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SHORT COMMUNICATION

Novel depsides as potential anti-inflammatory agents with potent inhibitory activity against *Escherichia coli*-induced interleukin-8 production

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Abstract

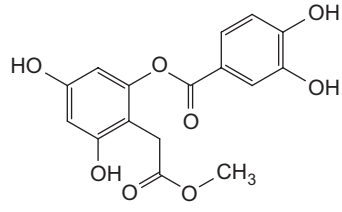
Sixteen novel depsides were synthesized for the first time. Their chemical structures were clearly determined by ^{1}H NMR, ESI mass spectra, and elemental analyses. All the compounds were assayed for antibacterial activities against three Gram-positive bacterial strains (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, and *Streptococcus faecalis* ATCC 9790) and three Gram-negative bacterial strains (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 13525, and *Enterobacter cloacae* ATCC 13047) by the MTT method. Compound 2-(2-methoxy-2-oxoethyl)phenyl 5-bromonicotinate (**5**) exhibited significant antibacterial activities against *E. coli* ATCC 35218 with an MIC of 0.78 μg/mL, which was superior to the positive control kanamycin B. In addition, compound **5** showed potent inhibitory activity against *E. coli*-induced interleukin-8 production.

Keywords: *Depsides; anti-inflammatory;* Escherichia coli*-induced interleukin-8*

Introduction

Depsides are compounds comprising two or more aromatic rings bound by a phenolic oxygen–ester linkage. It is reported that several well-characterized depsides exhibit antiproliferative, antibacterial, analgesic, antipyretic, anticancer, anti-human immunodeficiency virus (HIV)-1 integrase, and antiviral properties[1–9]. In addition, as inhibitors of prostaglandin biosynthesis and leukotriene B4 biosynthesis, depsides are potent nonsteroidal antiinflammatories[10]. Inflammation is a host response to a wide variety of tissue injuries characterized by the recruitment of leukocytes from the blood to the injured tissue. This movement is directed by chemokines, among which interleukin-8 (IL-8) plays an important role[11]. Interleukin-8 (IL-8) is produced by a number of cell types (e.g. T-lymphocytes, monocytes, endothelial cells, epithelial cells, and neutrophils) in response to a variety of stimuli, e.g. lipopolysaccharides (LPS), IL-1, and tumor necrosis factor (TNF)[12,13]. Raised levels of IL-8 have been detected in several disease states[14], and experiments using monoclonal antibodies have indicated that inhibition of the action of this chemokine in some models of disease can lead to beneficial effects[13,15]. Recently, a new depside, named jaboticabin (Figure 1), was demonstrated to inhibit chemokine IL-8 production before and after cigarette smoke treatment of cells[16].

In this study, we have synthesized 16 novel depsides and evaluated their antibacterial activity against six bacterial strains (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 9790, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 13525, and *Enterobacter cloacae* ATCC 13047), and found that compound **5** exhibited significant antibacterial activities against *E. coli* ATCC 35218 with a minimum inhibitory concentration (MIC) of 0.78 μg/mL, which was superior to the positive control kanamycin B. In addition, in view of its significant antibacterial activity, compound **5** was selected



jaboticabin

Figure 1. The structure of jaboticabin.

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to act against the IL-8 production induced by *E. coli* in gastric mucosal cells. Based on the data obtained, we found that compound **5** showed potent inhibitory activity against *E. coli*-induced interleukin-8 production.

Experimental

Chemistry

General

Melting points (uncorrected) were determined on an XT4 MP apparatus (Taike Corp., Beijing, China). Electrospray ionization (ESI) mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker PX500 or DPX300 spectrometer at 25°C with tetramethylsilane (TMS) and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4 % of the theoretical values.

Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM; E. Merck). The quantity of silica gel used was 50–100 times the weight charged on the column. Then, the eluates were monitored using thin layer chromatography (TLC).

