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RESEARCH ARTICLE

Synthesis of 6-deoxymollugins and their inhibitory activities on tyrosinase

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Abstract A series of 6-deoxymollugins were prepared five steps from benzaldehyde and its derivatives via phenylboronic acid-catalyzed chromenylation as a key step. Their inhibitory activities against tyrosinase from mushroom were evaluated to show that the parent, methyl 2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9a**) showed best and promising inhibitory activity at $IC_{50} = 18.3$ μM.

Keywords 6-Deoxymollugin · Chromenylation · Tyrosinase · 4-Hydroxynaphthoic acid

Introduction

Light especially ultraviolet (UV) irradiation on skin may result in skin damages such as sun-tanning, photoaging, and even photocarcinogenesis. Such damages can be protected by melanin endogenetically produced by a series of combined enzymatic and chemical reactions (Cullen 1998; Urabe et al. 1998). Accumulation of melanin at any specific sites of skin, however, resulted in many dermatological disorders including acquired hyperpigmentation such as melasma, freckles, postinflammatory melanoderma, solar lentigo, and age spot (Lerner et al. 1949; Kuzukami et al. 1993). Three melanocyte-specific enzymes, tyrosinase, tyrosinase-related protein-1 (TYRP-1) and tyrosinase-related protein-2 (TYRP-2), are involved in the process of forming melanin pigments from tyrosine (Park et al. 2004). Especially tyrosinase participates in two rate limiting steps of melanin synthesis, which are a hydroxylation of tyrosine and an oxidation L-2,4-dihydroxyphenylalanine (L-DOPA) (Halaban et al. 2002). Proper inhibition of tyrosinase, therefore, can be an effective tool for the treatment of such dermatological disorders (Seo et al. 2003; Okombi et al. 2006).

A series of tyrosinase inhibitors such as azelaic acid (Lemic-Stoicevic et al. 1995), arbutin (Maeda and Fukuda 1996; Nihei and Kubo 2003), hydroquinones (Sanchez and Vazquez 1982), kojic acid and its derivatives (Saruno et al. 1979), hydroxystilbenes represented by resveratrol (Satooka and Kubo 2012), methyl gentisate (Curto et al. 1999), *N*-phenylthioureas (Sawant et al. 2010), oxadiazoles (Khan et al. 2006a), oxazolones (Khan et al. 2006b), and tetraketone derivatives (Okombi et al. 2006) are developed as potential candidates for skin-decolorizing cosmetic agents. However, only few of these are successfully launched in practice mainly due to undesirable side effects.

Our interest in searching tyrosinase inhibitors from natural sources have led to find mollugin (methyl 6-hydroxy-2,2-dimethyl-2*H*-benzeno[*h*]chromene-5-carboxylate, **1**) as an strong inhibitor of tyrosinase. Mollugin was originally isolated from the herbal medicine Rubiaceae family such as *Putoria calabrica*, (Gonzalez et al. 1974) *Gallium mollugo* L. (Schildknecht et al. 1976a), and later from various *Rubia* species such as *R. cordifolia* and *R. akane* (Itokawa et al. 1983), and *R. tinctorum* (Marec et al. 2001).

Continuing study on mollugin revealed that mollugin inhibited cell adhesion between angioendothelial cells and lymphocytes thus having potentials for cosmetic additives with hair growth stimulant activity (Shibuya et al. 1999) and anti-aging activity (Murase et al. 1996; Murase et al. 1997).

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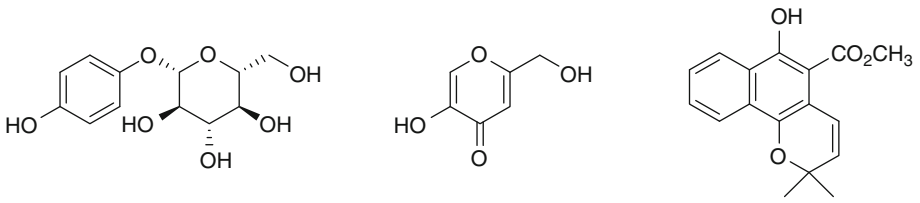


Although unique structure and intriguing biological properties of mollugin led to develop several total synthetic procedures (Schildknecht and Straub 1976b; Heide and Leistner 1981; Lumb and Trauner 2005; Habonimana et al. 2006; Claessens et al. 2006; Jung et al. 2007) systematic studies on specified biological properties were not yet pursued. We herein described a synthesis 6-deoxymollugins and their inhibitory activity on tyrosinase.

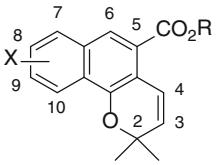
Materials and methods

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR and are reported as ppm from the internal standard TMS. Chemicals and solvents were commercial reagent grade and used without further purification. The derivatives of 2-(*E*)-benzylidenesuccinic acids, **6b** and **6d** (El-Abbady et al. 1961), and 4-hydroxynaphthoic acids, **7b** (Beech and Beech and Legg 1949), **7d** (El-Abbady et al. 1961) and **7j** (Sieglitz and Jordanides 1967) have been reported previously. Mollugin was prepared by employing previously reported methods (Jung et al. 2007). Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer. UV absorption experiment was performed from a JASCO-V550 spectrophotometer.

