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Facile Synthesis of Mollugin by Kinetic Control and *anti*-HCV (Hepatitis C Virus) Activity of Its Analogues

Da Hye Choi, Na Ri Lee, Cheol Gi Kim, Jong Woo Kim,† Sang Wook Lee,† and Jong-Gab Jun*

Department of Chemistry and Institute of Applied Chemistry, Hallym University, Chuncheon 200-702, Korea
**E-mail: jgjun@hallym.ac.kr*
†Advanced Institutes of Convergence Technology, B&C Biopharm Co. Ltd., Suwon 443-270, Korea
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Mollugin has been reported to have various biological activities including antineoplastic, antitumor, antiviral against the hepatitis B virus, anti-aging and antimutagenic activities. An effective and concise synthesis of mollugin in two steps including kinetic control from the cheap starting material 1,4-naphthoquinone has been introduced, and mollugin derivatives thus prepared are screened for their inhibition ability against the hepatitis C virus (HCV) and the dihydrobenzochromene structure might be an additional anti-HCV agent as a new leading compound.

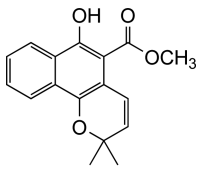
Key Words : Mollugin, Hepatitis C virus, Rubiaceae, Kinetic control, Dihydrobenzochromene

Introduction

Mollugin **1a** was isolated and characterized from the dried root of *Rubia cordifolia* L. (Rubiaceae),[1] which showed various biological properties, including antineoplastic,[2] antitumor,[1b] antiviral against the hepatitis B virus,[3] anti-aging[4] and antimutagenic[5] activities. Since the first synthesis of mollugin from 1,4-dihydroxy-2-naphthoic acid,[6] several reports have been known.[7] Here we report another concise synthesis of mollugin in two steps including kinetic control from a cheap starting material 1,4-naphthoquinone **2** (Scheme 1).

Results and Discussion

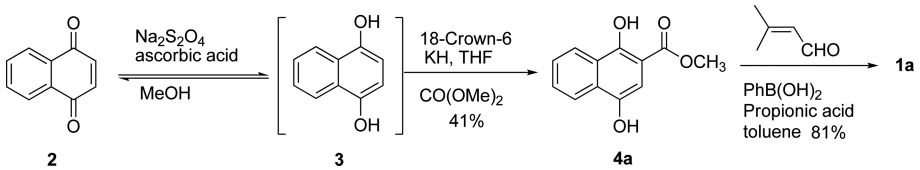
1,4-Naphthoquinone (**2**) is easily reduced to 1,4-dihydroxynaphthoquinone (**3**) with $Na_2S_2O_4$, and TLC indicated 100% conversion of this reduction in the reaction flask. However, the product **3** is highly unstable and easily oxidized to go back to starting material during work-up and isolation process. To give the desired ester **4a**, kinetic control condition is required in a one-flask reaction. After the reduction is completed, the methanol solvent is replaced by THF with caution under anhydrous condition. Reaction of **3** with dimethyl carbonate in THF in the presence of KH and 18-crown-6 provides **4a** in 41% yield from **2**. Phenyl boronic acid-mediated cyclization of phenol has been known to construct the benzopyran skeleton through a quinone-methide intermediate.[7g,8] Treatment of **4a** with 3-methyl-2-butenal in the presence of phenyl boronic acid and propionic acid in refluxing toluene gives mollugin in 81% yield.



Mollugin (1a)

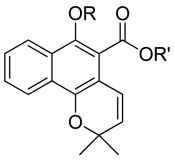
Figure 1. Structure of mollugin (**1a**).

Mollugin has been noticed showing anti-Hepatitis C virus (HCV) activity[9] and HCV is known to transfer *via* transfusion and community-acquired infection. It is also known that 20% of the infected patients develop acute hepatitis and about 80% suffered by chronic hepatitis lead into liver cirrhosis or liver cancer.[10] Worldwide approximately 200 million people are infected with HCV and the infection rates are increasing every year.[11] Satisfactory vaccine against hepatitis C or an effective therapeutic agent treating hepatitis C has not been developed. Therefore, a large number of pharmaceutical companies and research institutes all over the world have been trying to develop an effective hepatitis C treating agent. HCV patients are prevalent across the world and demonstrate much higher potential for chronic hepatitis compared with hepatitis B, and the mechanism for such progress has been still studied.[12] Combination therapy



Scheme 1. Synthesis of mollugin (**1**) from 1,4-naphthoquinone (**2**).

