JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
2023, VOL. 25, NO. 11, 1076–1084
https://doi.org/10.1080/10286020.2023.2254702

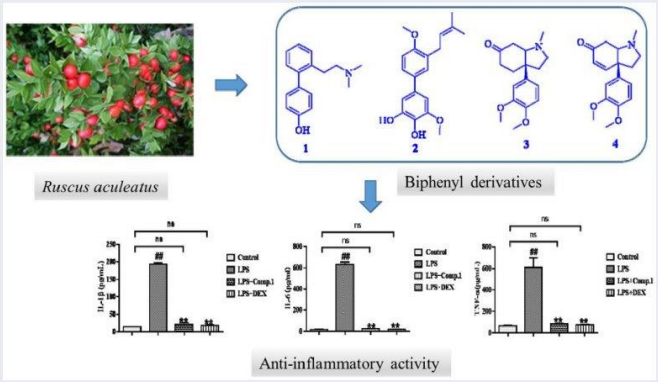
Aculebiphenyl A–B, new biphenyl derivatives from *Ruscus aculeatus*

Sheng-Li Niu[a], Jia-Hui Hao[a], Jing-Yi Xu[a], Qi Guan[a], Zhen-Chi Zhou[a], Tian-Meng Lv[a] and Ya-Ting Sun[b]

[a]Key Laboratory of Livestock Infectious Diseases, Ministry of Education, Key Laboratory of Ruminant Infectious Disease Prevention and Control (East), Ministry of Agriculture and Rural Affairs, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China; [b]School of Pharmacy, Shenyang Medical College, Shenyang 110034, China

ABSTRACT

The investigation of chemical constituents from the rhizomes of *Ruscus aculeatus* resulted in the isolation of two new biphenyl derivatives, aculebiphenyls A and B (**1-2**), together with two known analogs (**3-4**). Their chemical structures were elucidated based on extensive spectroscopic interpretation and HR-ESI-MS analysis. Compounds **3-4** were isolated from the *Ruscus* genus for the first time. The isolated compounds were tested for anti-inflammatory activities and antibacterial activities. Compound **1** exhibited significant inhibitory effects on LPS-induced NO production and COX-2 with IC_{50} values of 10.8 μM and 0.4 μM. Compound **1** also significantly down-regulated the levels of inflammatory cytokines IL-1β, IL-6, and TNF-α. Compound **1** showed moderate antibacterial activities.



ARTICLE HISTORY
Received 17 April 2023
Accepted 29 August 2023

KEYWORDS
Liliaceae; *Ruscus aculeatus*; biphenyl; anti-inflammatory activity; antibacterial activity

CONTACT Ya-Ting Sun sunyatingna@163.com School of Pharmacy, Shenyang Medical College, Shenyang 110034, China.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10286020.2023.2254702.

1. Introduction

Ruscus genus, belonging to the Liliaceae family, is native to the Mediterranean, Southern, and Western Europe, and is represented by perennial, rhizomatous, and evergreen shrubs [1]. Among approximately seven species spread throughout Europe up to Iran, *Ruscus aculeatus* Linn, locally referred to as "*Jia-Ye-Shu*" in Chinese is the most widely distributed and appreciated. As an ancient phlebo therapeutic agent, *R. aculeatus* is the most studied species. During the middle ages, the young shoots of *R. aculeatus* were used not only as food but also as medicinal agents for the treatment of urinary disorders and abdominal pain [2]. The hydroalcoholic extract of *R. aculeatus* rhizomes is traditionally used as a vascular preventive and tonic for venous system disorders, including venous fragility and varicose veins [3]. The underground parts of *R. aculeatus* have been also used as diuretic and anti-inflammatory agents for the treatment of hemorrhoids and atherosclerosis [4]. The most studied phytochemical investigations of this plant are steroidal saponins [5–7], while other constituents have been also isolated, including triterpenes, flavonoids, coumarins, sparteine, tyramine, and glycolic acid [8]. In order to exploit and utilize the compound resources of *R. aculeatus*, to provide a theoretical basis for exploring the chemical composition and biosynthetic pathways of the *Ruscus* genus, and to search for inflammatory inhibitors, we launched a systematic study to investigate the chemical constituents of *R. aculeatus*. Thus, we reported the isolation, structure elucidation, anti-inflammatory activities, and antibacterial activities of the biphenyl derivatives isolated from *R. aculeatus*. The structures of compounds **1–4** are shown in Figure 1.

