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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A structure–activity relationship study of phenyl sesquiterpenoids on efflux inhibition against *Staphylococcus aureus*

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Abstract

Sixteen natural acetophenone sesquiterpene derivatives were isolated from *Ferula ferulioides* and 11 synthetic acetophenone sesquiterpene analogues were evaluated to explore the structure–activity relationships (SARs) on their antibacterial activities against a panel of bacteria including drug-resistant *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values of the compounds were in the range of 0.5–128 mg/L. Preliminary SAR studies showed that subtle modifications on both the 2',4'-dihydroxyphenyl moiety and the side chain reduced their activity against drug-resistant *S. aureus*. All of the compounds that showed no or only weak direct antibacterial activity were tested for their efflux inhibitory effects, among which four compounds showed significant efflux inhibition against drug-resistant strains. Natural product **14** showed significant inhibitory effects for EtBr efflux in strain SA1199B, which has reduced susceptibility to fluoroquinolones by efflux. Compounds **5**, **14** and **F-3** moderately inhibited EtBr efflux in the macrolide-resistant strain RN4220 and compound **13** moderately inhibited efflux in an MRSA and effluxing tetracycline-resistant strain.

Keywords *Ferula ferulioides* · Acetophenone derivatives · Structure–activity relationships · Drug-resistant *Staphylococcus aureus* · Efflux pump inhibition

Introduction

Staphylococcus aureus is a commensal organism that is commonly cited as being a major hospital-acquired pathogen (Gibbons 2004; Sakoulas et al. 2012). Its capacity to acquire resistance to many different classes of antibacterial agents, such as β-lactams, quinolones and macrolides, is a significant issue that seriously impacts human health (Foster 2017). The number of new antimicrobial drugs has decreased over the past few decades (Sun et al. 2016) and the design and discovery of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today (Eom et al. 2016; Grare et al. 2007). A common mechanism of antibiotic resistance in *S. aureus* is efflux and it is a feasible strategy to search for efflux pump inhibitors to combat antibiotic resistance (Schindler et al. 2013; Agyei et al. 2014; Wang et al. 2016; Jamshidi et al. 2016).

Ferula ferulioides is distributed in western China and has been reported to contain a series of sesquiterpene coumarins (Isaka et al. 2001; Kojima et al. 2000; Meng et al. 2013; Liu et al. 2015) and acetophenone derivatives (Liu et al. 2015; Kojima et al. 1999; Kojima et al. 1999; Motai and Kitanaka 2005; Meng et al. 2013; Kojima et al. 1998). In terms of pharmacological research, these natural products have been found to possess many biological effects including cytotoxic activities against a panel of cancer cell lines (Meng et al. 2013), inhibition of nitric oxide production (Motai and Kitanaka 2005) and pesticidal activity (Liu et al. 2013).

In our screening for new antibacterial agents, an extract of *F. ferulioides* was found to possess antibacterial activity against a panel of bacteria including drug-resistant *Staphylococcus aureus*. Our preliminary research resulted in the isolation of two new antibacterial acetophenone sesquiterpene derivatives that inhibit efflux pumps of *S. aureus* strains (Liu et al. 2013). This was the first discovery of this class of natural product possessing efflux pump-inhibitory activity against drug-resistant *S. aureus*. In an extensive

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investigation of this type of natural compound, four further new acetophenone derivatives together with ten known acetophenones were isolated and shown to demonstrate noteworthy antibacterial activities against drug-resistant *S. aureus* (Liu et al. 2015). In the current work, 11 synthetic acetophenone sesquiterpene analogues together with 16 natural acetophenone sesquiterpene derivatives isolated from *F. ferulioides* were obtained to explore the structure–activity relationships (SARs) of this class. The natural and synthetic compounds, **1**–**6**, **13**, **14**, **F-2**–**F-4**, **F-6**–**F-11**, which showed no or weak direct antibacterial activity were tested for their inhibitory effects of efflux in five different drug-resistant strains.

Materials and methods

General experimental instruments and materials

All reagents used were of analytical quality. Column chromatography (CC) was performed on silica gel (300–400 mesh, Qingdao Marine Chemical Plant) and Sephadex LH-20 (GE Healthcare). Analytical thin-layer chromatography (TLC) was performed on precoated silica gel F254 (Qingdao Marine Chemical Plant). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Varian Mercury Plus 400 MHz spectrometer. Electrospray ionization (ESI)-mass spectrometry (MS) spectra were measured with an Agilent 5973N MSD mass spectrometer. Optical density was recorded on a Multiskan FC instrument (Thermo Co. Ltd, USA), and fluorescence was recorded on a Tecan M 1000 plate reader (Tecan Co. Ltd., Switzerland).

Chemistry

The isolation and identification of the 16 natural acetophenone sesquiterpene derivatives isolated from *F. ferulioides* were reported in our previous publication (Liu et al. 2015).

