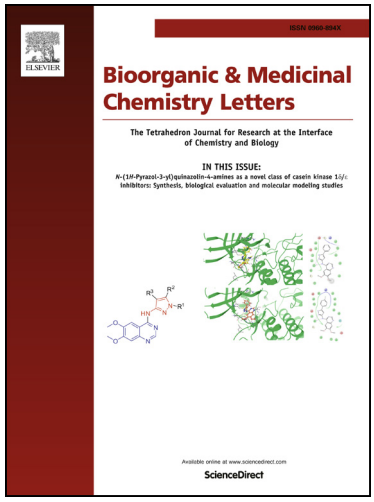

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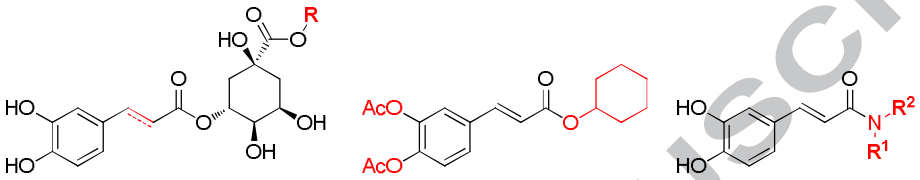

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Synthesis and Biological Evaluation of Caffeic Acid Derivatives as Potent Inhibitors of α-MSH-stimulated Melanogenesis

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ABSTRACT

We have disclosed our effort to develop caffeic acid derivatives as potent and non-toxic inhibitors of α-MSH-stimulated melanogenesis to treat pigmentation disorders and skin medication including a cosmetic skin-whitening agent. The SAR studies revealed that cyclohexyl ester and secondary amide derivatives of caffeic acid showed significant inhibitory activities.

Melanogenesis is a complex and multistep biosynthetic process that results in the production of melanin pigment, which plays a protecting role against harmful effects of ultra violet (UV) induced skin damages.[1-3] Upon exposure to UV radiation, alpha-melanocyte stimulating hormone (α-MSH) is produced and it stimulates melanocortin 1 receptor that results in the activation of adenylyl cyclase. The production of cAMP affects the pathway of cAMP-responsive element-binding protein (CREB), MAP kinase, and PI3K, finally resulting in the formation of melanin. cAMP leads to the phosphorylation of CREB transcription factors, which induces microphthalmia-associated transcription factor (MITF). MITF is the core controller in the expression of melanogenesis, which regulates the melanogenesis enzymes tyrosinase (TYR), catalyzing the rate-limiting step in melanogenesis. This key enzyme catalyses the hydroxylation of tyrosine into L-dopa and the subsequent oxidation to generate dopaquinone, which leads to the synthesis of pheomelanin and eumelanin; the basic melanin pigments which are responsible of human skin and color.[4]

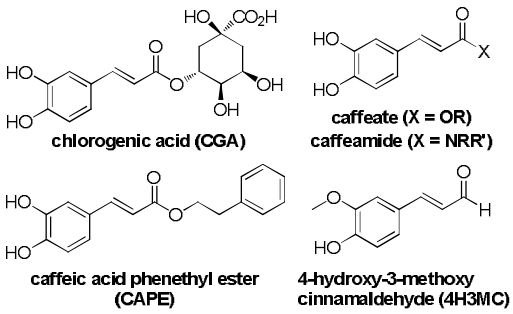

Fig. 1. Structures of caffeic acid-related derivatives to inhibit α-MSH-induced melanogenesis.

However, excessive accumulations of epidermal pigmentation can cause various hyperpigmentation disorders, such as, melasma, age spots, and sites of actinic damage and reducing the synthesis of melanin is important in cosmetic whitening.[5-8] Therefore, it is very important to control the related enzyme in