2. Results and discussion

Compound **1** was obtained as a white needle crystal (MeOH). Its molecular formula $C_{16}H_{19}NO$ was determined by HR-ESI-MS at *m/z* 242.1527 $[M+H]^+$. Its UV spectrum showed absorption maxima at 212 and 245 nm. The IR absorption band at 3378 cm^{-1} revealed the presence of hydroxyl. The ^1H NMR spectrum (Table 1) showed characteristic proton signals for 1,2-disubstituted benzene at δ 7.36 (1H, dd, $J=7.2$, 1.8 Hz, H-3), 7.33 (1H, td, $J=7.2$, 1.8 Hz, H-4), 7.30 (1H, td, $J=7.2$, 1.8 Hz, H-5), 7.19 (1H, dd, $J=7.2$, 1.8 Hz, H-6), proton signals for 1,4-disubstituted benzene

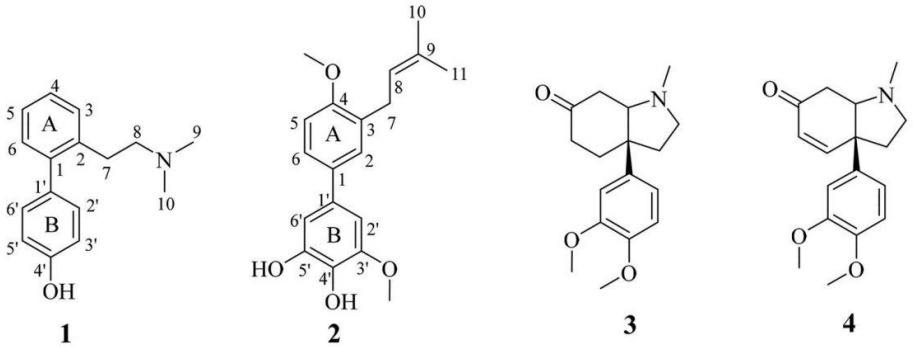


Figure 1. Structures of compounds 1 — 4 isolated from *Ruscus aculeatus*.

Table 1. The ^{1}H NMR and ^{13}C NMR spectral data of compounds **1** and **2**.

	1[a]		**2**[b]	
No.	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	141.8	—	133.7	—
2	133.9	—	128.0	7.31, 1H, (brs)
3	129.6	7.36, 1H, (dd, 7.2, 1.8)	130.4	—
4	127.3	7.33, 1H, (td, 7.2, 1.8)	156.7	—
5	127.0	7.30, 1H, (td, 7.2, 1.8)	110.5	6.89, 1H, (d, 8.4)
6	130.3	7.19, 1H, (dd, 7.2, 1.8)	133.7	7.33, 1H, (dd, 8.4, 1.9)
7	27.2	2.92, 2H, (m)	28.6	3.37, 2H, (d, 7.0)
8	57.1	3.16, 2H, (m)	144.0	5.35, 1H, (t, 7.0)
9	42.1	2.68, 3H, (s)	132.6	
10	42.1	2.68, 3H, (s)	25.8	1.75, 3H, (s)
11			17.8	1.74, 3H, (s)
1′	131.0	—	131.5	—
2′	130.0	7.14, 1H, (d, 8.4)	102.1	6.65, 1H, (brs)
3′	115.2	6.85, 1H, (d, 8.4)	147.0	—
4′	156.7	—	133.5	—
5′	115.2	6.85, 1H, (d, 8.4)	144.0	—
6′	130.0	7.14, 1H, (d, 8.4)	107.4	6.81, 1H, (brs)
4-OCH_3			55.5	3.87, 3H, (s)
3′-OCH_3			56.2	3.94, 3H, (s)

[a]recorded in DMSO-d_6, ^{1}H NMR, 600 MHz and ^{13}C NMR 150 MHz.
[b]recorded in $CDCl_3$, ^{1}H NMR, 400 MHz and ^{13}C NMR 100 MHz.

