Bioorganic Chemistry 52 (2014) 77–82

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis of novel and diverse mollugin analogues and their antibacterial and antioxidant activities

Akber Idhayadhulla [a], Likai Xia [a], Yong Rok Lee [a,*], Sung Hong Kim [b], Young-Jung Wee [c], Chong-Soon Lee [d]

[a] School of Chemical Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[b] Analysis Research Division, Daegu Center, Korea Basic Science Institute, Daegu 702-701, Republic of Korea
[c] Department of Food Science and Technology, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[d] School of Biotechnology, Yeungnam University, Gyeongsan 712-749, Republic of Korea

ARTICLE INFO

Article history:
Received 25 September 2013
Available online 7 December 2013

Keywords:
Mollugin
3,4-Dihydromollugin
Antioxidant activity
Antibacterial activity

ABSTRACT

Novel and diverse mollugin analogues (**1**–**12**) were synthesized using $PhB(OH)_2$/AcOH-mediated electrocyclization reaction as a key step. The newly synthesized compounds were screened for antioxidant and antibacterial activities. Compounds **1**, **2**, **5**, **6**, **8**, and **10**–**12** showed high antioxidant activities in DPPH inhibition (IC_{50} = 0.52–1.11 μM) compared with BHT (IC_{50} = 9.67 μM). Compounds **3** exhibited potent antibacterial activity against *Staphylococcus aureus* (KCTC-1916) bacterial strain at 100 μg/mL. Structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR data and high-resolution mass spectrometry.

1. Introduction

Rubia cordifolia has an extremely large area of distribution ranging from Africa to tropical Asia, India, China, Japan and Australia. In China, it has been used as a traditional Chinese medicine for centuries and is officially listed in the Chinese Pharmacopoeia [1]. The dried roots and rhizomes of this plant are used officially as a component in herbal medicines for the treatment of arthritis, dysmenorrhea, hemostasis, and other diseases [1]. In India, this plant has been used to treat rheumatism, menstrual pain, and urinary disorders [2]. Mollugin (**1**), 3,4-dihydromollugin (**2**), and naphthohydroquinone derivatives are major constituents isolated from *R. cordifolia* (Fig. 1) [3–12]. The pharmacological studies demonstrated that mollugin (**1**) and 3,4-dihydromollugin (**2**) exhibited various interesting biological properties, such as, antitumor [3], antimutagenic [13,14], antileukemia [15], anti-inflammatory [16], and antiallergic activities [16]. In particular, mollugin (**1**) has a potent antiviral activity with an IC_{50} value of 2.0 μg/mL in human hepatoma Hep3B cells [17], and antiproliferative activity with an IC_{50} value of 3.5 μg/mL in a human colon cancer cell line [18]. 3,4-Dihydromollugin (**2**) has also been shown to possess potent antiviral activity with an IC_{50} value of 2.0 μg/mL in human hepatoma Hep3B cells [17]. Furthermore, mollugin (**1**) strongly inhibits arachidonic acid-induced and collagen-induced platelet aggregation [19]. Although several biological activities and properties of naturally occurring mollugin (**1**) and 3,4-dihydromollugin (**2**) have been reported [13–19], but antioxidant and antibacterial activities of synthetic mollugin analogues have not been screened. Herein, we report the syntheses of novel and diverse mollugin analogues and evaluation of their antioxidant and antibacterial activities.

2. Experimental

2.1. General

All experiments were carried out in a nitrogen atmosphere. Merck, pre-coated silica gel plates (Art. 5554) with a fluorescent indicator were used for analytical TLC. Flash column chromatography was performed using silica gel 9385 (Merck). ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker Avance DPX 300 MHz spectrometer in $CDCl_3$ as the solvent; chemical shifts (δ values) were measured in ppm relative to tetramethylsilane, and coupling constants (J values) are in Hz. IR spectra were recorded on a Jasco FTIR 5300 spectrophotometer. All melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. HRMS was carried out at the Korea Basic Science Institute.

2.2. Organic synthesis

2.2.1. Preparation of alkyl 1,4-dioxo-1,4-dihydronaphthalene-2-carboxylates

To a solution of 1,4-dihydroxy-2-naphthoic acid (2.042 g, 10.0 mmol) in DMF (20 mL) was added sodium bicarbonate (0.840 g, 10.0 mmol) and iodomethane (1.419 g, 10.0 mmol) or (2-bromoethyl)benzene (1.851 g, 10.0 mmol) at room tempera-

* Corresponding author. Fax: +82 53 810 4631.
E-mail address: yrlee@yu.ac.kr (Y.R. Lee).

0045-2068/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bioorg.2013.11.008

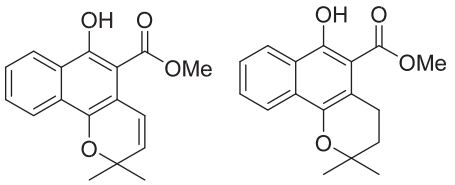


Fig. 1. Naturally occuring mollugin (**1**) and 3,4-dihydromollugin (**2**).

ture. The reaction mixture was stirred at room temperature for 10 h. The reaction mixture was quenched by addition of 1 N HCl (30 mL) solution and the aqueous solution was extracted with ethyl acetate (40 mL × 3). The combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated under vacuum. Flash chromatography on silica gel using hexane/ethyl acetate (3:1) afforded methyl 1,4-dihydroxy-2-naphthoate or phenethyl 1,4-dihydroxy-2-naphthoate.

