Bioorganic & Medicinal Chemistry 18 (2010) 4269–4274

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and antibacterial activity of novel andrographolide derivatives

Zhongli Wang, Pei Yu *, Gaoxiao Zhang, Lipeng Xu, Dingyuan Wang, Liang Wang, Xiangping Zeng, Yuqiang Wang *

Institute of New Drug Research and Guangdong Province Key Laboratory of Pharmacodynamic Constituents of Traditional Chinese Medicine & New Drug Research, Jinan University College of Pharmacy, Guangzhou 510632, China

ARTICLE INFO

Article history:
Received 23 March 2010
Revised 27 April 2010
Accepted 28 April 2010
Available online 20 May 2010

Keywords:
Andrographolide derivatives
Synthesis
Antibacterial
Quorum sensing

ABSTRACT

A series of andrographolide derivatives were synthesized through a facile condensation reaction with different carboxylic acids. The new compounds were characterized and screened for their antibacterial activities. A number of the new compounds significantly reduced bacterial quorum sensing virulence factors production in *Pseudomonas aeruginosa*, essential for pathogenesis. Compound **11b** showed the best activity among all the new compounds.

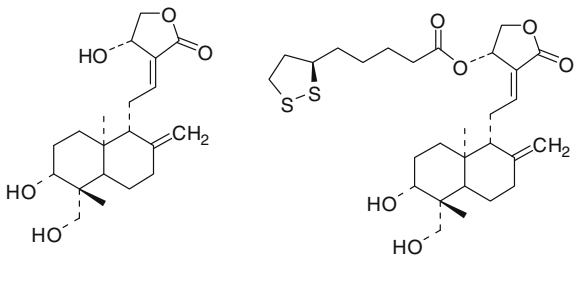
1. Introduction

Bacteria communicate with each other by means of different classes of signal molecules. In general, each bacterial cell produces a small amount of one or more signal molecules, which are subsequently released into the environment. When the signal molecule(s) reach a certain threshold, they promote the transcription of genes necessary for bacterial group functions.[1–4] These group phenomena are largely under the control of a cell–cell signaling pathway called quorum sensing (QS), which plays a considerable role in the establishment of both symbiotic and pathogenic relationships with eukaryotic hosts.[5] As quorum sensing represents a strategy to control bacterial growth, there is significant interest in the development of QS-targeting agents.[6,7]

The Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*) is a model bacterium for studying QS and biofilm formation. *P. aeruginosa* produces two different acyl homoserine lactones (AHLs) as QS signal molecules, that is, *N*-3-oxododecanoylhomoserine lactone (3-oxo-C_{12}-HSL) and *N*-butanoylhomoserine lactone (C_4-HSL). These molecules are sensed by three receptors: LasR, QscR, and RhlR. LasR and OscR commonly receive 3-oxo-C_{12}-HSL but operate distinct regulons. RhlR also regulate its own regulon by sensing C_4-HSL.[8,9] QS controls secondary metabolism, bioluminescence, protein secretion, motility, virulence factor production, plasmid transfer and biofilm formation in *P. aeruginosa*.[10–12] Therefore, finding QS antagonists that attenuate bacterial virulence rather than growth is a novel way for antibacterial drug research.

Andrographolide (Andro, **1**, Fig. 1), a natural bicyclic diterpeniod lactone, is extracted as the main phytoconstituent from *Andrographis paniculata* Nees, a herb commonly used in India, China and southeast Asia for the treatment of a large variety of illness, such as gastric disorders, cold, influenza and other infectious diseases.[13,14] Andro exhibits several pharmacological activities, including antiinflammatory, immunomodulatory, antibacterial and antiviral infections.[15–22] Although the herb, Andro and its derivatives have been used to treat bacterial infections for many years, these drugs have been found to have minimal or no direct inhibition on bacterial growth, especially on *P. aeruginosa*.[18–20]

Our laboratory recently developed Andro derivatives as autoinducer mimics that can inhibit the growth of both Gram-positive and the Gram-negative bacteria.[19] We have demonstrated that



1 (Andro) AL-1

Figure 1. Structures of Andro and AL-1.

* Corresponding authors. Tel.: +86 20 8522 8481; fax: +86 20 8522 4766.
E-mail addresses: pennypeiyu@yahoo.com.cn (P. Yu), yuqiangwang2001@yahoo.com (Y. Wang).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.04.094

some of these compounds, such as AL-1 (Fig. 1), a conjugate of *R*-(α)-lipoic acid and Andro, had significant effects on the QS system in *P. aeruginosa*, leading to strong inhibition of biofilm formation.[19] In search for more effective new compounds and uncover the mechanism of drug action, we herein report a new series of Andro derivatives and their preliminary evaluation for activity in inhibiting the QS.

2. Results and discussion

2.1. Chemistry

In this study, we designed new Andro derivatives (Scheme 1), structurally similar to natural AHLs to develop QS inhibitors of *P. aeruginosa* and biofilm formation. The LasI of *P. aeruginosa* primarily catalyzes synthesis of 3-oxo-C_{12}-HSL, and RhlI directs the synthesis of C_4-HSL. The length of acyl side chain and its substitutions provide signal specificity.[23] Thus, we coupled acyl side chains with different length to Andro's C14 position (**3b–7b**) to find out whether substitution on the side chain making any difference to their biological activity.

