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Natural and semisynthetic oxyprenylated aromatic compounds as stimulators or inhibitors of melanogenesis

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ABSTRACT

It has been very recently shown how naturally occurring oxyprenylated coumarins are effective modulators of melanogenesis. In this short communication we wish to generalize the potentialities as skin tanning or whitening agents of a wider panel of natural and semisynthetic aromatic compounds, including coumarins, cinnamic and benzoic acids, cinnamaldehydes, benzaldehyde, and anthraquinone derivatives. A total number of 43 compounds have been tested assaying their capacity to inhibit or stimulate melanin biosynthesis in cultured murine Melan A cells. The wider number of chemicals herein under investigation allowed to depict a detailed structure-activity relationship, as the following: (a) benzoic acid derivatives are slightly pigmenting agent, for which the effect is more pronounced in compounds with longer *O*-side chains; (b) independently from the type of substitution, cinnamic acids are able to increase melanin biosynthesis, while benzaldehydes are able to decrease it; (c) coumarins with a 3,3-dimethylallyl or shorter skeletons as substituents in position 7 are tanning agents, while coumarins with farnesyloxy groups are whitening ones; (d) double oxyprenylation in position 6 and 7 and 3,3-dimethylallyl or geranyl skeletons have slight depigmenting capacities, while farnesyl skeletons tend to marginally increase the tanning effect; (e) the presence of electron withdrawing groups (acetyl, COOH, and -Cl) and geranyl or farnesyl oxyprenylated chains respectively in positions 3 and 7 of the coumarin nucleus lead to a whitening effect, and finally (f) oxyprenylated anthraquinones have only a weak depigmenting capacity.

1. Introduction

Melanin is the main pigment in humans and other mammals found in skin, eyes, nasal cavity, inner ear, and hair. It is responsible for skin color and represents the most effective defense for these tissues and organs against overexposure to ultraviolet (UV)-B radiations [1]. The biosynthesis in humans (known as melanogenesis) occurs in specialized neural-crest derived cells called "melanocytes", located in the stratum basale of skin epidermis, uvea, inner ear, but also in the vaginal epithelium, meninges, bone tissues, and heart [2]. Melanin is obtained in humans by a metabolic process catalyzed in sequence by three enzymes: tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2. The first promotes the rate-limiting step of the overall melanin biosynthesis consisting in the hydroxylation of tyrosine (Tyr) to dihydroxyphenylalanine (DOPA), followed by the oxidation of the latter to DOPAquinone [3]. Once synthesized, melanin is temporarily stored in subcellular organelles, called "melanosomes", and transported to nearby keratinocytes leading to tissue pigmentation [4]. The main factor regulating melanogenesis is α-melanocyte stimulating hormone (α-MSH), a hormone secreted by the pituitary gland, that stimulates the phosphorylation of cyclic adenosine monophsosphate (cAMP)-responsive element binding protein (CREB), able in turn to interact with the CRE binding site of microphthalmia-associated transcription factor (MITF), finally increasing melanin biosynthesis. Other endogenous and exogenous stimuli able to promote melanogenesis are represented by eicosanoids, retinoids, estrogens, endothelins, psoralens, hydantoins, forskolin, cholera toxin, and xanthines [5]. Inhibitors of the melanogenic machinery are represented by several natural products including

Abbreviations: cAMP, cyclic adenosine monophosphate; CREB, cAMP-responsive element binding protein; DMSO, dimethylsulfoxide; DOPA, dihydroxyphenylalanine; MITF, microphthalmia-associated transcription factor; MSH, melanocyte stimulating hormone; TRP, tyrosinase-related protein; Tyr, tyrosine; UV, ultraviolet

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arbutin, kojic acid, flavonoids, catechins, and triterpenes [6]. Dysfunctions in melatonin biosynthesis lead to benign to severe acute and chronic syndromes mainly affecting skin like, melisma, freckles, senile lentigines, vitiligo, albinism, Griscelli's disease, scalp troubles (e.g. dandruff, lice, cradle cap, ringworms), and others [7,8]. The use of natural and synthetic remedies currently at disposition to cure such syndromes and/or for cosmetic and/or therapeutic purposes is often limited by several side effects. As explicative examples to this concern, hydroquinones, like arbutin, and kojic acid may cause skin irritation, contact dermatitis, allergic reactions, and sensitization [9], while psoralens are nowadays considered among the main causes of different skin disorders, including melanoma [10]. Therefore, the search for novel and alternative agents able to modulate melanogenesis is a field of current and growing interest, also in view of wide possibilities for practical applications for cosmetic purposes. In the course of ongoing studies aimed at better depicting the pharmacological potential of naturally occurring and semisynthetic oxyprenylated aromatic compounds, it has recently been put in evidence how the alkylation of the phenol function of umbelliferon with dimethylallyl, geranyl, and farnesyl chains have a deep influence on melanogenesis in cultured non-tumorigenic murine melanocyte Melan A cells. Such investigations pointed out how the longer is the chain the more pronounced is the whitening effect and vice versa [11]. In this manuscript we wish to report the properties as tanning or whitening agents of a wider panel of natural and semisynthetic aromatic compounds, including coumarins, cinnamic and benzoic acids, cinnamaldehydes, benzaldehydes, and anthraquinone derivatives. A total of 43 chemicals have been assayed to this purpose, the structures of which are illustrated in Fig. 1.