2.2.1.1. Methyl 1,4-dihydroxy-2-naphthoate. See Ref. [20].

2.2.1.2. Phenethyl 1,4-dihydroxy-2-naphthoate. Yellow solid, 2.31 g, 75% yield. Mp 156–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.08 (s, OH), 11.41 (s, OH), 8.27 (d, J = 8.1 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.1, 6.9 Hz, 1H), 7.43 (dd, J = 8.1, 6.9 Hz, 1H), 7.28–7.12 (m, 5H), 7.05 (s, 1H), 4.47 (t, J = 6.9 Hz, 2H), 3.00 (t, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 154.4, 144.7, 137.5, 129.5, 128.8 (2C, Phenyl-CH), 128.5 (2C, Phenyl-CH), 128.4, 126.5, 125.8, 125.3, 123.5, 122.1, 104.6, 104.4, 65.5, 34.9; IR (KBr): ν 3382, 3055, 2987, 2925, 1732, 1650, 1599, 1453, 1405, 1345, 1265, 1153, 1095, 1071, 895, 743 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{19}H_{16}O_4$: 308.1049; Found: 308.1052.

*2.2.2. General procedure for the synthesis of mollugin (**1**) and its analogues **5**, **7**, **9**, **11***

A solution of alky 1,4-dioxo-1,4-dihydronaphthalene-2-carboxylates (10.0 mmol), aldehyde (10.0 mmol), phenylboronic acid (10 mmol) and glacial AcOH (10 mL) in anhydrous toluene (200 mL) was refluxed for 8 h under N_2 in an apparatus fitted with a Dean–Stark trap. The mixture was cooled, concentrated under vacuum, and the residue was extracted with several portions of CH_2Cl_2 (30 mL). The combined extract was washed successively with H_2O (30 mL), $NaHCO_3$ (50 mL), and brine (30 mL), dried (Na_2SO_4), the solvent was evaporated under vacuum, and the crude product was purified by flash chromatography on silica gel hexane/ethyl acetate (5:1) to give the corresponding products.

*2.2.2.1. Mollugin (**1**).* See Refs. [20–25].

*2.2.2.2. Methyl 6-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)-2H-benzo[h]chromene-5-carboxylate (**5**).* Yellow liquid, 3.24 g, 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.21 (1H, s), 8.38 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.4, 7.2 Hz, 1H), 7.50 (dd, J = 8.4, 7.2 Hz, 1H), 7.14 (d, J = 9.9 Hz, 1H), 5.65 (d, J = 9.9 Hz, 1H), 5.13 (t, J = 7.2 Hz, 1H), 4.00 (s, 3H), 2.24–2.16 (m, 2H), 1.85–1.78 (m, 2H), 1.66 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.47 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 156.3, 141.4, 131.6, 129.2, 128.8, 128.1, 126.2, 125.0, 124.1, 124.0, 122.5, 121.8, 112.4, 102.1, 76.7, 52.2, 39.8, 25.6 (CH_3), 24.7 (CH_3), 22.5, 17.5 (CH_3); IR (neat) ν 2968, 2922, 1733, 1651, 1579, 1442, 1363, 1332, 1236, 1101, 1013, 808, 771 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{22}H_{24}O_4$: 352.1675. Found: 352.1677.

*2.2.2.3. Methyl 6-hydroxy-2-phenyl-4H-benzo[h]chromene-5-carboxylate (**7**).* Brown solid, 2.92 g, 88% yield, Mp 108–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.49 (s, 1H),), 8.36 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 7.2 Hz, 2H), 7.65 (dd, J = 8.4, 7.2 Hz, 1H), 7.48 (dd, J = 8.4, 7.2 Hz, 1H), 7.44–7.32 (m, 3H), 5.49 (t, J = 3.9 Hz, 1H), 3.93 (s, 3H), 3.83 (d, J = 3.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 158.7, 147.5, 139.4, 134.3, 129.8, 128.3 (2C, Ph-CH), 128.2, 128.0, 126.0, 124.3 (3C, 2Ph-CH, Naphthyl-C), 123.9, 121.2, 111.6, 104.1, 96.4, 52.2, 25.8; IR (KBr): ν 3069, 2953, 1734, 1651, 1590, 1444, 1337, 1238, 1166, 1097, 762 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{21}H_{16}O_4$: 332.1049. Found: 332.1050.

*2.2.2.4. Phenethyl 6-hydroxy-2,2-dimethyl-2H-benzo[h]chromene-5-carboxylate (**9**).* Yellow solid, 3.18 g, 85% yield. Mp 89–90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.06 (s, 1H), 8.25 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.48 (dd, J = 8.4, 7.2 Hz, 1H), 7.37 (dd, J = 8.1, 7.2 Hz, 1H), 7.24–7.09 (m, 5H), 6.78 (d, J = 9.9 Hz, 1H), 5.44 (d, J = 9.9 Hz, 1H), 4.53 (t, J = 6.9 Hz, 2H), 3.00 (t, J = 6.9 Hz, 2H), 1.37 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 156.4, 141.4, 137.5, 129.2, 128.9 (2C, Phenyl-CH), 128.9, 128.5 (2C, Phenyl-CH), 128.4, 126.7, 126.2, 125.0, 123.9, 122.4, 121.8, 112.6, 102.2, 74.5, 66.3, 34.9, 26.8 (2C, CH_3); IR (KBr): ν 3289, 3067, 3030, 2974, 2928, 1736, 1646, 1578, 1497, 1439, 1392, 1358, 1325, 1238, 1166, 1046, 1011, 808, 770, 744 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{24}H_{22}O_4$: 374.1518; Found: 374.1518.