(*E*)-3-(Furan-2-yl) acrylic acid, adamantaneacetic acid and (*E*)-3-cyclohexylacrylic acid were conjugated to Andro making compounds **8b**, **9b** and **10b**. This is to understand whether different cyclic-substitution is important in biological activity. Our previously synthesized AL-1 is superior to the natural Andro in inhibiting QS of *P. aeruginosa* and biofilm formation,[19,20] and is recently found to significantly reduces the expression of two regulators, LasI and RhlI in *P. aeruginosa*.[20] In this report, we design and synthesized compound **11b**, similar to AL-1, but without the disulfide bond in the ring to investigate the biological functions of the disulfide bond to its anti-QS activity.

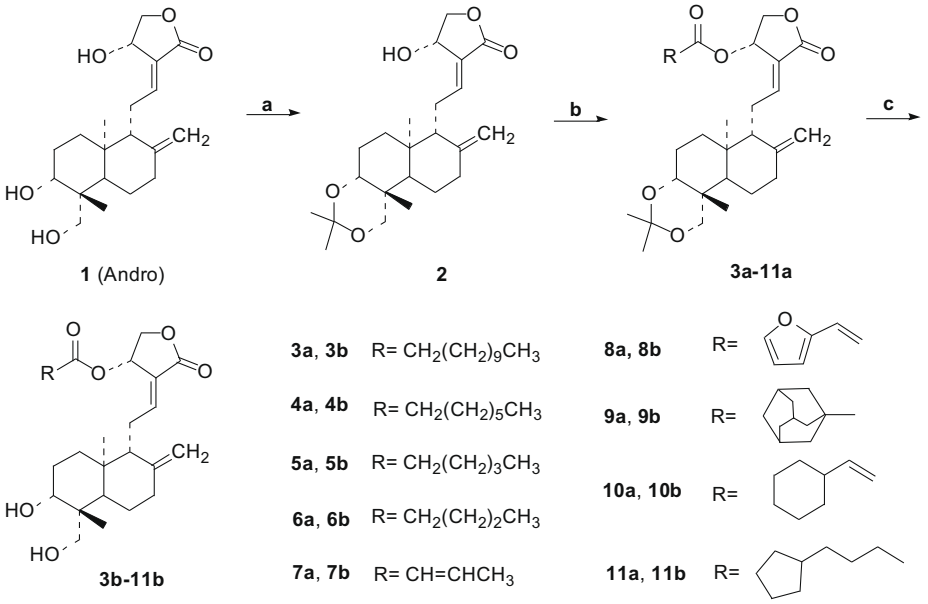
2.2. Biological activity evaluations

We have previously found that Andro compounds did not directly inhibit bacterial growth.[19,20] To find if the new compounds directly inhibit bacterial growth or not, we used the zone of inhibition method to measure the inhibition of *P. aeruginosa* growth. We found that the new compounds were not active against *P. aeruginosa* growth at a concentration of up to 2 mM (data not show), and the result is consist with our previous findings.[19]

Production of pyocyanin by *P. aeruginosa* is positively regulated by quorum sensing signals including AHLs.[24–26] AL-1 and Andro have been shown to inhibit the *P. aeruginosa* QS system.[19,20] We therefore examined the effect of the new Andro derivatives on pyocyanin production. *P. aeruginosa* were grown for overnight to mid log phase (OD_{600} 0.5) and were then diluted to an OD_{600} of 0.05, aliquoted to test tubes containing the new compounds. Following another 10 h of growth, pyocyanin was extracted, and was then quantified. The results are shown in Figure 2. We found that all of the tested compounds, **3b–11b,** Andro and AL-1, inhibited pyocyanin production. Among them, compound **11b** and AL-1 were the most potent. Compounds **3b–10b** also significantly inhibited pyocyanin production, and all were more potent than the natural Andro.

Protease is another virulence factor produced and excreted by *P. aeruginosa* under QS control. We then tested the new compounds for their anti-protease activity. A colorimetric method employing azocasein as a substrate was used to measure protease activity, and the results are shown in Figure 3. Compounds AL-1, **5b**, **8b** and **11b** almost completely suppressed protease production at 0.5 mM. Compounds **7b**, **9b, 10b** and Andro had similar activity.

Analysis of the SAR of the new compounds reveals that: (1) a side chain, aliphatic straight or cyclic, at the C14 of the natural Andro increases activity inhibiting the production of virulence factors; (2) Compounds such as **5b** and **8b** significantly inhibit the production of protease, but are much less potent inhibiting production of pyocyanin, suggesting that the compounds act through different pathways on inhibition of the productions of these two virulence factors; (3) In contrast to compounds **5b** and **8b** which inhibits pyocyanin and proteinase productions with different potencies, AL-1 and compound **11b** potently inhibit the productions of both pyocyanin and protease, the discrepancies between these results remain to be elucidated; (4) The fact that AL-1, with a disulfide bond, and compound **11b**, without a disulfide bond, are equally effective inhibiting the productions of pyocyanin and protease suggests that the disulfide bond plays insignificant role for antibacterial activity. It is important to point out that AL-1, in addition to its antibacterial and antiviral activity,[19,20] also exhibits



Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane/benzene/DMSO/reflux/1 h; (b) RCOOH/DCM/ClCOOEt/NEt_3/15–24 h; (c) AcOH/H_2O/30 min.