2-(*E*)-benzylidenesuccinic acid (**6a**) (general procedure)

A solution of benzaldehyde (15 g, 141 mmol) and dimethyl succinate (25.76 g, 176 mmol) in *t*-BuOH (20 mL) was added drop wise within 3 h to a refluxing mixture of *t*-BuOK

Table 1 Physical properties of **8** and **9** and their inhibitory activities against tyrosinase



8 R = H
9 R = CH_3

Entry	X	Yield (%)	mp (°C)	IC_{50} (μM)[a]	Entry	X	Yield (%)	mp (°C)	IC_{50} (μM)
8a	H	79	192	>50	**9a**	H	95	40	18.3
8b	7-Cl	79	211	>50	**9b**	7-Cl	93	88	29.0
8c	8-Cl	82	218	>50	**9c**	8-Cl	96	82	32.8
8d	9-Cl	85	216	>50	**9d**	9-Cl	92	111	45.7
8e	7-Br	85	195	>50	**9e**	7-Br	92	98	26.5
8f	8-Br	80	204	>50	**9f**	8-Br	95	92	28.9
8g	9-Br	84	202	>50	**9g**	9-Br	90	113	34.2
8h	7-CH_3	80	209	>50	**9h**	7-CH_3	91	84	32.3
8i	8-CH_3	81	219	>50	**9i**	8-CH_3	90	78	44.6
8j	9-CH_3	84	212	>50	**9j**	9-CH_3	94	99	47.3
Mollugin				1.5	**10**			96–97	>50
Arbutin				180					

[a] The IC_{50} value was expressed as the mean of 50 % inhibitory concentration of triplicate determination

(17.4 g, 155 mmol) and *t*-BuOH (100 mL). Reflux was continued for another 3 h, and *t*-BuOH was removed in vacuo. The residue obtained was dissolved in 1 *N* aq. HCl (100 mL), and the aqueous solution was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to yield an oily product as methyl 2-(*E*)-benzylidenesuccinate (**5a**). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.91 (s, 1H, –C*H* =), 7.43–7.30 (m, 5H, phenyl H's), 3.82 (s, 3H, OCH_3), 3.57 (s, 2H, –CH_2–). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 177.11, 167.85, 142.59, 134.65, 129.04, 128.96, 128.67, 125.20, 52.38, 33.50. This oil was re-dissolved in MeOH (60 mL), a 15 % solution of NaOH in MeOH (100 mL, 375 mmol) was added and the mixture was refluxed for 12 h. The resulting suspension was concentrated under reduced pressure, and the residue dissolved in water (150 mL). The solution was washed with EtOAc (3 × 100 mL), acidified with concentrated HCl and extracted with EtOAc (3 × 50 mL). The organic extracts were dried (Na_2SO_4) and concentrated in vacuo to afford a solid which was crystallized from EtOAc/hexane to give **6a** (20.4 g, 73 %) as a white crystalline solid: mp 174 °C. [lit. (Caro et al. 2003) mp 174–176 °C]. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.57 (s, 2H, 2COOH), 7.73 (s, 1H, Ar–C<u>H</u>), 7.47–7.37 (m, 5H, Ar), 3.36 (s, 2H, C<u>H</u>$_2$-COOH). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.42, 168.72, 140.19, 135.11, 129.24, 129.12, 128.95, 127.53, 33.64.

2-(*E*)-(3-Chlorophenyl)methylidenesuccinic acid (**6c**)

White needles (71 %): mp 189 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.47 (br s, 2H, 2COOH), 7.70 (s, 1H, Ar–C<u>H</u>), 7.46–7.45 (m, 3H, Ar), 7.37–7.34 (m, 1H, Ar), 3.34 (s. 2H, C<u>H</u>$_2$–COOH). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.17, 168.39, 138.54, 137.29, 133.55, 130.74, 128.98, 128.79, 128.75, 127.70, 33.67.

2-(*E*)-(2-Bromophenyl)methylidenesuccinic acid (**6e**)

White needles (65 %): mp 189 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.55 (br. s, COOH), 7.72 (dd, 1H, J = 7.8, 1.0 Hz, H3), 7.68 (s, 1H, Ar–C<u>H</u>), 7.77 (dd, 1H, J = 7.8, 1.0 Hz, H6), 7.38–7.30 (m, 2H, H4 and H5), 3.23 (s, 2H, C<u>H</u>$_2$–COOH). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.07, 168.03, 138.98, 135.09, 130.81, 130.09, 129.16, 128.08, 123.56, 33.51.

2-(*E*)-(3-Bromophenyl)methylidenesuccinic acid (**6f**)

White needles (69 %): mp 163 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.70 (s, 1H, Ar–C<u>H</u>), 7.60–7.57 (m, 2H, Ar–H), 7.41–7.39 (m, 2H, Ar–H), 3.35 (s, 2H, C<u>H</u>$_2$-COOH). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 171.99, 168.16, 138.40, 137.38, 131.51, 131.41, 130.81, 128.75, 127.87, 121.93, 33.43.

2-(*E*)-(4-Bromophenyl)methylidenesuccinic acid (**6g**)

White needles (72 %): mp 180 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.63 (s, 2H, 2COOH), 7.68 (s, 1H, Ar–C<u>H</u>), 7.63 (dm, 2H, J = 8.3 Hz, H3′ and H5′), 7.35 (dm, 2H, J = 8.3 Hz, H2′ and H6′), 3.34 (s, 2H, C<u>H</u>$_2$–COOH). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.07, 168.40, 138.85, 134.22, 131.77, 131.10, 128.21, 122.26, 33.47.

2-(*E*)-(2-Methylphenyl)methylidenesuccinic acid (**6h**)

White needles (65 %): mp 186 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.45 (s, 2H, 2COOH), 7.78 (s, 1H, Ar–C<u>H</u>), 7.27–7.17 (m, 4H, Ar), 3.20 (s, 2H, C<u>H</u>$_2$–COOH), 2.24 (s, 3H, Ar–C<u>H</u>$_3$). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 171.97, 168.10, 139.20, 136.41, 134.27, 129.99, 128.53, 128.02, 127.79, 125.77, 33.27, 19.31.

2-(*E*)-(3-Methylphenyl)methylidenesuccinic acid (6i)

White needles (67 %): mp 171 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.50 (s, 2H, 2COOH), 7.70 (s, 1H, Ar–C<u>H</u>), 7.36–7.30 (m, 1H, Ar), 7.21 (m, 3H, Ar), 3.37 (s, 2H, C<u>H</u>$_2$–COOH), 2.32 (s, 3H, Ar-C<u>H</u>$_3$). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.11, 168.45, 139.98, 137.85, 134.84, 129.59, 129.47, 128.55, 127.18, 125.98, 33.40, 20.94.