2. Materials and methods

2.1. Chemistry

Oxyprenylated compounds **1–31** and **38–43** have been synthesized from parent commercially available phenols in 88–98% yield by a single-step Williamson reaction using 3,3-dimethyallyl, geranyl, and farnesyl bromides as the alkylating agents as previously described [12] and detailed in Scheme 1. Compounds **32–34** and **35–37** have been obtained from 3-acetyl-7-hydroxycoumarin **44** and 7-hydroxycoumarin-3-carboxylic acid **45** respectively, in turn synthesized from commercially available 2,4-dihydroxybenzaldehyde and methyl acetoacetate, and 2,4-dihydroxybenzaldehyde and Meldrum's acid and lemon juice as the solvent/promoter following the already reported processes, as described in Schemes 2 and 3 [13–15]. Overall yields were in the range 61–66%. Purity (>98.1%) of all intermediates and desired adducts was assessed by HPLC, by application of the well validated data methodology previously set up for the qualitative and quantitative analysis of oxyprenylated compounds [16,17]. The same general procedures for NMR and elemental analysis experiments as already reported were followed [12]. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

2.2. Analytical data

2.2.1. 4-Isopentenyloxybenzaldehyde (1)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [18]. Anal. Calcd. for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42; O, 16.82. Found: C, 75.69; H, 7.37; O, 16.81.

2.2.2. 4-Geranyloxybenzaldehyde (2)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [19]. Anal. Calcd. for $C_{17}H_{22}O_2$: C, 79.03; H, 8.58; O, 12.39. Found: C, 79.09; H, 8.54; O, 12.33.

2.2.3. 4-Farnesyloxybenzaldehyde (3)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [20]. Anal. Calcd. for $C_{22}H_{30}O_2$: C, 80.94; H, 9.26; O, 9.80. Found: C, 80.88; H, 9.22; O, 9.74.

2.2.4. 4-Isopentenyloxy-3-methoxybenzaldehyde (4)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [21]. Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32; O, 21.79. Found: C, 70.88; H, 7.27; O, 21.77.

2.2.5. 4-Geranyloxy-3-methoxybenzaldehyde (5)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [22]. Anal. Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39; O, 16.64. Found: C, 74.92; H, 8.33; O, 16.62.

2.2.6. 4′-Isopentenyloxycinnamic acid (6)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [22]. Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.42; H, 6.91; O, 20.69.

2.2.7. 4′-Isopentenyloxy-3-methoxycinnamic acid (7)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [22]. Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92; O, 24.40. Found: C, 68.63; H, 6.89; O, 24.36.

2.2.8. 4′-Geranyloxycinnamic acid (8)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [22,23]. Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05; O, 15.98. Found: C, 75.93; H, 8.08; O, 15.99.

2.2.9. 4′-Geranyloxy-3-methoxycinnamic acid (9)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [24]. Anal. Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93; O, 19.37. Found: C, 72.68; H, 7.88; O, 19.32.

2.2.10. 4′-Farnesyloxycinnamic acid (10)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [25]. Anal. Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75; O, 13.02. Found: C, 78.16; H, 8.79; O, 12.98.

2.2.11. 4′-Farnesyloxy-3-methoxycinnamic acid (11)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [26]. Anal. Calcd. for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60; O, 16.06. Found: C, 75.31; H, 8.55; O, 16.07.

2.2.12. 1,8-Dihydroxy-6-isopentenyloxy-3-methylanthraquinone (12)

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [27]. Anal. Calcd. for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64. Found: C, 70.93; H, 5.41; O, 23.58.

2.2.13. 1,8-Dihydroxy-6-geranyloxy-3-methylanthraquinone (13)

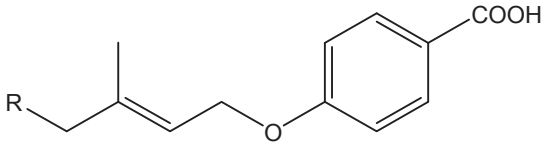
^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [27]. Anal. Calcd. for $C_{25}H_{26}O_5$: C, 73.87; H, 6.45; O, 19.68. Found: C, 73.82; H, 6.47; O, 19.64.

*2.2.14. 7-Isopentenyloxycoumarin (**14**)*

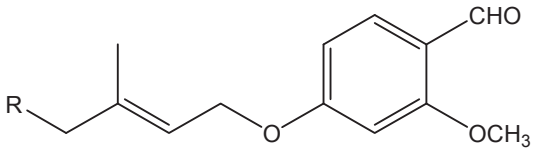
^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [28]. Anal. Calcd. for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13; O, 20.85. Found: C, 73.08; H, 6.11; O, 20.80.

*2.2.15. 7-Isopentenyloxy-4-methylcoumarin (**15**)*

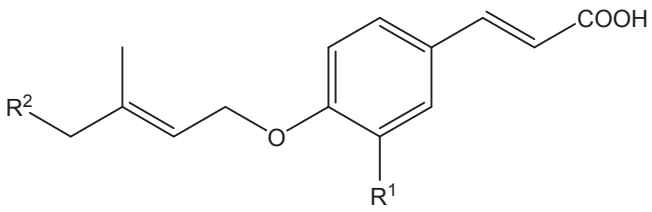
^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [29]. Anal. Calcd. for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60; O, 19.65. Found: C, 73.70; H, 6.56; O, 19.59.