Table 1. Mollugin derivatives



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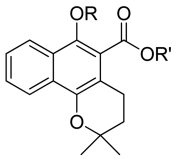

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R	R'	Benzochromenes	Dihydrobenzochromenes
H	Me	**1a**	**7a**
H	Et		**7b**
H	Ph	**1c**	**7c**
H	4-$MeOC_6H_4$		**7d**
H	4-$NO_2C_6H_4$		**7e**
H	4-IC_6H_4		**7f**
H	Bn		**7g**
H	4-MeOBn		**7h**
H	4-NO_2Bn		**7i**
H	4-ClBn		**7j**
H	2-Furylmethyl		**7k**
Me	Me	**1l**	**7l**
Ac	Me	**1m**	**7m**
Ac	Ph		**7n**
Ac	Bn		**7o**

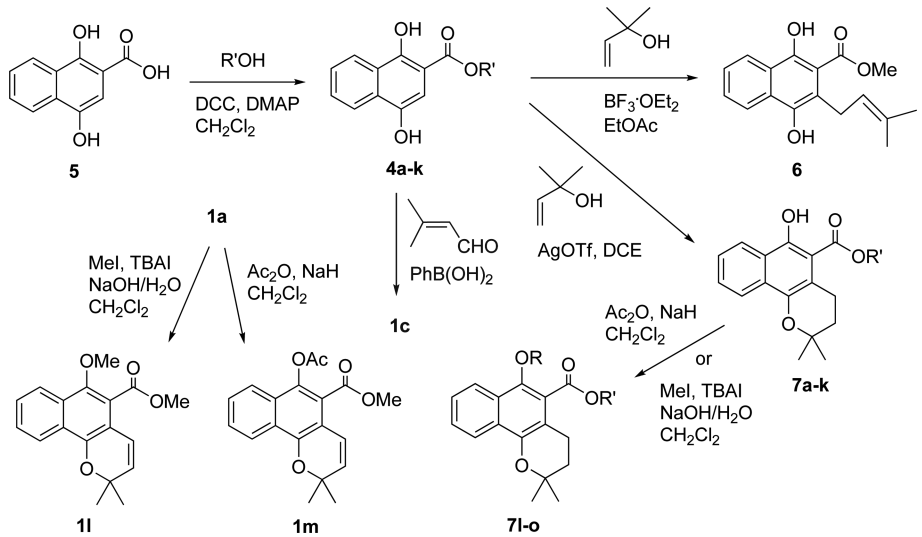
Table 2. HCV inhibition activity of mollugin derivatives

Compounds	Replicon Activity EC_{50} (μg/mL)	Cytotoxicity CC_{50} (μg/mL)
GS 9190	0.02-0.03	25
1a	–	12.5
1c	–	25
1l	–	25
1m	–	50
6	5-10	6.25
7a	1-2	6.25
7b	–	25
7c	5	12.5
7d	–	25
7e	7	50
7f	5	100
7g	0.5-1	25
7h	–	50
7i	0.5-0.8	12.5
7j	5	50
7k	–	100
7l	25	25
7m	2-4	25
7n	5	12.5
7o	0.5-1	25

The results are reported as mean value ± SEM for n = 3.

of Interferon-α with Ribavirin is the current treatment for hepatitis C.[13] But, this treatment demonstrates very low rate of cure and brings severe side effects. HCV has 6 genotypes, and genotype 1b is most common but does not respond to Interferon-α well, compared to genotype 2 or genotype 3.[14] Alternative therapies such as milk thistle, ginseng, and colloidal silver are claimed to be helpful for HCV. However, no alternative therapy has been shown to improve outcomes in HCV, and an effective antiviral agent that is specially targeted to HCV by directly inhibiting the replication has not been developed yet.[15]

Mollugin analogues such as 3-hydroxymollugin,[16] 3-methoxymollugin,[16] *cis* or *trans*-3,4-dihydroxymollugin,[17] have been found in nature and were synthesized by other groups. However, systematic structure-activity relationship has not been well reported despite of the growing importance of mollugin structure. In order to find an effective anti-HCV agent, mollugin derivatives were synthesized from **1a** by methylation and acetylation to give **1l** and **1m**, respectively (Scheme 2). Mollugin derivative **1c** was prepared from **4c** using 3-methyl-2-butenal in the presence of phenyl boronic acid as shown in Scheme 1. Prenyl derivative **6** and di-



Scheme 2. Synthesis of mollugin derivatives.

hydrobenzochromene **7a** were also prepared from **4a** with 2-methylbuten-2-ol using borontrifluoride[18] or AgOTf.[19] These derivatives were compared with reference GS 9190 (Tegobuvir), which is a novel imidazopyridine inhibitor of HCV in a Phase II study by Gileads,[20] for their anti-HCV activity (EC_{50}) and cytotoxicity (CC_{50}) as shown in Table 2, and it was found that dihydrobenzochromene **7a** showed a promising replicon activity (1-2 μg/mL) with a lower cytotoxicity (6.25 μg/mL). Therefore, dihydrobenzochromene derivatives (**7b-k**) were prepared from 1,4-dihydroxy-2-naphthoic acid **5** through DCC coupling with the corresponding alcohol followed by cyclization with 2-methylbuten-2-ol using AgOTf in 1,2-dichloroethane (DCE). Methylation (**7l**) or acetylations (**7m-o**) of the corresponding dihydrobenzochromene were also carried out. Structure change from the methyl (**7a**) to phenyl ester **7c** showed no advantage in activity and cytotoxicity. In addition, compound **7d** with electron donating substituent showed no activity compared to electron withdrawing substituents (**7e** and **7f**). The benzyl derivative **7g** showed higher activity (0.5-1 μg/mL) than **7a**, and *p*-NO_2 substituent **7i** showed better activity (0.5-0.8 μg/mL) with lower cytotoxicity (12.5 μg/mL) compared to **7g** (25 μg/mL). Chloro (**7j**) or methoxy (**7h**) substituents showed less or no activity with higher cytotoxicity. 2-Furylmethyl substituent **7k** compared to benzyl or phenyl ester showed no activity with higher cytotoxicity. Methyl (**7l**) or acetyl (**7m-o**) derivatives of dihydrobenzochromene showed no better advantage in activity and cytotoxicity.