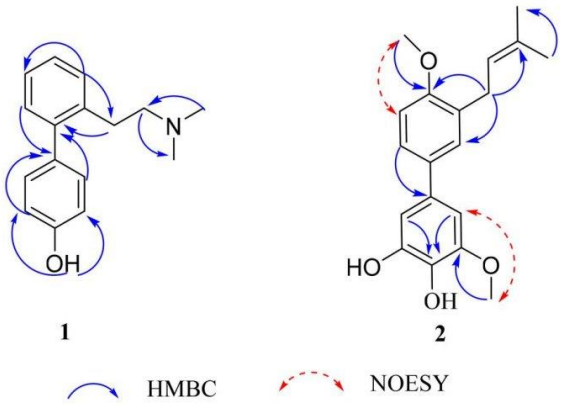


Figure 2. Key HMBC and NOESY correlations of compounds **1** and **2**.

at δ 7.14 (2H, d, $J = 8.4$ Hz, H-2′, 6′) and 6.85 (2H, d, $J = 8.4$ Hz, H-3′, 5′), two methyl proton signals at δ 2.68 (6H, s, CH_3-9, 10), and two methylene proton signals at δ 3.16 (2H, m, H-8) and 2.92 (2H, m, H-7). The ^{13}C NMR spectrum (Table 1) displayed sixteen carbon signals categorized as twelve aromatic carbons, two identical methyl carbons at δ 42.1 (C-9, 10), and two methylene carbons at δ 57.1 (C-8) and 27.2 (C-7). The ^{1}H and ^{13}C NMR spectra were similar to those of aminoethyl biphenyls, indicating the presence of two connected benzene rings, two methylene groups, and a N,N-dimethyl fragment [9]. The HSQC spectrum permitted the assignment of all the protons to their bonding carbons. The HMBC correlations (Figure 2) from H-7 (δ 2.92) to C-1 (δ 141.8), C-2 (δ 133.9), and C-3 (δ 129.6) indicated that the methylene of C-7 was linked at C-2 of the benzene ring A. The HMBC correlations from CH_3-9 (δ 2.68) and CH_3-10 (δ 2.68) to C-8 (δ 57.1) indicated that the

N,N-dimethyl fragment was linked at C-8. Therefore, the structure of compound **1** was deduced (Figure 1) and named as aculebiphenyl A.

Aculebiphenyl B (**2**) was obtained as a colorless crystal (MeOH), and this structure exhibited a protonated molecular ion peak at *m/z* 315.1579 $[M+H]^+$ in HR-ESI-MS, corresponding to the molecular formula $C_{19}H_{22}O_4$, which indicated 9 degrees of unsaturation. The UV spectrum of compound **2** was similar to that of compound **1**. The ^1H and ^{13}C NMR spectral data (Table 1) indicated that compound **2** had the same biphenyl skeleton as **1**, except for the absence of aminoethyl moiety, which was replaced by an isoprenyl substituent. The ^1H NMR spectrum (Table 1) showed the signals of a set of ABX-coupled aromatic protons at δ 6.89 (1H, d, $J = 8.4$ Hz, H-5), 7.33 (1H, dd, $J = 8.4$, 1.9 Hz, H-6), and 7.31 (1H, brs, H-2), a pair of *meta*-coupled aromatic protons at δ 6.65 (1H, brs, H-2′) and 6.81 (1H, brs, H-6′), and two methoxy groups at δ 3.87 (3H, s, OCH_3-4) and 3.94 (3H, s, OCH_3-3′). In addition, isoprenyl moiety 3-methyl-but-2-en-1-yl [δ 3.37 (2H, d, $J = 7.0$ Hz, H-7), 5.35 (1H, t, $J = 7.0$ Hz, H-8), 1.75 and 1.74 (each 3H, s, H-10, 11)] was observed. The ^{13}C NMR spectrum (Table 1) exhibited nineteen carbon signals identified as twelve aromatic carbon signals, including four oxygenated aromatic carbons at δ 156.7 (C-4), 147.0 (C-3′), 144.0 (C-5′), and 133.5 (C-4′), an isoprenyl moiety at δ 144.0 (C-8), 132.6 (C-9), 28.6 (C-7), 25.8 (C-10), and 17.8 (C-11), and two methoxy carbons at δ 56.2 (3′-OCH_3) and 55.5 (4-OCH_3).