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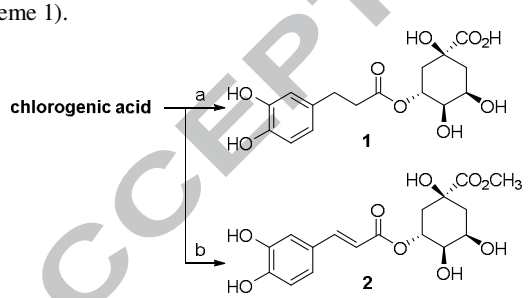
treating pigmentation disorders as well as in the development of skin medication including a cosmetic skin-whitening agent. This has led to the discovery of several tyrosinase inhibitors such as kojic acid, arbutin, and various herbal extracts.[9-19]

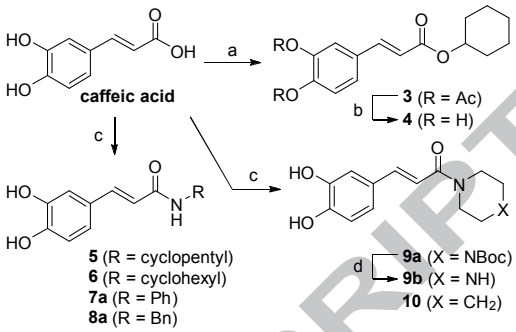
Hydroxycinnamic acids are class of phenolic acid derivatives, which are widely found in various plants with numerous biological activities; widely utilized in food, medicine, and cosmetics industries.[20] Of them caffeic acid occurs in foods mainly as an ester with L-quinic acid called chlorogenic acid (5-caffeoylquinic acid, CGA). CGA and caffeic acid are potent anti-oxidants in vitro/in vivo which present anti-inflammatory and immune-modulatory effects. Also, they might inhibit the formation of mutagenic and carcinogenic *N*-nitroso compounds and inhibit DNA damage. Recently, there are many efforts in which caffeic acid derivatives were found to inhibit α-MSH-induced melanogenesis (Figure 1).[21-23] Thus ester and amide derivatives of caffeic acid, isolated from plants or synthesized examined with respect to their abilities to control human melanogenesis.[24,25] Ha group reported that caffeic acid phenethyl ester (CAPE), found in various plants and propolis, has an anti-melanogenic activity in α-MSH-stimulated B16-F10 melanoma cells by suppression of melanogenic enzyme expression.[26] In contrast to the CAPE analogs, phenethyl alcohol, caffeic acid, and phenethyl cinnamate were not inhibitors of α-MSH-stimulated melanogenesis. On the other hands, Kim group recently discovered that 4-hydroxy-3-methoxycinnamaldehyde (4H3MC) inhibits α-MSH-induced melanin production in B16 mouse melanoma cells and primary human melanocytes, whereas 4H3MC analogs such as cinnamaldehyde, cinnamic acid, 4-hydroxycinnamic acid, 3-methoxycinnamic acid, 4-hydroxy-3-methoxycinnamic acid and 3-(4-hydroxyphenyl)propionic acid have the lower inhibitory activity of α-MSH-induced melanin production than 4H3MC.[27]

In this report, we have disclosed our effort to develop potent and non-toxic inhibitors of α-MSH-stimulated melanogenesis through the ester and amide derivatives of caffeic acid. Thus we first chose to synthesize two chlorogenic acid derivatives; the saturated compound **1** was prepared from chlorogenic acid by catalytic hydrogenation using 10% Pd/C, and methyl ester **2** by the treatment of (trimethylsilyl)diazomethane, repectively (Scheme 1).



Scheme 1. Synthesis of dihydrochlorogenic acid and methyl ester analogs. Reagents and conditions: (a). 10% Pd/C, H_2, EtOH, 12 h, rt, 95%; (b). $TMSCHN_2$, MeOH, rt, 12 h, 70%.

We turned our attention to the synthesis of various derivatives of caffeic acid such as esters, secondary and tertiary amides as depicted in Scheme 2. In order to prepare cyclohexyl ester, sequences of reactions were performed. Acetylation of the caffeic acid using Ac_2O, followed by the treatment of thionyl chloride in benzene at 50 ºC and finally esterification using DMAP and cyclohexanol afforded the compound **3**. Deacetylation of **3** using 2% sodium carbonate in methanol afforded the cyclohexyl ester of caffeic acid **4**. Caffeamides were prepared from caffeic acid with appropriate amines by using HOBt, EDCI, and DIPEA in DMF. Cycloalkylamines, phenylamines, benzylamines were selected for the secondary amides, whereas Boc-piperazine and piperidine were chosen for tertiary amide synthesis. Free amino group was obtained by treating Boc-piperazyl amide **9a** with 4 M HCl to afford **9b**.