From the structure-based results, we can conclude that the dihydrobenzochromene structure might be an additional anti-HCV agent as a new leading compound, however, the further study on structure and activity relationship is required for better clarification.

Culture of HCV RNA Replicon Cell Line. To screen a compound that is capable of inhibiting HCV replication, each compound was added to Huh-7 human hepatoma cell line harboring HCV RNA replicon, followed by culture. Then, expression level of HCV RNA was quantified and its inhibitory activity was measured. HCV replicon used in this invention was derived from HCV-Ib hepatitis C virus gene that was bicistronic replicon composed of HCV IRES, neomycin resistant gene, EMCV (encephalomyocarditis virus) IRES. HCV non-structural proteins were composed of the sequences comprising NS3-NS5B and HCV 3'end. An expression vector harboring HCV replicon proceeded to *in vitro* transcription, and the obtained HCV replicon was transfected into the Huh-7 cells by electroporation. To select only those cells having HCV replicon, Huh-7 cells were cultured with medium containing the antibiotic G418 (500 μg/mL). The selected cells were cultured with DMEM (Dulbecco's modified Eagles media) containing 10% FBS, non essential amino acids and 500 μg/mL of G418.

HCV RNA Replication Inhibitory Activity in HCV Replicon Cells of Compounds. Huh-7 cells harboring HCV subgenomic RNA replicon were cultured overnight in 6 well plate (3×10^5 cells/well), at 37 °C and 5% CO_2 with DMEM containing 10% FBS, non-essential amino acids and 500 μg/mL of G418. Medium of each well was replaced with DMEM containing 2% FBS, non-essential amino acids and 500 μg/mL of G418. Test compound was dissolved in DMSO, which was added to each well at different concentrations, followed by culture in a 5% CO_2 incubator at 37 °C for 72 h. Equal amount of DMSO (negative control) and Interferon-α (positive control) were added as controls. Upon completion of the culture, medium of each well was eliminated, followed by washing with 1 mL of PBS. 250 μL/well of trypsin/EDTA was added thereto and cells were separated from the plate and washed with PBS again to eliminate medium. Total RNA was isolated from the cells by using SV total RNA isolation system (Promega corporation), followed by quantification using GeneQuant pro (Amersham bioscience). EC_{50} against HCV replicon of each compound was investigated by RT-PCR and the result was compared with those of controls. RT-PCR was performed with the primer targeting HCV Ib NS5B region and by AccessQuick™ RT-PCR system (Promega corporation). To obtain more accurate EC_{50} values, quantitative real-time PCR was performed along with RT-PCR. cDNA was obtained from the isolated RNA by using Reverse transcription system (Promega corporation), followed by quantitative real-time PCR using iQ SYBR Green Supermix (Bio-rad). At the same time, one-step real time RT-PCR was performed using Taqman probe to investigate the inhibitory activity of each compound. At this time, the primer targeting HCV 5'-UTR was used and GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) gene was used as a reference gene for correction. Real time RT-PCR was performed by using iCycler iQ5 system (Bio-rad). The EC50 value was calculated by iCycler iQ5 optical system software (Bio-rad) program to determine the inhibitory activity. HCV replicon inhibitory activity of the compounds is shown in Table 2.

Cytotoxicity Test. To confirm cytotoxicity of the compounds, *in vitro* MTT assay was performed with HepG2 cells.

Experimental

All chemicals were purchased from Sigma-Aldrich Chemicals and were used without further purification unless noted otherwise. NMR spectra were recorded at Varian Mercury-300 MHz FT-NMR and 75 MHz for ^{13}C, with the chemical shift (δ) reported in parts per million (ppm) relative to TMS and the coupling constants (J) quoted in Hz. $CDCl_3$ was used as a solvent and an internal standard. Mass spectra were recorded using a JMS-700 (JEOL) spectrometer. Melting points were measured on a MEL-TEMP II apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F_{254} (Merck, layer thickness 0.2 mm) plastic-backed *silica* gel plates and visualized by UV light (254 nm) or staining with *p*-anisaldehyde.