Moreover, the isoprenyl moiety was located at C-3 of benzene ring A of biphenyl by HMBC correlations (Figure 2) of H-7 (δ 3.37) with C-2 (δ 128.0), C-3 (δ 130.4), and C-4 (δ 156.7), and H-8 (δ 5.35) with C-3 (δ 130.4). Furthermore, the HMBC correlations of methoxy group protons at δ 3.87 with C-4 (δ156.7) indicated that the methoxy group was located at C-4 of benzene ring A, adjacent to isoprenyl moiety. Additionally, the HMBC correlations of another methoxy group protons at δ 3.94 with C-3′ (δ147.0) suggested that the methoxy group was linked at C-3′ of benzene ring B of biphenyl. Moreover, the NOESY correlations (Figure 2) of 4-OCH_3 with H-5 and 3′-OCH_3 with H-2′ confirmed the positions of the methoxy groups. The structure of compound **2** was determined (Figure 1) and named as aculebiphenyl B.

By comparing the physico-chemical and spectroscopic properties with those reported in literature, two known compounds were elucidated mesembrine (**3**) [10] and mesembrenone (**4**) [11]. Compounds **3** and **4** were isolated from the *Ruscus* genus for the first time.

Considering the traditional use of *R. aculeatus* and a goal to obtain NO and COX-2 inhibitors for inflammatory disorders, all isolated compounds (**1**–**4**) were tested individually for NO production inhibition in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages and COX-2 inhibitory effects. The isolated compounds **1**–**4** showed remarkable NO inhibitory effects with IC_{50} values of 10.8 ± 0.1 μM, 41.2 ± 0.6 μM, 28.9 ± 0.4 μM, and 30.5 ± 1.3 μM. Compared with the positive control of aminoguanidine hydrochloride (IC_{50} = 25.8 μM), compound **1** exhibited more potent inhibitory activity against NO production. Compound **1** inhibited COX-2 activity in a dose-dependent manner in 0.031–0.5 μM concentration (Figure S1). The IC_{50} value of the inhibitory effect of compound **1** against COX-2 was 0.4 μM (Table S1, Figure S1). Using dexamethasone (DEX) as the positive control (1.0 μM), we tested the effects of

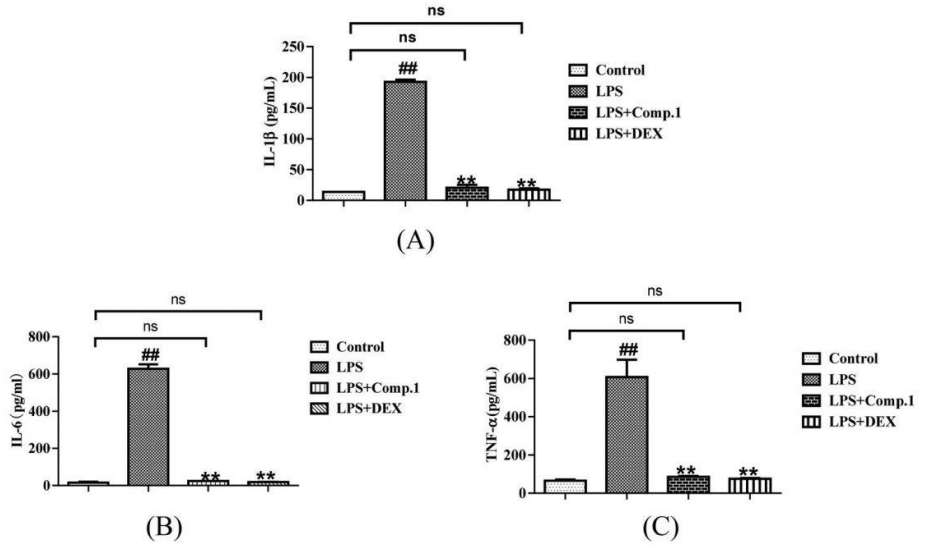


Figure 3. Results of the content of IL-1β (A), IL-6 (B), and TNF-α (C) in the supernatant of LPS-induced RAW 264.7 cells (mean ± SD, $n = 5$). Note: ## represents a significant difference from the blank control group ($P < 0.01$), ** Represents a significant difference from the model control group ($P < 0.01$), DEX: dexamethasone.

compound **1** on the concentration of inflammatory cytokines in RAW 264.7 cells. The results showed that compound **1** remarkably downregulated the levels of the inflammatory cytokines IL-1β, IL-6, and TNF-α at 2.0 μM (Table S2, Figure 3).

