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Synthesis and melanogenesis evaluation of 3′,4′,7-trihydroxyflavanone derivatives and characterization of flavanone–BODIPY

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ABSTRACT

A class of flavanone was found to improve melanogenesis in B16F10 mouse melanoma cell line, but little is known about its target. Herein we described the synthesis and bioevaluation of sixteen 3′,4′,7-trihydroxyflavanone analogues and further synthesized a novel fluorescent flavanone–BODIPY, which could improve melanogenesis in B16F10 cell line by selectively binding to its endoplasmic reticulum. The fluorescent flavanone–BODIPY was proved to be a valuable probe for studying the localization of intracellular flavanone on living cells.

Vitiligo is an acquired pigmentary disorder of unknown etiology that is clinically characterized by the development of white macules related to the selective loss of melanocytes.[1,2] The exact pathogenesis is unknown although many hypothesis have been postulated to explain its pathogenesis including the autoimmune theory, the autocytotoxic theory, the neural theory, the 'impaired epidermal cytokine' theory, the melanocythorragic hypothesis and the recent inflammatory theory. However, vitiligo has been considered currently as a complex disorder in which every above-mentioned hypothesis eventually converged in a synergistic theory.[3] Conventional agents treated for vitiligo contains photochemotherapy (PUVA), phototherapy (UVB), vitamin D3 analogues, topical corticosteroids, topical immunomodulators, excimer laser and surgery. These treatment options have limited success and show some present significant risks such as suspected increases in skin cancer risk by PUVA, skin atrophy with corticosteroids, and skin boils with UVB therapy.[4] Hence it is essential to find a safe and effective alternative approach for the treatment of vitiligo.

Natural occurring flavanones derivatives have been reported as effective, safe and well tolerated agents for the treatment of vitiligo.[5] In our preliminary study, we found that a series of flavanones derivatives affected the melanogenesis of B16F10 cells, but the specific mechanism of the action of flavanones derivatives in vitiligo is still unknown. As it was described previously, small-molecule fluorescent probes could be used for studying the targets of the active small molecule,[6–9] and the mode of action between small molecules and their targets.[10–13]

While attractive in principle, the development of protein selective, activity-based imaging probes poses substantial technical challenges.[14] Such an ideal probe should possess several features, including high selectivity for a single target, a reporter tag for imaging the probe-labeled target, and suitable cell permeability and pharmacokinetic properties for in vivo studies.[15]

To design the appropriate probe, a structure–activity relationship (SAR) analysis of the flavanone was conducted to confirm which positions were not critical to biological activity so that the fluorescent could be introduced into the molecule without affecting its bioactivity. Thus, a series of flavanone, **5a**–**5p**, were synthesized as outlined in Scheme 1. Compound **2** was obtained from 3,4-dihydroxybenzaldehyde (**1**) that reacted with 3,4-dihydropyran in the presence of pyridinium-*p*-toluenesulfonate (PPTS) as catalyst in CH_2Cl_2. **2** was treated with **3** under reflux in ethanol afforded **4** using barium hydroxide octahydrate ($Ba(OH)_2{\cdot}8H_2O$) as catalyst. Cyclization of **4** employing 20% sulfuric acid in methanol afforded compound **5a**. Modification of **5a** at 3′,4′,7-trihydroxy and carbonyl positions provided its derivatives **5b**–**5p**. Moreover, the structures of **5j** and **5k** were confirmed by ROESY spectrum (see Supplementary material). 16 of flavanones derivatives (**5a**–**5p**) and α-MSH (positive control) were evaluated their effectiveness on melanin synthesis in B16F10 cell line (shown as Table 1).[16] SAR analysis of these compounds (**5a**–**5p**) showed that

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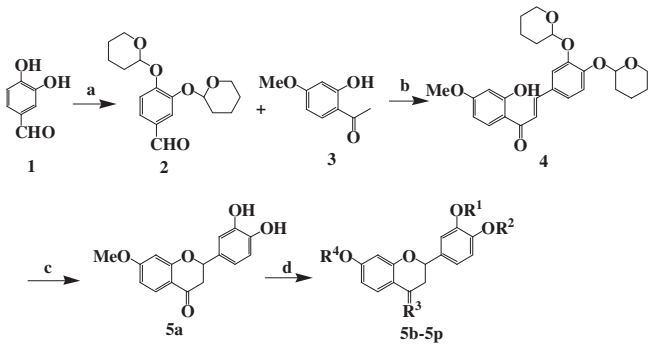


Scheme 1. Synthesis of **5a**–**5p**. Reagents and conditions: (a) 3,4-dihydropyran (PPTS), CH_2Cl_2, rt, overnight; (b) $Ba(OH)_2{\cdot}8H_2O$, ethanol, reflux, 7 h; (c) 20% H_2SO_4, methanol, reflux, 7 h; (d) different reaction conditions as Supporting information. For R^1, R^2, R^3 and R^4, see Table 1.

simultaneous introduction of relatively small substitute groups at the C3′ and C4′ positions such as propionyl (**5f**) and acetyl (**5l**) were favorable to improve the melanogenesis of B16F10 cells. Comparably, introduction of benzoyl (**5c**), substituted benzoyl (**5d**–**5e**, **5g**, **5o**), benzenesulfonyl (**5m**), furoyloxy (**5n**), allyl (**5p**) may have weak affect. However, monosubstitution at C3′ or C4′ position may considerably decrease the melanogenesis of B16F10 cell line (**5j**–**5k**). Demethylation at the C7 position (**5b**) could promote the melanoma synthesis of B16F10 cells. Notably, modification at C4 position affording ketoxime (**5h**) could also improve the melanin synthesis of B16F10 cells. Thus, **5b** and **5h** were picked out for the further in vivo study. They could significantly promote the melanin content of hair follicle on C57BL/6J depigmented skin (Fig. 1), the expression of tyrosinase, TRP-1, TRP-2, and microphthalmia transcription factor (MITF), shown as Supporting information.