Methyl 1,4-Dihydroxy-2-naphtoate (4a). To a 1,4-naphtoquinone (**2**) (200 mg, 1.26 mmol) in MeOH (10 mL) was added $Na_2S_2O_4$ (330 mg, 1.90 mmol) with ascorbic acid (178 mg, 1.01 mmol) under nitrogen atmosphere and stirred for 5 h at rt under light protection. After MeOH was removed by

evaporation in the reaction, potassium hydride (159 mg, 1.39 mmol) and 18-crown-6 (506 mg, 1.39 mmol) were added slowly to this reaction mixture with THF (20 mL) at 0 °C and stirred for 10 min. Dimethyl carbonate (0.117 mL, 1.39 mmol) was added slowly to this reaction mixture at 0 °C and stirred for 10 h at rt. The reaction mixture was filtered with Celite® 545, extracted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and purified by *silica* gel flash column chromatography (PetEther:EtOAc = 25:1) to give a yellow solid (112 mg, 41%). R_f 0.43 (EtOAc:hexane = 1:3); mp 192-193 °C (lit.[7g] mp 193-194 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (1H, d, *J* = 8.3 Hz), 8.21 (1H, d, *J* = 8.3 Hz), 7.76 (1H, t, *J* = 7.8 Hz), 7.71 (1H, t, *J* = 7.8 Hz), 6.72 (1H, s), 3.89 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 153.7, 147.1, 129.5, 128.0, 127.7, 124.6, 122.5, 147.1, 110.4, 105.2, 51.5.

Mollugin (1a). Procedure followed the method A in the reference.[7g] R_f 0.68 (EtOAc:hexane = 1:3); mp 129-131 °C (lit.[7g] mp 131-132 °C); ^{1}H NMR and ^{13}C NMR data are well matched with the reference.

Phenyl 6-Hydroxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (1c). 30%; R_f 0.40 (Pet. Ether/EtOAc = 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 4.5 Hz), 7.48 (2H, m), 7.33 (2H, m), 7.16 (1H, m), 7.00 (3H, m), 6.67 (1H, d, *J* = 4.5 Hz), 5.77 (1H, d, *J* = 4.5 Hz), 1.44 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7, 151.9, 141.5, 138.9, 129.6, 129.4, 127.4, 126.6, 126.1, 126.0, 125.2, 124.3, 119.0, 118.6, 117.4, 116.3, 106.5, 77.4, 28.1.

Methyl 6-Methoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (1l). 98%; R_f 0.48 (Pet. Ether/EtOAc = 5/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (1H, m), 8.02 (1H, m), 7.49 (2H, m), 6.40 (1H, d, *J* = 5.0 Hz), 5.68 (1H, d, *J* = 5.0 Hz), 3.97 (6H, d, *J* = 4.7 Hz), 1.51 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 166.3, 147.0, 139.1, 130.4, 127.9, 127.4, 126.9, 122.7, 122.6, 120.6, 119.9, 112.5, 63.8, 52.7, 30.0, 27.9.

Methyl 6-Acetoxy-2,2-dimethyl-2*H*-benzo[*h*]chromene-5-carboxylate (1m). 99%; R_f 0.20 (Pet. Ether/EtOAc = 5/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (1H, m), 7.72 (1H, m), 7.51 (2H, m), 6.70 (1H, d, *J* = 5.1 Hz), 5.69 (1H, d, *J* = 5.1 Hz), 3.94 (3H, s), 2.42 (3H, s), 1.52 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 166.3, 147.0, 139.1, 130.2, 127.6, 127.1, 126.9, 126.7, 122.6, 122.3, 120.3, 119.5, 113.0, 52.8, 30.1, 28.1, 21.1.

Ethyl 1,4-Dihydroxy-2-naphtoate (4b). To a 1,4-dihydroxy-2-naphtoic acid (**5**) (50 mg, 0.25 mmol) in CH_2Cl_2 (5 mL) was added EtOH (23 mg, 0.49 mmol), *N,N*,-dicyclohexylcarbodiimide (100 mg, 0.49 mmol), and dimethylaminopyridine (6 mg, 0.05 mmol) under nitrogen atmosphere and stirred for 40 min at rt. The reaction mixture was neutralized with 6 N HCl, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and purified by *silica* gel flash column chromatography (EtOAc:hexane = 1:3) to give a yellow solid (57 mg, 88%). R_f 0.43 (EtOAc:Hex = 1:3); mp 161-162 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (1H, d, *J* = 8.1 Hz), 8.10 (1H, d, *J* = 9.0 Hz), 7.61 (1H, t, *J* = 7.8 Hz), 7.53 (1H, t, *J* = 7.8 Hz), 7.09 (1H, s), 5.65 (1H, s), 4.38 (2H, q, *J* = 6.9 Hz), 1.38 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 152.9, 143.3, 129.0, 128.7, 125.7, 123.1, 122.6, 121.3, 111.5, 109.9, 62.9, 14.8.

