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To cite this article: Peng-Cheng Lv, Jing Xiong, Jin Chen, Kai-Rui Wang, Wen-Jun Mao & Hai-Liang Zhu (2010) Novel depsides as potential anti-inflammatory agents with potent inhibitory activity against *Escherichia coli*-induced interleukin-8 production, Journal of Enzyme Inhibition and Medicinal Chemistry, 25:4, 590-595, DOI: [10.3109/14756360903357551](https://doi.org/10.3109/14756360903357551)

To link to this article: <https://doi.org/10.3109/14756360903357551>



Published online: 17 Mar 2010.



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## SHORT COMMUNICATION

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

Peng-Cheng Lv, Jing Xiong, Jin Chen, Kai-Rui Wang, Wen-Jun Mao, and Hai-Liang Zhu

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**Abstract**

Sixteen novel depsides were synthesized for the first time. Their chemical structures were clearly determined by  $^1\text{H}$  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  $\mu\text{g}/\text{mL}$ , 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<sup>1–9</sup>. In addition, as inhibitors of prostaglandin biosynthesis and leukotriene B<sub>4</sub> biosynthesis, depsides are potent nonsteroidal antiinflammatories<sup>10</sup>. 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<sup>11</sup>. 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)<sup>12,13</sup>. Raised levels of IL-8 have been detected in several disease states<sup>14</sup>, 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<sup>13,15</sup>. Recently, a new depside, named jaboticabin (Figure 1), was demonstrated to inhibit chemokine IL-8

production before and after cigarette smoke treatment of cells<sup>16</sup>.

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  $\mu\text{g}/\text{mL}$ , which was superior to the positive control kanamycin B. In addition, in view of its significant antibacterial activity, compound **5** was selected

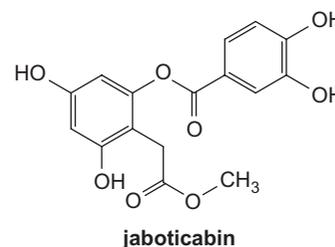


Figure 1. The structure of jaboticabin.

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(Received 16 June 2009; revised 18 August 2009; accepted 26 August 2009)

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  $^1\text{H}$  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 ( $\delta$ ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within  $\pm 0.4\%$  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  $\text{H}_2\text{SO}_4$  (5 mL) was refluxed overnight. Water (100 mL) was added, the organic phases were washed with saturated NaCl (100 mL) and dried over  $\text{Na}_2\text{SO}_4$ , 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{15}\text{H}_{13}\text{BrNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{12}\text{BrNO}_4$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{15}\text{H}_{13}\text{ClNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{12}\text{ClNO}_4$ : 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,  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{15}\text{H}_{13}\text{BrNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{12}\text{BrNO}_4$ : 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,  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{15}\text{H}_{13}\text{ClNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{12}\text{ClNO}_4$ : 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,  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{15}\text{H}_{14}\text{NO}_5$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{15}\text{H}_{13}\text{NO}_5$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{18}\text{H}_{19}\text{BrNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{BrNO}_4$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{18}\text{H}_{19}\text{ClNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{ClNO}_4$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{18}\text{H}_{19}\text{BrNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{BrNO}_4$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{18}\text{H}_{19}\text{ClNO}_4$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{18}\text{H}_{18}\text{ClNO}_4$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{18}\text{H}_{20}\text{NO}_5$ ,  $[\text{M} + \text{H}]^+$ ). Anal. Calcd for  $\text{C}_{18}\text{H}_{19}\text{NO}_5$ : 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%.  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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 ( $\text{C}_{19}\text{H}_{20}\text{FO}_4$ ,

[M + H]<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>19</sub>FO<sub>4</sub>: 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%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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<sub>19</sub>H<sub>20</sub>ClO<sub>4</sub>, [M + H]<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>19</sub>ClO<sub>4</sub>: 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%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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<sub>19</sub>H<sub>20</sub>BrO<sub>4</sub>, [M + H]<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>19</sub>BrO<sub>4</sub>: 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%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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<sub>19</sub>H<sub>20</sub>NO<sub>6</sub>, [M + H]<sup>+</sup>). Anal. Calcd for C<sub>19</sub>H<sub>19</sub>NO<sub>6</sub>: 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%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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<sub>20</sub>H<sub>23</sub>O<sub>4</sub>, [M + H]<sup>+</sup>). Anal. Calcd for C<sub>20</sub>H<sub>22</sub>O<sub>4</sub>: 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%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): 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<sub>20</sub>H<sub>23</sub>O<sub>5</sub>, [M + H]<sup>+</sup>). Anal. Calcd for C<sub>20</sub>H<sub>22</sub>O<sub>5</sub>: 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<sup>-1</sup> penicillin G, and 100 mg mL<sup>-1</sup> streptomycin. Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub>.

### 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<sup>8</sup> CFU mL<sup>-1</sup>. 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<sup>-1</sup> 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<sup>-1</sup> for 48 h with the concentration of DMSO lower than 1%. Then MTT (5 mg L<sup>-1</sup>) 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<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>Cl<sub>2</sub>. 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.