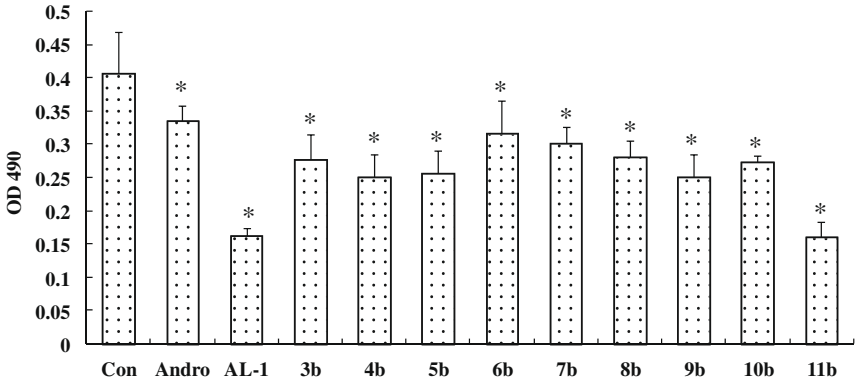


Figure 2. Inhibition of pyocyanin production in *P. aeruginosa* PAO1. Con, control; the concentration of all compounds was 0.5 mM. $^{*}P < 0.05$ compared to control group.

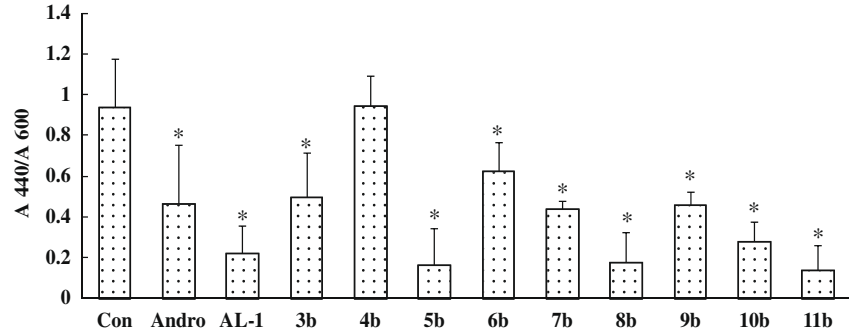


Figure 3. Inhibition of protease production in *P. aeruginosa* PAO1. Con, control; the concentration of all compounds was 0.5 mM. $^{*}P < 0.05$ compared to control group.

significant anti-diabetic activity both in vitro and in experimental animal model.[27] In contrast, we found that compound **11b** had minimal activity in experimental diabetic animal model (unpublished data). These results indicate that the disulfide bond of AL-1 plays a critical role in its anti-diabetic activity, and AL-1 acts through different mechanisms for its antimicrobial and anti-diabetic activity. We have recently found that AL-1 significantly suppressed the gene expressions of LasI and RhlI in *P. aeruginosa*.[20]

3. Conclusions

We have designed and synthesized a series of new Andro derivatives as the AHL mimics, and tested them for inhibition of virulence factors production. None of the compounds were active against *P. aeruginosa* growth up to 2 mM concentration, but all of them inhibited pyocyanin production and protease expression. Compounds **5b**, **8b**, **11b** and AL-1 almost completely suppressed protease expression. The fact that compound **11b** and AL-1 are almost equally potent inhibiting productions of both pyocyanin and protease suggests that antioxidative activity of AL-1 is not important for inhibition of virulence factors production in *P. aeruginosa*. To explore the mechanism of action for this novel class of antibacterial agents, we are conducting further experiments, and will report the results in due course.

4. Experimental

^{1}H NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz. Electrospray ionization mass spectra (ESI-MS) were obtained on a Finnigan LCQ Advantage MAX mass spectrometer (ABI company, 4000 Q TRAP). High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-700 mass spectrometer. Lauric acid, octanoic acid, hexanoic acid, pentanoic acid, *E*-2-butenoic acid, (*E*)-3-(furan-2-yl) acrylic acid and adamantaneacetic acid were purchased from Alfa Aesar Company (Beijing). Silica gel plates (Merck F_{254}) were used for thin layer chromatography. Dichloromethane was distilled over calcium hydride prior to use. Chromatographic purification was performed with silica gel (200–400 mesh).