The antibacterial activities of four compounds isolated from *R. aculeatus* were evaluated by determining the minimum inhibitory concentration (MIC). The results showed that compound **1** had moderate antibacterial activity against the four standard strains, and MIC values against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, and *Escherichia coli* were 0.2, 0.4, 0.4, and 0.4 mg/ml, respectively. Compared with *S. typhimurium* and *E. coli*, the isolated compounds showed better antibacterial activity against *S. aureus* and *S. pneumoniae*, indicating that these compounds may have a better antibacterial effect against Gram-positive bacteria (Table S3).

In this study, two new biphenyl derivatives were isolated, which were rarely reported from *R. aculeatus*. Additionally, these derivatives enriched the structural resources of the *Ruscus* genus. The biological evaluation of the isolated biphenyl derivatives indicated that compound **1** has shown great application potential as a lead compound from natural sources in the research and development of anti-inflammatory drugs.

3. Experimental

3.1. General experimental procedures

UV-1700 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) was used to record UV spectra. The FT-IR Spectra of samples in KBr discs were recorded on a

Bruker Tensor-27 spectrometer (Bruker, Karlsruhe, Germany) with KBr pellets. Waters AutoSpec Premier P776 spectrometer (Waters Corp., Massachusetts, USA) was used to acquire HR-ESI-MS data. NMR (^{1}H NMR, ^{13}C NMR, HSQC, and HMBC) spectra were obtained on a Bruker-AV-600 NMR spectrometer (Bruker Corporation, Bremen, Germany). Qingdao Ocean Chemical Factory provided Silica gel (Qingdao, China). GE Healthcare offered Sephadex LH-20 (Stockholm, Sweden) and ODS was purchased from YMC Co. Ltd. (Kyoto, Japan). Semi-preparative HPLC was conducted on an Agilent 1260 (Agilent Technologies Inc., California, USA) with a DAD detector equipped with a C-18 column (10 × 250 mm, 5 μm, YMC Co. Ltd., Kyoto, Japan). Molecular Device microplate reader was performed on a SpectraMax plus 384 (MD, San Francisco, USA). COX-2 kit was manufactured by Shanghai Biyuntian Biotechnology Co., LTD (Shanghai, China). The ELISA kits of inflammatory cytokines (IL-1β, IL-6 and TNF-α) were manufactured by Thermo Fisher Scientific Co., LTD (Waltham, MA, USA). The standard strains, *S. aureus* (ATCC29213), *S. pneumoniae* (ATCC49619), *S. typhimurium* (C7731), and *E. coli* (ATCC25922), were purchased from type culture collection of China Veterinary Drug Control Institute (Beijing, China). Gentamicin sulfate was purchased from Dalian Meilun Biotechnology Co., LTD (Dalian, China).

3.2. Plant material

Dried rhizomes of *Ruscus aculeatus* were purchased in June 2016 from Anguo Market of Hebei Province, China. The plant material was authenticated by Prof. Wei Ning (College of Horticulture, Shenyang Agricultural University). The voucher specimen (RA-2016061502) was deposited at the Department of Animal Pharmacy of Shenyang Agricultural University.

3.3. Extraction and isolation

The air-dried powdered rhizomes of *R. aculeatus* (2.5 kg) were extracted three times with 95% EtOH under reflux. The EtOH extract was concentrated *in vacuo* to yield a brown-yellow residue (61 g), which was suspended in water (6 L) and partitioned successively with *n*-hexane (3 × 6 L), ethyl acetate (EtOAc, 3 × 6 L), and n-butanol (BuOH, 3 × 6 L). The EtOAc extract (22 g) was separated by chromatography over a silica gel column using a gradient system of increasing polarity of *n*-hexane – acetone (100:0 – 0:100, v/v). The collected 150 fractions were combined based on their TLC characteristics using UV light to yield ten fractions (Fr. A – Fr. J). Fr. G (8.2 g) was subjected to a silica gel column chromatography and eluted with *n*-hexane – acetone (100:0 – 100:30, v/v) to yield ten fractions (Fr. G1 – Fr. G10). Fr. G3 (1.2 g) was further purified by Sephadex LH-20 column chromatography eluted with MeOH to yield eight fractions (Fr. G3a – Fr. G3h). Fr. G3c (0.9 g) was further separated by an ODS column eluted with a gradient system of decreasing polarity of acetonitrile – water (0.1% trifluoroacetic acid) system (20:80 – 80:20, v/v) to obtain **3** (6.5 mg) and **4** (5.8 mg). Fr. G5 (4.8 g) was subjected to a silica gel column chromatography and eluted with *n*-hexane – acetone (100:0 – 100:30, v/v) to afford Fr. G5a – Fr. G5k. Fr.