*2.2.2.5. Phenethyl 6-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)-2H-benzo[h]chromene-5-carboxylate (**11**).* Yellow liquid, 3.89 g, 88% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.29 (s, 1H), 8.45 (d, J = 8.1 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H), 7.65 (dd, J = 8.1, 7.5 Hz, 1H), 7.54 (dd, J = 8.1, 7.5 Hz, 1H), 7.45–7.29 (m, 5H), 7.01 (d, J = 9.9 Hz, 1H), 5.61 (d, J = 9.9 Hz, 1H), 5.21 (t, J = 6.9 Hz, 1H), 4.68 (t, J = 6.9 Hz, 2H), 3.15 (t, J = 6.9 Hz, 2H), 2.32–2.41 (m, 2H), 1.92–1.86 (m, 2H), 1.75 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.54 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 156.3, 141.3, 137.5, 131.4, 129.1, 128.8 (2C, Phenyl-CH), 128.7, 128.4 (2C, Phenyl-CH), 127.6, 126.6, 126.0, 124.9, 124.1, 123.9, 122.7, 121.7, 112.4, 102.1, 76.6, 66.2, 39.7, 34.8, 25.6 (CH_3), 24.6 (CH_3), 22.4, 17.5 (CH_3); IR (neat): ν 3287, 3064, 3029, 2969, 2923, 1723, 1647, 1579, 1496, 1443, 1388, 1361, 1323, 1234, 1180, 1101, 1011, 905, 809, 770, 743, 700 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{29}H_{30}O_4$: 442.2144; Found: 442.2147.

*2.2.3. General procedure for the synthesis of 3,4-dihydroxy mollugin (**2**) and its analogues **6**, **8**, **10**, and **12***

To a solution of mollugin (**1**) or its analogues **5**, **7**, **9**, and **11** (1 mmol) in anhydrous ethyl acetate (20 mL) in a Parr bottle was added 10% Pd/C (30 mg). The bottle was shaken for 5 h at 20 psi of H_2. Removal of the solvent at reduced pressure left an oily residue, which was then purified by column chromatography on silica gel using hexane/ethyl acetate (15:1) to give the corresponding hydrogenated products.

*2.2.3.1. 3,4-Dihydroxy mollugin (**2**).* See Ref. [20].

*2.2.3.2. Methyl 6-hydroxy-2-methyl-2-(4-methylpentyl)-3,4-dihydro-2H-benzo[h]chromene-5-carboxylate (**6**).* Yellow liquid, 349 mg, 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.23 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.60 (1H, dd, J = 8.4, 7.5 Hz), 7.49 (1H, dd, J = 8.4, 7.5 Hz), 3.97 (s, 3H), 3.05 (t, J = 6.9 Hz, 2H,), 1.85–1.80 (m, 2H), 1.76–1.65 (m, 2H), 1.61–1.58 (m, 2H), 1.56–1.45 (m, 2H), 1.35 (s, 3H, CH_3), 1.26–1.17 (m, 1H), 0.89 (d, J = 6.6 Hz, 6H, 2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 156.1, 141.3, 129.5, 129.0, 125.6, 124.3, 123.7, 121.5, 111.7, 105.1, 74.9, 52.0, 39.7, 39.4, 31.6, 27.8 (CH_3), 23.4, 23.0, 22.6 (CH_3), 22.5 (CH_3), 21.3; IR (neat) ν 3071, 2949, 2869, 1734, 1651,

1589, 1445, 1380, 1336, 1237, 1170, 1101, 997, 770 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{22}H_{28}O_4$: 356.1988. Found: 356.1987.

*2.2.3.3. Methyl 6-hydroxy-2-phenyl-3,4-dihydro-2H-benzo[h]chromene-5-carboxylate (**8**).* Yellow solid, 327 mg, 98% yield. Mp 131–132 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 12.19 (s, 1H), 8.21 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.38 (dd, J = 8.1, 6.9 Hz, 1H), 7.32–7.14 (m, 6H), 4.79 (d, J = 9.9 Hz, 1H), 3.70 (s, 3H), 2.88–2.66 (m, 2H), 2.02–1.98 (m, 1H), 1.80–1.72 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 156.6, 142.6, 141.9, 129.1, 128.8, 128.3 (2C, Phenyl-CH), 127.5, 125.7 (2C, Phenyl-CH), 125.6, 124.0, 123.6, 121.3, 112.8, 104.8, 76.3, 51.8, 30.2, 25.7; IR (KBr) ν 3055, 2952, 2867, 1726, 1645, 1589, 1444, 1382, 1333, 1240, 1170, 1097, 997, 763, 743, 702, 649 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{21}H_{18}O_4$: 334.1205. Found: 334.1204.

*2.2.3.4. Phenethyl 6-hydroxy-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-5-carboxylate (**10**).* Yellow solid, 361 mg, 96% yield. Mp 74–75 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 12.13 (s, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.49 (dd, J = 8.1, 6.9 Hz, 1H), 7.39 (dd, J = 8.4, 6.9 Hz, 1H), 7.27–7.16 (m, 5H), 4.57 (t, J = 6.9 Hz, 2H), 3.04 (t, J = 6.9 Hz, 2H), 2.71 (t, J = 6.9 Hz, 2H), 1.61 (t, J = 6.9 Hz, 2H), 1.28 (s, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 156.3, 141.4, 137.7, 129.4, 129.0, 128.8 (2C, Phenyl-CH), 128.6 (2C, Phenyl-CH), 126.7, 125.6, 124.3, 123.7, 121.5, 111.7, 105.2, 72.9, 66.0, 35.0, 33.1, 26.4 (2C, CH_3), 23.2; IR (KBr): ν 3395, 3067, 3028, 2970, 2935, 1736, 1642, 1587, 1445, 1387, 1327, 1235, 1174, 1123, 1097, 989, 807, 770, 700 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{24}H_{24}O_4$: 376.1675; Found: 376.1676.