2-(*E*)-(4-Methylphenyl)methylidenesuccinic acid (**6j**)

White needles (70 %): mp 211 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.45 (s, 2H, 2COOH), 7.70 (s, 1H, Ar–C<u>H</u>), 7.30 (d, 2H, J = 8.3 Hz, H3′, H5′), 7.25 (d, 2H, J = 8.3 Hz, H2′, H6′), 3.40 (s, 2H, C<u>H</u>$_2$-COOH), 2.33 (s, 3H, Ar–C<u>H</u>$_3$). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 172.04, 168.49, 139.85, 138.54, 132.01, 129.23, 129.00, 126.45, 33.37, 20.82.

4-Hydroxy-2-naphthoic acid (**7a**)

2-(*E*)-Benzylidenesuccinic acid **6a** (206 mg, 1.0 mmol) was dissolved in conc. H_2SO_4 (464 mg) and stirred at room temperature for 8 h. The reaction mixture was carefully poured over cold water (1 mL) and allowed to crystallize overnight at 5 °C. The resulting crystals were filtered, washed with water, dried *in vacuo* and recrystallized from 95 % EtOH to give 144 mg (77 %) of **7a**: mp 223–224 °C. [lit. (Cason 1941) 225–226 °C]. IR (KBr) 3447, 1656, 1579 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 10.48 (s, 1H, OH), 8.17–8.13 (dt, 1H, J = 8.5, 2.0 Hz, H5), 8.05 (s, 1H, H4), 7.99 (dt, 1H, J = 8.5, 2.0 Hz, H8), 7.56–7.51 (m, 2H, H6, H7), 7.37 (s, 1H, H3) ^{13}C NMR (DMSO-d_6) δ 167.82, 153.53, 133.67, 129.19, 128.76, 127.17, 127.12, 126.84, 122.19, 121.44, 107.11.

6-Chloro-4-hydroxy-2-naphthoic acid (**7b**)

Pale yellow solid (65 %): mp 298 °C [lit. (Beech and Legg 1949) mp 302–304 °C]. Unreported spectral data are as follows: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 10.72 (br. s, 1H), 8.08 (dd, 1H, J = 2.3 Hz, H5), 8.07 (s, 1H, H1), 8.05 (d, 1H, J = 8.8 Hz, H8), 7.56 (dd, 1H, J = 8.8, 2.2 Hz, H7), 7.40 (d. 1H. J = 1.3 Hz, H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 167.54, 152.83, 132.07, 132.05, 131.54, 129.39, 127.64, 127.43, 121.24, 121.10, 108.29.

7-Chloro-4-hydroxy-2-naphthoic acid (**7c**)

Pale yellow solid (57 %): mp 290 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 13.07 (br s, 1H, COOH), 10.69 (s, 1H, OH), 8.15 (d, 1H, J = 2.3 Hz, H8), 8.13 (d, 1H, J = 8.4 Hz, H5), 8.03 (s, 1H, H1), 7.56 (dd, 1H, J = 8.4, 2.0 Hz, H7), 7.36 (s, 1H, H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 167.55, 153.75, 134.61, 131.88, 130.23, 127.73, 127.55, 125.12, 124.61, 120.56, 107.69.

6-Bromo-4-hydroxy2-naphthoic acid (**7e**)

Pale yellow solid (58 %): mp 305 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 12.99 (br s, 1H, COOH), 10.71 (s, 1H, OH), 8.29 (d, 1H, J = 2.3 Hz, H5), 8.07 (s, 1H, H1), 7.99 (d, 1H, J = 8.5 Hz, H8), 7.68 (d, 1H, J = 8.8, 2.3 Hz, H7), 7.42 (s, 1H, H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 167.34, 152.51, 132.01, 131.33, 129.93, 129.31, 127.61, 124.16, 121.08, 120.52, 108.10.

7-Bromo-4-hydroxy-2-naphthoic acid (**7f**)

Pale yellow solid (55 %): mp 283 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 12.99 (br s, 1H, COOH), 10.65 (s, 1H, OH), 8.31 (d, 1H, J = 1.8 Hz, H8), 8.08 (d, 1H, J = 8.9 Hz, H5), 8.03 (s, 1H, H1), 7.68 (dd, 1H, J = 8.9, 2.0 Hz, H6), 7.39 (d, 1H, J = 1.0 Hz H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 167.36, 153.63, 134.87, 130.75, 129.98, 129.86, 125.12, 124.47, 120.45, 120.31, 107.61.

8-Bromo-4-hydroxy-2-naphthoic acid (**7g**)

Pale yellow solid (61 %): mp 289 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.94 (br s, 1H, COOH), 10.82 (s, 1H, OH), 8.27 (d, 1H, J = 1.8 Hz, H1), 8.22 (d, 1H, J = 8.4 Hz, H7), 7.94 (dd, 1H, J = 7.4, 0.9 Hz, H5), 7.51–7.45 (m, 2H, H6 and H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 167.21, 153.96, 131.76, 131.21, 130.08, 127.93, 127.52, 122.90, 122.37, 119.27, 107.94.

6-Methyl-4-hydroxy-2-naphthoic acid (**7h**)

Pale yellow solid (60 %), mp 257 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 812.79 (br. s, COOH), 10.34 (s, 1H, OH), 7.98 (s, 1H, H5), 7.91 (s, 1H, H1), 7.87 (d, J = 8.4 Hz, H8), 7.37 (dd, 1H, J = 8.4, 1.8 Hz, H6), 7.31 (d, 1H, J = 1.2 Hz, H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 167.72, 152.79, 136.63, 131.71, 129.06, 128.93, 127.60, 126.82, 121.18, 120.98, 106.99, 21.64.

7-Methyl-4-hydroxy-2-naphthoic acid (7i)

Pale yellow solid (63 %): mp 239 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 10.32 (br s, 1H, OH), 8.06 (d, 1H, J = 8.5 Hz, H5), 7.94 (s, 1H, H1), 7.75 (s, 1H, H8), 7.40 (d, 1H, J = 8.5 Hz, H7), 7.30 (s, 1H, H3). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 167.67, 153.22, 136.13, 133.68, 128.96, 128.72, 127.69, 124.83, 121.86, 120.52, 106.25.