Based on the in vitro and in vivo assay data and the SAR analysis, we hypothesized that the C7, C3′ and C4′ positions were critical for activity, whereas the C4 position would tolerate additional functional groups. Therefore, **5b**, the most active compound among

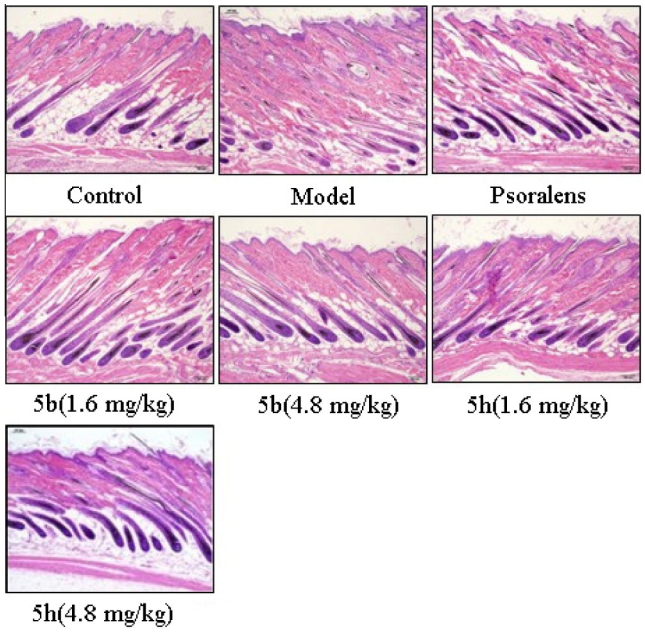
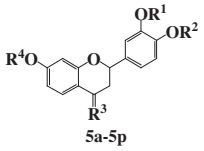


Figure 1. Effect of **5b** and **5h** treat on hair follicle melanogenesis in C57BL/6J depigmentation mouse stained by H&E (original magnification 100×).

the tested ones, was further combined with a fluorescent moiety boron-dipyrromethene (BODIPY) at C4 position to generate a flavanone–BODIPY probe.[17,18] This probe could be used for studying the targets of compound **5b** to promote the melanin synthesis of B16F10 cells.[19–22]

Carbonyl moieties may react with hydrazide in the presence of acetic acid under reflux afforded acylhydrazone.[23,24] BODIPY hydrazide is an ideal fluorescent reagent, which is widely used in in vivo fluorescence experiments.[25–27] Therefore, BODIPY hydrazide was firstly synthesized. Refluxing of a mixture of 2 equiv of succinic anhydride and 1 equiv of 2,4-dimethylpyrrole provided the BODIPY acid (**6**).[28–30] HOBt/EDCI was then used to activate **6**,

Table 1
The effect on melanin synthesis in B16F10 cells about a series of flavonoids (1 μM)[a]



Compound	R^1	R^2	R^3	R^4	Melanin content (%)
5a	H	H	O	Methyl	110.31 ± 7.17
5b	H	H	O	H	136.83 ± 7.55**
5c	Benzoyl	Benzoyl	O	Methyl	121.43 ± 4.23
5d	*p*-Methylbenzoyl	*p*-Methylbenzoyl	O	Methyl	113.49 ± 3.37
5e	*p*-Chlorobenzoyl	*p*-Chlorobenzoyl	O	Methyl	121.98 ± 4.26
5f	Propionyl	Propionyl	O	Methyl	133.65 ± 6.09**
5g	*p*-Fluorobenzoyl	*p*-Fluorobenzoyl	O	Methyl	111.79 ± 3.47
5h	H	H	Ketoximino	Methyl	134.71 ± 9.41**
5i	Dodecanoyl	Dodecanoyl	O	Methyl	89.54 ± 3.23*
5j	H	Dodecanoyl	O	Methyl	74.13 ± 5.96**
5k	H	Tetradecanoyl	O	Methyl	44.63 ± 8.40***
5l	Acetyl	Acetyl	O	Methyl	132.30 ± 2.26**
5m	*p*-Toluenesulfonyloxy	*p*-Toluenesulfonyloxy	O	Methyl	100.52 ± 9.09
5n	2-Furoyloxy	2-Furoyloxy	O	Methyl	96.11 ± 5.81
5o	*o*-Chlorobenzoyl	*o*-Chlorobenzoyl	O	Methyl	119.46 ± 4.30
5p	Allyl	Allyl	O	Methyl	91.19 ± 16.21
α-MSH	—	—	—	—	187.60 ± 2.11***

[a] Assay details are described in Supporting information. *P<0.05. **P<0.01. ***P<0.001 versus **5a**. The data are the mean ± SD, n = 3.