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

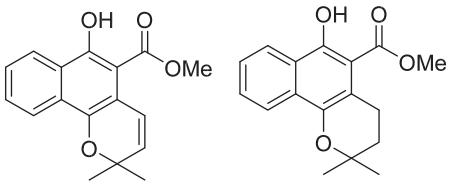

Fig. 1. Naturally occuring mollugin (**1**) and 3,4-dihydromollugin (**2**).

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,