1 R = H, **2** R = $CH_2CH{=}C(CH_3)_2$, **3** R = $CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$



4 R = H, **5** R = $CH_2CH{=}C(CH_3)_2$



6 $R^1 = R^2 = H$

7 R^1 = H, $R^2 = OCH_3$

8 $R^1 = CH_2CH{=}C(CH_3)_2$, R^2 = H

9 $R^1 = CH_2CH{=}C(CH_3)_2$, $R^2 = OCH_3$

10 $R^1 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$, R^2 = H

11 $R^1 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$, $R^2 = OCH_3$

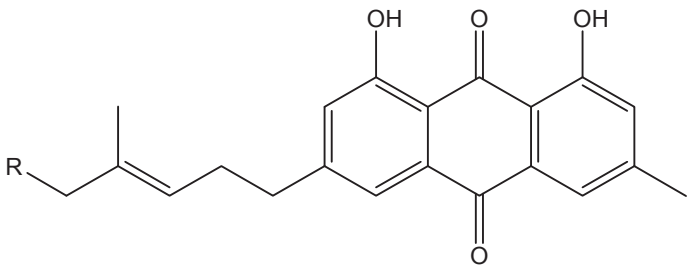
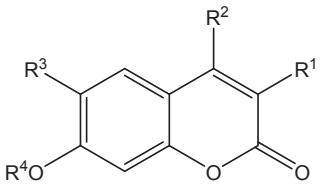


Fig. 1. Illustration of the chemical structures studied. The 43 compounds under investigation belong to five chemical groups: benzoic acids (compounds **1–3**), benzaldehydes (compounds **4** and **5**), cinnamic and ferulic acids derivatives (compounds **6–11**), anthraquinones (compounds **12** and **13**), and coumarins (compounds **14–43**).

12 R = H, **13** R = $CH_2CH=C(CH_3)_2$



14 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH=C(CH_3)_2$

15 $R^1 = R^3 = H$, $R^2 = CH_3$, $R^4 = CH_2CH=C(CH_3)_2$

16 $R^1 = R^3 = H$, $R^2 = CH_3$, $R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

17 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

18 $R^1 = R^3 = H$, $R^2 = CH_3$, $R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

19 $R^1 = R^2 = H$, $R^3 = R^4 = CH_2CH=C(CH_3)_2$

20 $R^1 = R^2 = H$, $R^3 = R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

21 $R^1 = R^2 = H$, $R^3 = R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

22 $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$

23 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH_3$

24 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH_2CH_3$

25 $R^1 = R^2 = R^3 = H$, $R^4 = OCH_2CH=CH_2$

26 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH=CHCH_3$

27 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH_2CH(CH_3)_2$

28 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2C\equiv CCH_2CH_3$

29 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2Ph$

30 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH=CHPh$

31 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2C(CH_3)_3$

32 $R^1 = COCH_3$, $R^2 = R^3 = H$, $R^4 = CH_2CH=C(CH_3)_2$

33 $R^1 = COCH_3$, $R^2 = R^3 = H$, $R^4 = CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

Fig. 1. (*continued*)

*2.2.16. 7-Geranyloxy-4-methylcoumarin (**16**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [30]. Anal. Calcd. for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74; O, 15.36. Found: C, 76.83; H, 7.77; O, 15.32.

*2.2.17. Umbelliprenin (**17**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [30]. Anal. Calcd. for $C_{24}H_{30}O_3$: C, 78.65; H, 8.25; O, 13.10. Found: C, 78.66; H, 8.23; O, 13.06.

*2.2.18. 7-Farnesyloxy-4-methylcoumarin (**18**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [30]. Anal. Calcd. for $C_{25}H_{32}O_3$: C, 78.91; H, 8.48; O, 12.61. Found: C, 78.88; H, 8.43; O, 12.64.

*2.2.19. 6,7-Diisopentenyloxycoumarin (**19**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [15]. Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05; O, 20.36. Found: C, 72.54; H, 7.09; O, 20.31.

34 $R^1 = COCH_3$, $R^2 = R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

35 $R^1 = COOH$, $R^2 = R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)_2$

36 $R^1 = COOH$, $R^2 = R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

37 $R^1 = COOH$, $R^2 = R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

38 $R^1 = R^2 = H$, $R^3 = CH_2CH{=}C(CH_3)_2$, $R^4 = CH_3$

39 $R^1 = R^2 = H$, $R^3 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$, $R^4 = CH_3$

40 $R^1 = R^2 = H$, $R^3 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$, $R^4 = CH_3$

41 $R^1 = Cl$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)_2$

42 $R^1 = Cl$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

43 $R^1 = Cl$, $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

Fig. 1. (*continued*)

2.2.20. 6,7-Digeranyloxycoumarin ***(20)***

^{1}H NMR δ 1.68 (s, 3H), 1.70 (s, 3H), 1.71 (s, 3H), 1.72 (s, 3H), 1.74 (s, 3H), 1.76 (s, 3H), 2.01–2.15 (m, 8H), 4.69–4.74 (m, 4H), 5.05–5.11 (m, 1H), 5.31–5.38 (m, 4H), 5.37–5.43 (m, 2H), 6.33 (d, 1H, J = 9.6 Hz), 6.74 (s, 1H), 6.91 (s, 1H), 7.68 (d, 1H, J = 9.6 Hz); ^{13}C NMR δ 16.1, 17.4, 17.6, 23.9, 25.6, 25.8, 26.3, 26.7, 32.3, 39.5, 65.8, 66.1, 102.9, 109.6, 112.7, 113.6, 120.0, 121.9, 123.7, 123.9, 131.3, 131.8, 141.6, 143.1, 143.5, 148.0, 149.9, 160.5. Anal. Calcd. for $C_{29}H_{38}O_4$: C, 77.30; H, 8.50; O, 14.20. Found: C, 77.24; H, 8.55; O, 14.21.

