Bioorganic Chemistry 86 (2019) 501–506

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

New bis-thioglycosyl-1,1′-disulfides from *Nasturtium officinale* R. Br. and their anti-neuroinflammatory effect

Tae Hyun Lee[a], Zahra Khan[b,c], Lalita Subedi[b,c], Sun Yeou Kim[b,c], Kang Ro Lee[a,*]

[a] Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea
[b] Gachon Institute of Pharmaceutical Science, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea
[c] College of Pharmacy, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea

ARTICLE INFO

Keywords:
Nasturtium officinale
Brassicaceae
Bis-thioglycosides
Anti-neuroinflammation
Microglia

ABSTRACT

As a part of our continuing search for bioactive constituents from Brassicaceae family, three new bis-thioglycosides (**1–3**) were isolated from the 80% MeOH extract of *Nasturtium officinale*, together with 13 known compounds (**4–16**). The chemical structures of three new bis-thioglycosides (**1–3**) were elucidated using NMR techniques (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC), HRESIMS, and a chemical method. All the compounds were evaluated for their inhibitory effects on nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cells. Among the isolates, compound **5** exhibited a strong inhibitory effect on NO production, and compounds **4** and **15** showed moderate inhibitory activities, suggesting the neuroprotective and anti-neuroinflammatory effects of bis-thioglycosides from *N. officinale*.

1. Introduction

Chronic activation of microglia leading to excessive production of Nitric oxide (NO) can induce the inflammatory signal which can further induce the oxidative stress like the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can cause neuronal cell death and neurodegeneration [1,2]. These processes are selectively involved in the pathologies of multiple neurodegenerative disorders like Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), stroke/excitotoxicity, multiple sclerosis (MS), and Alzheimer's disease (AD) [3]. Finding the active phytochemicals that inhibit the NO production could be a better alternative for the management of the aforementioned disease conditions.

Nasturtium officinale R. Br. (Brassicaceae) is a hardy perennial herb native to Europe, and commonly called as "Watercress" or "Cresson", which is one of the brassica vegetables, such as broccoli, cabbage, wasabi, and horseradish. The plant has aroused interest because of the presence of sulfur-containing compounds and their important role in protecting human health [4,5]. The characteristics mentioned above are speculated to be due to the some kinds of sulfur compounds deriving from myrosinase-catalyzed hydrolysis of glucosinolates in Brassicaceae family [6]. Glucosinolates could exert neuroprotective activity by regulating inflammatory responses in the central nervous system [7,8]. The extract of *N. officinale* not only possesses a variety of biological activities, including anti-inflammatory, antioxidant, anticancer, hepatoprotective, and anti-hypercholesterolemia and anti-hyperlipidemia effects [9,10]. In our continuing efforts to study the bioactive constituents from Brassicaceae plants [11,12], we investigated MeOH extract of *N. officinale*, which was resulted in the isolation and characterization of three new bis-thioglycosides (**1–3**) along with 13 known compounds (**4–16**). The structures of the new bis-thioglycosides (**1–3**) were elucidated by conventional NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC), HRESIMS and a chemical method. All the compounds (**1–16**) were evaluated for their potential inhibitory effects on nitric oxide levels in LPS-induced murine microglia BV-2 cells.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. Infrared (IR) and ultraviolet (UV) spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer and a Shimadzu UV-1601 UV visible spectrophotometer, respectively. NMR spectra were recorded on a Bruker AVANCE III 700 MNR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C) with chemical shifts given in ppm (δ). HRESIMS was acquired on a Waters SYNAPT G2 Q-TOF mass spectrometer. LC/MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C_{18} 100 Å column (100 × 2.1 mm,

* Corresponding author.
E-mail address: krlee@skku.edu (K.R. Lee).

https://doi.org/10.1016/j.bioorg.2019.01.062
Received 18 October 2018; Received in revised form 14 January 2019; Accepted 27 January 2019
Available online 31 January 2019

5 μm; flow rate 0.3 ml/min; Phenomenex, Torrance, CA, USA). The semi-preparative high performance liquid chromatography (HPLC) was conducted utilizing systems of a Gilson 306 pump with a Shodex refractive index detector. Low-pressure liquid chromatography (LPLC) was performed over a LiChroprep Lobar-A Si 60 (240 × 10 mm) column with an FMI QSY-0 pump (ISCO). Open column chromatography was implemented with silica gel 60 (70–230 and 230–400 mesh; Merck, Darmstadt, Germany) and RP-C_{18} silica gel (230–400 mesh, Merck, Germany). Ion exchange resin (Dowex® 50WX8 hydrogen form, Sigma-Aldrich) was used for alkali elimination. Thin layer chromatography (TLC) was carried out with pre-coated silica gel F_{254} plates and reversed-phase (RP)-C_{18} F_{254s} plates. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

2.2. Plant material

N. officinale was collected in Namyangju-si, Gyeonggi-do, Republic of Korea in October 2014, and the plant was identified by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL 1408) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.