2,2-Dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8a**) (general procedure for phenylboronic acid-mediated chromenylation)

To a mixture of 4-hydroxy-2-naphthoic acid (**7a**, 940 mg, 5 mmol), 3-methyl-2-butenal (505 mg, 6 mmol), phenylboronic acid (732 mg, 6 mmol), and propionic acid (0.1 mL) in toluene (50 mL) was refluxed using Dean-Stark trap for 24 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (50 mL). The organic layer was separated, washed with brine (3 × 50 mL), dried, and evaporated in vacuo. The resultant residue was purified by column chromatography. The early fractions were recrystallized from pet. hexane:EtOAc to afford **8a** (1.0 g, 79 %) as yellow-green flakes: mp 192 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.24 (s, 1H, H6), 8.22 (d, 1H, J = 8.0 Hz, H10), 7.84 (d, 1H, J = 7.8 Hz, H7), 7.59–7.49 (m, 2H, H8, H9), 7.41 (d, 1H, J = 10.0 Hz, H4), 5.75 (d, 1H, J = 10.0 Hz, H3), 1.53 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 172.70, 149.36, 132.39, 129.80, 129.00, 128.02, 127.57, 127.03, 125.51, 123.21, 122.25, 120.88, 114.76, 76.08, 27.57.

7-Chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8b**)

The same procedure described for **8a** was applied to 8-chloro-4-hydroxy-2-naphthoic acid (**7d**) (El-Abbady et al. 1961) to afford **8b** (320 mg, 79 %) as yellow-green flakes: mp 211 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.62 (s, 1H, H6), 8.16 (d, 1H, J = 8.3 Hz, H10), 7.57 (d, 1H, J = 6.8 Hz, H8), 7.44 (t, 1H, J = 7.9 Hz, H9), 7.40 (d, 1H, J = 10.0 Hz, H4), 5.78 (d, 1H, J = 10.0 Hz, H3), 1.52 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.92, 149.50, 133.05, 130.62, 129.80, 128.63, 127.71, 127.27, 124.13, 121.51, 121.38, 120.56, 115.53, 77.21, 27.53.

8-Chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (8c)

The same procedure described for **8a** was applied to 7-chloro-4-hydroxy-2-naphthoic acid (**7c**) to afford **8c**

(330 mg, 82 %) as yellow-green flakes: mp 218 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.16 (d, 1H, J = 9.0 Hz, H10), 8.11 (s, 1H, H6), 7.80 (d, 1H, J = 2.0 Hz, H7), 7.47 (dd, 1H, J = 9.0, 2.0 Hz, H9), 7.37 (d, 1H, J = 10.0 Hz, H4), 5.76 (d, 1H, J = 10.0 Hz, H3), 1.52 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.29, 149.51, 133.04 (2 C's), 131.17, 128.76, 127.49, 125.67, 124.28, 124.12 (2 C's), 120.56, 115.11, 77.21, 27.60.

9-Chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (8d)

The same procedure described for **8a** was applied to 6-chloro-4-hydroxy-2-naphthoic acid (**7b**) to afford **8d** (345 mg, 85 %) as yellow-green flakes: mp 216 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.18 (s, 1H, H6), 8.18 (d, 1H, J = 2.0 Hz, H10), 7.76 (d, 1H, J = 8.8 Hz, H7), 7.42 (dd, 1H, J = 8.8, 2.0 Hz, H8), 7.38 (d, 1H, J = 10.3 Hz, H4), 5.77 (d, 1H, J = 10.0 Hz, H3), 1.52 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.96, 148.52, 130.52 (2 C's), 130.19, 128.50, 128.03, 127.95, 125.01, 121.41, 124.21, 120.55, 115.69, 77.21, 27.60.

7-Bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (8e)

The same procedure described for **8a** was applied to 8-bromo-4-hydroxy-2-naphthoic acid (**7g**) to afford **8e** (250 mg, 85 %) as yellow-green flakes: mp 195 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.58 (s, 1H, H6), 8.21 (d, 1H, J = 8.5 Hz, H10), 7.77 (d, 1H, J = 7.3 Hz, H8), 7.39 (d, 1H, J = 10.3 Hz, H4), 7.37 (t, 1H, J = 7.9 Hz, H9), 5.78 (d, 1H, J = 10.0 Hz, H3), 1.52 (s, 6H, CMe_2).

8-Bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8f**)

The same procedure described for **8a** was applied to 7-bromo-4-hydroxy-2-naphthoic acid (**7f**) to afford **8f** (235 mg, 80 %) as yellow-green flakes: mp 204 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.07 (s, 1H, H6), 8.06 (d, 1H, J = 8.5 Hz, H10), 7.95 (d, 1H, J = 1.8 Hz, H7), 7.58 (dd, 1H, J = 9.1, 1.9 Hz, H9), 7.34 (d, 1H, J = 10.0 Hz, H4), 5.75 (d, 1H, J = 10.0 Hz, H3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 172.05, 149.46, 133.36, 131.16, 130.75, 130.19, 125.78, 124.46, 124.11, 124.04, 121.24, 120.59, 115.17, 76.36, 27.52.

9-Bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8g**)

The same procedure described for **8a** was applied to 6-bromo-4-hydroxy-2-naphthoic acid (**7e**) to afford **8g** (245 mg, 84 %) as yellow-green flakes: mp 246 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.35 (s, 1H, H10), 8.15 (s, 1H, H6), 7.68 (d, 1H, J = 8.8 Hz, H7), 7.54 (dd, 1H, J = 8.8, 1.8 Hz, H8), 7.36 (d, 1H, J = 10.0 Hz, H4), 5.76 (d, 1H, J = 10.0 Hz, H3), 1.52 (s, 6H, CMe_2).