2.2.21. 6,7-Difarnesyloxycoumarin ***(21)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [31]. Anal. Calcd. for $C_{39}H_{54}O_4$: C, 79.82; H, 9.27; O, 10.91. Found: C, 79.78; H, 9.23; O, 10.95.

2.2.22. 7-Methoxycoumarin ***(22)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.58; O, 27.25. Found: C, 68.13; H, 4.59; O, 27.21.

2.2.23. 7-Ethoxycoumarin ***(23)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; O, 25.24. Found: C, 69.44; H, 5.34; O, 25.20.

2.2.24. 7-Propoxycoumarin ***(24)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92; O, 23.50. Found: C, 70.51; H, 5.95; O, 23.53.

2.2.25. 7-Allyloxycoumarin ***(25)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98; O, 23.74. Found: C, 71.22; H, 4.97; O, 23.75.

2.2.26. 7-(2′-Butenyloxy)coumarin ***(26)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59; O, 22.20. Found: C, 72.17; H, 5.54; O, 22.17.

2.2.27. 7-(3′-Methyl)butoxycoumarin ***(27)***

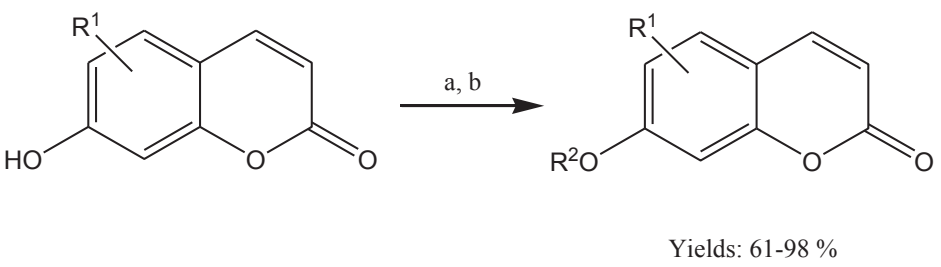
^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [31]. Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.38; H, 6.91; O, 20.69.

2.2.28. 7-(2′-Pentinyloxy)coumarin ***(28)***

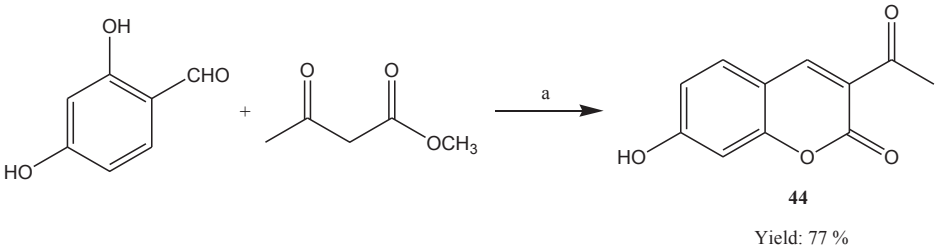
^{1}H and data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30; O, 21.03. Found: C, 73.62; H, 5.24; O, 20.99.

2.2.29. 7-Benzyloxycoumarin ***(29)***

^{1}H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79; O, 19.03. Found: C, 76.14; H, 4.81; O, 19.09.



Scheme 1. General synthetic scheme for the prenylation of hydroxycoumarins. Reagents and conditions: (a) alkyl bromide (1.1. equiv.), K_2CO_3 (1.2 equiv.), acetone, 80 °C, 1 h; (b) acid-base work-up, crystallization (*n*-hexane).



44

Yield: 77 %

Scheme 2. Synthesis of 3-acetyl-7-hydroxycoumarin **44.** Reagents and conditions: (a) EtOH, Et_2NH, 80 °C, 5 h.

*2.2.30. 7-Styryloxycoumarin (**30**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07; O, 17.25. Found: C, 77.64; H, 5.05; O, 17.26.

*2.2.31. 7-(3′,3′-Dimethyl)propoxycoumarin (**31**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [32]. Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.66. Found: C, 72.44; H, 6.97; O, 20.62.

*2.2.32. 3-Acetyl-7-isopentenyloxycoumarin (**32**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [15]. Anal. Calcd. for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; O, 23.50. Found: C, 70.55; H, 5.91; O, 23.56.

*2.2.33. 3-Acetyl-7-geranyloxycoumarin (**33**)*

^1H NMR δ 1.59 (s, 3H), 1.62 (s, 3H), 1.71 (s, 3H), 2.06–2.12 (m, 4H), 2.59 (s, 3H), 4.60–4.64 (m, 2H), 5.08–5.12 (m, 1H), 5.38–5.43 (m, 1H), 6.82–7.24 (m, 3H), 8.23 (s, 1H); ^{13}C NMR δ 17.6, 24.9, 25.6, 31.7, 32.2, 64.6, 100.9, 110.0, 112.3, 121.9, 123.8, 125.5, 130.2, 131.9, 141.4, 146.0, 156.5, 158.2, 162.3, 201.7. Anal. Calcd. for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11; O, 18.80. Found: C, 74.12; H, 7.07; O, 18.85.