Scheme 2. Synthesis of caffeate and caffeamide derivatives. Reagents and conditions: (a). i) Ac_2O, Py, DMAP, rt, 12 h, 42%; ii) $SOCl_2$, benzene, 50 ºC, 3 h; iii) DMAP, cyclohexanol, DCM, rt, 12 h, 20%; (b). 2% Na_2CO_3, MeOH, rt, 4 h, 50%; (c). HOBt, EDCI, DMF, amine, DIPEA, rt, 12 h; (d) 4 M HCl, 1,4-dioxane, rt, 3 h, 85%.

Synthetic analogs of CGA and caffeic acid derivatives were evaluated for their inhibitory potential on the production of melanin in α-MSH-activated B16 melanoma cells in Tables 1~3. Arbutin and kojic acid were used as positive controls. This assay was to determine the inhibitory activity of melanin formation in B16 melanoma cells in the presence of α-MSH (100 nM) throughout three-day incubation. The amount of melanin released into the culture media was determined by measuring sample absorbance at 405 nm against a synthetic melanin standard.[28-33] Both the saturated compound **1** and methyl ester **2** of CGA are inactive even with 40 μM.[22]

Table 1. The inhibitory activity of caffeic acid analogs

Compound	IC_{50} (μM)[a]	cell viability % (μM)	clogP[c]
Arbutin[b]	400 ± 9.50	ND[d]	
Kojic acid[b]	57 ± 1.0	ND	
1	> 40	ND	
2	> 40	ND	
3	0.20 ± 0.05	24 (2.5)	2.91
4	7.0 ± 1.0	30 (10)	2.96
5	30 ± 2.0	0 (40)	1.87
6	2.8 ± 0.10	23 (10)	2.29
7a	7.6 ± 0.85	21 (10)	2.4
8a	8.0 ± 1.0	23 (10)	2.47
9a	5.8 ± 0.26	31 (20)	1.46
9b	> 40	31 (20)	0.35
10	24 ± 2.0	0 (40)	1.71

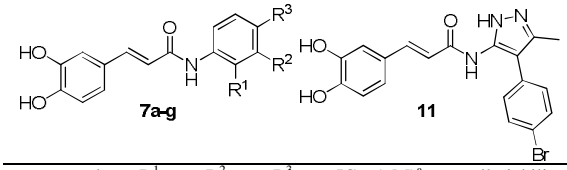
[a] Data are taken as a mean from 3 independent experiments; [b] Arbutin and kojic acid were used as the positive control; [c] clogP value was calculated by chemdraw ver. 13; [d] ND: not determined.

However, cyclohexyl ester **3** of caffeic acid with diacetate on catechol moiety exhibited potent inhibitory activity with IC_{50} of 0.2 μM, whereas reduced inhibitory activity (7 μM) was observed in the case of deacetylated ester **4**. Of secondary amides (**5**, **6**, **7a**, and **8a**), the cyclohexyl amide **6** exhibited more potent, than the cyclopentyl amide **5**. The inhibitory activity of tertiary amides

having piperidine and piperazine is lower than that of secondary amides. Ring size (**5** vs **6**) and secondary amide are critical to have the promising activity.

Next, the effect of substitution on phenyl and benzyl amide derivatives which are expected to have the improved activity and drug-like properties. Aryl and benzyl amides having substituents on phenyl ring were prepared like as the synthesis of amides such as **7a** and **8a** in Scheme 2. Caffeamides (**7a~7g**) containing substituted-phenyl as well as 3-methyl-4-(4-bromophenyl)-5-pyrazolyl caffeamide (**11**) as a heteroaromatic analog were evaluated for their inhibitory effect on the melanin production in α-MSH-activated B16 melanoma cells (Table 2); all the tested compounds except phenyl amide **7a** and *p*-isoproylphenyl amide **7d**, inhibited the melanin production with the IC_{50} values ranging from 0.7 to 2.1 μM. The cell viability of them was measured using the MTT assay, even though the tested compounds had no significant cytotoxic activity; some of these derivatives exhibited the cytotoxicity with higher concentration than IC_{50} value of inhibitory effect on the melanin production. Interestingly, irrespective of electronic and steric effect, substituent on phenyl ring is beneficial, displays higher activity than unsubstituted phenyl amide **7a**.