G5e (0.8 g) was further purified by HPLC on a semi-preparative YMC C-18 column using acetonitrile – water (0.1% trifluoroacetic acid) system (40:60, v/v) as the mobile phase to provide **1** (8.9 mg, t_R = 25.2 min). Fr.J (7.5 g) was subjected to a silica gel column chromatography and eluted with *n*-hexane – acetone (100:0 – 100:30, v/v) to yield eight fractions (Fr. J1 – Fr. J8). Fr. J6 (4.8 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to yield six fractions (Fr. J6a – Fr. J6f). Fr. J6c (1.1 g) was further purified by HPLC on a semi-preparative YMC C-18 column using acetonitrile – water (0.1% trifluoroacetic acid) system (30:70, v/v) as the mobile phase to yield **2** (6.2 mg, t_R = 18.5 min).

3.3.1. Aculebiphenyl A (1)

White needle crystal; UV (MeOH) λ_{max}: 212 and 245 nm; IR (KBr) ν_{max}: 3378, 2924, 1735, 1655, 1451, 1421, 1246, 1032, 830 cm^{-1}; ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 242.1527 $[M+H]^+$ (calcd for $C_{16}H_{20}NO$, 242.1539).

3.3.2. Aculebiphenyl B (2)

White needle crystal; UV (MeOH) λ_{max}: 213 and 261 nm; ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 315.1579 $[M+H]^+$ (calcd for $C_{19}H_{23}O_4$, 315.1596).

3.4. Anti-inflammatory assays

3.4.1. NO production inhibition assays

The nitrite concentration in the medium was measured as an indicator of NO production in accordance with the Griess reaction [12]. The NO inhibitory effects of **1–4** were tested in LPS-activated RAW 264.7 cells. Then, RAW 264.7 cells were cultured in LPS and phenol red–free RPMI 1640 medium with 10% FBS at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded in 96-well plates at 1×10^5 cells/well and incubated for 24 h. After stimulation with LPS at 1 μg/ml, the compounds were added at different concentrations; then, cells were incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. The level of nitrite formation in supernatants was measured using a Griess reagent by measuring the absorbance at 540 nm. The percent inhibition for nitrite production was determined by comparing it with the vehicle control. IC_{50} values were calculated from dose curves, and aminoguanidine hydrochloride was used as the positive control. Nitrite concentrations and inhibitory rates were calculated by the calibration curve prepared with sodium nitrite standards. Experiments were conducted in triplicate, and data were presented as mean ± SD of three independent experiments.

3.4.2. COX-2 inhibition assays

COX-2 assay buffer, Milli-Q grade water, DMSO, and other appropriate solvents were used to prepare appropriate concentrations of compound **1**. The effect of compound **1** on COX-2 enzyme activity was determined by fluorescence assay. Groups included the blank control group, 100% enzyme active control group, compound **1**

with different concentrations (31.25, 62.5, 125, 250, and 500 nM) groups, and positive control group (50 nM Celecoxib). Corresponding reagents were added to the 96-well plate successively. Referring to the instruction manual of the COX-2 inhibitor screening kit for the other steps, the relative fluorescence unit was measured by using a microplate reader. The inhibition of COX-2 enzyme activity by compound **1** was analyzed by GraphPad Prism 8, and the IC_{50} value was obtained.

3.4.3. Inflammatory cytokine assays

RAW 264.7 cells (5×10^5 cell/ml) were cultured in a 96-well plate under 5% CO_2 at 37 °C for 48 h and were incubated with LPS (1 μg/ml) for 1 h followed by the addition of compound **1** (2 μM) and DEX (1 μM) for 1 h. The cell supernatant was collected, and the concentration of the cytokines IL-1β, IL-6, and TNF-α was determined by ELISA. Experimental data were presented as mean ± SD of five independent experiments.