General experimental procedure for the synthesis of compounds 3–18

A solution of 2-hydroxyphenylacetic acid (7.6 g, 50 mmol) in methanol or isobutyl alcohol (50 mL) containing concentrated H_2SO_4 (5 mL) was refluxed overnight. Water (100 mL) was added, the organic phases were washed with saturated NaCl (100 mL) and dried over Na_2SO_4, and the solvents were evaporated. This step yielded compound **2**. To a stirred solution of compound **2** (10 mmol) in dichloromethane (50 mL) was added differently substituted nicotinic acid or benzoic acid, *N*,*N*-dimethylaminopyridine (248 mg, 2.03 mmol), and *N*,*N*-dicyclohexylcarbodiimide (2.26 g, 11 mmol). Then, the mixture was refluxed overnight. Flash chromatography (acetate:petroleum ether 1:5 or 1:2) afforded the corresponding depsides (compounds **3–18)** as powder or oil.

*2-(2-Methoxy-2-oxoethyl)phenyl 2-bromonicotinate (**3**)* White oil. Yield 80%. ^{1}H NMR (300 MHz, DMSO-*d6*): 3.52 (s, 3H); 3.71 (s, 2H); 7.27–7.37 (m, 2H); 7.36–7.42 (m, 2H); 7.64–7.72 (m, 1H); 8.47–8.56 (m, 1H); 8.69–8.71 (m, 1H). MS (ESI): 349.9 ($C_{15}H_{13}BrNO_4$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{12}BrNO_4$: C, 51.45; H, 3.45%; Found: C, 51.27; H, 3.58%.

*2-(2-Methoxy-2-oxoethyl)phenyl 2-chloronicotinate (**4**)* White oil. Yield 83%. ^{1}H NMR (300 MHz, DMSO-*d6*): 3.50 (s, 3H); 3.72 (s, 2H); 7.28–7.36 (m, 2H); 7.39–7.42 (m, 2H); 7.66–7.70 (m, 1H); 8.49–8.53 (m, 1H); 8.68–8.70 (m, 1H). MS (ESI): 306.0 ($C_{15}H_{13}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{12}ClNO_4$: C, 58.93; H, 3.96%; Found: C, 58.78; H, 3.87%.

*2-(2-Methoxy-2-oxoethyl)phenyl 5-bromonicotinate (**5**)* White powder. Yield 82%, mp: 67–68°C, ^{1}H NMR (300 MHz, DMSO-*d6*): 3.48 (s, 3H); 3.75 (s, 2H); 7.27–7.44 (m, 4H); 8.60–8.61 (t, *J* = 2.01 Hz, 1H); 9.06–9.07(d, *J* = 2.01 Hz, 1H); 9.19–9.20 (d, *J* = 2.01 Hz, 1H). MS (ESI): 349.1 ($C_{15}H_{13}BrNO_4$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{12}BrNO_4$: C, 51.45; H, 3.45%; Found: C, 51.32; H, 3.61%.

*2-(2-Methoxy-2-oxoethyl)phenyl 5-chloronicotinate (**6**)* White powder. Yield 77%, mp: 67–68°C, ^{1}H NMR (300 MHz, DMSO-*d6*): 3.45 (s, 3H); 3.73 (s, 2H); 7.26–7.42 (m, 4H); 8.61–8.63 (t, *J* = 2.01 Hz, 1H); 9.07–9.08 (d, *J* = 2.01 Hz, 1H); 9.18–9.20 (d, *J* = 2.01 Hz, 1H). MS (ESI): 306.0 ($C_{15}H_{13}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{12}ClNO_4$: C, 58.93; H, 3.96%; Found: C, 58.81; H, 3.76%.