*2.2.3.5. Phenethyl 6-hydroxy-2-methyl-2-(4-methylpentyl)-3,4-dihydro-2H-benzo[h]chromene-5-carboxylate (**12**).* Yellow liquid, 437 mg, 98% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 12.13 (s, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.45 (dd, J = 8.1, 6.9 Hz, 1H), 7.34 (dd, J = 8.1, 6.9 Hz, 1H), 7.25–7.08 (m, 5H), 4.51 (t, J = 6.9 Hz, 2H), 2.98 (t, J = 6.9 Hz, 2H), 2.66 (t, J = 6.9 Hz, 2H), 1.61–1.32 (m, 8H), 1.19 (s, 3H), 1.15–1.05 (m, 1H), 0.78 (d, J = 6.6 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 156.2, 141.2, 137.6, 129.4, 129.0, 128.8 (2C, Phenyl-CH), 128.5 (2C, Phenyl-CH), 126.7, 125.5, 124.3, 123.7, 121.5, 111.9, 105.1, 74.9, 65.9, 39.6, 39.4, 34.9, 31.5, 27.8, 23.4, 22.9, 22.6 (CH_3), 22.5 (CH_3), 21.3; IR (neat): ν 3272, 3066, 3028, 2948, 2870, 1723, 1642, 1588, 1496, 1445, 1386, 1323, 1235, 1172, 1099, 988, 806, 769, 743, 700, 649 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{29}H_{34}O_4$: 446.2457; Found: 446.2453.

*2.2.4. General procedure for the synthesis of carboxylic acid **3** and **4***

To a solution of KOH (168 mg, 3.0 mmol) in DMSO (4 mL), **1** (284 mg, 1.0 mmol) or **2** (286 mg, 1.0 mmol) was added. The reaction mixture was heated at 60 °C for 8 h, after which was quenched by addition of 1 N HCl (10 mL) solution and the aqueous solution was extracted with ethyl acetate (15 mL × 3). The combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated under vacuum. Flash chromatography on silica gel using hexane/ethyl acetate (9:1) afforded **3** or **4**.

*2.2.4.1. 6-Hydroxy-2,2-dimethyl-2H-benzo[h]chromene-5-carboxylic acid (**3**).* See Refs. [26,27].

*2.2.4.2. 6-Hydroxy-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-5-carboxylic acid (**4**).* See Refs. [25,28].

2.2.5. 6-O-Methylmollugin

See Refs. [23,29].

2.2.6. 6-O-Acetylmollugin

To a solution of **1** (142 mg, 0.5 mmol) in CH_2Cl_2 (10 mL), *N,N*-diisopropylethylamine (DIPEA, 129 mg, 1.0 mmol), 4-(dimethylamino)pyridine (DMAP, 6.1 mg, 0.05 mmol) were added followed by acetyl chloride (47 mg, 0.6 mmol). The reaction mixture was stirred at room temperature for 8 h. Then, the reaction was quenched by the addition of 1 N HCl (10 mL) solution and the aqueous solution was extracted with ethyl acetate (15 mL × 3). The combined organic extracts were washed with water, dried ($MgSO_4$), and evaporated in vacuo. Flash chromatography on silica gel using hexane/ethyl acetate (90:10) afforded 115 mg of product **9** (95%) as a yellow liquid. ^1H NMR(300 MHz, $CDCl_3$) δ 8.23–8.20 (m, 1H), 7.75–7.72 (m, 1H), 7.54–7.47 (m, 2H), 6.62 (d, J = 9.9 Hz, 1H), 5.68 (d, J = 9.9 Hz, 1H), 3.93 (s, 3H), 2.39 (s, 3H), 1.51 (s, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 166.0, 146.7, 138.9, 129.9, 127.3, 127.2, 126.8, 126.5, 122.3, 122.0, 120.0, 119.3, 112.7, 76.5, 52.2, 27.6 (2C, CH_3), 20.5; IR (KBr) ν 3071, 2976, 1773, 1727, 1440, 1368, 1295, 1227, 1195, 1131, 1080, 1021, 768 cm^{-1}, HRMS m/z (M^+) calcd for $C_{19}H_{18}O_5$: 326.1154. Found: 326.1153.

2.3. Antioxidant activity

General procedure for evaluation of antioxidant activity: Free radical-scavenging activity was measured by the method proposed by Sharma and Bhat, using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [30–32]. The methanolic solution of DPPH exhibits a strong purple colour with a strong absorption at 517 nm, which is reduced in the presence of an antioxidant agent, giving a yellowish solution. Therefore, there is an inverse relationship between the remaining DPPH concentration and the anti-radical activity of the antioxidant. The synthesized compounds **1**–**12** were well dissolved in methanol. These methanolic solutions of compounds were measured from the bleaching of the purple coloured methanol solution of DPPH (0.004%, w/v). Various concentrations of the test compounds (10–100 μg/mL) in methanol were added to methanolic solution of DPPH. The absorbance was read against blank at 517 nm. The inhibition (*I*%) of free radical production from DPPH was calculated by the following equation.

$$\text{Inhibition}(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100$$

The absorbance at 517 nm by DPPH was measured by UV–vis spectrophotometry. Where A_{control} is the absorbance of the DPPH solution and A_{sample} is the test sample. IC_{50} values denote the concentration (in μM) of sample required to scavenge 50% DPPH free radicals (Table 1). The mean of the results was calculated based on at least 3 independent evaluations and the standard deviations (SD) were also calculated using Microsoft Excel. All IC_{50} values were calculated from the corresponding sigmoidal dose–response curve according to their best fit shapes based on at least five reaction points using the Office Excel 2007 software (Microsoft, Redmond, WA, USA).