*2.2.34. 3-Acetyl-7-farnesyloxycoumarin (**34**)*

^1H NMR δ 1.58 (s, 3H), 1.63 (s, 3H), 1.71 (s, 3H), 1.78 (s, 3H), 2.01–2.14 (m, 8H), 2.60 (s, 3H), 4.59–4.63 (m, 2H), 5.02–5.10 (m, 2H), 5.38–5.42 (m, 1H), 6.82–7.22 (m, 3H), 8.21 (s, 1H); ^{13}C NMR δ 14.9, 17.8, 23.9, 25.4, 25.6, 26.9, 31.8, 32.6, 39.7, 65.0, 100.9, 109.8, 112.3, 121.9, 124.3, 124.7, 125.4, 130.3, 131.0, 135.0, 141.7, 145.6, 156.4, 158.2, 162.3, 202.4. Anal. Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90; O, 15.67. Found: C, 76.42; H, 7.91; O, 15.70.

*2.2.35. 7-Isopentenyloxycoumarin-3-carboxylic acid (**35**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [15]. Anal. Calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15; O, 29.17. Found: C, 65.66; H, 5.18; O, 29.21.

*2.2.36. 7-Geranyloxycoumarin-3-carboxylic acid (**36**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [33]. Anal. Calcd. for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48; O, 23.36. Found: C, 70.20; H, 6.49; O, 23.39.

*2.2.37. 7-Farnesyloxycoumarin-3-carboxylic acid (**37**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [33]. Anal. Calcd. for $C_{25}H_{30}O_5$: C, 73.15; H, 7.37; O, 19.49. Found: C, 73.11; H, 7.35; O, 19.50.

*2.2.38. 6-Isopentenyloxy-7-methoxycoumarin (**38**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [15]. Anal. Calcd. for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20; O, 24.59. Found: C, 69.24; H, 6.19; O, 24.54.

*2.2.39. 6-Geranyloxy-7-methoxycoumarin (**39**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [15]. Anal. Calcd. for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37; O, 19.49. Found: C, 73.10; H, 7.38; O, 19.53.

*2.2.40. 6-Farnesyloxy-7-methoxycoumarin (**40**)*

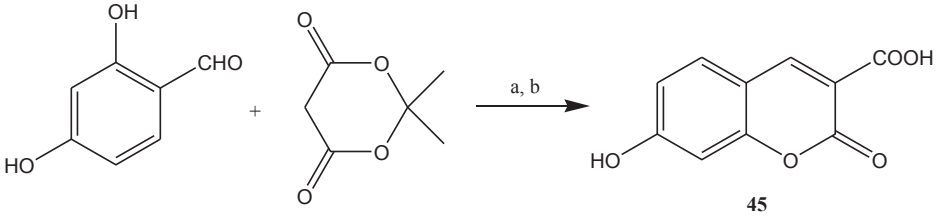
^1H NMR δ 1.60 (s, 3H), 1.64 (s, 3H), 1.72 (s, 3H), 1.80 (s, 3H), 2.03–2.13 (m, 8H), 3.88 (s, 3H), 4.71–4.75 (m, 2H), 5.08–5.14 (m, 2H), 6.33 (d, 1H, J = 9.5 Hz), 6.91–6.94 (m, 2H), 7.69 (d, 1H, J = 9.5 Hz); ^{13}C NMR δ 13.7, 16.2, 17.8, 25.6, 25.9, 26.7, 39.8, 56.2, 65.9, 99.9, 109.6, 112.7, 113.7, 119.8, 123.9, 124.4, 131.3, 135.4, 141.7, 142.7, 143.5, 148.8, 150.0, 160.5. Anal. Calcd. for $C_{25}H_{32}O_4$: C, 75.73; H, 8.13; O, 16.14. Found: C, 75.69; H, 8.17; O, 16.15.

*2.2.41. 3-Chloro-4-methyl-7-isopentenyloxycoumarin (**41**)*

^1H and ^{13}C NMR data were in full agreement with those already reported in the literature for the same compound [34]. Anal. Calcd. for $C_{15}H_{15}ClO_3$: C, 64.64; H, 5.42; O, 17.22. Found: C, 64.67; H, 5.41; O, 17.24.

*2.2.42. 3-Chloro-4-methyl-7-geranyloxycoumarin (**42**)*

^1H NMR δ 1.65 (s, 3H), 1.69 (s, 3H9, 1.77 (s, 3H), 2.08–2.14 (m, 4H), 2.52 (s, 3H), 4.47–4.51 (m, 2H), 5.06–5.11 (m, 1H), 5.49–5.53 (m,



45

Yield: 96 %

Scheme 3. Synthesis of 7-hydroxycoumarin-3-carboxylic acid **45.** Reagents and conditions: (a) lemon juice, r.t., (b) acid-base work-up, crystallization (H_2O).

1H), 7.52–7.80 (m, 3H); ^{13}C NMR δ 16.2, 17.3, 18.6, 25.6, 26.2, 39.1, 65.1, 102.0, 106.1, 111.0, 112.4, 119.9, 123.9, 126.3, 131.3, 142.0, 144.8, 152.9, 154.8, 162.3. Anal. Calcd for $C_{20}H_{23}ClO_3$: C, 69.26; H, 6.68; O, 13.84. Found: C, 69.29; H, 6.64; O, 13.87.