*2-(2-Methoxy-2-oxoethyl)phenyl 5-hydroxynicotinate (**7**)* White powder. Yield 79%, mp: 106–108°C, ^{1}H NMR (300 MHz, DMSO-*d6*): 3.48 (s, 3H); 3.69 (s, 2H); 7.27–7.34 (m, 2H); 7.39– 7.44 (m, 2H); 7.72–7.74 (m, 1H); 8.43–8.45 (m, 1H); 8.70–8.71 (m, 1H); 10.51 (s, 1H). MS (ESI): 288.0 ($C_{15}H_{14}NO_5$, $[M + H]^+$). Anal. Calcd for $C_{15}H_{13}NO_5$: C, 62.72; H, 4.56%; Found: C, 62.61; H, 4.41%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 5-bromonicotinate (**8**)* White oil. Yield 83%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.83 (s, 3H); 0.85 (s, 3H); 1.77–1.90 (m, 1H); 3.54 (s, 2H); 3.79–3.81 (d, *J* = 6.6 Hz, 2H); 6.71–6.82 (m, 2H); 7.04–7.11 (m, 2H); 7.64–7.72 (m, 1H); 8.47–8.56 (m, 1H); 8.69–8.71 (m, 1H). MS (ESI): 392.0 ($C_{18}H_{19}BrNO_4$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{18}BrNO_4$: C, 55.12; H, 4.63%; Found: C, 55.31; H, 4.49%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 5-chloronicotinate (**9**)* White oil. Yield 78%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.82 (s, 3H); 0.84 (s, 3H); 1.75–1.88 (m, 1H); 3.52 (s, 2H); 3.77–3.80 (d, *J* = 6.6 Hz, 2H); 6.74–6.83 (m, 2H); 7.06–7.13 (m, 2H); 7.64–7.73 (m, 1H); 8.49–8.56 (m, 1H); 8.67–8.70 (m, 1H). MS (ESI): 348.0 ($C_{18}H_{19}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{18}ClNO_4$: C, 62.15; H, 5.22%; Found: C, 62.28; H, 5.41%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 2-bromonicotinate (**10**)* White oil. Yield 76%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.83 (s, 3H); 0.84 (s, 3H); 1.74–1.82 (m, 1H); 3.50 (s, 2H); 3.75–3.83 (d, *J* = 6.6 Hz, 2H); 6.71–6.81 (m, 2H); 7.08–7.14 (m, 2H); 7.62–7.71 (m, 1H); 8.47–8.53 (m, 1H); 8.66–8.72 (m, 1H). MS (ESI): 392.0 ($C_{18}H_{19}BrNO_4$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{18}BrNO_4$: C, 55.12; H, 4.63%; Found: C, 55.28; H, 4.41%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 2-chloronicotinate (**11**)* White oil. Yield 80%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.84 (s, 3H); 0.86 (s, 3H); 1.72–1.80 (m, 1H); 3.53 (s, 2H); 3.73–3.89 (d, *J* = 6.6 Hz, 2H); 6.74–6.84 (m, 2H); 7.14–7.19 (m, 2H); 7.56–7.68 (m, 1H); 8.34–8.46 (m, 1H); 8.62–8.71 (m, 1H). MS (ESI): 348.0 ($C_{18}H_{19}ClNO_4$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{18}ClNO_4$: C, 62.15; H, 5.22%; Found: C, 62.31; H, 5.36%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 5-hydroxynicotinate (**12**)* White oil. Yield 77%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.82 (s, 3H); 0.84 (s, 3H); 1.68–1.74 (m, 1H); 3.54 (s, 2H); 3.68–3.79 (d, *J* = 6.6 Hz, 2H); 6.62–6.78 (m, 2H); 7.11–7.18 (m, 2H); 7.53–7.69 (m, 1H); 8.52–8.63 (m, 1H); 8.67–8.78 (m, 1H); 10.55 (s, 1H). MS (ESI): 330.1 ($C_{18}H_{20}NO_5$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81%; Found: C, 65.49; H, 5.67%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-fluorobenzoate (**13**)* White oil. Yield 76%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.82 (s, 3H); 0.85 (s, 3H); 1.68–1.74 (m, 1H); 3.54 (s, 2H); 3.68–3.79 (d, *J* = 6.6 Hz, 2H); 6.62–6.78 (m, 2H); 7.11–7.18 (m, 2H); 7.41–7.47 (m, 2H); 8.15–8.23 (m, 2H). MS (ESI): 331.1 ($C_{19}H_{20}FO_4$,

