2004**, *24*, 2783–2784.
17. Mendes, J. B. E.; Riekes, M. K.; Oliveira, V. M.; Michel, M. D.; Stulzer, H. K.; Khalil, N. M.; Zawadzki, S. F.; Mainardes, R. M.; Farago, P. V. *ScientificWorldJournal.* **2012**, *2012*, 542937.
19. Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
20. Calil, N. O.; Carvalho, G. S. G.; Silva, A. F.; Silva, A. D.; Raposo, N. R. B. *Lett. Drug Des. Discov.* **2012**, *9*, 676.
21. Food and Drug Administration. 21 CFR Parts 347 and 352. Sunscreen Drug Products for Over-the-CounterHuman Use, Proposed Amendment of Final Monograph, Proposed Rule, Food and Drug Administration, Silver Spring, 2007.
22. Springsteen, A.; Yurek, R.; Frazier, M.; Carr, K. F. *Anal. Chim. Acta* **1999**, *380*, 155.
23. Boots the Chemist Ltd *The Revised Guidelines to the Practical Measurement Of UVA/UVB Ratios according to the Boots Star Rating System*; The Boots Co, PLC: Notthingham, 2008.
24. Diffey, B. L. In *Sunscreens, Development, Evaluation and Regulatory Aspects*; Lowe, N. J., Shaath, N. A., Pathak, M. A., Eds.; Marcel Dekker: New York, 1997.
25. Flor, J.; Davolos, M. R.; Correa, M. A. *Quím. Nova* **2007**, *30*, 153.