4.1. 14-Lauroyl andrographolide (3b)

A stirred solution of the lauric acid (400 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added, and the reaction mixture was stirred for 1 h. Compound **2**[28] (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was then stirred at room temperature for 18 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and were concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **3a** as a colorless liquid (150 mg, 51.2% yield). ^{1}H NMR ($CDCl_3$): 7.01 (t, J = 6.08 Hz, 1H), 5.92 (d, J = 6.0 Hz, 1H), 4.86 (s, 1H), 4.54 (m, J = 10 Hz, 1H), 4.48 (s, 1H), 4.18 (m, J = 3.6 Hz, 2H), 3.42 (t, J = 7.6 Hz, 2H), 3.28 (d, J = 10.8 Hz, 1H), 2.48–2.30 (m, 5H), 2.0–1.95 (m, 1H), 1.88–1.74 (m, 4H), 1.74–1.54 (m, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 0.92 (s, 3H), 0.86 (t, J = 6.6 Hz, 3H). MS (EI) $[M+H]^+$ m/z 573.8. Without further purification, compound **3a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **3b** as a colorless liquid (54 mg, 38.7% yield). MS (EI) $[M+H]^+$ m/z 533.9. ^{1}H NMR ($CDCl_3$): 7.01 (t, J = 6.08 Hz, 1H), 5.92 (d,

J = 6.0 Hz, 1H), 4.86 (s, 1H), 4.54 (m, J = 10 Hz, 1H), 4.48 (s, 1H), 4.18 (m, 2H), 3.42 (t, J = 7.6 Hz, 2H), 3.28 (d, J = 10.8 Hz, 1H), 2.48–2.30 (m, 5H), 2.0–1.95 (m, 1H), 1.88–1.74 (m, 4H), 1.74–1.54 (m, 3H), 0.86 (t, J = 6.6 Hz, 3H), 0.64 (s, 3H). HRMS (ESI, m/z) calcd for $C_{32}H_{52}O_6Na$ 555.3662, found 555.3659.

4.2. 14-Octanoyl andrographolide (4b)

A stirred solution of the octanoic acid (300 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere, ethyl chloroformate (0.33 mL, 3.36 mmol) was added, and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 18 h. After quenching with water (30 mL), the organic layer was separated, and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **4a** (153.5 mg, 58% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$): 6.99 (t, J = 6.8 Hz, 1H), 5.92 (d, J = 6.0 Hz, 1H), 4.88 (s, 1H), 4.56–4.52 (m, 2H), 4.18 (dd, J = 2.0, 9.2 Hz, 2H), 3.93 (d, J = 11.6 Hz, 1H), 3.48 (m, 1H), 3.16 (d, J = 11.6 Hz, 1H), 2.45–2.37 (m, 2H), 2.34 (t, J = 7.6 Hz, 2H), 2.02–1.93 (m, 2H), 1.80–1.58 (m, 6H), 1.38 (s, 3H), 1.34 (s, 3H), 1.18 (s, 3H), 0.92 (s, 3H), 0.87 (t, J = 6.8 Hz, 3H). MS (EI) $[M+H]^+$ m/z 517.8. Without further purification, compound **4a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **4b** (53.5 mg, 37.9% yield) as a colorless liquid. MS (EI) $[M+Na]^+$ m/z 499.7. ^{1}H NMR ($CDCl_3$): 6.97 (t, J = 6.0 Hz, 1H), 5.90 (d, J = 6.0 Hz, 1H), 4.85 (s, 1H), 4.53 (m, 1H), 4.47 (s, 1H), 4.20–4.13 (m, 2H), 3.43 (t, J = 8.0 Hz, 2H), 3.28 (d, J = 10.8 Hz, 1H), 2.41–2.31 (m, 5H), 1.21 (s, 3H), 0.86 (t, J = 6.8 Hz, 3H), 0.64 (s, 3H). HRMS (ESI, m/z) calcd for $C_{28}H_{44}O_6Na$ 499.3036, found 499.3033.

4.3. 14-Caproyl andrographolide (5b)

A stirred solution of the hexanoic acid (0.26 mL, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere, ethyl chloroformate (0.33 mL, 3.36 mmol) was added, and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 20 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3×10 mL). The combined organic extracts were dried with Na_2SO_4 and were then concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **5a** (150.1 mg, 60% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$): 7.03 (t, J = 6.08 Hz, 1H), 5.94 (d, J = 6.0 Hz, 1H), 4.91 (s, 1H), 4.59–4.55 (m, 2H), 4.22 (dd, J = 2.0, 9.2 Hz, 1H), 4.13 (dd, J = 7.2, 7.2 Hz, 2H), 3.96 (d, J = 11.6 Hz, 1H) 3.51 (m, 1H), 3.19 (d, J = 11.6 Hz, 1H), 2.47–2.35 (m, 5H), 1.88–1.74 (m, 4H), 1.74–1.54 (m, 3H), 1.42 (s, 3H), 1.37 (s, 3H), 1.21 (s, 3H), 0.95 (s, 3H), 0.92 (t, J = 7.2 Hz, 3H). MS (EI) $[M+H]^+$ m/z 489.7. Without further purification, compound **5a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **5b** (57.4 mg, 41.7% yield) as a colorless liquid. MS (EI) $[M+Na]^+$ m/z 471.7. ^{1}H NMR ($CDCl_3$): 7.0 (t, J = 6.8 Hz, 1H), 5.93 (d, J = 6.0 Hz, 1H), 4.86 (s, 1H), 4.56 (m, 1H), 4.50 (s, 1H), 4.22–4.16 (m, 2H), 3.47 (m, 1H), 3.32 (d, J = 10.8 Hz, 1H), 2.48–2.34 (m, 5H), 1.35–1.28 (m, 5H), 1.25 (s, 3H), 0.90 (t, J = 6.6 Hz, 3H), 0.67 (s, 3H). HRMS (ESI, m/z) calcd for $C_{26}H_{40}O_6Na$ 471.2723, found 471.2717.