$[M + H]^+$). Anal. Calcd for $C_{19}H_{19}FO_4$: C, 69.08; H, 5.80%; Found: C, 69.22; H, 5.62%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-chlorobenzoate (**14**)* White oil. Yield 80%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.81 (s, 3H); 0.84 (s, 3H); 1.62–1.71 (m, 1H); 3.53 (s, 2H); 3.56–3.72 (d, J = 6.6 Hz, 2H); 6.61–6.77 (m, 2H); 7.15–7.24 (m, 2H); 7.45–7.49 (m, 2H); 8.12–8.22 (m, 2H). MS (ESI): 347.1 ($C_{19}H_{20}ClO_4$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{19}ClO_4$: C, 65.80; H, 5.52%; Found: C, 65.68; H, 5.65%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-bromobenzoate (**15**)* White oil. Yield 74%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.82 (s, 3H); 0.85 (s, 3H); 1.61–1.74 (m, 1H); 3.52 (s, 2H); 3.59–3.71 (d, J = 6.6 Hz, 2H); 6.58–6.73 (m, 2H); 7.09–7.22 (m, 2H); 7.37–7.42 (m, 2H); 8.19–8.25 (m, 2H). MS (ESI): 391.0 ($C_{19}H_{20}BrO_4$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{19}BrO_4$: C, 58.33; H, 4.89%; Found: C, 58.46; H, 4.71%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-nitrobenzoate (**16**)* White oil. Yield 72%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.81 (s, 3H); 0.84 (s, 3H); 1.57–1.68 (m, 1H); 3.54 (s, 2H); 3.62–3.78 (d, J = 6.6 Hz, 2H); 6.49–6.66 (m, 2H); 7.13–7.27 (m, 2H); 7.31–7.42 (m, 2H); 8.23–8.28 (m, 2H). MS (ESI): 358.1 ($C_{19}H_{20}NO_6$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36%; Found: C, 63.71; H, 5.19%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-methylbenzoate (**17**)* White oil. Yield 77%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.83 (s, 3H); 0.85 (s, 3H); 1.52–1.64 (m, 1H); 2.38 (s, 1H); 3.58 (s, 2H); 3.67–3.83 (d, J = 6.6 Hz, 2H); 6.37–6.56 (m, 2H); 7.17–7.24 (m, 2H); 7.37–7.43 (m, 2H); 8.27–8.31 (m, 2H). MS (ESI): 327.1 ($C_{20}H_{23}O_4$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79%; Found: C, 73.48; H, 6.91%.

*2-(2-Isobutoxy-2-oxoethyl)phenyl 4-methoxybenzoate (**18**)* White oil. Yield 81%. ^{1}H NMR (300 MHz, DMSO-*d6*): 0.82 (s, 3H); 0.84 (s, 3H); 1.57–1.64 (m, 1H); 3.47 (s, 3H); 3.56 (s, 2H); 3.59–3.66 (d, J = 6.6 Hz, 2H); 6.61–6.75 (m, 2H); 7.13–7.24 (m, 2H); 7.35–7.41 (m, 2H); 8.24–8.27 (m, 2H). MS (ESI): 343.1 ($C_{20}H_{23}O_5$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48%; Found: C, 70.32; H, 6.61%.

Biological activity

Cell culture

The human gastric epithelial cancer cell line AGS (ATCC, WA, USA) was grown in Ham's F12 containing 10% fetal bovine serum (FBS), L-glutamine, 100 U mL^{-1} penicillin G, and 100 mg mL^{-1} streptomycin. Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

E. coli culture

The strain used in this study was Gram-negative bacteria *E. coli* ATCC 35218, which was cultured on MH medium (casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL) for 1 day at 37°C before use.

Preparation of E. coli water extract

E. coli ATCC 35218 was harvested from agar plants and then suspended in distilled water at a concentration of 2. 5×10^8 CFU mL^{-1}. After vortex-mixing for 1 min, the suspension was incubated at room temperature for 40 min and then centrifuged at 20,000 *g* for 20 min. Finally the supernatant was filtered through a 0.2 mm filter and stored at –20°C until use.

IL-8 assessment

Briefly, AGS cells, grown for 2 days on 24-well plates to ~80% confluence in Ham's F12 medium, were washed three times with serum-free Ham's F12 and then preincubated with compound **5** and aspirin in serial concentrations of 15, 30, and 60 μmol L^{-1} with a concentration of dimethylsulfoxide (DMSO) lower than 1% for 1 h. After that, cells were incubated with 10% *E. coli* water extract (v/v) for 12 h. The supernatant was aspirated, centrifuged at 500 *g* for 10 min, and then stored at –80°C. The levels of IL-8 in the culture supernatant were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Rapidbio, USA) according to the instructions of the manufacturer.