Phenyl 1,4-Dihydroxy-2-naphtoate (4c). 84%; R_f 0.70 (EtOAc/hexane = 1/2); mp 156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (1H, d, *J* = 8.2 Hz), 8.14 (1H, d, *J* = 8.2 Hz), 7.67 (1H, t, *J* = 7.8 Hz), 7.46 (5H, m), 7.31 (1H, t, *J* = 7.5 Hz), 7.09 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 159.7, 151.6, 151.3, 132.5, 129.6, 126.6, 126.5, 123.4, 122.5, 120.2, 119.7, 117.1, 111.6, 108.9.

4-Methoxyphenyl 1,4-Dihydroxy-2-naphthoate (4d). 49%; R_f 0.37 (EtOAc/hexane = 1/3); mp 155-156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (1H, d, *J* = 8.4 Hz), 8.16 (1H, d, *J* = 8.4 Hz), 7.67 (1H, t, *J* = 8.4 Hz), 7.58 (1H, t, *J* = 8.1 Hz), 7.26 (1H, s), 7.12 (2H, d, = 9.3 Hz), 6.95 (2H, d, *J* = 9.3 Hz), 3.83 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 157.4, 156.9, 143.2, 141.6, 129.7, 129.2, 125.6, 124.4, 123.7, 122.3, 121.5, 114.5, 111.1, 104.5, 55.6.

4-Nitrophenyl 1,4-Dihydroxy-2-naphthoate (4e). 35%; R_f 0.54 (EtOAc/hexane = 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (1H, d, *J* = 8.4 Hz), 8.32 (2H, d, *J* = 8.4 Hz), 8.14 (1H, d, *J* = 8.7 Hz), 7.68 (1H, t, *J* = 8.1 Hz), 7.59 (1H, t, *J* = 8.1 Hz), 7.40 (2H, d, *J* = 9.3 Hz), 7.21 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8, 154.8, 145.8, 145.4, 142.2, 130.1, 127.0 125.6, 125.5, 124.4, 122.9, 122.8, 122.1, 107.8, 104.7.

4-Iodophenyl 1,4-Dihydroxy-2-naphthoate (4f). 22%; R_f 0.57 (EtOAc/hexane = 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (1H, d, *J* = 8.4 Hz), 8.14 (1H, d, *J* = 8.4 Hz), 7.73 (2H, d, *J* = 9.0 Hz), 7.67 (1H, t, *J* = 6.6 Hz), 7.58 (1H, t, *J* = 6.9 Hz), 7.22 (1H, s), 6.96 (2H, d, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.0, 155.5, 150.2, 143.7, 138.6, 129.9, 126.9, 125.7, 124.3, 124.1, 122.0, 118.0, 105.1, 103.7, 90.8.

Benzyl 1,4-Dihydroxy-2-naphthoate (4g). 51%; R_f 0.51 (EtOAc/hexane = 1/4); mp 154-156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (1H, d, *J* = 8.1 Hz), 8.11 (1H, d, *J* = 7.8 Hz), 7.65 (1H, t, *J* = 7.2 Hz), 7.57 (1H, t, *J* = 7.2 Hz), 7.45 (5H, m), 7.12 (1H, s), 5.41 (2H, s), 4.96 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 155.9, 143.4, 135.6, 129.3, 129.2, 128.9, 128.7, 128.5, 126.6, 125.8, 124.2, 121.9, 105.4, 104.6, 67.3.

4-Methoxybenzyl 1,4-Dihydroxy-2-naphthoate (4h). 49%; R_f 0.51 (EtOAc/hexane = 1/3); mp 134-135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (1H, d, *J* = 7.8 Hz), 8.09 (1H, d, *J* = 8.1 Hz), 7.63 (1H, t, *J* = 8.4 Hz), 7.55 (1H, t, *J* = 8.4 Hz), 7.39 (2H, d, *J* = 8.4 Hz), 7.08 (1H, s), 6.92 (2H, d, *J* = 8.4 Hz), 5.33 (2H, s), 3.82 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 159.6, 155.5, 142.9, 130.3, 128.8, 128.7, 127.2, 126.1, 125.4, 123.8, 121.4, 113.9, 105.0, 104.3, 66.8, 55.2.

4-Nitrobenzyl 1,4-Dihydroxy-2-naphthoate (4i). 54%; R_f 0.31 (EtOAc/hexane = 1/3); mp 158-160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.92 (1H, s), 8.40 (1H, t, *J* = 7.8 Hz), 8.33 (1H, d, *J* = 8.1 Hz), 8.25 (2H, d, *J* = 7.8 Hz), 8.02 (2H, d, *J* = 7.8 Hz), 7.63 (3H, m), 7.42 (1H, s), 4.95 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8, 158.2, 145.8, 142.2, 130.4, 130.1, 126.3, 125.6, 124.4, 124.2, 124.1, 123.0, 122.4, 108.2, 104.8, 73.1.