2.4. Antibacterial activity

The bacterial strains used for the analysis were *Escherichia coli* (*E. coli*, KCTC-1924) and *Staphylococcus aureus* (*S. aureus*, KCTC-1916) obtained from the Korean Collection for Type Cultures (KCTC). The compounds (**1**–**12**) were evaluated for their *in vitro* antibacterial activity against *E. coli* and *S. aureus* by agar-disc diffusion method [33]. Each compound was tested at a concentration of 100 μg/mL in DMSO and samples were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. Ciprofloxacin was used as a standard. The plates were incubated for 24 h at 37 °C, and the diameter of the growth inhibition zones was measured and recorded.

Table 1
The *in vitro* DPPH antioxidant scavenging activity of mollugin (**1**), 3,4-dihydromollugin (**2**), and their analogues **3–12**.[a]

Compound	Concentration (μg/mL) (inhibition of free radicals in percentage)							IC_{50} μM
	0.1	1	10	25	50	75	100	
1	49.87 ± 0.23	53.42 ± 0.78	75.74 ± 0.17	79.09 ± 0.26	84.54 ± 1.21	88.89 ± 0.75	93.03 ± 1.08	0.70 ± 0.07
2	49.90 ± 0.36	58.47 ± 0.95	77.03 ± 0.43	79.70 ± 0.67	81.44 ± 1.09	89.19 ± 0.87	95.62 ± 0.47	0.52 ± 0.05
3	–	26.52 ± 0.86	48.78 ± 0.20	59.43 ± 0.30	61.40 ± 0.25	67.01 ± 0.31	71.91 ± 0.21	42.50 ± 1.02
4	–	29.85 ± 0.36	49.81 ± 0.31	57.53 ± 0.29	58.53 ± 1.20	63.77 ± 0.71	72.15 ± 0.26	41.23 ± 0.87
5	48.58 ± 0.24	53.46 ± 0.23	58.01 ± 0.20	65.17 ± 0.25	74.15 ± 0.30	79.04 ± 0.37	81.92 ± 0.25	0.97 ± 0.02
6	49.67 ± 0.84	53.43 ± 0.11	59.50 ± 0.15	66.31 ± 0.29	78.36 ± 1.00	81.41 ± 0.15	83.28 ± 0.31	0.84 ± 0.03
7	–	49.84 ± 0.68	54.80 ± 0.24	59.36 ± 0.65	68.74 ± 0.16	74.31 ± 0.23	78.68 ± 0.84	5.99 ± 0.08
8	47.61 ± 0.62	53.04 ± 0.52	59.04 ± 0.34	66.19 ± 0.23	76.25 ± 0.56	78.09 ± 0.76	81.88 ± 0.76	1.11 ± 0.01
9	–	25.03 ± 0.21	49.52 ± 0.30	58.60 ± 1.05	65.30 ± 1.40	70.70 ± 1.75	73.92 ± 1.65	28.62 ± 0.06
10	49.82 ± 0.57	54.69 ± 0.33	60.31 ± 0.20	68.79 ± 0.30	75.47 ± 0.25	86.82 ± 0.20	87.98 ± 1.15	0.77 ± 0.04
11	48.61 ± 0.17	53.30 ± 0.19	57.50 ± 0.60	64.80 ± 0.55	72.90 ± 0.70	76.50 ± 1.28	82.20 ± 1.15	0.79 ± 0.05
12	49.72 ± 0.55	51.20 ± 0.64	59.20 ± 1.30	69.40 ± 1.41	73.60 ± 1.85	78.01 ± 1.80	83.50 ± 1.55	0.74 ± 0.09
BHT	–	48.82 ± 0.78	53.90 ± 0.35	61.40 ± 0.32	61.20 ± 0.56	67.90 ± 0.73	74.80 ± 0.77	9.67 ± 0.61