MTT assay for cellular viability

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay. Briefly, AGS cells, grown for 24 h on 24-well plates, were incubated with compound **5** and aspirin at serial concentrations of 15, 30, and 60 μmol L^{-1} for 48 h with the concentration of DMSO lower than 1%. Then MTT (5 mg L^{-1}) was added to each well for a 4 h period. After the formation of formazan crystals, the culture medium supernatant was removed from the wells without disruption of the precipitate. The formazan crystals were then dissolved in 150 μL DMSO/well. The absorbance was measured at 570 nm using a microplate spectrophotometer (Dynex Technologies, Chantilly, VA).

Statistical analysis

One-way analysis of variance was performed to compare differences between groups. Two-tailed probability values were derived, and a p value of < 0.05 was considered statistically significant.

Results and discussion

Chemistry

Sixteen depsides were synthesized for the first time. The synthesis of compounds **3–18** followed the general pathway outlined in Scheme 1. They were prepared through two steps. First, a solution of 2-hydroxyphenylacetic acid (compound **1**) in methanol or isobutyl alcohol containing concentrated H_2SO_4 was refluxed overnight. This step yielded the corresponding ester (compound **2**). Second, the coupling reaction between the obtained esters and the differently substituted nicotinic acid or benzoic acid was performed by using *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in anhydrous CH_2Cl_2. Then, compounds **3–18** were obtained by subsequent purification with flash chromatography. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

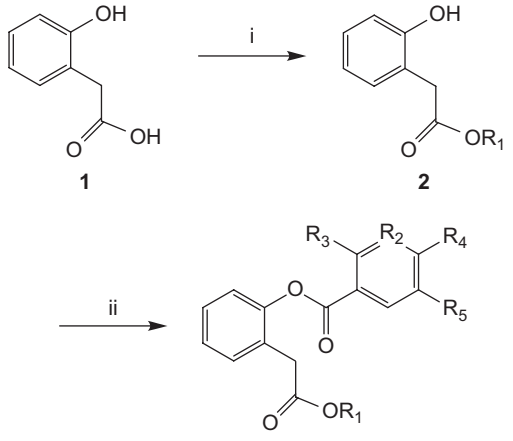
Antibacterial activity

All the compounds prepared were evaluated for their antibacterial activities against three Gram-positive bacterial strains (*B. subtilis* ATCC 6633, *S. aureus* ATCC 6538, and *S. faecalis* ATCC 9790) and three Gram-negative bacterial strains (*E. coli* ATCC 35218, *P. aeruginosa* ATCC 13525, and *E. cloacae* ATCC 13047) by the MTT method, and the results are shown in Table 1.

As shown in Table 1, in general, compounds **3–12** exhibited better antibacterial activity than compounds **13–18** against *E. coli* ATCC 35218, which indicated that nicotinic acid derivatives had better antibacterial activity than that of benzoic acid derivatives. Among them, compounds **3**, **5**, and **6** displayed potent activity, with MIC values of 1.562 μg/mL, 0.78 μg/mL, and 1.562 μg/mL against *E. coli* ATCC 35218, which were superior to the positive control kanamycin B. Compound **4** exhibited significant activity, with an MIC value of 3.125 μg/mL against *E. coli* ATCC 35218, which was comparable to the positive control kanamycin B. In view of its significant antibacterial activity, compound **5** (MIC: 0.78 μg/mL) was selected to act against the IL-8 production induced by *E. coli* in gastric mucosal cells.

IL-8 assessment

Gram-negative bacterial infections rapidly induce an inflammatory response in which the cytokine network plays a major role. LPS released from these bacteria may be a major immunogen contributing to the cytokine burst by the LPS ± LPS binding protein (BP) ± CD14 complex formation[17,18]. IL-8 is a cytokine implicated in some cancers and a wide range of chronic inflammatory conditions, including rheumatoid