4-Chlorobenzyl 1,4-Dihydroxy-2-naphthoate (4j). 54%; R_f 0.54 (EtOAc/hexane = 1/3); mp 122-124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.5 (1H, s), 8.37 (1H, d, *J* = 8.7 Hz), 8.10 (1H, d, *J* = 7.8 Hz), 7.63 (1H, t, *J* = 7.8 Hz), 7.55 (1H, t, *J* = 7.5 Hz), 7.37 (4H, m), 7.07 (1H, s), 5.34 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 156.0, 143.5, 134.7, 134.1, 129.9, 129.3, 129.2, 129.1, 127.1, 126.6, 124.2, 121.9, 105.2, 104.4, 66.4.

Furan-2-ylmethyl 1,4-Dihydroxy-2-naphthoate (4k). 33%; R_f 0.40 (EtOAc/hexane = 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.42 (1H, s), 8.35 (1H, d, *J* = 7.8 Hz), 8.08 (1H, d, *J* = 8.4 Hz), 7.61 (1H, t, *J* = 6.3 Hz), 7.53 (1H, t, *J* = 6.9 Hz), 7.44 (1H, d, *J* = 1.8 Hz), 7.04 (1H, s), 6.50 (1H, t, *J* = 3.3 Hz), 6.39 (1H, d, *J* = 2.1 Hz), 5.32 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.9, 157.9, 143.8, 138.0, 125.1, 124.8, 123.1, 123.0, 122.2, 121.1, 120.9, 111.8, 110.9, 104.8, 104.4, 69.2.

Methyl 1,4-Dihydroxy-3-(3-methylbut-2-en-1-yl)-2-naphthoate (6). To a methyl 1,4-dihydroxy-2-naphtoate (**4a**) (0.05 g, 0.22 mmol) in ethyl acetate (3 mL) was added BF_3OEt_2 (0.03 mL, 0.27 mmol), 2-methyl-3-buten-2-ol (0.03 mL, 0.25 mL) under nitrogen atmosphere and stirred for 15 min at −78 °C. The reaction mixture was extracted with EtOAc, dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by *silica* gel flash column chromatography (Pet Ether/EtOAc = 30/1) to give a brown liquid (0.05 mg, 75%). R_f 0.2 (Pet Ether/EtOAc = 25/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (2H, m), 7.74 (2H, m), 5.07 (1H, m), 3.92 (3H, s), 3.30 (2H, d, *J* = 3.6 Hz), 1.73 (3H, s), 1.69 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 157.9, 145.5, 131.8, 129.3, 129.0, 126.9, 125.7, 123.5, 123.1, 121.9, 117.4, 105.0, 51.5, 25.7, 24.6, 18.6.

Methyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7a). To a methyl 1,4-dihydroxy-2-naphthoate (**4a**) (0.07 g, 0.32 mmol) in 1,4-dichloroethane (2 mL) was added AgOTf (3 mg, 10 μmol), 2-methyl-3-buten-2-ol (0.03 mL, 0.30 mmol) under nitrogen atmosphere and stirred for 4 h at rt. The reaction mixture was extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and purified by *silica* gel flash column chromatography (EtOAc/hexane = 1/5) to give a yellow solid (32 mg, 35%). R_f 0.83 (Pet. Ether/EtOAc = 5/1); mp 96-97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.15 (1H, s), 8.33 (1H, d, *J* = 4.1 Hz), 8.15 (1H, d, *J* = 4.2 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.47 (1H, t, *J* = 7.5 Hz), 3.97 (3H, s), 3.03 (2H, t, *J* = 6.8 Hz), 1.81 (2H, t, *J* = 6.9 Hz), 1.39 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 156.4, 141.8, 129.4, 125.9, 124.6, 124.0, 121.9, 120.1, 111.9, 105.5, 73.4, 62.4, 33.5, 26.9, 23.8.

Ethyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7b). 25%; R_f 0.55 (EtOAc/hexane = 1/6); mp 78-80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (1H, d, *J* = 7.8 Hz), 8.15 (1H, d, *J* = 8.4 Hz), 7.58 (1H, t, *J* = 7.2 Hz), 7.47 (1H, t, *J* = 7.2 Hz), 4.46 (2H, q, *J* = 6.9 Hz), 3.07 (2H, t, *J* = 6.9 Hz), 1.82 (2H, t, *J* = 6.9 Hz), 1.46 (3H, t, *J* = 7.2 Hz), 1.40 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 155.9, 141.3, 129.2, 128.7, 125.4, 124.2, 123.5, 122.6, 121.3, 111.5, 72.8, 61.4, 33.2, 26.3, 23.3, 14.3.

Phenyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7c). 32%; R_f 0.80 (EtOAc/hexane = 1/2); mp 158-160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (1H, d, *J* = 8.1 Hz), 8.20 (1H, d, *J* = 8.4 Hz), 7.62 (1H, t, *J* = 7.5 Hz), 7.47 (4H, m), 7.31 (1H, t, *J* = 7.5 Hz), 7.22 (1H, s), 3.24 (2H, t, *J* = 6.6 Hz), 1.87 (2H, t, *J* = 6.9 Hz), 1.43 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 159.7, 151.6, 151.4, 132.7, 128.9, 126.7, 126.5, 123.3, 123.1, 120.1, 119.7, 117.3, 112.9, 111.8, 80.9, 33.5, 26.9, 22.9.

4-Methyoxyphenyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7d). 20%; R_f 0.80 (EtOAc/hexane = 1/2); mp 143-144 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (1H, d, *J* = 8.4 Hz), 8.19 (1H, d, *J* = 8.4 Hz), 7.62 (1H, t, *J* = 6.9 Hz), 7.50 (1H, t, *J* = 6.9 Hz), 7.15 (2H, d, *J* = 9.3 Hz), 6.96 (2H, d, *J* = 9.9 Hz), 3.84 (3H, s), 3.32 (2H, t, *J* = 6.9 Hz), 1.87 (2H, t, *J* = 6.9 Hz). 1.43 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 157.4, 156.9, 143.2, 141.6, 129.7, 129.2, 125.6, 124.4, 123.7, 122.3, 121.5, 114.5, 111.1, 104.5, 73.0, 55.6, 33.2, 26.5, 23.5.

4-Nitrophenyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7e). 66%; R_f 0.54 (EtOAc/hexane = 1/6); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (1H, d, *J* = 8.3 Hz), 8.34 (2H, d, *J* = 9.3 Hz), 8.20 (1H, d, *J* = 8.3 Hz), 7.65 (1H, t, *J* = 7.9 Hz), 7.52 (1H, t, *J* = 7.9 Hz), 7.43 (2H, d, *J* = 8.7 Hz), 3.20 (2H, t, *J* = 6.9 Hz), 1.88 (2H, t, *J* = 6.9 Hz), 1.45 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 158.2, 154.8, 145.8, 142.2, 130.4, 130.1, 126.3, 125.6, 124.4, 124.2, 123.0, 121.9, 110.6, 104.1, 73.5, 33.4, 26.8, 23.8.

4-Iodophenyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7f). 45%; R_f 0.71 (EtOAc/hexane = 1/6); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (1H, d, *J* = 7.5 Hz), 8.18 (1H, d, *J* = 7.5 Hz), 7.76 (2H, d, *J* = 8.1 Hz), 7.62 (1H, t, *J* = 7.8 Hz), 7.50 (1H, t, *J* = 8.1 Hz), 7.00 (2H, d, *J* = 8.1 Hz), 3.19 (2H, t, *J* = 6.6 Hz), 1.87 (2H, t, *J* = 6.6 Hz), 1.43 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 150.1, 142.1, 141.1, 138.9, 130., 129.9, 126.2, 124.2, 124.1, 121.9, 121.5, 111.6, 104.5, 90.7, 73.5, 33.5, 26.9, 23.9.

Benzyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (6g). 56%; R_f 0.57 (EtOAc/hexane = 1/6); mp 76-78 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (1H, d, *J* = 8.1 Hz), 8.14 (1H, d, *J* = 8.4 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.44 (6H, m), 5.44 (2H, s), 3.04 (2H, t, *J* = 6.9 Hz), 1.78 (2H, t, *J* = 6.9 Hz), 1.37 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 156.1, 141.3, 135.2, 129.3, 128.8, 128.5, 128.3, 127.1, 125.4, 124.1, 123.5, 121.3, 111.4, 105.9, 72.8, 67.1, 33.2, 26.4, 23.4.

4-Methoxybenzyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7h). 35%; R_f 0.49 (EtOAc/hexane = 1/6); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (1H, d, *J* = 8.1 Hz), 8.22 (1H, d, *J* = 9.0 Hz), 7.65 (1H, t, *J* = 6.9 Hz), 7.52 (1H, t, *J* = 7.2 Hz), 7.41 (2H, d, *J* = 8.7 Hz), 6.91 (2H, d, *J* = 9.0 Hz), 5.36 (2H, s), 3.80 (3H, s), 3.34 (2H, t, *J* = 7.2 Hz), 1.90 (2H, t, *J* = 6.9 Hz), 1.45 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 159.6, 155.5, 142.9, 130.0, 128.8, 128.7, 127.2, 126.1, 125.4, 123.8, 121.4, 113.9, 105.0, 104.3, 71.4, 66.8, 55.2, 26.9, 23.3, 14.7.

4-Nitrobenzyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7i). 50%; R_f 0.38 (EtOAc/hexane = 1/6); mp 105-106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (1H, d, *J* = 8.4 Hz), 8.24 (2H, d, *J* = 8.4 Hz), 8.15 (1H, d, *J* = 8.4 Hz), 7.61 (3H, m), 7.48 (1H, t, *J* = 7.8 Hz), 5.50 (2H, s), 3.03 (2H, t, *J* = 6.6 Hz), 1.80 (2H, t, *J* = 6.9 Hz), 1.39 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 156.9, 148.0, 142.8, 141.9, 129.6, 129.0, 126.0, 124.4, 124.3, 124.2, 124.1, 123.9, 121.8, 73.3, 66.0, 33.5, 26.8, 23.9.