2,2,7-Trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8h**)

The same procedure described for **8a** was applied to 8-methyl-4-hydroxy-2-naphthoic acid (**7j**) to afford **8h** (340 mg, 80 %) as yellow-green flakes: mp 209 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.40 (s, 1H, H6), 8.11 (d, 1H, J = 8.3 Hz, H10), 7.44 (t, 1H, J = 7.8 Hz, H9), 7.42 (d, 1H, J = 10.3 Hz, H4), 7.33 (d, 1H, J = 6.8 Hz, H8), 5.75 (d, 1H, J = 10.0 Hz, H3), 2.70 (s, 3H, CH_3), 1.52 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 172.66, 149.71, 135.72, 131.66, 129.95, 127.84, 127.81, 127.79, 122.65, 121.76, 120.89, 120.47, 114.63, 76.04, 27.52, 19.44.

2,2,8-Trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (8i)

The same procedure described for **8a** was applied to 7-methyl-4-hydroxy-2-naphthoic acid (**7i**) to afford **8i** (345 mg, 81 %) as yellow-green flakes: mp 227 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.14 (s, 1H, H6), 8.12 (d, 1H, J = 9.8 Hz, H10), 7.59 (s, 1H, H7), 7.39 (d, 1H, J = 10.0 Hz, H4), 7.38 (dd, 1H, J = 8.5, 1.5 Hz, H9), 5.71 (d, 1H, J = 10.0 Hz, H3), 2.50 (s, 3H, CH_3), 1.51 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 172.14, 149.42, 136.91, 132.74, 130.29, 129.37, 127.96, 125.81, 124.86, 123.21, 122.15, 120.96, 114.13, 76.01, 27.56, 21.63.

2,2,9-Trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8j**)

The same procedure described for **8a** was applied to 6-methyl-4-hydroxy-2-naphthoic acid (**7h**) to afford **8j** (350 mg, 83 %) as yellow-green flakes: mp 246 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.20 (s, 1H, H6), 7.98 (s, 1H, H10), 7.73 (d, 1H, J = 8.5 Hz, H7), 7.41 (d, 1H, J = 10.0 Hz, H4), 7.33 (d, 1H, J = 8.3 Hz, H8), 5.74 (d, 1H, J = 10.0 Hz, H3), 2.54 (s, 3H, CH_3), 1.52 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 171.73, 148.76, 138.32, 130.61, 129.69, 129.32, 128.88, 127.68, 125.43, 122.06, 121.13, 120.95, 114.88, 75.95, 27.50, 22.13.

Methyl 2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (9a)

To a solution of 2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid (**8a**, 254 mg, 1.0 mmol) in CH_3OH

(50 mL) was slowly added conc. H_2SO_4 (0.5 mL) and resulting mixture was refluxed for 10 h and poured to water. The white precipitate formed was collected and washed with CH_3OH and water to give **9a** (80 mg, 95 %) as yellow flakes after recrystallization from hexane:EtOAc (1:1): mp 40 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.19 (d, 1H, J = 8.1 Hz, H10), 8.05 (s, 1H, H6), 7.78 (d, 1H, J = 8.4 Hz, H7), 7.55–7.42 (m, 2H, H8, H9), 7.28 (d, 1H, J = 10.1 Hz, H4), 5.71 (d, 1H, J = 10.1 Hz, H3), 3.93 (s, 3H, CO_2CH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 167.73, 149.15, 132.44, 129.66, 128.71, 127.52, 126.99, 126.88, 124.52, 124.01, 122.16, 120.81, 114.55, 76.04, 52.15, 27.54. *Anal*. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 4.99. Found C, 74.98; H, 5.06.

Methyl 7-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9b**)

The same procedure described for **9a** was applied to 7-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9b** (66 mg, 93 %) as yellow-green flakes: mp 88 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.41 (s, 1H, H6), 8.13 (d, 1H, J = 8.3 Hz, H10), 7.53 (dd, 1H, J = 7.4, 1.1 Hz, H8), 7.40 (t, 1H, J = 7.9 Hz, H9), 7.28 (d, 1H, J = 10.3 Hz, H4), 5.75 (d, 1H, J = 10.3 Hz, H3), 3.95 (s, 3H, OCH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.47, 149.31, 132.75, 130.45, 129.83, 128.09, 127.20, 127.11, 125.51, 121.32, 120.49, 120.03, 115.30, 76.45, 52.31, 27.54. *Anal*. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found C, 67.67; H, 4.85.

Methyl 8-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9c**)

The same procedure described for **9a** was applied to 8-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9c** (73 mg, 96 %) as yellow flakes: mp 82 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.14 (d, 1H, J = 9.0 Hz, H10), 7.93 (s, 1H, H6), 7.76 (d, 1H, J = 2.0 Hz, H7), 7.44 (dd, 1H, J = 9.0, 2.0 Hz, H9), 7.27 (d, 1H, J = 10.3 Hz, H4), 5.73 (d, 1H, J = 10.0 Hz, H3), 3.94 (s, 3H, OCH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 167.37, 149.31, 133.14, 132.83, 129.96, 128.24, 127.24, 125.84, 125.14, 124.05, 124.76, 120.57, 114.89, 76.38, 52.22, 27.60. *Anal*. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found C, 68.36; H, 4.80.

Methyl 9-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9d**)

The same procedure described for **9a** was applied to 9-chloro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9d** (55 mg, 92 %) as yellow flakes: mp 111 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.16 (dd, 1H, J = 2,1, 0.7 Hz, H10), 8.00 (s, 1H, H6), 7.71 (d, 1H, J = 8.8 Hz, H7), 7.38 (dd, 1H, J = 8.7, 2.1 Hz, H8), 7.27 (d, 1H, J = 10.1 Hz, H4), 5.73 (d, 1H, J = 10.1 Hz, H3), 3.93 (s, 3H, OCH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 167.41, 148.38, 133.73, 130.66, 130.34, 130.27, 127.80, 127.57, 124.88, 123.66, 121.36, 120.56, 115.50, 76.42, 52.17, 27.61. *Anal*. Calcd for $C_{17}H_{15}ClO_3$: C, 67.44; H, 4.99. Found C, 68.54; H, 4.82.