4.4. 14-Valeryl andrographolide (6b)

A stirred solution of the pentanoic acid (0.23 mL, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 20 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and were then concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **6a** (126.4 mg, 51.8% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$): 6.98 (t, J = 6.8 Hz, 1H), 5.91 (d, J = 6.0 Hz, 1H), 4.87 (s, 1H), 4.55–4.51 (m, 2H), 4.18 (dd, J = 2.0, 9.2 Hz, 1H), 3.92 (d, J = 11.6 Hz, 1H), 3.46 (m, 1H), 3.15 (d, J = 11.6 Hz, 1H), 3.28 (d, J = 10.8 Hz, 1H), 2.49–2.28 (m, 5H), 1.37 (s, 3H), 1.33 (s, 3H), 1.17 (s, 3H), 1.27–1.21 (m, 3H), 0.92–0.88 (m, 6H). MS (EI) $[M+H]^+$ m/z 475.7. Without further purification, compound **6a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **6b** (48.96 mg, 42.57% yield) as a colorless liquid. MS (EI) $[M+Na]^+$ m/z 457.7. ^{1}H NMR ($CDCl_3$): 7.0 (t, J = 6.8 Hz, 1H), 5.92 (d, J = 6.0 Hz, 1H), 4.88 (s, 1H), 4.55 (m, 1H), 4.50 (s, 1H), 4.22–4.16 (m, 2H), 3.48 (m, 1H), 3.33 (d, J = 11.2 Hz, 1H), 2.44–2.34 (m, 5H), 2.04–1.95 (m, 9H), 1.38–1.21 (m, 8H), 0.92 (t, J = 7.2 Hz, 3H), 0.66 (s, 3H). HRMS (ESI, m/z) calcd for $C_{25}H_{38}O_6Na$ 457.2561, found 457.2566.

4.5. 14-(*E*-2-Crotonoyl) andrographolide (7b)

A stirred solution of the *E*-2-butenoic acid (180 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 20 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and were then concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **7a** (100 mg, 42.59% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$): 7.05 (t, J = 6.8 Hz, 1H), 6.0 (d, J = 6.0 Hz, 1H), 5.8 (dd, J = 1.6, 14 Hz, 1H), 4.9 (s, 1H), 4.58 (dd, J = 4.8, 6.4 Hz, 1H), 4.55 (s, 1H), 4.26 (dd, J = 2.0, 9.2 Hz, 1H), 3.97 (d, J = 3.6 Hz, 1H), 3.5 (dd, J = 4.0, 4.8 Hz, 2H), 3.18 (d, J = 11.6 Hz, 1H), 2.5–2.40 (m, 5H), 2.04–1.95 (m, 2H), 1.88–1.6 (m, 6H), 1.74–1.54 (m, 3H), 1.42 (s, 3H), 1.38 (s, 3H), 1.21 (s, 3H), 0.94 (s, 3H). MS (EI) $[M+Na]^+$ m/z 481.7. Without further purification, compound **7a**

was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **7b** (37 mg, 40.54% yield) as a colorless liquid. MS (EI) $[M+Na]^+$ *m/z* 441.6. ^1H NMR ($CDCl_3$): 7.05 (t, *J* = 6.08 Hz, 1H), 5.99 (d, *J* = 6.0 Hz, 1H), 5.87 (dd, *J* = 1.6, 14 Hz, 1H), 4.88 (s, 1H), 4.58 (dd, *J* = 5.2, 6.0 Hz, 1H), 4.52 (s, 1H), 4.26 (dd, *J* = 2.0, 9.2 Hz, 1H), 4.18 (d, *J* = 11.2 Hz, 1H), 3.49 (m, 1H), 3.33 (d, *J* = 11.2 Hz, 1H), 2.51–2.34 (m, 3H), 1.25–1.10 (s, 7H), 0.9–0.8 (m, 5H), 0.67 (s, 3H). HRMS (ESI, *m/z*) calcd for $C_{24}H_{34}O_6Na$ 441.2253, found 441.2248.

4.6. 14-(*E*-3-Furan-2-yl-acryloyl) andrographolide (8b)