4-Chlorobenzyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7j). 22%; R_f 0.69 (EtOAc/hexane = 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (1H, d, *J* = 8.7 Hz), 7.63 (1H, t, *J* = 7.8 Hz), 7.55 (1H, t, *J* = 7.5 Hz), 8.10 (1H, d, *J* = 8.7 Hz), 7.41 (2H, d, *J* = 8.7 Hz), 7.10 (2H, d, *J* = 9.0 Hz), 5.41 (2H, s), 3.10 (2H, t, *J* = 6.7 Hz), 1.92 (2H, t, *J* = 6.9 Hz), 1.41 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 156.0, 148.2, 143.5, 134.7, 134.1, 129.9, 129.4, 129.2, 129.1, 126.6, 124.2, 121.9, 105.2, 104.4, 74.1, 66.4, 33.3, 23.7, 23.4.

Furan-2-ylmethyl 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5-carboxylate (7k). 54% R_f 0.51 (EtOAc/hexane = 1/6); mp 109-110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (1H, d, *J* = 8.7 Hz), 8.23 (1H, d, *J* = 8.4 Hz), 7.61 (1H, t, *J* = 6.9 Hz), 7.53 (1H, t, *J* = 7.2 Hz), 7.46 (1H, d, *J* = 2.1 Hz), 6.55 (1H, t, *J* = 3.9 Hz), 6.40 (1H, d, *J* = 1.2 Hz), 5.39 (2H, s), 3.35 (2H, t, *J* = 6.9 Hz), 1.91 (2H, t, *J* = 6.6 Hz), 1.46 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 159.9, 143.8, 130.6, 129.9, 126.8, 126.2, 124.8, 124.2, 121.9, 121.4, 116.4, 111.8, 110.9, 73.4, 59.2, 33.6, 26.9, 24.0.

Methyl 6-Methoxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo-[*h*]chromene-5-carboxylate (7l). 85%; R_f 0.34 (Pet. Ether/EtOAc = 5/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (1H, m), 8.00 (1H, m), 7.47 (2H, m), 3.97 (3H, s), 3.94 (3H, s), 2.78 (2H, t, *J* = 6.6 Hz), 1.87 (2H, t, *J* = 6.6 Hz), 1.41 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 149.2, 141.0, 125.2, 124.5, 122.1, 120.9, 119.2, 118.2, 100.6, 99.9, 48.5, 35.9, 35.7, 35.4, 35.2, 34.9, 34.6.

Methyl 6-Acetoxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo-[*h*]chromene-5-carboxylate (7m). 88%; R_f 0.38 (Pet. Ether/EtOAc = 5/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (1H, m), 7.69 (1H, m), 7.48(2H, m), 3.92 (3d, s), 2.88 (2H, t, *J* = 6.6 Hz), 1.86 (2H, t, *J* = 6.6 Hz), 1.42 (6H, s).

Phenyl 6-Acetoxy-2,2-dimethyl-3,4-dihyro-2*H*-[*h*]chromene-5-carboxylate (7n). 83%; R_f 0.14 (Pet. Ether/EtOAc = 25/1); mp 152-154 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (1H, d, *J* = 9.3 Hz), 7.78 (1H, d, *J* = 9.3 Hz), 7.53 (2H, m), 7.45 (2H, t, *J* = 7.5 Hz), 7.29 (2H, d, *J* = 7.8 Hz), 7.22 (1H, t, *J* = 7.8 Hz), 3.06 (2H, t, *J* = 6.9 Hz), 2.41 (3H, s), 1.92 (2H, t, *J* = 6.9 Hz), 1.46 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 159.7, 151.6, 151.4, 132.7, 128.9, 126.7, 126.5, 123.3, 123.1, 120.1, 119.7, 117.3, 112.9, 111.8, 80.9, 33.5, 26.9, 22.9, 13.8.

Benzyl 6-Acetoxy-2,2-dimethyl-3,4-dihydro-2*H*-[*h*]chromene-5-carboxylate (7o). 99%; R_f 0.22 (Pet. Ether/hexane = 25/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (1H, d, *J* = 6.9 Hz), 7.63 (1H, d, *J* = 8.4 Hz), 7.44 (7H, m), 5.38 (2H, s), 2.86 (2H, t, *J* = 6.3 Hz), 2.07 (3H, s), 1.85 (2H, t, *J* = 6.3 Hz), 1.42 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 169.9, 156.1, 141.3, 135.2, 129.3, 128.9, 128.8, 128.3, 127.1, 125.4, 124.1, 123.5, 121.3, 114.0 111.4, 72.8, 67.1, 33.2, 26.4, 23.4, 17.2.

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