2.3. Extraction and isolation

The whole plant (3.7 kg) was extracted three times with 80% aqueous MeOH at room temperature and filtered. The MeOH extract (350 g) was suspended in distilled water (2.4 L) and successively partitioned with hexane, $CHCl_3$, EtOAc, and BuOH, yielding 34, 7, 5, and 30 g of dried organic extracts, respectively. The BuOH soluble layer was chromatographed over a normal-phase silica gel column chromatography eluting with mixtures of $CHCl_3$-MeOH-H_2O (7:1:0.1, 5:1:0.1, 3:1:0.1, and 1:1:0.1) to obtain six fractions (A-F). Fraction B (1.2 g) was applied to an RP-C_{18} silica gel column chromatography eluting with 20% aqueous MeOH to give seven subfractions (B1-B7). Compounds **11** (30 mg) and **12** (5 mg) were purified from the subfraction B4 (78 mg), using semi-preparative reversed-phase silica gel HPLC eluting with an isocratic mixture of 27% aqueous MeOH (flow rate of 2.0 ml/min). The subfraction B5 (111 mg) was further separated into three subfractions (B51-B53) by a Lobar-A Si 60 (240 × 10 mm) column ($CHCl_3$-MeOH-H_2O, 7:1:0.1). The subfraction B52 (68 mg) was purified by semi-preparative reversed-phase silica gel HPLC (28% aqueous MeOH, flow rate of 2.0 ml/min), yielding compound **13** (10 mg). The subfraction B6 (198 mg) was loaded on a Lobar-A Si 60 (240 × 10 mm) column ($CHCl_3$-MeOH-H_2O, 6:1:0.1) to obtain five subfractions (B61-B65). Compound **2** (6 mg) was purified from the subfraction B64 (34 mg) with semi-preparative reversed-phase silica gel HPLC (38% aqueous MeOH, flow rate of 2.0 ml/min). Fraction C (1.4 g) was separated over an RP-C_{18} silica gel column (230–400 mesh) eluting with 25% aqueous MeOH to yield six subfractions (C1-C6). The subfraction C4 (488 mg) was subjected to separation on a silica gel ($CHCl_3$-MeOH-H_2O, 3.5:1:0.1 → 1:1:0.1) to afford five subfractions (C41-C45). The subfraction C42 (35 mg) was purified by semi-preparative reversed-phase silica gel HPLC (47% aqueous MeOH, flow rate of 2.0 ml/min) to acquire compound **14** (13 mg). Compounds **1** (10 mg) and **3** (8 mg) were purified from the subfraction C5 (136 mg) using semi-preparative reversed-phase silica gel HPLC (47% aqueous MeOH, flow rate of 2.0 ml/min). Fraction D (2.6 g) was further divided into eight subfractions (D1-D8) by passage over an RP-C_{18} silica gel column chromatography (230–400 mesh) eluting with 25% aqueous MeOH. Compounds **4** (3 mg), **15** (6 mg), and **16** (5 mg) were purified from the subfraction D2 (51 mg), using semi-preparative reversed-phase silica gel HPLC (24% aqueous MeOH, flow rate of 2.0 ml/min). The $CHCl_3$ soluble layer was subjected to a silica gel open column (hexane-$CHCl_3$-MeOH, 10:2:1 → 1:1:1), which resulted in six fractions (A-F). The fraction C (1.0 g) was isolated using an RP-C_{18} silica gel column chromatography eluting with 50% aqueous MeOH to give eight subfractions (C1-C8). The subfraction C1 (485 mg) was separated on a normal-phase silica gel column chromatography ($CHCl_3$-MeOH, 60:1 → 1:1) to yield five subfractions (C11-C15). Compounds **7** (18 mg) and **8** (6 mg) were purified from the subfraction C11 (36 mg), using semi-preparative reversed-phase silica gel HPLC with 40% aqueous MeOH (flow rate of 2.0 ml/min). Fraction D (1.1 g) was implemented to an RP-C_{18} silica gel column chromatography eluting with 45% aqueous MeOH to obtain seven subfractions (D1-D7). The subfraction D1 (217 mg) was further separated on a silica gel column chromatography ($CHCl_3$-MeOH, 60:1 → 1:1), which resulted in five subfractions (D11-D15). The subfraction D12 (85 mg) was purified by semi-preparative reversed-phase silica gel HPLC with 40% aqueous MeOH (flow rate of 2.0 ml/min), yielding compounds **9** (5 mg), **10** (61 mg), and **11** (8 mg). The EtOAc soluble layer (4.0 g) was separated over a silica gel column chromatography (hexane-EtOAc, 7:1, 5:1, 3:1, and 1:1) to acquire six fractions (A-F). Fraction B (2.1 g) was subjected to an RP-C_{18} silica gel column chromatography eluting with 53% aqueous MeOH to acquire ten sub-fractions (B1-B10). The subfraction B5 (487 mg) was applied to a silica gel column chromatography ($CHCl_3$-MeOH-H_2O, 5:1:0.1 → 1:1:0.1) to afford six subfractions (B51-B56). Compound **5** (5 mg) was isolated from the subfraction B52 (69 mg) by Sephadex LH-20 column chromatography (100% MeOH) and purified with semi-preparative reversed-phase silica gel HPLC with an isocratic solvent system of 25% aqueous acetonitrile (flow rate of 2.0 ml/min).