*2.2.43. 3-Chloro-4-methyl–7-farnesyloxycoumarin (**43**)*

^{1}H NMR δ 1.61 (s, 3H), 1.65 (s, 3H), 1.69 (s, 3H), 1.73 (s, 3H), 2.04–2.16 (m, 8H), 2.54 (s, 3H), 4.48–4.52 (m, 2H), 5.08–5.13 (m, 2H), 5.41–5.44 (m, 1H), 6.31–6.33 (m, 1H), 7.52–7.77 (m, 2H); ^{13}C NMR δ 16.7, 17.8, 18.6, 23.3, 25.7, 26.5, 27.0, 39.6, 39.9, 65.2, 101.9, 106.0, 111.1, 112.4, 118.2, 124.4, 124.8, 126.3, 131.2, 135.0, 141.7, 144.9, 152.9, 154.7, 162.3. Anal. Calcd. for $C_{25}H_{31}ClO_3$: C, 72.36; H, 7.53; O, 11.57. Found: C, 72.32; H, 7.53; O, 11.51.

2.3. Cell culture

Melan-a cells, an immortalized mouse melanocyte cell line, were obtained from the Wellcome Trust Functional Genomics Cell Bank (London, UK). Cells were maintained in RPMI 1640 (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum, 50 U/mL penicillin, 50 U/mL streptomycin (PS Lonza) and 200 nM PMA (phorbol 12-myristate 13-acetate; Sigma). Cells were incubated at 37 °C in a humidified 5% CO_2/air atmosphere. The stock solutions of oxyprenylated compounds were prepared in dimethylsulfoxide (DMSO) (1000X) and were stored at −20 °C until use. The concentration used for the study was 20 μM, which were freshly prepared for each experiment with a final DMSO concentration of 0.1%. Controls were always treated with the same amount of DMSO (0.1%, v/v) as used in the corresponding experiments.

2.4. Cell viability measurements

Non-tumoral murine melanocytes were seeded at 60,000 cells on 6 plate wells and treated for 48 h with the oxyprenylated compound at 20 μM or DMSO. Cells were detached by trypsinization, collected in phosphate buffer saline and centrifuged at 1500 rpm for 5 min at 4 °C. Cells pellets were resuspended in the trypan blue solution (0.25%, w/v in PBS) and counted in a Malassez cell counter under a light microscope. The percentage of cell viability was calculated using the following formula: % cell viability [1 − (blue cells/total cells)] × 100.

2.5. Melanin content measurements

Non-tumoral murine melanocytes were seeded at 60,000 cells on 6 plate wells and treated for 48 h with the oxyprenylated compound at the indicated concentration of 20 μM or carrier solvent (DMSO). 5×10^6 cells were centrifuged at 1500 rpm for 5 min at 4 °C. The cell pellet was washed twice with phosphate buffer saline, transferred in an Eppendorf vial and centrifuged at 5000*g* for 5 min at 4 °C. The supernatant was discarded. 200 μL of H_2O and 1 mL of EtOH/Et_2O (1/1) were added to remove opaque contaminants. The mixture was incubated for 15 min at r.t., centrifuged at 5000*g* for 5 min, and the supernatant discarded. The precipitate containing melanin was dissolved in 300 μL of a mixture of 1 M NaOH (aq)/DMSO 9:1 after heating at 80 °C for 1 h. The absorbance was measured at 405 nm. The melanin content was expressed as a percentage of control (=100%). UV experiments have been performed following the method reported by Liebermann and Hopkins in 2004 [35] and using a UVX radiometer (UVP, Inc., Upland, CA, USA).

2.6. Statistical analysis

Values are the mean ± S.E. of three independent experiments, each carried out in duplicate. Statistical analysis was carried out with GraphPad using a Student's *t*-test for unpaired variables. *, **, and *** in the figures refer to P values of <0.05, <0.01, $p < 0.001$ respectively, compared with control cells that received the solvent vehicle alone.

3. Results and discussion

Previous studies suggested that the presence of prenyl chains linked to a coumarin ring via ethereal bonds have a deep influence on the stimulation and or inhibition of melanin biosynthesis in non-tumorigenic Melan-a cells, an immortalized mouse melanocyte cell line. The length of these chains are crucial structural determinants to observe a tanning or a depigmenting effect, in case of compounds with shorter and longer chains respectively [11]. In the present work it was investigated and discussed in more details such results using a wider panel of oxyprenylated chemicals, consisting in 41 natural and semisynthetic compounds plus 7-isopentenyloxycoumarin **14** and umbelliprenin **17** used as references for the tanning and whitening effects respectively in the above-mentioned murine cell line, in order to study the impact of the aromatic ring substitutions on the overall melanogenesis. Samples herein under investigation can be grouped into five groups, namely benzoic acids (compounds **1–3**), benzaldehydes (compounds **4** and **5**), cinnamic acid and ferulic acid derivatives (compounds **6–11**), anthraquinones (compounds **12** and **13**), and coumarins (compounds **14–43**), as illustrated in Fig. 1. Among the compounds synthesized, 29, namely **1–18**, **22**, and **32–40,** have been found in nature as minority phytochemicals of plants mainly belonging to Apiaceae, Asteraceae, Rhamnaceae, and Rutaceae families [36], as well as components of some fungi and marine organisms [37]. The remaining 14, namely **19–21**, **23–30**, and **41–43,** are of semisynthetic origin. Compounds **20**, **33**, **34**, **40**, **42**, and **43** are described herein for the first time. Prenylation and alkylation in general to obtain all chemical samples have been accomplished following a well validated route previously reported to provide terpenyl ethers of naturally occurring phenols [32] as depicted in Scheme 1. Briefly, the substrate was dissolved in acetone and then dry K_2CO_3 was suspended and the suitable alkyl bromide or iodide were added. The reaction mixture was let to react 1 h at 80 °C, poured into icy water and extracted twice with diethyl ether to get, after evaporation of the organic solvent to complete dryness, a raw solid that was further purified by crystallization to provide the desired compound in pure form and high yield (88%–98%) without the need for chromatographic separations.