Methyl 7-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9e**)

The same procedure described for **9a** was applied to 7-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9e** (71 mg, 92 %) as yellow-green flakes: mp 98 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.38 (s, 1H, H6), 8.19 (d, 1H, J = 8.3 Hz, H10), 7.75 (d, 1H, J = 7.3 Hz, H8), 7.33 (t, 1H, J = 8.3 Hz, H9), 7.28 (d, 1H, J = 10.3 Hz, H4), 5.75 (d, 1H, J = 9.8 Hz, H3), 3.96 (s, 3H, OCH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 167.46, 149.29, 131.07, 130.90, 130.50, 128.19, 127.60, 125.77, 123.59, 122.71, 122.06, 120.50, 115.30, 76.49, 52.33, 27.56. *Anal*. Calcd for $C_{17}H_{15}BrO_3$: C, 58.81; H, 4.35. Found C, 59.41; H, 4.21.

Methyl 8-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9f**)

The same procedure described for **9a** was applied to 8-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9f** (63 mg, 95 %) as yellow flakes: mp 92 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.06 (d, 1H, J = 8.8 Hz, H10), 7.93 (s, 1H, H6), 7.93 (d, 1H, J = 2.5 Hz, H7), 7.56 (dd, 1H, J = 8.9, 1.9 Hz, H9), 7.25 (d, 1H, J = 10.0 Hz, H4), 5.73 (d, 1H, J = 10.3 Hz, H3), 3.93 (s, 3H, OCH_3), 1.49 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.35, 149.34, 133.52, 130.72, 130.54, 130.07, 125.74, 125.33, 124.19, 122.71, 121.11, 120.56, 115.00, 76.38, 52.26, 27.57. *Anal*. Calcd for $C_{17}H_{15}BrO_3$: C, 58.81; H, 4.35. Found C, 58.33; H, 4.43.

Methyl 9-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9g**)

The same procedure described for **9a** was applied to 9-bromo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9g** (57 mg, 90 %) as yellow flakes: mp 113 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.34 (dd, 1H, J = 2,0, 0.7 Hz, H10), 7.99 (s, 1H, H6), 7.63 (d, 1H, J = 8.8 Hz, H7), 7.51 (dd, 1H, J = 8.8, 2.0 Hz, H8), 7.27 (d, 1H, J = 10.0 Hz, H4), 5.73 (d, 1H, J = 10.3 Hz, H3), 3.93 (s, 3H, OCH_3), 1.51 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$,

62.5 MHz) δ 167.39, 148.28, 130.83, 130.35, 130.29 (two C's), 127.90, 125.01, 124.66, 123.69, 122.07, 120.53, 115.51, 76.44, 52.19, 27.61. *Anal*. Calcd for $C_{17}H_{15}BrO_3$: C, 58.81; H, 4.35. Found C, 59.33; H, 4.24.

Methyl 2,2,7-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9h**)

he same procedure described for **9a** was applied to 2,2,7-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9h** (63 mg, 91 %) as yellow flakes: mp 84 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.19 (d, 1H, $J = 0.5$ Hz, H6), 8.09 (d, 1H, $J = 8.3$ Hz, H10), 7.41 (t, 1H, $J = 7.6$ Hz, H9), 7.30 (t, 1H, $J = 6.8$ Hz, H8), 7.28 (d, 1H, $J = 10.0$ Hz, H4), 5.72 (d, 1H, $J = 10.0$ Hz, H3), 3.97 (s, 3H, OCH_3), 2.68 (s, 3H, CH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.98, 149.52, 135.39, 131.74, 129.77, 127.67, 127.23 (two C's), 124.14, 120.84, 120.39, 120.22, 114.36, 76.00, 52.12, 27.53. *Anal*. Calcd for $C_{18}H_{18}O_3$: C, 78.57; H, 6.43. Found C, 79.67; H, 6.22.

Methyl 2,2,8-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9i**)

The same procedure described for **9a** was applied to 2,2,8-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9i** (58 mg, 90 %) as yellow-green flakes: mp 78 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.09 (d, 1H, $J = 8.5$ Hz, H10), 7.96 (s, 1H, H6), 7.55 (s, 1H, H7), 7.35 (d, 1H, $J = 8.3$ Hz, H9), 7.27 (d, 1H, $J = 10.3$ Hz, H4), 5.69 (d, 1H, $J = 10.0$ Hz, H3), 3.93 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3), 1.49 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.84, 149.19, 136.72, 132.75, 129.80, 129.22, 127.69, 125.21, 124.55, 123.42, 122.04, 120.89, 113.89, 75.94, 52.09, 27.51. *Anal*. Calcd for $C_{18}H_{18}O_3$: C, 78.57; H, 6.43. Found C, 79.46; H, 6.29.

Methyl 2,2,9-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9j**)

The same procedure described for **9a** was applied to 2,2,9-trimethyl-2*H*-benzo[*h*]chromene-5-carboxylic acid to afford **9j** (67 mg, 94 %) as yellow-green flakes: mp 99 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.02 (s, 1H, H6), 7.96 (d, 1H, $J = 0.7$ Hz, H10), 7.69 (d, 1H, $J = 8.3$ Hz, H7), 7.33–7.26 (m, 1H, H8), 7.29 (d, 1H, $J = 10.0$ Hz, H4), 5.70 (d, 1H, $J = 10.3$ Hz, H3), 3.92 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3), 1.50 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 167.80, 148.59, 137.71, 130.72, 129.52, 129.17, 128.60, 127.16, 123.98, 123.58, 121.05, 120.93, 114.86, 75.90, 52.03, 27.51, 22.05. *Anal*. Calcd for $C_{18}H_{18}O_3$: C, 78.57; H, 6.43. Found C, 78.97; H, 6.38.

