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Synthesis, antioxidant and photoprotection activities of hybrid derivatives useful to prevent skin cancer

Juliana Santana Reis, Marcos Antonio Corrêa, Man Chin Chung, Jean Leandro dos Santos *

Lapdesf—Laboratório de Pesquisa e Desenvolvimento de Fármacos, Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista–UNESP, Rodovia Araraquara Jaú Km 01, 14801-902 Araraquara, SP, Brazil

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ABSTRACT

Chronic ultraviolet (UV) radiation exposure is a major cause of skin cancer. A novel series of hybrid derivatives (**I**–**VIII**) for use in sunscreen formulations were synthesized by molecular hybridization of *t*-resveratrol, avobenzone, and octyl methoxycinnamate, and were characterized. The antioxidant activity values for **VIII** were comparable than to those of *t*-resveratrol. Compounds **I**–**III** and **VI** demonstrated Sun Protector Factor superior to that of *t*-resveratrol. Compounds **I** and **IV**–**VIII** were identified as new, broad-spectrum UVA filters while **II**–**III** were UVB filters. In conclusion, novel hybrid derivatives with antioxidant effects have emerged as novel photoprotective agents for the prevention of skin cancer.

1. Introduction

The incidence and mortality of skin cancer are increasing worldwide, and skin cancer now accounts for one in three of all diagnosed cancers.[1] The most common skin cancers are melanoma and non-melanoma (basal cell carcinoma and squamous cell carcinoma), with malignant melanoma being the main cause of death.[2] It was estimated that 1 in 49 people will be diagnosed with malignant melanoma during their lifetime, particularly in people exposed to certain risk factors, including constant exposure to ultraviolet (UV) radiation.[3,4]

Although there is widespread concern over the importance of using sunscreen formulations to prevent skin cancer, epidemiologic data are still showing progressive increases in its morbidity and mortality rates. In the United States of America, for example, over 3.5 million cases of skin cancer are diagnosed annually.[5] The preventive behavior is complex and, in most cases, the incorrect use of sunscreen formulations results in inadequate photoprotection. It is well established that chronic exposure to UV radiation is one of the main causes of skin cancer, and clinical studies have demonstrated that increased frequency of sunburns increase the risk of melanoma.[6] UV radiation can directly damage DNA through the formation of DNA adducts and/or ROS generation, which promote tumor formation through different pathways.[7]

UV radiation is divided into ultraviolet A (UVA, 320–400 nm), ultraviolet B (UVB, 280–320 nm), and ultraviolet C (UVC, 100–280 nm). UVA and UVB have direct effects on genetic material.[8] The direct genotoxic effects of UV radiation are due to the formation of dimeric photoproducts between adjacent bases in DNA.[9] UV radiation also has indirect effects mediated by increased Reactive Oxygen Species (ROS) levels, which (a) regulate cell processes associated with malignant transformation; (b) cause DNA damage-induced mutations; (c) alter the activity of the pro-survival pathway; and (d) promote skin aging.[10,11]

It was reported that antioxidants could prevent ROS-induced DNA damage.[12] In particular, *t*-resveratrol has potent antioxidant activity exceeding those of vitamins E and C,[13] and might also protect against UV radiation. Because antioxidant compounds, such as *t*-resveratrol, have chemoprotective properties,[14] the development of novel photoprotectors with antioxidant properties is a

Abbreviations: DPPH·, 2,2-diphenyl-1-picrylhydrazyl radical; *t*-resveratrol, *trans*-resveratrol; FDA, Food and Drug Administration; ROS, Reactive Oxygen Species; UVR, Ultraviolet Radiation; SPF, Sun Protector Factor.

* Corresponding author. Tel.: +55 16 33016972; fax: +55 16 33016960.
E-mail address: santosjl@fcfar.unesp.br (J.L. dos Santos).

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promising strategy for the development of sunscreen formulations with improved photoprotective properties.

The mechanisms involved in the protective effects of resveratrol and other polyphenols against UV radiation are complex, but seem to involve the modulation of cellular signaling pathways, anti-inflammatory activities, induction of cytokines such as interleukin-12 (IL-12), prevention of UVB-induced immunosuppression, and upregulation of genes encoding nucleotide excision repair (NER) enzymes.[15–18] In an in vivo study using SKH-1 hairless mice, *t*-resveratrol inhibited UVB-induced skin edema and the production of hydrogen peroxide.[19] In another study, *t*-resveratrol protected mouse skin against UVB-induced skin damage by modulating survival pathways.[20] Furthermore, it has been suggested that *t*-resveratrol blocks UVB radiation, in particular.[21]

Avobenzone and octyl methoxycinnamate are widely used in sunscreen formulations to protect against UVA and UVB, respectively. However, these compounds have some limitations, including photo-instability, low efficacy if used alone, absence of antioxidant effects, potential allergenic effects, and incompatibility with other components used in commercial sunscreen formulations.[22–24] Molecular modification strategies, such as hybridization, represent a powerful tool that might facilitate the discovery of new photoprotective compounds that are effective, stable and safe.

In a continuing effort to develop new candidate sunscreens to prevent skin cancer and radiation-induced oxidative stress, this report describes the synthesis, antioxidant activity, and photoprotective effects of novel hybrid derivatives (**I**–**VIII**), which were obtained by molecular hybridization of the prototypes *t*-resveratrol (**1**), octyl methoxycinnamate (**2**), and avobenzone (**3**) (Fig. 1). The selections of each subunit are due contributions to (a) antioxidant effect (phenolic hydroxyl in A, B and D subunits); (b) ability to promote electronic conjugation and shift the absorption maximum to higher wavelengths (aryl-α,β-insaturation in subunit C); and (c) free radical stabilization (subunit E). Therefore, this approach aims to combine UVA and UVB photoprotection and antioxidant activity in the same molecule to improve the efficacy of sunscreen formulations.