2.3.1. 6-O-Hydrocinnamoyl-bis(1-deoxy-1-thio-β-D-glucopyranosyl)-1,1′-disulfide (1)

Yellowish gum; $[\alpha]_D^{25}$ −11.8 (*c* = 1.0, MeOH); IR (KBr) ν_{max} 3726, 3336, 2941, 2830, 1553, 1445, 1108, 1022 cm^{-1}; UV (MeOH) λ_{max} (log ε) 212, 337 nm; ^{1}H MNR (700 MHz) data, see Table 1; ^{13}C NMR (175 MHz) data, see Table 2; HRESIMS (positive-ion mode) *m/z*: 545.1119 $[M + Na]^+$ (calcd. for $C_{21}H_{30}O_{11}S_2$, 545.1127).

2.3.2. 3-O-Hydrocinnamoyl-bis(1-deoxy-1-thio-β-D-glucopyranosyl)-1,1′-disulfide (2)

Yellowish gum; $[\alpha]_D^{25}$ −4.2 (*c* = 1.0, MeOH); IR (KBr) ν_{max} 3763, 3370, 2944, 2832, 1707, 1449, 1118, 1025 cm^{-1}; UV (MeOH) λ_{max} (log ε) 214, 341 nm; ^{1}H MNR (700 MHz) data, see Table 1; ^{13}C NMR (175 MHz) data, see Table 2; HRESIMS (positive-ion mode) *m/z*: 545.1133 $[M + Na]^+$ (calcd. for $C_{21}H_{30}O_{11}S_2$, 545.1127).

2.3.3. 2-O-Hydrocinnamoyl-bis(1-deoxy-1-thio-β-D-glucopyranosyl)-1,1′-disulfide (3)

Yellowish gum; $[\alpha]_D^{25}$ −17.8 (*c* = 1.0, MeOH); IR (KBr) ν_{max} 3722, 3368, 2945, 2830, 1390, 1029 cm^{-1}; UV (MeOH) λ_{max} (log ε) 210, 338 nm; ^{1}H MNR (700 MHz) data, see Table 1; ^{13}C NMR (175 MHz) data, see Table 2; HRESIMS (positive-ion mode) *m/z*: 545.1111 $[M + Na]^+$ (calcd. for $C_{21}H_{30}O_{11}S_2$, 545.1127).

2.3.4. β-D-Glucopyranoside-6-O-β-D-glucopyranosyl-1-[3-(4-hydroxy-3,5-dimethoxyphenyl)-2propanate] (15)

Yellowish gum; $[\alpha]_D^{25}$ −1.9 (*c* = 1.0, MeOH); IR (KBr) ν_{max} 3346, 2943, 2831, 1682, 1590, 1419, 1033 cm^{-1}; UV (MeOH) λ_{max} (log ε) 211, 239, 329 nm; ^{1}H MNR (700 MHz) data, see Table 1; ^{13}C NMR (175 MHz) data, see Table 2; ESIMS (positive-ion mode) *m/z*: 549 $[M + H]^+$.

2.4. Alkaline hydrolysis of compounds 1, 2, and 3

Compound **1** (1.0 mg) was treated with 0.1 N KOH in H_2O at room temperature for 1 h. The hydrolysate was passed through an ion exchange column (Dowex® 50WX8 hydrogen form, Sigma-Aldrich) to remove KOH, and partitioned with $CHCl_3$ and H_2O (1 ml, 3 times). **1a** (0.4 mg) and **1b** (0.3 mg) were obtained from the H_2O and $CHCl_3$ layers, respectively, which were identified as bis(1-deoxy-1-thio-β-D-glucopyranosyl)-1,1′-disulfide (**1a**) [13] and hydrocinnamic acid (**1b**)

Table 1
^{1}H (700 MHz) spectral data of **1**, **2**, **3**, and **15** in CD_3OD (δ in ppm).[a]