All phenols were commercially available, with the only exception of the starting products for the synthesis of compounds **32–34** and **35–37**, for which preliminary condensation steps between 2,4-dihydroxybenzaldehyde and methyl acetoacetate to yield 3-acetyl-7-hydroxycoumarin **44** (Scheme 2), and between the same aromatic aldehyde and Meldrum's acid to yield 7-hydroxycoumarin-3-carboxylic acid **45** (Scheme 3) respectively were carried out.

All synthesized chemicals were then assayed for their capacity to modulate melanin biosynthesis in Melan-a murine cell line. The more usefulness and better responsiveness of this line as a pharmacological model in this context respect to other strains, like malignant melanocyte cell lines B16F10 and SK-MEL 28 has been well explained in our previously published manuscript reporting the modulatory effects of four naturally occurring coumarins, comprising 7-isopentenyloxycoumarin **14** and umbelliprenin **17**, on melanogenesis in the same non tumoral cell line employed in the present investigation. It has been preliminarily assayed the impact of oxyprenylated natural and semisynthetic compounds on proliferation and viability applying a dose of 20 μM, the same used to carry out tests on the modulation of melanin biosynthesis and corresponding to the highest solubility of such products into the medium employed to accomplish biological assays. All synthesized chemicals displayed no significant impact on these two parameters (data not shown). Thus, all were selected to perform further experiments. Melanin content was recorded on Melan-a cells exposed to a concentration of 20 μM of oxyprenylated compounds for 48 h. Results are reported in Fig. 2. The melanin content of untreated Melan-a cells

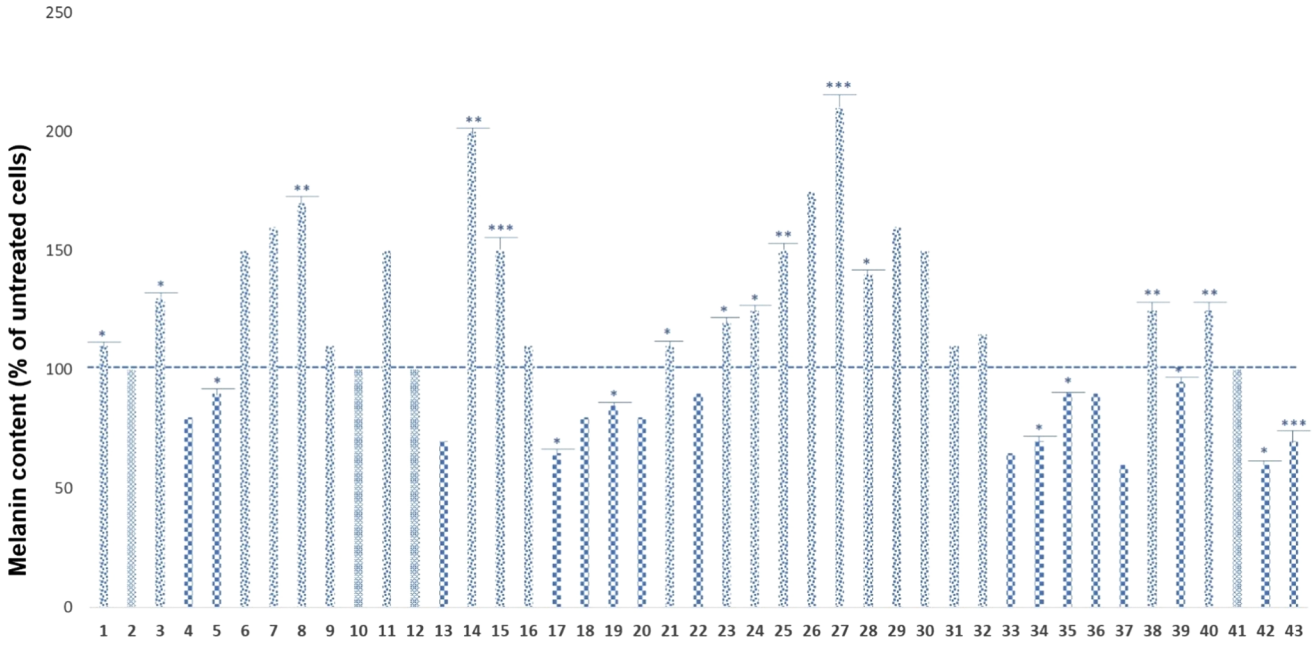


Fig. 2. Effect of oxyprenylated compounds in modulating melanin biosynthesis in Melan-a cells. *, **, and *** stand for P values of < 0.05, <0.01, p < 0.001 respectively.

was taken as the reference value of 100%. 7-Isopentenyloxycoumarin **14**, a tanning agent, and umbelliprenin **17**, a whitening agent, were used as control substances. Parent phenols were not assayed as in the course of previous investigations it was revealed that they do not exert any appreciable modulatory effect on the melanogenic machinery [11].