3	$R_1 = CH_3$	$R_2 = N$	$R_3 = Br$	$R_4 = H$	$R_5 = H$
4	$R_1 = CH_3$	$R_2 = N$	$R_3 = Cl$	$R_4 = H$	$R_5 = H$
5	$R_1 = CH_3$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = Br$
6	$R_1 = CH_3$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = Cl$
7	$R_1 = CH_3$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = OH$
8	$R_1 = CH_2CH(CH_3)_2$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = Br$
9	$R_1 = CH_2CH(CH_3)_2$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = Cl$
10	$R_1 = CH_2CH(CH_3)_2$	$R_2 = N$	$R_3 = Br$	$R_4 = H$	$R_5 = H$
11	$R_1 = CH_2CH(CH_3)_2$	$R_2 = N$	$R_3 = Cl$	$R_4 = H$	$R_5 = H$
12	$R_1 = CH_2CH(CH_3)_2$	$R_2 = N$	$R_3 = H$	$R_4 = H$	$R_5 = OH$
13	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = F$	$R_5 = H$
14	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = Cl$	$R_5 = H$
15	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = Br$	$R_5 = H$
16	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = NO_2$	$R_5 = H$
17	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = CH_3$	$R_5 = H$
18	$R_1 = CH_2CH(CH_3)_2$	$R_2 = C$	$R_3 = H$	$R_4 = OCH_3$	$R_5 = H$

Scheme 1. (i) CH_3OH or $(CH_3)_2CHCH_2OH$; H_2SO_4; reflux. (ii) DCC, DMAP, CH_2Cl_2, reflux.

Table 1. MICs (minimum inhibitory concentrations) (μg/mL) of the synthetic compounds.

	Microorganism					
	Gram-positive			Gram-negative		
Compound	*B. subtilis* ATCC 6633	*S. aureus* ATCC 6538	*S. faecalis* ATCC 9790	*P. aeruginosa* ATCC 13525	*E. coli* ATCC 35218	*E. cloacae* ATCC 13047
3	6.25	6.25	6.25	6.25	1.562	3.125
4	6.25	12.5	12.5	12.5	3.125	6.25
5	12.5	6.25	6.25	3.125	0.78	3.125
6	12.5	25	6.25	6.25	1.562	6.25
7	25	>50	>50	6.25	6.25	6.25
8	>50	6.25	3.125	12.5	12.5	>50
9	12.5	25	25	12.5	6.25	6.25
10	6.25	6.25	6.25	6.25	6.25	>50
11	>50	12.5	25	>50	12.5	>50
12	25	>50	12.5	25	12.5	25
13	1.562	>50	>50	6.25	>50	>50
14	3.125	25	25	>50	25	12.5
15	3.125	6.25	12.5	25	>50	6.25
16	12.5	12.5	>50	25	>50	6.25
17	6.25	6.25	25	>50	25	25
18	6.25	6.25	>50	25	>50	6.25
Penicillin G	1.562	1.562	1.562	6.25	6.25	3.125
Kanamycin B	0.39	1.562	3.125	3.125	3.125	1.562

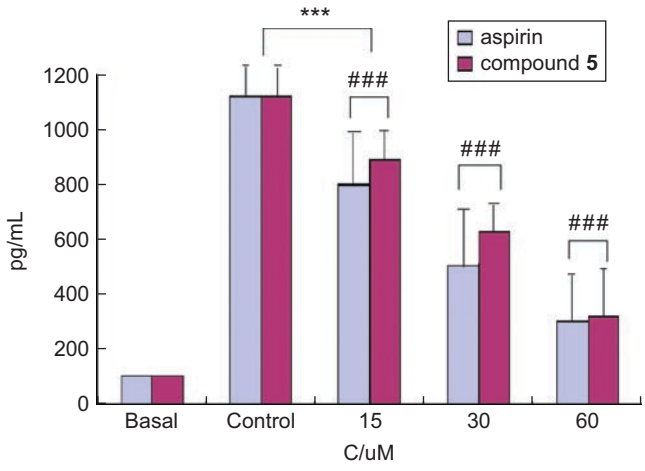


Figure 2. Inhibitory activities of compound **5** on IL-8 production (pg/mL) induced by *E. coli* water extract. Results are mean ± SEM of 3–5 experiments. Comparison 15, 30, and 60 μmol L^{-1} of all agents versus control: ***$p < 0.001$; comparison compound **5** versus aspirin: ###$p < 0.001$. C, concentration of agent.