Position	1	2	3	Position	15
Glc-1	4.31 (d, 9.7)	4.38 (d, 9.6)	4.52 (d, 10.0)	1	
2	3.45 (t, 9.2)	3.63 (t, 9.4)	4.82 (t, 9.6)	2	6.95 (s)
3	3.30 (d, 8.4)	4.90 (t, 9.3)	3.46 (t, 8.9)	3	
4	3.20, (m)	3.38 (t, 9.6)	3.32 (m)	4	
5	3.37 (dd, 6.2, 2.0)	3.30 (dd, 5.8, 2.4)	3.21 (m)	5	
6a	4.32 (dd, 12.0, 2.0)	3.79 (dd, 12.0, 1.8)	3.81 (dd, 12.0, 1.8)	6	6.95 (s)
6d	4.10 (dd, 12.0, 6.2)	3.60 (dd, 12.1, 5.5)	3.63 (dd, 12.0, 5.3)	7	7.75 (d, 15.8)
Glc-1′	4.31 (d, 9.4)	4.33 (d, 9.6)	4.33 (d, 9.6)	8	6.45, (d, 15.8)
2′	3.34 (t, 9.1)	3.33 (t, 9.2)	3.41 (t, 9.2)	9	
3′	3.27 (d, 8.9)	3.27 (t, 8.7)	3.29 (m)	OCH_3-3	3.90 (s)
4′	3.21 (m)	3.19 (m)	3.20 (m)	OCH_3-5	3.90 (s)
5′	3.23 (m)	3.21 (m)	3.21 (m)	Glc-1′	5.57 (d, 7.8)
6′a	3.78 (m)	3.78 (dd, 12.1, 1.8)	3.76 (dd, 12.0, 1.5)	2′	3.45 (m)
6′b	3.59 (dd, 11.9, 5.2)	3.54 (dd, 12.0, 5.9)	3.55 (dd, 12.0, 5.3)	3′	3.36 (d, 7.8)
1″				4′	3.47 (m)
2″	7.13 (m)	7.15 (m)	7.15 (m)	5′	3.61 (m)
3″	7.17 (m)	7.15 (m)	7.15 (m)	6′a	4.19 (dd, 11.4, 1.9)
4″	7.07 (t, 7.2)	7.07 (t, 7.1)	7.07 (t, 7.1)	6′b	3.80 (dd, 11.5, 5.3)
5″	7.17 (m)	7.15 (m)	7.15 (m)	Glc-1″	4.36 (d, 7.8)
6″	7.13 (m)	7.15 (m)	7.15 (m)	2″	3.23 (dd, 9.1, 7.9)
7″	2.85 (t, 7.6)	2.86 (m)	2.85 (m)	3″	3.30 (d, 8.7)
8″	2.59 (t, 7.7)	2.61 (m)	2.60 (m)	4″	3.48 (m)
9″				5″	3.27 (m)
				6″a	3.86 (dd, 11.9, 2.1)
				6″b	3.67 (dd, 12.0, 5.6)

[a] δ is in ppm, and J values are in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

Table 2
^{13}C (175 MHz) spectral data of **1**, **2**, **3**, and **15** in CD_3OD (δ in ppm).[a]

Position	1	2	3	Position	15
Glc-1	91.7	90.7	89.7	1	126.5
2	73.1	71.4	73.8	2	107.3
3	79.6	80.5	77.4	3	149.7
4	71.4	69.5	71.1	4	140.4
5	79.7	82.4	82.6	5	149.7
6	65.0	62.7	62.8	6	107.3
Glc-1′	91.3	92.4	91.8	7	148.8
2′	73.4	73.6	73.3	8	115.3
3′	79.5	79.7	79.7	9	167.8
4′	71.3	71.4	71.5	OCH_3-3	57.0
5′	82.5	82.8	82.6	OCH_3-5	57.0
6′	63.0	63.1	63.1	Glc-1′	96.0
1″	142.1	142.4	142.1	2′	74.2
2″	129.6	129.5	129.5	3′	78.0
3″	129.7	129.6	129.7	4′	71.7
4″	127.4	127.3	127.4	5′	78.2
5″	129.7	129.6	129.7	6′	69.7
6″	129.6	129.5	129.5	Glc-1″	104.7
7″	32.1	32.0	31.9	2″	75.3
8″	37.0	37.2	37.1	3″	78.0
9″	174.7	174.6	173.7	4″	71.2
				5″	78.1
				6″	62.8

[a] δ is in ppm, and J values are in parentheses. The assignments were based on ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.

[14] by ^{1}H NMR and MS data. Compounds **2** and **3** were also hydrolyzed by using aforementioned method and identified by MS data and co-TLC ($CHCl_3$-MeOH-H_2O, 2:1:0.1) with **1a** and **1b**.

2.5. Measurement of nitric oxide production and cell viability in LPS-stimulated BV-2 cells

Murine microglia BV-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). After 24 h, the BV-2 cells were pretreated with compounds (**1**–**16**) for 30 min and stimulated with 100 ng/mL LPS for another 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media by the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). The absorbance at 540 nm was measured after 10 min using a microplate reader. Cell viability was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. N^G-monomethyl-L-arginine (L-NMMA, Sigma, St, Louis, MO, USA) that is a well-known nitric oxide synthesis (NOS) inhibitor was tested as a positive control [15].