As shown in Fig. 2, not considering the already known 7-isopentenyloxycoumarin **14** and umbelliprenin **17**, 15 out of 41 samples were able to decrease melanin biosynthesis from a moderate to a good extent, 22 out of 41 compounds had an appreciable capacity to increase melanogenesis leading to a tanning effect. Compounds **2**, **10**, **12**, and **41** did not exhibit any appreciable differences respect to untreated control. In 3 cases the recorded inhibition or stimulation of melanogenesis were equal or even higher than values obtained for the reference products **14** and **17**. In particular 3-acetyl-7-geranyloxycoumarin **33** shares with umbelliprenin the capacity to inhibit melanogenesis around 65%, while 7-farnesylcoumarin-3-carboxylic acid **37** provided a slightly lower percentage (60%). On the other hand, the saturated derivative of compound **14**, namely 7-(3′-methyl)butoxycoumarin **27**, recorded a more than 2-fold stimulation of melanogenesis in Melan-a cells. Our results can be also well rationalized in terms of structure-activity relationship considerations. The following statements can be formulated (a) benzoic acid derivatives are slightly pigmenting agent. In this group of products, the tanning effect is more pronounced in compounds with longer *O*-side chains, thus exhibiting a profile exactly contrary recorded for prenyloxycoumarin derivatives (vide infra); (b) independently from the type of substitution, cinnamic acids are able to increase melanin biosynthesis, with the only exception of 4′-farnesyloxycinnamic acid **10**. Again, in this case, the pattern exhibited by such samples is partially different from that obtained for oxyprenylated coumarins in that 4′-farnesyl-3′-methoxycinnamic acid **11**, having a longer chain, provided a more than appreciable tanning effect (170%). (c) benzaldehydes are able to slightly decrease melanogenesis exhibiting values in the range 80%–90% of untreated controls, but such numbers are too close to allow to hypothesize a correlation between the individual structure and the recorded effect. (d) Coumarins with a 3,3-dimethylally or shorter skeletons as substituents in position 7 are tanning agents, while coumarins with farnesyloxy groups are whitening ones, in this resembling what we have already preliminarily observed in our previous investigation [11]. As a confirmation a similar pattern of results has been obtained also in the case of partially alkylated luteolin derivatives [38]. (e) The presence of two ortho oriented oxyprenylated chains in position 6 and 7 led to opposite effects. 3,3-dimehtyl or geranyl skeletons are slightly depigmenting agents, while farnesyl skeletons tend to marginally increase the tanning effect. (f) Electron withdrawing groups (acetyl, COOH, and -Cl) as substitutents of *O*-geranyl and *O*-farnesylcoumarins led to a whitening effect, and finally (g) oxyprenylated anthraquinones have only weak depigmenting capacities. Thus, for polyketides like compounds **12** and **13**, also considering the inhibitory properties of parent emodine [39], it may be hypothesized that, in an opposite way respect to phenylpropanoids, free phenolics are largely more effective as modulators of melanogenesis than prenylated counterparts.

In this paper the current status of knowledge about the modulatory properties of naturally occurring and semisynthetic oxyprenylated aromatic compounds using a non-tumoral cell line was widened. Studies on products able to modulate skin color are of great importance not only for curing patients with skin issue, but also for cosmetic industry purposes. Indeed, the market of tanning activators and skin whitening pharmaceutical preparations is experiencing a rapid increase over recent times. In this context pure natural products and plant extracts are nowadays valid, economically important, and effective means, as witnessed by the high number of publications per week reported in the literature focused on this topic. Results described herein may greatly contribute to consider such oxyprenylated aromatic compounds as putative novel remedies for skin diseases featured by hyper- or hypopigmentation of dermal tissues as well as new ingredients for cosmetic formulations and cosmeceuticals in both cases with a great potential. Furthermore, it has been herein clearly demonstrated that more classes of oxyprenylated compounds, other than coumarins, can be effectively considered agents able to modulate melanin biosynthesis. This is particularly true for cinnamates as well pointed out by Gunia-Krzyzak and coworkers in their explicative and excellent recently published review [40]. Data detailed in the present manuscript demonstrate that oxyprenylated cinnamic acid and ferulic derivatives are efficient modulators of melanogenesis, and surely represent a completion of

records reported by these Authors. Similar considerations can be formulated also in the case of oxyprenylated benzaldehydes respect to the in so far reported effects [41]. The capacity to interfere with melanin biosynthesis have been revealed at a relatively low concentration for all chemical samples (20 μM). Such a concentration value is very close to those of applied for arbutin and kojic acid, both representing common ingredients of widely used commercially available cosmetic formulations. So, it may be regarded as a safe concentration also considering that at 20 μM all compounds exhibited an appreciable effect on Melan-a cell viability and proliferation.

4. Conclusions

Examining data reported in the present manuscript, it can be concluded that first it has been provided further evidences on the role of prenylation of aromatic nuclei on the extent and course of melanogenesis in a non-cancer cell line. For the most products, it seems that inhibitory or stimulating properties are very strictly depending more on the structure of the prenyl side chain rather than the aromatic core itself. Enlargement of the number of chemical categories of natural and semisynthetic compounds with capacities to interfere to a different extent with melanin biosynthesis may be helpful in identifying new pharmacophores and/or to select novel lead compounds and templates to draw and synthesize a wider panel of new and hopefully more effective and less risky skin tanning and whitening agents. Finally, as largely pointed out in our recent papers, the data presented herein confirm and enforce the concept that a simple metabolic reaction like prenylation, widely occurring in all-natural kingdoms, does improve the pharmacological potential of natural compounds. Prenylated derivatives are in fact subject of an increasingly growing interest especially during the last five years [36,42–45].

Declaration of interest

None.

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