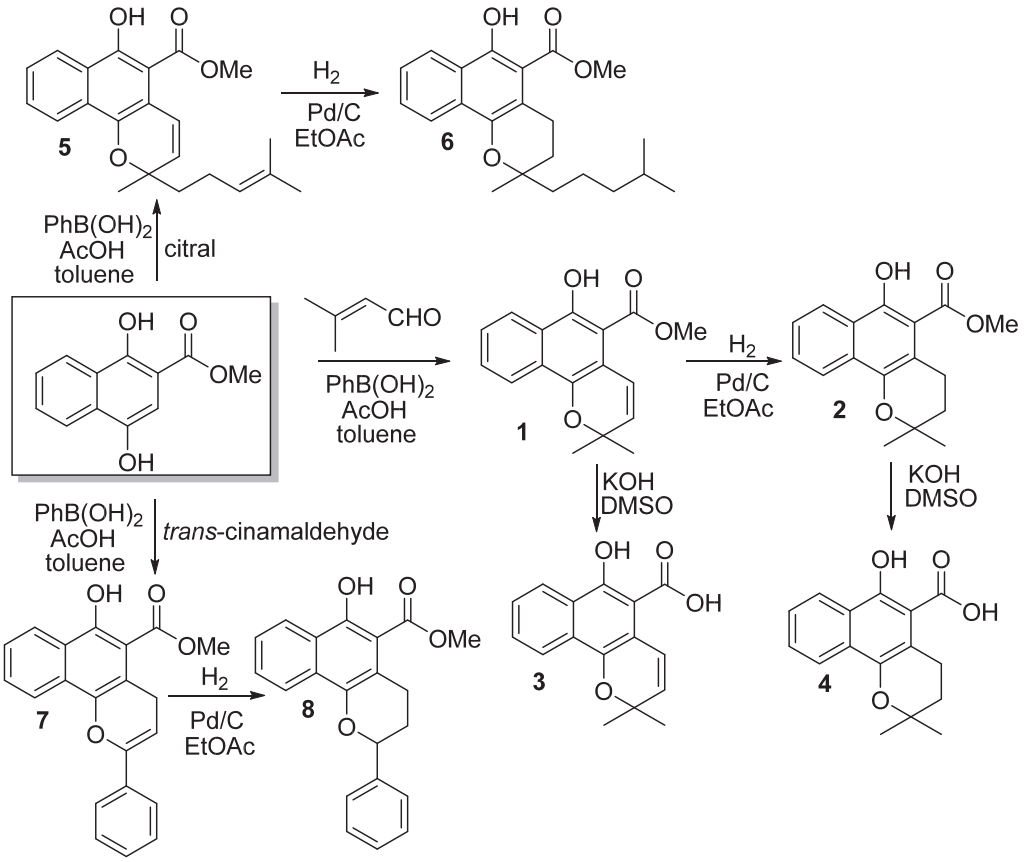
[a] Value were the means of three replicates ± SD.

3. Results and discussion

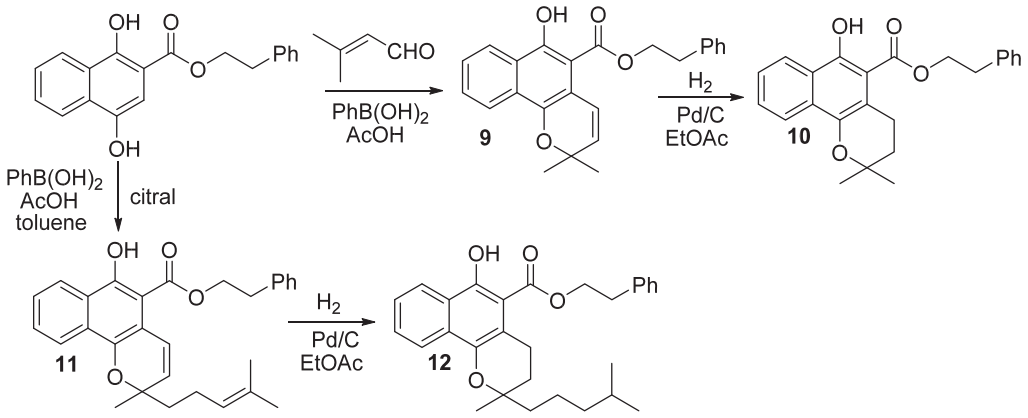
3.1. Chemistry

The syntheses of mollugin (**1**) and 3,4-dihydromollugin (**2**) have been reported by several groups [20–25]. Although several synthetic approaches to mollugin (**1**) and 3,4-dihydromollugin (**2**) have been reported, other mollugin analogues **4–12** have not been synthesized. We previously reported the syntheses of mollugin (**1**) and 3,4-dihydromollugin (**2**) using an electrocyclization reaction [34–42].

In the present study, using this technique as a key step, diverse mollugin analogues **3–8** were prepared (Scheme 1). Reaction between methyl 1,4-dihydroxy-2-naphthoate and 3-methyl-2-butenal using $PhB(OH)_2$/AcOH in refluxing toluene for 8 h afforded mollugin (**1**) in 91% yield [20]. Subsequent catalytic hydrogenation of **1** over Pd/C (20 psi) in ethyl acetate for 5 h gave 3,4-dihydromollugin (**2**) in 96% yield [20]. Hydrolysis of mollugin (**1**) or 3,4-dihydromollugin (**2**) in the presence of KOH in DMSO at 60 °C for 8 h afforded **3** and **4** at yields of 76% and 72%, respectively. Treatment of methyl 1,4-dihydroxy-2-naphthoate with citral in the presence of $PhB(OH)_2$/AcOH in refluxing toluene for 7 h provided compound **5** (92%), which was hydrogenated over Pd/C (20 psi) in ethyl acetate to give **6** (98%). Similarly, treatment of methyl 1,4-dihydroxy-2-naphthoate with *trans*-cinamaldehyde in $PhB(OH)_2$/AcOH in refluxing toluene for 8 h afforded compound **7** in 88% yield. Interestingly, in this case, double bond migration occurred. Finally, catalytic hydrogenation of **7** gave **8** in 98% yield.



Scheme 1. Synthesis of mollugin (**1**) dihydromolugin (**2**), and their novel analogues **3–8**.



Scheme 2. Synthesis of mollugin analogues **9**–**12**.

Other analogues **9**–**12** were synthesized as shown in Scheme 2. Reaction of phenethyl 1,4-dihydroxy-2-naphthoate with 3-methyl-2-butenal or citral provided **9** (85%) and **11** (88%), respectively, which were then hydrogenated over Pd/C to afford the corresponding compounds **10** (96%) and **12** (98%), respectively.