A stirred solution of the (*E*)-3-(furan-2-yl) acrylic acid (283 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 20 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **8a** (112.3 mg, 43% yield) as a yellow liquid. ^1H NMR ($CDCl_3$): 7.53 (s, 1H), 7.46 (d, *J* = 12.4 Hz, 1H), 7.06 (t, *J* = 5.6 Hz, 1H), 6.69 (d, *J* = 2.8 Hz, 1H), 6.51 (m, 1H), 6.3 (d, *J* = 12.8 Hz, 1H), 6.05 (d, *J* = 3.2 Hz, 1H), 4.89 (s, 1H), 4.66 (dd, *J* = 4.0, 4.8 Hz, 1H), 4.57 (s, 1H), 4.30 (dd, *J* = 1.2, 7.6 Hz, 1H), 3.95 (d, *J* = 9.2 Hz, 1H), 3.49 (m, 1H), 3.46 (s, 1H), 3.16 (d, *J* = 9.6 Hz, 1H), 2.52–2.39 (m, 3H), 2.04–1.68 (m, 8H), 1.39 (s, 3H), 1.36 (s, 3H), 1.20 (s, 3H), 1.31–1.23 (m, 5H), 0.92 (s, 3H). MS (EI) $[M+Na]^+$ *m/z* 533.6. Without further purification, compound **8a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **8b** (47.6 mg, 46% yield) as a yellow power. MS (EI) $[M+H]^+$ *m/z* 471.6. ^1H NMR ($CDCl_3$): 7.50 (s, 1H), 7.43 (d, *J* = 12.1 Hz, 1H), 7.01 (t, *J* = 5.8 Hz, 1H), 6.69 (d, *J* = 2.8 Hz, 1H), 6.49 (m, 1H), 6.25 (d, *J* = 12.3 Hz, 1H), 6.15 (d, *J* = 3.5 Hz, 1H), 4.92 (s, 1H), 4.61 (dd, *J* = 4.0, 4.8 Hz, 1H), 4.59 (s, 1H), 4.30 (dd, *J* = 1.2, 7.6 Hz, 1H), 3.98 (d, *J* = 9.2 Hz, 1H), 3.45 (s, 1H), 3.18 (d, *J* = 10 Hz, 1H), 2.07–1.65 (m, 6H), 1.26 (s, 3H), 1.35–1.20 (m, 7H), 0.89 (s, 3H). HRMS (ESI, *m/z*) calcd for $C_{23}H_{33}O_7$ 469.2206, found 469.2263.

4.7. 14-Adamantaneacetyl andrographolide (9b)

A stirred solution of the adamantaneacetic acid (400 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 20 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and were then concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **9a** (110 mg, 38% yield) as a colorless liquid. ^1H NMR ($CDCl_3$): 7.05 (t, *J* = 6.08 Hz, 1H), 5.90 (d, *J* = 6.0 Hz, 1H), 4.91 (s, 1H), 4.55 (m, *J* = 10 Hz, 1H), 4.45 (s, 1H), 4.19 (m, *J* = 3.6 Hz, 2H), 3.40 (t, *J* = 7.6 Hz, 2H), 3.25 (d, *J* = 10.8 Hz, 1H), 2.40–2.30 (m, 5H), 1.9–1.70 (m, 5H), 1.70–1.55 (m, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 0.92 (s, 3H). MS (EI) $[M+H]^+$ *m/z* 567.4. Without further purification, compound **9a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloro methane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **9b** (27 mg, 26.5% yield) as a colorless liquid. MS (EI) $[M+Na]^+$ *m/z* 549.6. ^1H NMR ($CDCl_3$): 7.03 (t, *J* = 6.11 Hz, 1H), 6.01 (d, *J* = 5.7 Hz, 1H), 4.85 (s, 1H), 4.54 (m, *J* = 9.8 Hz, 1H), 4.48 (s, 1H), 4.18 (m, *J* = 4.0 Hz, 2H), 3.42 (t, *J* = 7.3 Hz, 2H), 3.28 (d, *J* = 9.8 Hz, 1H), 2.48–2.30 (m, 5H), 2.0–1.95 (m, 1H), 1.88–1.74 (m, 4H), 1.74–1.54 (m, 3H), 0.64 (s, 3H). HRMS (ESI, *m/z*) calcd for $C_{32}H_{46}O_6Na$ 549.3192, found 549.3186.

4.8. 14-(*E*-3-Cyclohexylacryloyl) andrographolide (10b)

A stirred solution of the (*E*)-3-cyclohexylacrylic acid[29] (315 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg, 0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 24 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and were then concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **10a** (113 mg, 43% yield) as a colorless liquid. ^1H NMR ($CDCl_3$): 7.04 (t, *J* = 6.12 Hz, 1H), 6.0 (d, *J* = 4.8 Hz, 1H), 5.78 (dd, J = 1.2, 11.6 Hz, 1H), 4.89 (s, 1H), 4.60 (dd, *J* = 4.0, 4.8 Hz, 1H), 4.52 (s, 1H), 4.28 (dd, *J* = 1.6, 7.2 Hz, 1H), 4.0 (d, *J* = 9.6 Hz, 1H), 3.50 (m, 1H), 3.15 (d, *J* = 9.2 Hz, 1H), 2.48–2.42 (m, 4H), 1.43 (s, 3H), 1.38 (s, 3H), 1.32–1.27 (m, 9H), 1.22 (s, 3H), 0.93 (s, 3H). MS (EI) $[M+H]^+$ *m/z* 527.7. Without further purification, compound **10a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **10b** (51.2 mg, 49% yield) as a colorless liquid. MS (EI) $[M+H]^+$ *m/z* 487.6. ^1H NMR ($CDCl_3$): 7.01 (t, *J* = 6.10 Hz, 1H), 5.96 (d, *J* = 5.2 Hz, 1H), 5.77 (dd, *J* = 1.2, 11.6 Hz, 1H), 4.86 (s, 1H), 4.58 (dd, *J* = 4.0, 4.8 Hz, 1H), 4.49 (s, 1H), 4.26 (dd, *J* = 1.6, 7.2 Hz, 1H), 4.17 (d, *J* = 8.8 Hz, 1H), 3.47 (m, 1H), 3.31 (d, *J* = 8.8 Hz, 1H), 2.44–2.37 (m, 4H), 2.2–2.15 (m, 1H), 2.0–1.95 (m, 1H), 1.35–1.27 (m, 4H), 1.25 (s, 3H), 0.91–0.79 (m, 2H), 0.64 (s, 3H). HRMS (ESI, *m/z*) calcd for $C_{29}H_{41}O_6$ 485.2881, found 485.2903.