3. Results and discussion

3.1. Structure elucidation of isolated compounds

Compound **1** was obtained as a yellowish gum. The molecular formula of **1** was determined as $C_{21}H_{30}O_{11}S_2$ based on the positive ion peak at *m/z*: 545.1119 $[M + Na]^+$ in the HRESIMS spectra (calcd. for $C_{21}H_{30}O_{11}NaS_2$, 545.1127). The ^{1}H and ^{13}C spectra of **1** displayed the presence of hydrocinnamic acid moiety [14] [δ_H 7.17 (2H, m, H-3″ and H-5″), 7.13 (2H, m, H-2″ and H-6″), 7.07 (1H, t, J = 7.2 Hz, H-4″), 2.85 (2H, t, J = 7.6, H-7″), and 2.59 (2H, t, J = 7.7, H-8″); δ_C 174.7 (C-9″), 142.1 (C-1″), 129.6 (C-2″ and C-6″), 129.7 (C-3″ and C-5″), 127.4 (C-4″), 37.0 (C-7″), and 32.1(C-8″)], and two sugars [δ_H 4.31 (1H, d, J = 9.7 Hz, H-1), 3.45 (1H, t, J = 9.2 Hz, H-2), 3.30 (1H, d, J = 8.4 Hz, H-3), 3.20 (1H, m, H-4), 3.37 (1H, dd, J = 6.2, 2.0 Hz, H-5), 4.32 (1H, dd, J = 12.0, 2.0 Hz, H-6a), 4.10 (1H, dd, J = 12.0, 6.2 Hz, H-6b), 4.31 (1H, d, J = 9.4 Hz, H-1′), 3.34 (1H, t, J = 9.1 Hz, H-2′), 3.27 (1H, d, J = 8.9 Hz, H-3′), 3.21 (1H, m, H-4′), 3.23 (1H, m, H-5′), 3.78 (1H, m, H-6′a), and 3.59 (1H, dd, J = 11.9, 5.2 Hz, H-6′b); δ_C 91.67 (C-1), 73.08 (C-2), 79.58 (C-3), 71.43 (C-4), 79.72 (C-5), 65.02 (C-6), 91.31 (C-1′), 73.43 (C-2′), 79.51 (C-3′), 71.25 (C-4′), 82.52 (C-5′), and 62.99 (C-6′)]. The large coupling constants [9.7 ($^3J_{H\text{-}1/H\text{-}2}$) and 9.4 ($^3J_{H\text{-}1'/H\text{-}2'}$) Hz] of anomeric proton signals of two sugars implied that **1** was to be thioglycoside and β-form [11]. Two sugars were to be connected through a disulfide bond on the basis of the anomeric carbon chemical shifts ($\delta_{C\text{-}1}$ 91.7 and $\delta_{C\text{-}1'}$ 91.3) [16]. Thus, **1** was suggested as a bis-thioglycoside containing the hydrocinnamic acid. The position of hydrocinnamic acid in sugar moiety was identified to be at C-6 based on the HMBC cross-

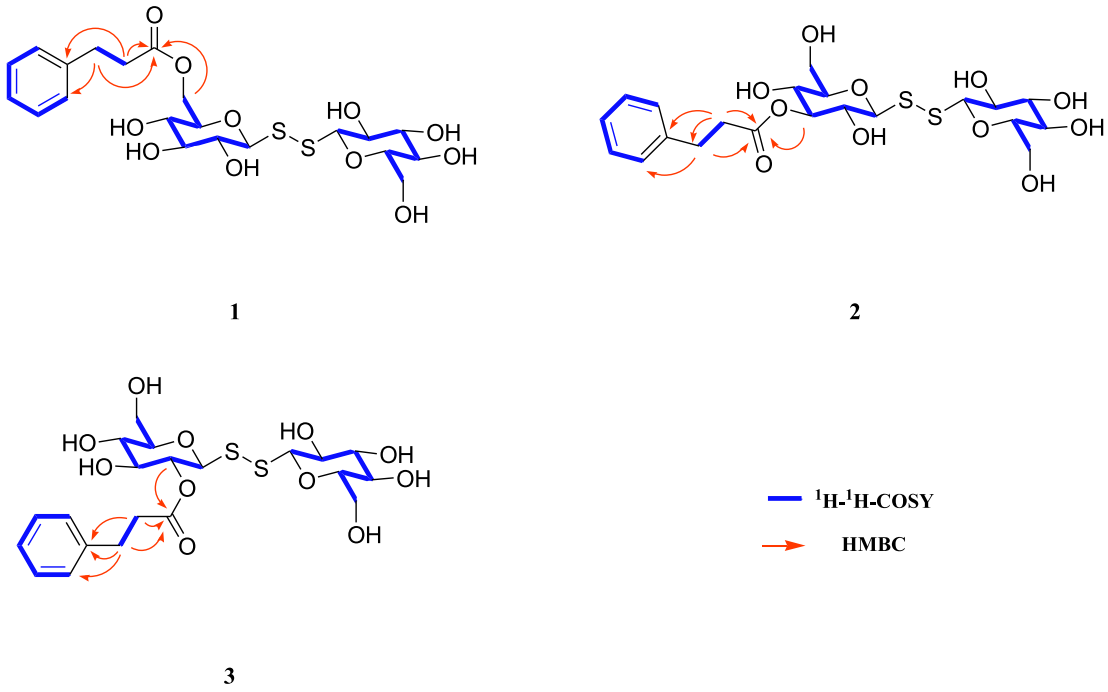

Fig. 1. COSY (*bold line*) and HMBC (*arrow*) correlation of compounds **1**, **2**, and **3**.

Table 3
Inhibitory effects of compounds **1–16** on NO production in LPS-activated BV-2 cells.