3.2. Biological activity

Synthesized mollugin (**1**), 3,4-dihydromollugin (**2**), and their analogues (**3–12)** were assayed for their antioxidant and antibacterial activities. The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) method provides a rapid and convenient technique for the screening of antioxidant activity [30–32]. Compounds **1**–**12** were first examined at five different concentrations, namely, 10, 25, 50, 75, and 100 μg/mL; antioxidant activities are expressed as DPPH inhibition%. IC_{50} was defined as the concentration of sample necessary to inhibit DPPH by 50%.

Results are summarized in Table 1. Synthesized compounds **1**, **2**, **5**, **6**, **8**, **10**, **11**, and **12** showed better antioxidant activities than compounds **3**, **4**, **7**, or **9** at concentrations of 10–100 μg/mL. Importantly, 3,4-dihydro mollugin (**2**) exhibited potent radical scavenging activity with an IC_{50} value of 0.52 μM, whereas the IC_{50} value of the 2,6-di-*tert*-butyl-4-methylphenol (BHT) standard was 9.67 μM.

The present study also revealed the importance and influence of the 6-hydroxy and naphthopyran structure in the mollugin analogues on their radical scavenging activity. A 6-hydroxy group on a mollugin skeleton is essential to exhibit the antioxidant activity while the 6-*o*-acetylmollugin and 6-*o*-methylmollugin showed no activity in DPPH assay. Interestingly, compounds **2**, **4**, **6**, **8**, **10** and **12** reduced by catalytic hydrogenation reactions have a little better antioxidant activities than corresponding compounds **1**, **3**, **5**, **7**, **9** and **11** due to stabilization of the phenolic radicals. This result was well in agreement with antioxidant activity between benzochromenol and dihydrobenzochromenol reported in the literature [43]. On the other hand, the order of inhibitory activity of compounds of **2** > **6** > **8**, based on their IC_{50} values, is also largely explainable in terms of stereoelectronic constraints and thus reflects their intrinsic antioxidant properties [44]. Finally, compounds **3** and **4** exhibited weak radical scavenging activity because of the intramolecular hydrogen bond, formed by between hydrogen atom of a carboxylic acid group and the adjacent oxygen atom on the naphthalene ring, which have a specific deactivating effect [45].

Next, we examined the *in vitro* antimicrobial activities of compounds **1–12** by examining their abilities to inhibit the growths of the standard strains *E. coli*, and *S. aureus*. Primary screening was performed using the disc diffusion method and Müller–Hinton agar medium [33]. Preliminary antibacterial activity results of the tested compounds (100 μg/mL) and of the ciprofloxacin standard (100 μg/mL) are shown in Table 2.

Table 2
The *in vitro* antibacterial activities of compounds **1**–**12** (100 μg/5 mm disc) and of ciprofloxacin (100 μg/5 mm disc) against *E. coli* and *S. aureus*.

Compound	Diameter of growth inhibition zone (mm)	
	E. coli gram-negative	*S. aureus* gram-positive
1	15	18
2	13	30
3	21	38
4	19	18
5	21	18
6	18	20
7	20	15
8	14	28
9	17	15
10	20	18
11	19	20
12	21	14
Ciprofloxacin	30	35

The results revealed that the synthesized compounds inhibited the tested microorganisms to different extents. Compounds **3**, **5**, **7**, **10**, and **12** showed strong activity (growth inhibition zones 20–21 mm) against the *E. coli* bacterial strain, whereas compounds **4**, **6**, **9** and **11** exhibited moderate activity (growth inhibition zones 17–19 mm) and the remainder weak activity. Compound **3** was found to be more active (growth inhibition zone 38 mm) against the *S. aureus* strain than ciprofloxacin (growth inhibition zone 35 mm); compounds **2** and **8** exhibited strong activity (growth inhibition zones 28–30 mm). Against the *S. aureus* strain, compounds **1**, **4**, **5**, **10**, and **11** exhibited moderate activity (growth inhibition zones 18–20 mm), and the remainder low activity.

Our antibacterial activity results suggest that mollugin analogues bearing a naphthopyran ring and a 6-hydroxy group are generally active. In addition, 3,4-dihydroxymollugin **(2)** and compound **8** were found to be highly active against the *S. aureus* strain but only weakly active against the *E. coli* strain. On the other hand, compound **12** was highly active against the *E. coli* strain, and weakly active against the *S. aureus* strain. However, in the antibacterial activity study, compound **3** was found to be highly active against both the gram-negative *E. coli* and the gram-positive *S. aureus* bacterial strains.

4. Conclusion

In summary, we described the synthesis of mollugin (**1**), 3,4-dihydromollugin (**2**), and their analogues **3–12**, and screened their

antioxidant and antibacterial activities. Compound **2** was found to have greater antioxidant activity than BHT, and compound **3** was highly active against the *S. aureus* bacterial strain at a level of ciprofloxacin. Our results suggest that these substances represent interesting lead compounds for the development of novel antioxidant and antibacterial agents.

Acknowledgments

This research was supported by the Nano Material Technology Development Program through the Korean National Research Foundation (NRF) funded by the Korean Ministry of Education, Science, and Technology (2012M3A7B4049675).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.11.008.