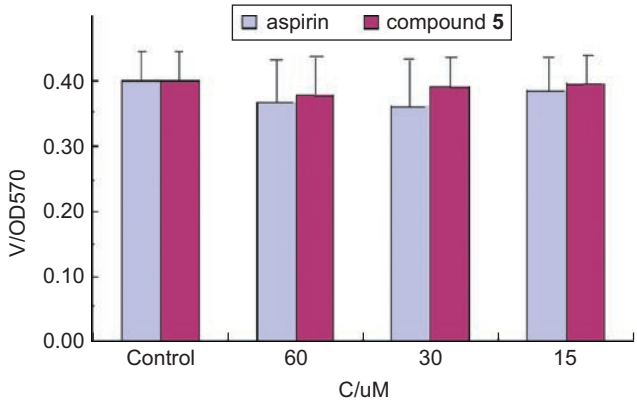


Figure 3. Effects of different agents on cell viability. Results are mean ± SEM of 4–6 experiments. Comparison 15, 30, and 60 μmol L^{-1} of all agents versus control: $p > 0.05$. V, cell viability; C, concentration of agent.

arthritis, heart disease, and gastritis. In addition, IL-8 has been shown previously to be derived after stimulation with *E. coli* in transformed epithelial cell-lines[18,19]. In this study, Gram-negative bacteria *E. coli* were used to stimulate the human gastric epithelial cancer cell line AGS to produce IL-8, then compound **5** was assessed by enzyme-linked immunosorbent assay (ELISA).

Fortunately, we found that compound **5** exhibited a strong attenuation of IL-8 production induced by *E. coli* water extract in AGS cells, as shown in Figure 2. Aspirin was used as a reference. AGS cells were preincubated with or without aspirin or compound **5** in serial concentrations of 15, 30, and 60 μmol L^{-1} for 1 h and then stimulated by 10% *E. coli* water extract (v/v) for 12 h. The levels of IL-8 in the culture supernatant were determined by ELISA. "Basal" represents cells without incubation of *E. coli* water extract, and "Control" shows cells incubated with *E. coli* water extract but without any agent. The data obtained indicated that *E. coli* alone stimulated AGS cells to produce as much as 1100 pg/mL IL-8. Compound **5** showed a strong attenuation of IL-8 production induced by *E. coli* in AGS cells.

Different dose-dependent attenuations of *E. coli*-induced IL-8 production were seen with the addition of aspirin and compound **5**. Compound **5** significantly decreased the IL-8 level in a dose-dependent way at concentrations of 15, 30, and 60 μmol L^{-1} compared with control, and the lowest IL-8 production was observed when the concentration of compound **5** was 60 μmol L^{-1}. The same inhibition of *E. coli*-induced IL-8 production could be observed when aspirin was preincubated with AGS cells followed by the addition of *E. coli* water extract. However, the IL-8 production was decreased to 890 pg/mL and 610 pg/mL when the concentrations of aspirin were 15 μmol L^{-1} and 30 μmol L^{-1} respectively, which were lower than those of compound **5**, indicating that aspirin showed a slightly stronger inhibition of *E. coli*-induced IL-8 production than compound **5** at the two concentrations. However, there was scarcely any difference at the concentration 60 μmol L^{-1}.

In addition, cell viability was assessed using the MTT assay to see whether compound **5** and aspirin affected cell viability at the concentrations tested (15, 30, and 60 μmol L^{-1}), as shown in Figure 3. AGS cells were incubated with aspirin or compound **5** at serial concentrations of 15, 30, and 60 μmol L^{-1}. The MTT assay was performed 48 h later. The results shown in Figure 3 demonstrate that compound **5** and aspirin did not affect cell viability at the concentrations tested (15, 30, and 60 μmol L^{-1}). Based on the data obtained in this study, it can be concluded that compound **5** would be a potential and promising anti-inflammatory agent.

Conclusion

In this study, 16 novel depsides were synthesized for the first time. All the compounds were assayed for antibacterial activities against three Gram-positive bacterial strains and three Gram-negative bacterial strains by the MTT method. Compound **5** exhibited significant antibacterial activities against *E. coli* ATCC 35218, with an MIC of 0.78 μg/mL, and was selected to act against the IL-8 production induced by *E. coli* in gastric mucosal cells. Compound **5** significantly decreased the IL-8 level in a dose-dependent manner at concentrations of 15, 30, and 60 μmol L^{-1} compared with control, and the lowest IL-8 production was observed when the concentration of compound **5** was 60 μmol L^{-1}.

Declaration of interest

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