Methyl 2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (**10**)

Method A

A mixture of **9a** (134 mg, 0.5 mmol) and 5 % Pd/C (25 mg) in CH_3OH(15 mL) was stirred in a H_2 atmosphere for 5 h at 50 psi. The reaction mixture was filtered through Celite. Evaporation of the solvent gave pale yellow oily material which was flash column chromatographed on silica gel eluting with CH_2Cl_2 to give an thick oil (130 mg, 96 %). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.21 (dd, 1H, $J = 7.8$, 1.0 Hz, H10), 8.03 (s, 1H, H6), 7.78 (dd, 1H, $J = 7.8$, 1.3 Hz, H7), 7.52 (td, 1H, $J = 7.8$, 1.3 Hz, H9), 7.45 (td, 1H, $J = 7.8$, 1.3 Hz, H8), 3.92 (s, 3H, OCH_3), 3.16 (t, 2H, $J = 6.8$ Hz, H3), 1.87 (t, 2H, $J = 6.8$ Hz, H4), 1.41 (s, 6H, CMe_2). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 168.24, 149.42, 131.59, 130.25, 128.41, 127.45, 126.24, 123.17, 122.72, 121.84, 114.13, 74.25, 51.93, 32.70, 26.84, 21.59. *Anal*. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found 75.68; H, 6.68.

Method B

The same procedure, described above for Method A, was applied to **9b** (151 mg, 0.5 mmol) to give thick oil (132 mg, 98 %). The spectral data were identical to those obtained above from Method A.

Inhibitory activity on tyrosinase from mushroom (Yang and Robb 2005)

The activity of tyrosinase from mushroom (Sigma-Aldrich) was spectrophotometrically determined by evaluating the formation of dopachrome at 30 °C in a 50 mM phosphate buffer (pH 6.0) by employing previously reported method. An enzyme solution (50 μL) was added to a cuvette containing 50 mM phosphate buffer (1.2 mL) and 10 mM L-DOPA (0.8 mL), the solution was immediately mixed. The increase in absorbance at 475 nm (indicating the formation of dopachrome) was recorded with a JASCO-V550 spectrophotometer, which was compared to that of a blank cuvette. One enzyme unit was defined as the volume of enzyme solution producing a rate of absorbance change of one per minute.

Inhibition of melanin production (Siegrist and Eberle 1986)

B-16 mouse melanoma cell lines were obtained from ATCC (Manassas, USA) and cultured in Dulbecco's modified Eagle's medium, supplemented with 10 % fetal bovine serum, 143 M/mL benzylpenicillin potassium and 10 mg/

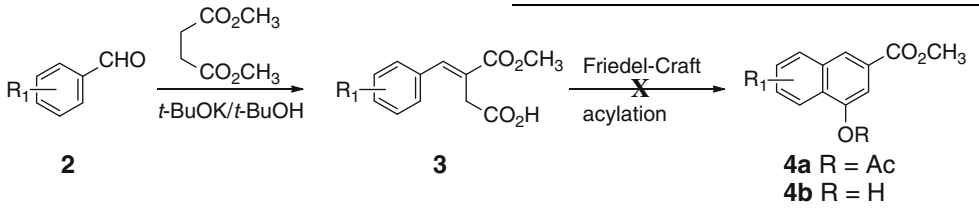
mL streptomycin sulfate at 37 °C under the 5 % CO_2 atmosphere. The B-16 cells were seeded at a density of 2.5 × 103 cells per well in 96-well culture plates, and incubated at 37 °C under the 5 % CO_2 atmosphere for 24 h. The cells were then treated with the samples and 100 μM IBMX for 72 h, with the amounts of melanin in the culture media were spectrophotometrically measured at 405 nm.

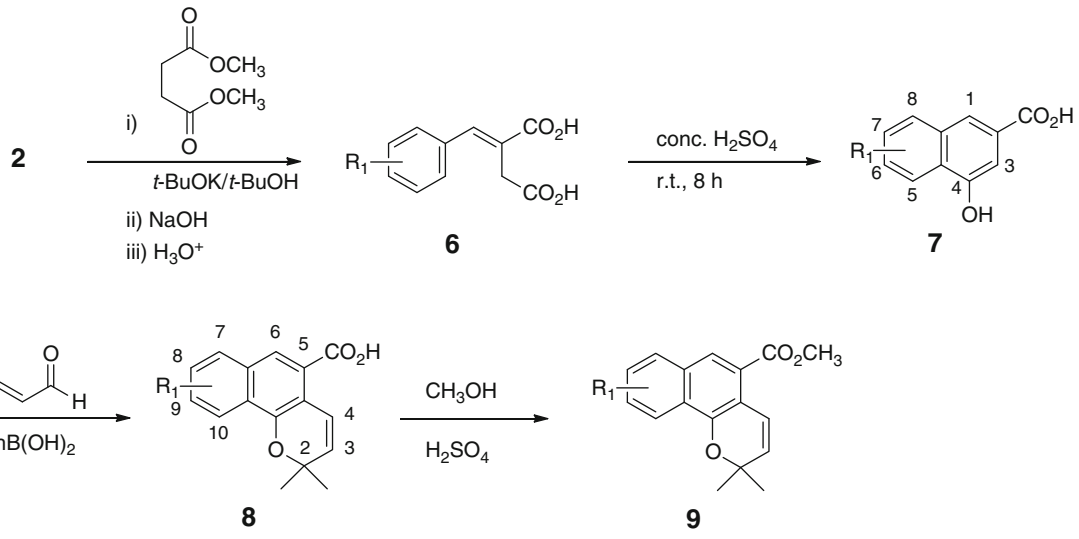
Results and discussion

Chemistry

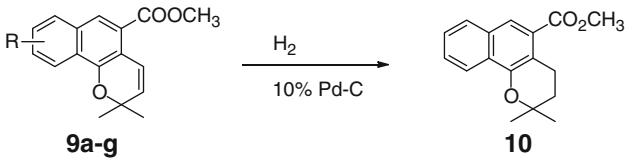
The 6-deoxymollugin derivatives were prepared 5 steps from benzaldehyde and its derivatives. The Stobbe reaction of (substituted)benzaldehydes (**2**) and dimethyl succinate afforded 2-(*E*)-(substituted-phenyl)methylidenesuccinic acid mono methyl esters (**3**) (El-Abbady et al. 1961; El-Assal et al. 1963). Although previous reports claimed that compounds **3** were readily cyclized with Ac_2O in the presence of anhydrous NaOAc to lead a series of methyl (substituted)-4-acetoxy-2-naphthoates (**4a**) (El-Abbady et al. 1961), all the attempts for direct Friedel–Crafts acylation were failed (Jahng 2013).