2. Chemistry

The synthetic routes for preparing the hybrid derivatives (**I**–**VIII**) are summarized in Scheme 1. The coupling reactions between the previously selected aldehydes and hydrazides were catalyzed with 37% hydrochloric acid, and generated the corresponding hydrazone derivatives (**I**–**VIII**) with yields of 70–99%. The structures of all of the compounds were established by elemental analysis, infrared (IR) spectroscopy, and ^{1}H- and ^{13}C-nuclear magnetic resonance. All compounds were analyzed by high-performance liquid chromatography, and the purities were >98.5%. The ^{1}H-nuclear magnetic resonance spectra of all hybrid derivatives exhibited one peak, corresponding to the ylidenic hydrogen of the *E*-diastereomer.[25]

3. Results and discussion

The new hybrid compounds were generated from hydrazone derivatives (**I**–**VIII**), as *E* diastereoisomers, with excellent yields (70–99%). All hybrid compounds were obtained with a purity >98.5% and were characterized using standard analytical methods.

The antioxidant activities of the hybrid compounds (**I**–**VIII**) and positive controls were evaluated by measuring their free radical scavenging capacities against test compounds using the adapted DPPH· microplate assay (Table 1). This assay measures the hydrogen-donating ability of antioxidants to convert the stable DPPH free radical to 2,2-diphenyl-1-picrylhydrazyl, which is accompanied by a change in color from deep-violet to light-yellow. All compounds were incubated in the microplate for 60 min at concentrations of 1000, 300, 100, and 35 μM. Although the test was also conducted for 30 min, the results were similar for both times (results not shown). All compounds, except **VI**, had antioxidant properties. The most potent compounds were **I** (inhibitory concentration 50% [IC_{50}] = 275 μM), **VII** (IC_{50} = 88.2 μM), and **VIII** (IC_{50} = 109.6 μM). The compounds **VII** and **VIII** were more potent than *t*-resveratrol (IC_{50} = 110 μM) but were less potent than ascorbic acid (IC_{50} = 64.2 μM), which was used as a control. The presence of α,β-unsaturated carbonyl moieties for **V**–**VIII** seems to increase the compound's antioxidant activity if at least one of the aryl rings is substituted with a hydroxyl group at the *para* position. As previously reported by some authors, *N*-acyl hydrazone spacer could extend delocalized π-electron in the structure. This effect allows conjugated electrons to flow between the aromatic rings and for this subunit to react with high-energy oxygen species.[26–29] This effect is particularly relevant because UV radiation induces ROS, which promote skin cancer development and photoaging.

In addition, in vitro photoprotection analyses were determined using an Optometric 290S analyzer (SPF-290S; Optometrics, Ayer, MA, USA), and the results were manipulated using WinSPF software version 4.1 (Optometrics). High correlations between SPF-290S in vitro measurements and in vivo tests were reported for a variety of formulations, including creams, lotions, gels, and sprays.[30] Sun Protector Factor (SPF), UVA Protection Factor, UVA/UVB ratio, and the critical wavelength (λ_C) were determined using a stable neutral cream containing 7% of compounds **I**–**VIII**, avobenzone, benzophenone-3, *t*-resveratrol and octyl methoxycinnamate (Table 2). Avobenzone and benzophenone-3 as UVA filters and resveratrol and octyl methoxycinnamate as UVB filters were used as reference. Table 2 shows that the SPF values for compounds **I**–**III** and **VI** were superior to that of resveratrol (SPF = 2). All compounds demonstrated superior activity to that of benzophenone-3 (SPF = 1). Interestingly, the most active compound (**II**) (SPF = 5) is structurally related to resveratrol (1), having an *N*-acyl-hydrazone subunit that extends electron conjugation (Scheme 1). Generally, organic UV filters contain a chromophore that is conjugated by the π-electron system. Therefore, increasing the conjugation, until certain limits, shifts the absorption maximum to higher wavelengths, increasing the molecule's ability to absorb UV radiation. Very large conjugation systems could have maxima of absorption bathochromic shifted out of the UV range.

All synthesized compounds were less active than octyl methoxycinnamate (SPF = 13), however this filter has a lot of inconveniences such as photo-instability and absence of antioxidant effects. Compounds **IV**, **V**, **VII** and **VIII** had similar activities to that of *t*-resveratrol. Polonini et al. developed several *t*-resveratrol analogs as potential photoprotectors, but the SPF value of the most active compound was only 1.42 times greater than that of *t*-resveratrol.[31] Despite the apparent reductions in SPF values of *t*-resveratrol analogs, it is important to acknowledge that sunscreen formulations contain multiple filters to achieve high SPF values.

The UVA protection factor values of compounds **II**, **III**, and **VI** were 1.5–2 times greater than that of *t*-resveratrol, but were less than that of avobenzone. Meanwhile, the UVA protection factor values of compounds **I**, **IV**, **V**, **VII**, and **VIII** were similar to that of resveratrol. Sunscreen formulations that can block UVA are desirable because this type of radiation has immunosuppressive and mutagenic effects in humans and animals.[32,33] The critical wavelength (λ_C), another UVA parameter, is classified by the United States Food and Drug Administration into five categories, as follows: 0 (λ_C <325 nm), 1 (325–335), 2 (335–350), 3 (350–370), and 4 ($\geqslant$370).[34] According these categories, compounds **I** and **IV**–**VIII** were scored as '4', like avobenzone and benzophenone-3,