Compound	IC_{50} (μM)[a]	Cell viability (%)[b]	
		25 μM	50 μM
1	64.89	97.09 ± 2.13	91.01 ± 6.72
2	244.34	89.80 ± 1.31	88.89 ± 8.15
3	92.88	85.02 ± 3.20	83.44 ± 2.76
4	46.70	100.51 ± 6.82	103.92 ± 7.72
5	21.30	87.24 ± 0.90	78.32 ± 0.48
6	126.50	90.79 ± 7.09	78.72 ± 3.09
7	98.43	99.64 ± 2.16	93.93 ± 6.97
8	> 500	89.32 ± 2.64	79.30 ± 3.9
9	205.63	83.32 ± 8.50	80.51 ± 2.05
10	82.19	87.56 ± 7.46	89.93 ± 5.74
11	233.12	81.62 ± 6.82	73.36 ± 4.77
12	> 500	81.43 ± 3.20	87.71 ± 5.37
13	146	86.42 ± 3.02	74.02 ± 0.48
14	62.99	80.70 ± 5.07	73.91 ± 0.55
15	39.20	99.22 ± 0.74	84.60 ± 4.70
16	> 500	91.59 ± 5.63	86.50 ± 8.43
L-NMMA	30.06	95.47 ± 1.49	95.47 ± 1.49

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.

[b] Cell viability after treatment with 25 and 50 μM of each compound was determined by MTT assay and is expressed in percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD.

peak between H-6 [δ_H 4.32 (1H, dd, J = 12.0, 2.0 Hz, H-6a) and 4.10 (1H, dd, J = 12.0 Hz, 6.2, H-6b)] to C-9″ [δ_C 174.7] (Fig. 1). Thus, the structure of **1** was inferred as 6-*O*-hydrocinnamoyl-bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide. Alkaline hydrolysis of **1** afforded both bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide (**1a**) [13] and hydrocinnamic acid (**1b**) [14], which were verified by their ^{1}H NMR and MS data. Therefore, the structure of **1** was determined as 6-*O*-hydrocinnamoyl-bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide (see Table 3).

Compound **2** was purified as a yellowish gum and identified the same molecular formula, $C_{21}H_{30}O_{11}S_2$, as **1** based on the positive ion peak at *m/z*: 545.1133 $[M + Na]^+$ in the HRESIMS spectra (calcd. for $C_{21}H_{30}O_{11}NaS_2$, 545.1127). The ^{1}H and ^{13}C NMR spectroscopic data of **2** were very similar to **1** with the main differences in signals of downfield shifted proton at H-3 (δ_H 4.90, **2**; δ_H 3.30, **1**) and up-field shifted carbon at C-6 (δ_C 62.7, **2**; δ_C 65.0, **1**), suggesting that hydrocinnamic acid (**1b**) was to be attached in the other position of **1a**. The HMBC correlation of H-3 [δ_H 4.90 (1H, t, J = 9.3 Hz, H-3)] with C-9″ [δ_C 174.6] assured that the hydrocinnamic acid (**1b**) was located at C-3 of **1a** (Fig. 1). Thus, the structure of **2** was determined as 3-*O*-hydrocinnamoyl-bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide.

Compound **3** was obtained as a yellowish gum. The molecular formula of **3** was also exhibited the same molecular formula, $C_{21}H_{30}O_{11}S_2$, as **1** and identified from the positive ion peak at *m/z*: 545.1111 $[M + Na]^+$ in the HRESIMS spectra (calcd. for $C_{21}H_{30}O_{11}NaS_2$, 545.1127). Inspection of the ^{1}H and ^{13}C NMR spectra of **3** was suggestive of those of **1**, except for the signals of H-2 (δ_H 4.82, **3**; δ_H 3.30, **1**) and C-6 (δ_C 62.8, **3**; δ_C 65.0, **1**), which indicated that **3** is also a positional isomer of **1**. Analysis of the HMBC correlation of H-2 [δ_H 4.82 (1H, t, J = 9.6 Hz, H-2)] with C-9″ [δ_C 173.7] confirmed that the hydrocinnamic acid (**1b**) to be connected at C-2 of **1a** (Fig. 1). Hence, the structure of **3** was determined as 2-*O*-hydrocinnamoyl-bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide.

The thirteen reported compounds (**4–16**) were confirmed by comparison of their recorded spectroscopic data as bis(1-deoxy-1-thio-*β*-D-glucopyranosyl)-1,1′-disulfide (**4**) [13], indole-3-acetonitrile-4-methoxy-2-*S*-*β*-D-glucopyranoside (**5**) [17], 8-(methylsulfonyl)octanonitrile (**6**) [18], 9-(methylsulfonyl)nonanenitrile (**7**) [18], 7-(methylsulfinyl)heptanenitrile (**8**) [18], 8-(methylsulfinyl)heptanenitrile (**9**) [18], 9-(methylsulfinyl)heptanenitrile (**10**) [18], syringin (**11**) [19], sinapic aldehyde 4-*O*-*β*-D-glucopyranoside (**12**) [20], 1-sinapoyl-*β*-D-glucopyranoside (**13**) [21], 1,2-di-*O*-*E*-sinapoyl-*β*-gentiobiose (**14**) [22], *β*-D-glucopyranoside-6-*O*-*β*-D-glucopyranosyl-1-[3-(4-hydroxy-3,5-dimethoxyphenyl)-2propanate] (**15**) [23], and lycibarbarphenylpropanoid C (**16**) [24] (Fig. 2). The full assignments of ^{1}H and ^{13}C NMR data of **15** are first reported in this study (Tables 1 and 2).