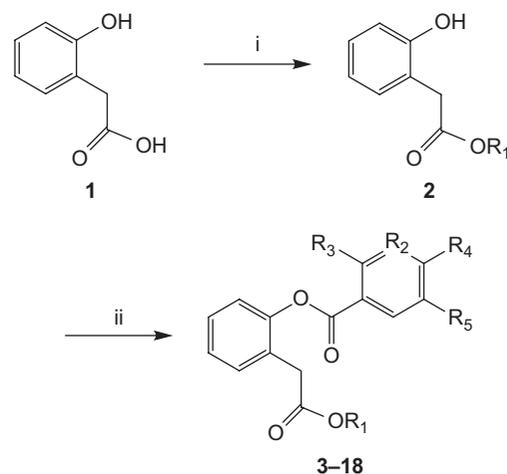
**Antibacterial activity**

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<sup>17,18</sup>. IL-8 is a cytokine implicated in some cancers and a wide range of chronic inflammatory conditions, including rheumatoid

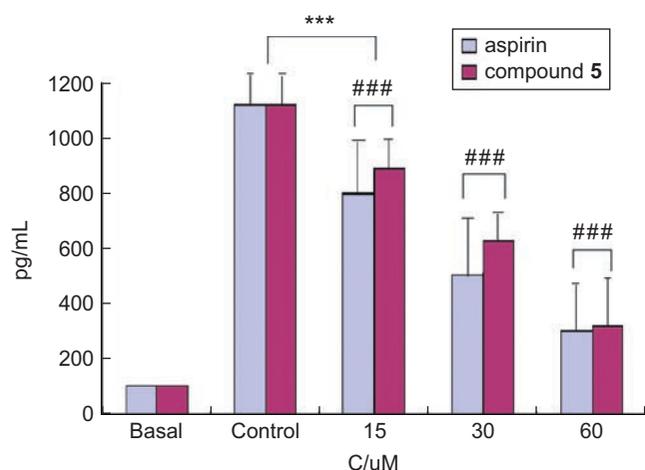


<b>3</b> R <sub>1</sub> = CH <sub>3</sub>	R <sub>2</sub> = N	R <sub>3</sub> = Br	R <sub>4</sub> = H	R <sub>5</sub> = H
<b>4</b> R <sub>1</sub> = CH <sub>3</sub>	R <sub>2</sub> = N	R <sub>3</sub> = Cl	R <sub>4</sub> = H	R <sub>5</sub> = H
<b>5</b> R <sub>1</sub> = CH <sub>3</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = Br
<b>6</b> R <sub>1</sub> = CH <sub>3</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = Cl
<b>7</b> R <sub>1</sub> = CH <sub>3</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = OH
<b>8</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = Br
<b>9</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = Cl
<b>10</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = N	R <sub>3</sub> = Br	R <sub>4</sub> = H	R <sub>5</sub> = H
<b>11</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = N	R <sub>3</sub> = Cl	R <sub>4</sub> = H	R <sub>5</sub> = H
<b>12</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = N	R <sub>3</sub> = H	R <sub>4</sub> = H	R <sub>5</sub> = OH
<b>13</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = F	R <sub>5</sub> = H
<b>14</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = Cl	R <sub>5</sub> = H
<b>15</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = Br	R <sub>5</sub> = H
<b>16</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = NO <sub>2</sub>	R <sub>5</sub> = H
<b>17</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = CH <sub>3</sub>	R <sub>5</sub> = H
<b>18</b> R <sub>1</sub> = CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	R <sub>2</sub> = C	R <sub>3</sub> = H	R <sub>4</sub> = OCH <sub>3</sub>	R <sub>5</sub> = H

**Scheme 1.** (i) CH<sub>3</sub>OH or (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>OH; H<sub>2</sub>SO<sub>4</sub>; reflux. (ii) DCC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, reflux.

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

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

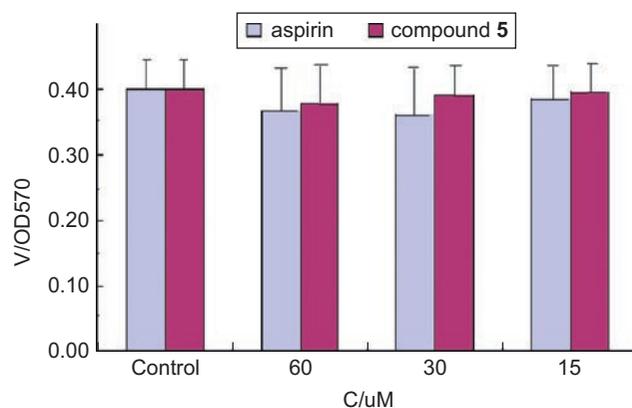


**Figure 2.** Inhibitory activities of compound **5** on IL-8 production (pg/mL) induced by *E. coli* water extract. Results are mean  $\pm$  SEM of 3–5 experiments. Comparison 15, 30, and 60  $\mu\text{mol L}^{-1}$  of all agents versus control: \*\*\* $p < 0.001$ ; comparison compound **5** versus aspirin: ### $p < 0.001$ . 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<sup>18,19</sup>. 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  $\mu\text{mol L}^{-1}$  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  $\mu\text{mol L}^{-1}$  compared with control, and the lowest IL-8 production was observed when the concentration of compound **5** was 60  $\mu\text{mol L}^{-1}$ . 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  $\mu\text{mol L}^{-1}$  and 30  $\mu\text{mol L}^{-1}$  respectively, which were lower than those of compound **5**, indicating that aspirin showed a slightly stronger inhibition



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

of *E. coli*-induced IL-8 production than compound **5** at the two concentrations. However, there was scarcely any difference at the concentration 60  $\mu\text{mol L}^{-1}$ .

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  $\mu\text{mol L}^{-1}$ ), as shown in Figure 3. AGS cells were incubated with aspirin or compound **5** at serial concentrations of 15, 30, and 60  $\mu\text{mol L}^{-1}$ . 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  $\mu\text{mol L}^{-1}$ ). 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  $\mu\text{g/mL}$ , 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  $\mu\text{mol L}^{-1}$  compared with control, and the lowest IL-8 production was observed when the concentration of compound **5** was 60  $\mu\text{mol L}^{-1}$ .

## Declaration of interest

The work was financed by grants (Project 30772627) from the National Natural Science Foundation of China.

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