References

[1] Pharmacopeia of the People's Republic of China; Guangdong Science and Technology Press: Guangzhou, PR China, 1992; p. 179.
[2] G.M.A. Hocking, Dictionary of Natural Products, Plexus Publishing, Medford, NJ, 1997. p. 679.
[3] H. Itokawa, T. Mihar, K. Takeya, Chem. Pharm. Bull. 31 (1983) 2353.
[4] H. Itokawa, Y. Qiao, K. Takeya, Phytochemistry 28 (1989) 3465.
[5] M. Wang, Z. Wang, L. Yue, J. Cai, Xibei Yaoxue Zazhi 27 (2012) 307.
[6] K. Wang, W. Sun, M. Shan, A. Ding, Zhongguo Shiyan Fangjixue Zazhi 18 (2012) 113.
[7] Z.-Y. Tan, Y. Yao, J.-H. Wang, Z.-L. You, Zhongguo Zhongyiyao Xinxi Zazhi 18 (2011) 65.
[8] Z.-L. Zhang, Y. Zhou, X.-F. Huang, Zhongchengyao 29 (2007) 1803.
[9] S.-C. Kuo, P.-R. Chen, S.-W. Lee, Z.-T. Chen, J. Chin. Chem. Soc. 42 (1995) 869.
[10] J. Koyama, T. Ogura, K. Tagahara, T. Konoshima, M. Kozuka, Phytochemistry 31 (1992) 2907.
[11] Y. Liu, Y. Bai, Yaoxue Xuebao 20 (1985) 53.
[12] K. Inoue, Y. Shiobara, H. Nayeshiro, H. Inouye, G. Wilson, M.H. Zenk, Phytochemistry 23 (1984) 307.
[13] Y. Kawasaki, Y. Goda, K. Yoshihira, Chem. Pharm. Bull. 40 (1992) 1504.
[14] F. Marec, I. Kollarova, A. Jegorov, Planta Med. 67 (2001) 127.
[15] P.P. Gutpa, R.C. Srimal, N. Verma, J.S. Tandon, Pharm. Biol. 37 (1999) 46.
[16] Jpn. Kokai Tokkyo Koho, JP 081s13536, 1996.
[17] L.-K. Ho, M.-J. Don, H.-C. Chen, S.-F. Yeh, J.-M. Chen, J. Nat. Prod. 59 (1996) 330.
[18] L.C. Chang, D. Chavez, J.J. Gills, H.H.S. Fong, J.M. Pezzuto, D. Kinghorn, Tetrahedron Lett. 41 (2000) 7157.
[19] M.I. Chung, S.J. Jou, T.H. Cheng, C.N. Lin, F.N. Ko, C.M. Teng, J. Nat. Prod. 57 (1994) 313.
[20] Y.R. Lee, X. Wang, Y.M. Kim, J.J. Shim, B.N. Kim, D.H. Han, Bull. Korean Chem. Soc. 28 (2007) 1735.
[21] M.N.V. Sastry, S. Claessens, P. Habonimana, K.N. De, J. Org. Chem. 75 (2010) 2274.
[22] P. Habonimana, S. Claessens, K.N. De, Synlett (2006) 2472.
[23] S. Claessens, B. Kesteleyn, V.T. Nguyen, N. De Kimpe, Tetrahedron 62 (2006) 8419.
[24] J.-P. Lumb, D. Trauner, Org. Lett. 7 (2005) 5865.
[25] L.-K. Ho, H.-J. Yu, C.-T. Ho, M.-J. Don, J. Chin. Chem. Soc. 48 (2001) 77.
[26] H.W. Jung, J.S. Oh, S.H. Lee, J.L. Liang, D.H. Kim, A.F.M.M. Rahman, Y. Jahng, Bull. Korean Chem. Soc. 28 (2007) 1863.
[27] H. Schildknecht, F. Straub, Justus Liebigs Ann. Chem. (1976) 1307.
[28] L. Heide, E. Leistner, J. Biosci. 37 C (1982) 354.
[29] N.V.S. Mudiganti, S. Claessens, P. Habonimana, N. De Kimpe, J. Org. Chem. 73 (2008) 3867.
[30] G. Cefarelli, B. D'Abrosca, A. Fiorentino, A. Izzo, C. Mastellone, S. Pacifico, V. Piscopo, J. Agric. Food Chem. 54 (2006) 803.
[31] M. Burits, F. Bucar, Phytother. Res. 14 (2000) 323.
[32] O.P. Sharma, T.K. Bhat, Food Chem. 113 (2009) 1202.
[33] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Am. J. Clin. Pathol. 45 (1966) 493.
[34] R.P. Pandit, Y.R. Lee, Synthesis 44 (2012) 2910.
[35] B.H. Park, H.J. Lee, Y.R. Lee, J. Nat. Prod. 74 (2011) 644.
[36] E.J. Jung, B.H. Park, Y.R. Lee, Green Chem. 12 (2010) 2003.
[37] B.H. Park, Y.R. Lee, W.S. Lyoo, Synthesis (2009) 2146.
[38] L. Xia, Y.R. Lee, Synlett (2008) 1643.
[39] Y.R. Lee, L. Xia, Tetrahedron Lett. 49 (2008) 3283.
[40] Y.R. Lee, J.H. Kim, Synlett (2007) 2232.
[41] X. Wang, Y.R. Lee, Tetrahedron Lett. 48 (2007) 6275.
[42] Y.R. Lee, X. Wang, Org. Biomol. Chem. 3 (2005) 3955.
[43] L.R.C. Barclay, C.D. Edwards, K. Mukai, Y. Egawa, T. Nishi, J. Org. Chem. 60 (1995) 2739.
[44] G.W. Burton, T. Doba, E. Gabe, L. Hughes, F.L. Lee, L. Prasad, K.U. Ingold, J. Am. Chem. Soc. 107 (1985) 7053.
[45] M. Charton, Prog. Phys. Org. Chem. 13 (1981) 119.