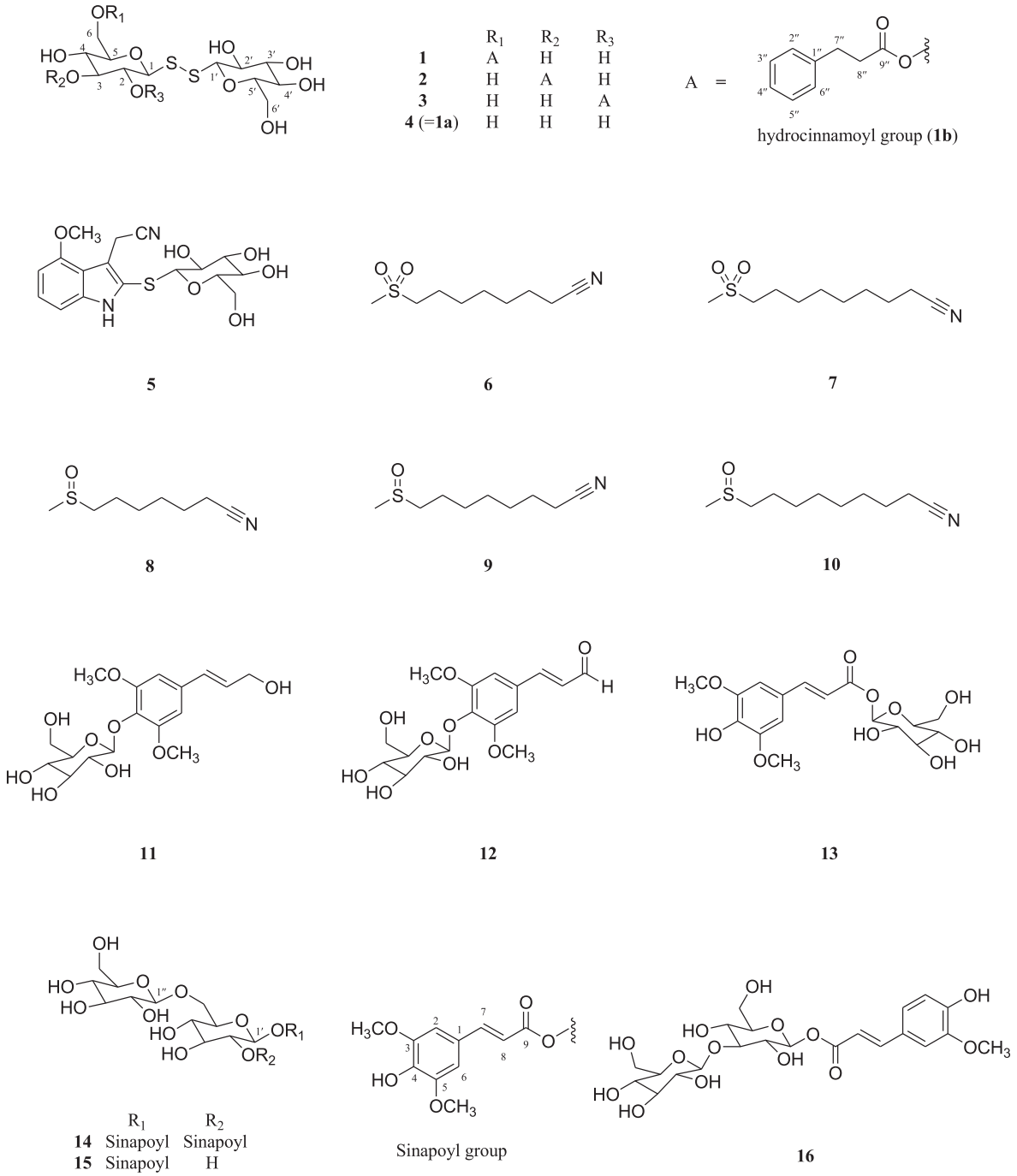

Fig. 2. Structures of compounds **1–16**.

3.2. Anti-inflammatory activities of the isolated compounds

In this study, we evaluted the role of the isolated compounds (**1–16**) in inhibiting the NO production against LPS-activated murine microglia BV-2 cells. Among the isolates, compound **5** significantly inhibited the production of NO in LPS-induced BV-2 cells with an IC_{50} value of 21.30 μM, compared with positive control, L-NMMA (IC_{50} 30.06 μM). Additionally, compounds **4** and **15** showed moderate activities with IC_{50} value of 46.70 and 39.20 μM, respectively. These results make a suggestion that compound **5** exhibits better potency than the well-known iNOS inhibitor, L-NMMA.