4.9. 14-(5-Cyclopentylvaleryl) andrographolide (11b)

A stirred solution of the 5-cyclopentylpentanoic acid[30] (350 mg, 2.0 mmol) and triethylamine (0.63 mL, 4.58 mmol) in dry dichloromethane (16 mL) was maintained at 0 °C under a nitrogen atmosphere. Ethyl chloroformate (0.33 mL, 3.36 mmol) was added and the reaction mixture was stirred for 1 h. Compound **2** (200 mg,

0.51 mmol) was added dropwise to the reaction mixture, which was stirred at room temperature for 24 h. After quenching with water (30 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (3× 10 mL). The combined organic extracts were dried with Na_2SO_4 and concentrated in vacuo. The product was purified by column chromatography using ethyl acetate–petroleum ether (1:2), affording compound **11a** (132 mg, 47.6% yield) as a colorless liquid. MS (EI) $[M+H]^+$ *m/z* 543.7. ^1H NMR ($CDCl_3$): 7.02 (t, *J* = 6.10 Hz, 1H), 5.96 (d, *J* = 5.2 Hz, 1H), 4.91 (s, 1H), 4.59–4.55 (m, 2H), 4.22 (dd, *J* = 1.6, 7.2 Hz, 1H), 3.97 (d, *J* = 9.2 Hz, 1H), 3.50 (m, 1H), 3.18 (d, *J* = 9.2 Hz, 1H), 2.45–2.36 (m, 4H), 2.25–2.14 (m, 3H), 2.07–1.97 (m, 3H), 1.87–1.82 (m, 4H), 1.71–1.60 (m, 7H), 1.32–1.22 (m, 7H), 1.38 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 0.92 (s, 3H). Without further purification, compound **11a** was added to a diluted acetic acid solution ($AcOH/H_2O$ = 7:3, 10 mL), and the solution was stirred at room temperature for 30 min. Water was added, and the solution was neutralized with $NaHCO_3$. The product was extracted with dichloromethane, and the organic phase was dried over Na_2SO_4 and was then filtered. The product was purified by column chromatography using ethyl acetate–petroleum ether (2:1), affording compound **11b** (61 mg, 50% yield) as a colorless liquid. MS (EI) $[M+H]^+$ *m/z* 503.6. ^1H NMR ($CDCl_3$): 7.03 (t, *J* = 5.20 Hz, 1H), 5.94 (d, *J* = 5.2 Hz, 1H), 4.90 (s, 1H), 4.58 (dd, *J* = 4.4, 4.8 Hz, 1H), 4.52 (s, 1H), 4.26 (dd, *J* = 1.6, 7.2 Hz, 1H), 4.20 (d, *J* = 8.8 Hz, 1H), 3.50 (m, 1H), 3.2 (d, *J* = 8.8 Hz, 1H), 2.45–2.36 (m, 4H), 2.25–2.14 (m, 3H), 2.07–1.97 (m, 3H), 1.87–1.82 (m, 4H), 1.71–1.60 (m, 7H), 1.27 (s, 3H), 0.68 (s, 3H). HRMS (ESI, *m/z*) calcd for $C_{30}H_{45}O_6$ 501.3199, found 501.3216.

4.10. Virulence factor assays

For assay of pyocyanin, cells were grown overnight in LB medium, washed in fresh media and diluted to an OD_{600} of 0.05. This culture was aliquoted to test tubes containing appropriate amount of test compounds. Following 24 h of growth, pyocyanin was extracted from the filtered culture supernatants, and was quantified using a reported methods.[31] For assay of protease activity, cells were grown overnight in PB media (20% Protease Peptone, 1.4% $MgCl_2$, 10% K_2SO_4) at 37 °C. Cells were washed, and diluted to an OD_{600} of 0.05. After growth reached midlog phase, cells were washed again, and resuspended to an OD_{600} of 0.05. This culture was then added to test tubes containing the test compounds, and was cultured for another 10 h. Protease activity was quantified by incubation of 100 μL of filtered culture supernatant with 5 mg azocasein substrate and 1 mL of 10 mM Tris (pH 7.2) and 1 mM of $CaCl_2$ for 4 h at 37 °C with agitation. Protease activity was represented by the OD_{440} of the enzyme assay following quenching with 1 mM NaOH and centrifugation to remove unreacted substrate, and was divided by the OD_{600} of the cell culture.[32]

Acknowledgements

This work was supported in part by grants from the China Natural Science Fund (30772642 and 30973618 to YW), The Guangdong Province (2008A030101007 to YW) and Guangzhou City (2008Z1-E691 to PY) Science and Technology Funds as well as the 211 Project of Jinan University. The authors thank Professor Lixin Zhang of the Institute of Microbiology, Chinese Academy of Sciences for his critical and valuable comments and Ms. Linda Wang of Yale University for her proofreading the manuscript.