Table 2. The inhibitory effect of aryl amide analogs

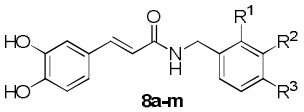


compound	R^1	R^2	R^3	IC_{50} (μM)[a]	cell viability % (μM)
7a	H	H	H	7.6 ± 0.85	21 (10)
7b	H	CF_3	H	1.4 ± 0.47	22-36 (2.5), 60-82 (5)
7c	H	H	CF_3	1.4 ± 0.45	56-58 (5), 77-90 (10)
7d	H	H	*i*Pr	3.4 ±0.058	15 (5), 82 (10)
7e	H	H	*n*-Bu	1.2 ± 0.46	33-49 (2.5), 68-96 (5)
7f	H	NO_2	H	1.9 ± 0.21	24-35 (5), 48 (10)
7g	H	H	NO_2	1.4 ± 0.17	30-35 (5), 51-62 (10)
11	-	-	-	1.0 ± 0.15	29-56 (2.5), 68-93 (5)

[a] Data are taken as a mean from 3 independent experiments.

Next, we have evaluated the inhibitory activity of the synthesized benzyl caffeamide analogues (**8a~8m**) on the melanin production in α-MSH-activated B16 melanoma cells (Table 3). Interestingly, electron-withdrawing group such as CF_3 on phenyl moiety exhibited more potent effects than electron-donating group except **8j**, **8k**, and **8m**. Later, cell viability of them was measured by using the MTT assay; all the compounds had no significant cytotoxic activity.

Table 3. The inhibitory effect of benzyl amide analogs



compound	R^1	R^2	R^3	IC_{50} (μM)[a]	cell viability (%, μM)[a]
8a	H	H	H	8.0 ± 1.0	23 (10)
8b	CH_3	H	H	20 ± 3.6	25 (10), 33-49 (20)
8c	H	CH_3	H	3.0 ± 0.90	20 (10), 45 (20)
8d	H	H	CH_3	3.7 ± 0.10	20 (10), 22-30 (20)
8e	CF_3	H	H	1.8 ± 0.26	22-28 (5), 33-49 (10)
8f	H	CF_3	H	1.4 ± 0.36	24 (5), 29-47 (10)
8g	H	H	CF_3	1.6 ± 0.40	26 (5), 39-48 (10)
8h	OCH_3	H	H	7.0 ± 2.6	20 (20), 35-46 (40)
8i	H	H	OCH_3	6.2 ± 1.7	20 (20), 37-54 (40)
8j	Cl	H	H	6.5 ± 0.60	0 (40)
8k	H	Cl	H	6.0 ± 1.6	22 (10), 59-62 (20)
8l	H	H	Cl	1.8 ± 0.26	23 (5), 39-53 (10)
8m	H	H	SO_2NH_2	>40	0 (40)

[a] Data are taken as a mean from 3 independent experiments.

In summary, a series of novel caffeate and caffeamide analogs were synthesized and evaluated inhibitory activity screening for the production of melanin in α-MSH-activated B16 melanoma cells identified potent and non-toxic agents such as **3**, **7c**, **7g**, **11**, **8g** and **8l**. SAR studies revealed that cyclohexyl ester and amide showed potent inhibitory effect and some substituents on phenyl group of secondary amides are very crucial. Tertiary amides are showing weaker inhibitory activity than secondary amides. Our ongoing efforts are to improve biological activities and drug-like properties. These findings provide important information of the structural features that influence the biological activities within this class of compounds, and offer new possibilities for further explorations in analog design. Based on SAR studies, further study for mode of action of these caffeates and caffeamide is under progress.

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi:

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