We, therefore, hydrolyzed the Stobbe adducts (**5**) to their corresponding free acids, 2-(substituted-phenyl)methylidenesuccinic acids (**6**) (Doulut et al. 1993; Caro et al. 2003) in 67–73 % yields. A direct Friedel–Crafts acylation of **6** with H_2SO_4 at room temperature led a series of expected (substituted)-4-hydroxy-2-naphthoic acids (**7**) (Cason, 1941), which were then subjected to phenylboronic acid-catalyzed intermolecular chromenylation (Murphy et al. 1992; Pettigrew et al. 2005) with 3-methyl-2-butenal to afford the corresponding 2*H*-naphtho[1,2-*b*]pyrans **8** in 79–85 % yields. Subsequent esterification of **8** with CH_3OH to afford a series of 6-deoxymollugin derivatives (**9**) in over 90 % yields (Table 1). It should be noted that 2,2-dimethyl-2*H*-pyranylation of 2-(*E*)-(3-substituted-phenyl)methylidenesuccinic acids (**6**) afforded two isomeric 4-hydroxy-2-naphthoic acids such as 8-substituted-4-hydroxy-2-naphthoic acids and 10-substituted-4-hydroxy-2-naphthoic acids, of which the ^{1}H NMR spectra showed a ratio of 8.5–9.0:1 in crude stage. Although careful chromatography provided the major products 8-substituted-4-hydroxy-2-naphthoic acids as pure state, the other congeners would only be obtained as mixtures. Thus, it was not able to prepare the compounds with a substituent at C10.





Additional homologous alkyl esters such as ethyl, propyl and isopropyl esters of **8a** were prepared by simple Fischer esterification, of which the inhibitory activities against tyrosinase were, however, not improved at all. Although catalytic hydrogenation of **9a** afforded corresponding series of 6-deoxydihydromollugin analogue (**10**) in quantitative yield, catalytic hydrogenation of **9** with halogens as a substituent (**9b–g**) suffered from hydrogenolysis to afford unsubstituted **10** in fairly good yields.



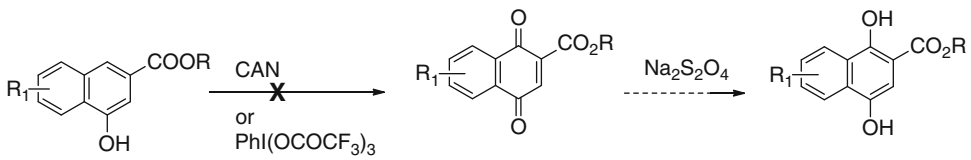
9a-g **10**

It should be noted that attempts to convert 4-hydroxynaphthalene-2-carboxylic acid (**7**) and their methyl ester (**11**) to corresponding 1,4-dihydroxynaphtalene-2-carboxylic acids and their methyl esters (**12**), possible synthetic precursors for substituted mollugins, by ceric ammonium nitrate (Jacob et al. 1976) or $PhI(OCOCF_3)_2$-mediated oxidation (Andrews et al. 1996) were failed. All attempts to prepare the derivatives of mollugin were, thus, failed.

showed the promising activity, no significant increase of activity was observed upon the introduction of substituent on benzene ring.

To prove a potential for the skin-decolorizing effect, the inhibitory activity of **9a**, the most potent congener, on melanin synthesis in mouse melanoma B-15 cells was evaluated by previously reported method (Siegrist and Eberle 1986) showing strong activity at 10 μM. It should be noted that all the compounds prepared did not show any significant toxicity against selected human cancer cell lines such as breast adenocarcinoma (MCF7), prostate tumor (DU-145), colorectal adrenocarcinoma (HCT60), and chronic myelogenous leukemia (HL60) up to 70 μM, while mollugin (**1**) showed strong cytotoxicity against several selected human cancer cell lines (Itokawa et al. 1993, Son et al. 2008).

In conclusion, a series of 6-deoxymollugins were prepared in 5-steps from benzaldehyde and its derivatives in fairly good yields. The inhibitory activity of the compounds prepared against tyrosinase were evaluated to show that the parent, methyl 2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (**9a**) showed best inhibitory activity (IC_{50} = 18.3 μM) with no significant cytotoxicity implying potential lead for skin-decolorizing agent.



7 R = H
11 R = CH_3

12

Biology

In vitro inhibitory activity of the compounds prepared on tyrosinase from mushroom was evaluated by measuring the formation of dopachrome employing previously reported method (Yang and Robb 2005). The results were summarized in Table 1. Although the parent 6-deoxymollugin

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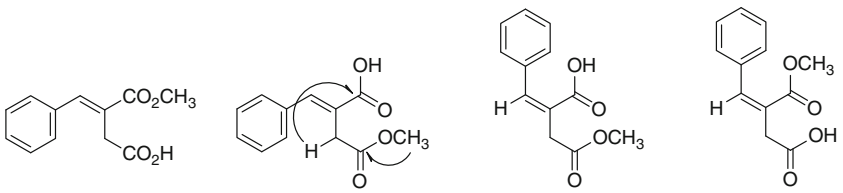
Jahng, Y. 2013. Unpublished results. Thus, we re-examined the Stobbe adduct, especially known as (***E***)-**3aa** ($R_1 = H$) by spectroscopic methods. HMBC correlation study showed the correlations between carbonyl carbon of ester moiety and methyl protons as well as C2–H and C1 = O of 2-phenylmethylidenesuccinic acid monomethyl ester were observed as shown in Figure 1 to define the structure **5** as a Stbbe adduct.



(*E*)-3aa **(*E*)-5a** **(*Z*)-5b** **(Z)-3ab**

Figure 1. Possible Structures of Stobbe Adduct and HMBC corelation

However, the structures (*Z*)-**5b** and (*Z*)-**3ab**, the less favored Stobbe adducts cannot be excluded, in which the geometry of the double bond is not properly oriented toward the acylation process. However, it should be noted that even such an inappropriate geometry in **9b** may adopt the Friedel-Craft acylation by isomerization induced by the acid present.

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