References and notes

1. Fuqua, C.; Parsek, M. R.; Greenberg, E. P. *Annu. Rev. Genet.* **2001**, *35*, 439.
2. Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319.
3. Whitehead, N. A.; Barnard, A. M.; Slater, H.; Simpson, N. J.; Salmond, G. P. *FEMS Microbiol. Rev.* **2001**, *25*, 365.
4. Withers, H.; Swift, S.; Williams, P. *Curr. Opin. Microbiol.* **2001**, *4*, 186.
5. Greenberg, E. P. *Nature* **2003**, *424*, 134.
6. Lyon, G. J.; Muir, T. W. *Chem. Biol.* **2003**, *10*, 1007.
7. Gonzalez, J. E.; Keshavan, N. D. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 859.
8. Fuqua, C. *J. Bacteriol.* **2006**, *188*, 3169.
9. Venturi, V. *FEMS Microbiol. Rev.* **2006**, *30*, 274.
10. Schuster, M.; Greenberg, E. P. *Int. J. Med. Microbiol.* **2006**, *296*, 73.
11. Fuqua, C.; Greenberg, E. P. *Nat. Rev. Microbiol.* **2002**, *3*, 685.
12. Williams, P. *Microbiology* **2007**, *153*, 3923.
13. Chakravarti, D.; Chakravarti, R. N. *J. Chem. Soc.* **1952**, 1697.
14. Mishra, S. K.; Sangwan, N. S.; Sangwan, R. S. *Pharmacol. Rev.* **2007**, *1*, 283.
15. Wang, T.; Liu, B.; Zhang, W.; Wilson, B.; Hong, J. S. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 975.
16. Xia, Y. F.; Ye, B. Q.; Li, Y. D.; Wang, J. G.; He, X. J.; Lin, X. *J. Immunol.* **2004**, *173*, 4207.
17. Iruretagoyena, M. I.; Tobar, J. A.; González, P. A. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 366.
18. Li, H. T.; Tan, H. M.; Wang, W. H. *Chin. J. Chin. Mater. Med.* **2006**, *12*, 1015.
19. Jiang, X. J.; Yu, P.; Jiang, J.; Wang, Y. Q. *Eur. J. Med. Chem.* **2009**, *44*, 2936.
20. Zeng, X. P.; Wang, Y. Q. Antimicrob. Agents Chemother., submitted for publication.
21. Calabrese, C.; Berman, S. H.; Babish, J. G.; Ma, X.; Shinto, L.; Dorr, M.; Wells, K.; Wenner, C. A.; Standish, L. J. *Phytother. Res.* **2000**, *14*, 333.
22. Chen, J. X.; Xue, H. J.; Wang, Y. Q. *Biol. Pharm. Bull.* **2009**, *8*, 1385.
23. Dong, Y. H.; Wang, L. H.; Xu, J. L.; Zhang, H. B.; Zhang, X. F.; Zhang, L. H. *Nature* **2001**, *411*, 813.
24. Bianchi, S. M.; Prince, L. R.; McPhillips, K.; Allen, L.; Marriott, H. M.; Taylor, G. W.; Hellewell, P. G.; Sabroe, I.; Dockrell, D. H.; Henson, P. W.; Whyte, M. K. *Am. J. Respir. Crit. Care Med.* **2008**, *177*, 35.
25. Wilson, R.; Sykes, D. A.; Watson, D.; Rutman, A.; Taylor, G. W.; Cole, P. J. *Infect. Immun.* **1988**, *56*, 2515.
26. Lau, G. W.; Hassett, D. J.; Ran, H.; Kong, F. *Trends Mol. Med.* **2004**, *10*, 599.
27. Zhang, Z. J.; Jiang, J.; Yu, P.; Zeng, X. P.; Wang, Y. Q. *J. Transl. Med.* **2009**, *7*, 62.
28. Nanduri, S.; Nyavanandi, V. K.; Thunuguntla, S. S.; Kasu, S.; Pallerla, M. K.; Ram, P. S.; Rajagopal, S.; Kumar, R. A.; Ramanujam, R.; Babu, J. M.; Vyas, K.; Devi, A. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4711.
29. Alhamadsheh, M. M.; Palaniappan, N.; Chouduri, S. D.; Reynolds, K. A. *J. Am. Chem. Soc.* **2007**, *129*, 1910.
30. Maria, K.; Theodora, C.; Anastasia, D. *J. Med. Chem.* **2001**, *44*, 4300.
31. Essar, D. W.; Eberly, L.; Hadero, A.; Crawford, I. P. *J. Bacteriol.* **1990**, *172*, 884.
32. Hentzer, M.; Riedel, K.; Rasmussen, T. B.; Heydorn, A.; Andersen, J. B.; Parsek, M. R.; Rice, S. A.; Eberl, L.; Molin, S.; Hiby, N.; Kjelleberg, S.; Givskov, M. *Microbiology* **2002**, *148*, 87.