3.5. Antibacterial activity assays

Broth microdilution [13] was used to determine the antibacterial activities of the four isolated compounds. *S. aureus*, *S. pneumoniae*, *S. typhimurium*, and *E. coli* were cultured in a nutrient broth medium at 37 °C and 150 rpm until $OD_{600} = 0.4–0.6$. Then, the bacterial solution was diluted with an MHB medium at 1×10^5 CFU/ml as the using concentration. Using the continuous twofold dilution method, gentamicin sulfate (control antibiotic) was diluted to 256–0.5 μg/ml concentration gradient with MHB medium. Each compound was prepared as a concentration from 6400 to 12.5 μg/ml in MHB medium. The diluted compounds **1–4** and 100 μl of gentamicin sulfate were added to wells 1–10 of the 96-well plate in descending order of concentration. Then, 100 μl of diluted bacterial solution (1×10^5 CFU/ml) was added to wells 1–10 of each row. At this point, the concentration of compounds in wells 1–10 was 3200, 1600, 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μg/ml, respectively. Next, 100 μl of MHB medium was added in the 11^{th} well for blank control, and 100 μl of diluted bacterial solution was added in the 12^{th} well for control. Two parallel tests were conducted for each compound. The bacterial solution and medicinal solution in each well were mixed, covered, and then incubated at 37 °C for 18–24 h to observe the phenomenon. The MIC of the four compounds against *S. aureus*, *S. pneumoniae*, *S. typhimurium*, and *E. coli* were determined by using this method.

Acknowledgements

The authors are grateful to Mr. Jing-Chao Liu and Mr. Zi-Shuang Liu (Analytical Testing Center, Northeast University, Shenyang, China) for their measurements of the NMR data.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the Science Research Fund Project of Liaoning Provincial Department of Education (Grant No. LJKZ0667 and No. LJKZ1140), and the Scientific Research Foundation of Shenyang Agricultural University (No. 880415026).

References

[1] M. Masullo, C. Pizza, and S. Piacente, *Planta Med.* **82**, 1513 (2016).
[2] G. Balica, O. Vostinaru, M. Tamas, G. Crisan, and C. Mogosan, *J. Food Agric. Environ.* **11**, 106 (2013).
[3] E. Bouskela, F.Z. Cyrino, and G. Marcelon, *J. Cardiovasc. Pharmacol.* **22**, 225 (1993).
[4] G. Ozer, E. Guzelmeric, G. Sezgin, E. Ozyurek, A. Arslan, E. Sezik, and E. Yesilada, *J. Chem. Metrol.* **12**, 79 (2018).
[5] N. Hadžifejzović, J. Kukić-Marković, S. Petrović, M. Soković, J. Glamočlija, D. Stojković, and A. Nahrstedt, *Ind. Crop. Prod.* **49**, 407 (2013).
[6] T. Ivanova, C. Banciu, C. Gussev, Y. Bosseva, D. Dimitrova, T. Stoeva, and A. Manole, *Rom. Biotechnol. Lett.* **24**, 354 (2019).
[7] A. Mari, A. Napolitano, A. Perrone, C. Pizza, and S. Piacente, *Food Chem.* **134**, 461 (2012).
[8] J.P.B. Rodrigues, A. Fernandes, M.I. Dias, C. Pereira, T. C. S.P. Pires, R.C. Calhelha, A.M. Carvalho, I.C.F.R. Ferreira, and L. Barros, *Molecules* **26**, 1882 (2021).
[9] M. Paillet-Loilier, F. Fabis, A. Lepailleur, R. Bureau, S. Butt-Gueulle, F. Dauphin, A. Lesnard, C. Delarue, H. Vaudry, and S. Rault, *Bioorg. Med. Chem. Lett.* **17**, 3018 (2007).
[10] E.A. Ilardi, M.J. Isaacman, Y.C. Qin, S.A. Shelly, and A. Zakarian, *Tetrahedron* **65**, 3261 (2009).
[11] J. Bastida, F. Viladomat, J.M. Llabres, G. Ramirez, C. Codina, and M. Rubiralta, *J. Nat. Prod.* **52**, 478 (1989).
[12] V.M. Dirsch, H. Stuppner, and A.M. Vollmar, *Planta Med.* **64**, 423 (1998).
[13] P. Wangchuk, P.A. Keller, S.G. Pyne, M. Taweechotipatr, A. Tonsomboon, R. Rattanajak, and S. Kamchonwongpaisan, *J. Ethnopharmacol.* **137**, 730 (2011).