4. Conclusion

In the present study, three new bis-thioglycosides (**1–3**), together with thirteen known compounds (**4–16**) were isolated from *N. officinale*. Moreover, we evaluated the anti-inflammatory activity of the compounds (**1–16**). Anti-inflammatory assays of the compounds (**1–16**) revealed that the compound **5** exhibited significant inhibition of NO production in LPS-activated microglia BV-2 cells with an IC_{50} value of 21.30 μM, and compounds **4** and **15** showed moderate anti-inflammatory activity by inhibiting the NO levels in the cells (IC_{50} value of 46.70 and 39.20 μM, respectively). These results are in accordance with the previous studies which glucosinolates and sulfur-containing compounds were associated with neuroprotective activity, and this study showed that bis-thioglycosides are an important role for the

neuroprotective and anti-neuroinflammatory effects of *N. officinale*.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (2012R1A5A2A28671860). We are thankful to the Korea Basic Science Institute (KBSI) for the measurements of MS spectra.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.062.

References

[1] J.E. Yuste, E. Tarragon, C.M. Campuzano, F. Ros-Bernal, Front. Cell. Neurosci. 9 (2015) 322–334.
[2] D.A. Drechsel, A.G. Estevez, L. Barbeito, J.S. Beckman, Neurotox Res. 22 (2012) 251–264.
[3] J.R. Steinert, T. Chernova, I.D. Forsythe, Neuroscientist 16 (2010) 435–452.
[4] P. Rose, Y.K. Won, C.N. Ong, M. Whiteman, Nitric Oxide 12 (2005) 237–243.
[5] H. Sadeghi, M. Mostafazadeh, H. Sadeghi, M. Naderian, M.J. Barmak, M.S. Talebianpoor, F. Mehraban, Pharm. Biol. 52 (2014) 169–174.
[6] B. Holst, G. Williamson, Nat. Prod. Rep. 21 (2004) 425–447.
[7] V. Foti Cuzzola, M. Galuppo, R. Iori, G.R. De Nicola, G. Cassata, S. Giacoppo, P. Bramanti, E. Mazzon, Life Sci. 93 (2013) 288–305.
[8] S. Giacoppo, M. Galuppo, R. Iori, G.R. De Nicola, G. Cassata, P. Bramanti, E. Mazzon, CNS Neurosci. Ther. 19 (2013) 577–584.
[9] L.A. Boyd, M.J. McCann, Y. Hashim, R.N. Bennett, C.I. Gill, I.R. Rowland, Nutr. Cancer 55 (2006) 232–241.
[10] A.R. Ebadollahi Natanzi, M.H. Ghahremani, H.R. Monsef Esfehani, M.B. Minaei, H. Nazarian, O. Sabzevari, Int. J. Pharmacol. 6 (2010) 896–902.
[11] K.H. Kim, E. Moon, S.Y. Kim, S.U. Choi, J.H. Lee, K.R. Lee, J. Ethnopharmacol. 151 (2014) 503–508.
[12] C.S. Kim, L. Subedi, O.W. Kwon, H.B. Park, S.Y. Kim, S.U. Choi, K.R. Lee, J. Nat. Prod. 79 (2016) 2652–2657.
[13] M. Adinolfi, D. Capasso, S. Di Gaetano, A. Iadonisi, L. Leone, A. Pastore, Org. Biomol. Chem. 9 (2011) 6278–6283.
[14] A. Schmidt, C.B. Stark, Org. Lett. 13 (2011) 4164–4167.
[15] D.W. Reif, S.A. McCreedy, Arch. Biochem. Biophys. 1 (1995) 170–176.
[16] S.J. Kim, S. Jin, G. Ishii, Biosci. Biotechnol. Biochem. 68 (2004) 2444–2450.
[17] L. Yang, G. Wang, M. Wang, H. Jiang, L. Chen, F. Zhao, F. Qiu, Fitoterapia 95 (2014) 175–181.
[18] G. Spencer, M. Daxenbichler, J. Sci. Food Agric. 31 (1980) 359–367.
[19] E.J. Yang, S.I. Kim, H.Y. Ku, D.S. Lee, J.W. Lee, Y.S. Kim, Y.H. Seong, K.S. Song, Arch. Pharm. Res. 33 (2010) 531–538.
[20] Y. Ida, Y. Satoh, M. Ohtsuka, M. Nagasao, J. Shoji, Phytochemistry 35 (1994) 209–215.
[21] Y. Miyake, M. Mochizuki, M. Okada, M. Hiramitsu, Y. Morimitsu, T. Osawa, Biosci. Biotechnol. Biochem. 71 (2007) 1911–1919.
[22] A. Baumert, C. Milkowski, J. Schmidt, M. Nimtz, V. Wray, D. Strack, Phytochemistry 66 (2005) 1334–1345.
[23] F. Ferreres, F. Fernandes, C. Sousa, P. Valentao, J.A. Pereira, P.B. Andrade, J. Agric. Food Chem. 57 (2009) 8884–8892.
[24] Z.Q. Zhou, J. Xiao, H.X. Fan, Y. Yu, R.R. He, X.L. Feng, H. Kurihara, K.F. So, X.S. Yao, H. Gao, Food Chem. 214 (2017) 644–654.