Procedure for the synthesis of compounds F-2 and F-3

A mixture of compound **5** (**F-1**) (800 mg, 2.24 mmol dissolved in 20 mL acetone) and potassium carbonate (3.08 g, 10 equiv. suspended in 80 mL acetone) were stirred for 2 h at room temperature. Me_2SO_4 (2 equiv.) was then added and the reaction was monitored by TLC. The solution was then filtered and concentrated under vacuum after the reaction was complete. The residue was dissolved in ethyl acetate (30 mL), washed with water and dried with anhydrous sodium sulfate. The products were purified by silica gel CC to give compounds **F-2** (24.1%) and **F-3** (76.5%).

Compound F-2

Yellow resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65 (1H, d, $J = 9.5$ Hz, H-6'), 6.44 (1H, dd, $J = 2.5$, 7.3 Hz, H-5'), 6.42 (1H, d, $J = 2.5$ Hz, H-3'), 5.17 (1H, t, $J = 7.2$ Hz, H-4), 5.09 (2H, t, overlapping, H-8, 12), 3.85 (3H, s, 4'-OCH_3), 2.92 (2H, t, $J = 7.6$ Hz, H-2), 2.42 (2H, q, $J = 7.3$ Hz, H-3), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 13-CH_3), 1.59 (3H, s, 9-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 204.6 (C, C-1), 165.9 (C, C-4'), 165.4 (C, C-2'), 136.8 (C, C-5), 135.1 (C, C-9), 131.6 (CH, C-6'), 131.3 (C, C-13), 124.4 (CH, C-12), 124.0 (CH, C-8), 122.4 (CH, C-4), 113.5 (C, C-1'), 107.5 (CH, C-5'), 100.9 (CH, C-3'), 55.5 (4'-OCH_3), 39.7 (CH_2, C-6), 39.6 (CH_2, C-10), 38.1 (CH_2, C-2), 26.8 (CH_2, C-11), 26.5 (CH_2, C-7), 25.7 (CH_3, C-14), 23.2 (CH_2, C-3), 17.7 (13-CH_3), 16.1 (CH_3, 5-CH_3), 16.0 (CH_3, 9-CH_3); EIMS *m/z* 370 $[M]^+$ ($C_{24}H_{34}O_3$).

Compound F-3

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.79 (1H, d, $J = 8.6$ Hz, H-6'), 6.51 (1H, dd, $J = 2.0$, 8.6 Hz, H-5'), 6.43 (1H, d, $J = 2.4$ Hz, H-3'), 5.18 (1H, t, $J = 7.0$ Hz, H-4), 5.09 (2H, t, $J = 6.7$ Hz, H-8, 12), 3.88 (3H, s, 4'-OCH_3), 3.85 (3H, s, 2'-OCH_3), 2.97 (2H, t, $J = 7.4$ Hz, H-2), 2.38 (2H, q, $J = 7.5$ Hz, H-3), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 13-CH_3), 1.59 (3H, s, 9-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 200.6 (C, C-1), 164.4 (C, C-2'), 160.9 (C, C-4'), 135.8 (C, C-5), 135.1 (C, C-9), 132.9 (CH, C-6'), 131.5 (C, C-13), 124.6 (CH, C-12), 124.4 (CH, C-8), 123.8 (CH, C-4), 121.6 (C, C-1'), 105.2 (CH, C-5'), 98.9 (CH, C-3'), 55.8 (4'-OCH_3), 55.7 (2'-OCH_3), 44.0 (CH_2, C-2), 40.0 ($CH_2 \times 2$, C-6, 10), 27.0 (CH_2, C-7), 26.8 (CH_2, C-11), 25.9 (CH_3, C-14), 23.5 (CH_2, C-3), 17.9 (CH_3, 13-CH_3), 16.3 (CH_3, 5-CH_3), 16.2 (CH_3, 9-CH_3); ESI *m/z* 385.3 $[M+1]^+$ ($C_{25}H_{36}O_3$).

Procedure for the synthesis of compounds F-4 and F-5

Compound **F-1** (200 mg, 0.56 mmol), acetic anhydride (21.2 mL, 22.4 mmol) and sodium acetate (0.28 g, 3.36 mmol) were mixed and refluxed for 6 h. Methanol and sodium bicarbonate were added after the reaction was complete. The solution was then filtered and concentrated under vacuum and the residue was dissolved in ethyl acetate (30 mL), washed with water and dried with anhydrous

sodium sulfate. The products were purified by silica gel CC to give compounds **F-4** (63.6%) and **F**-5 (17.5%).

Compound F-4

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.79 (1H, d, $J = 8.8$ Hz, H-6'), 6.72 (1H, d, $J = 2.0$ Hz, H-3'), 6.67 (1H, dd, $J = 2.2$, 8.6 Hz, H-5'), 5.18 (1H, t, $J = 7.1$ Hz, H-4), 5.09 (2H, t, $J = 6.8$ Hz, H-8, 12), 2.97 (2H, t, $J = 7.6$ Hz, H-2), 2.43 (2H, q, $J = 7.3$ Hz, H-3), 2.31 (3H, s, 4'-OAc), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 9-CH_3), 1.60 (3H, s, 13-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 205.4 (C, C-1), 168.5 (C, 4'-OAc), 164.0 (C, C-2'), 156.3 (C, C-4'), 137.0 (C, C-5), 135.1 (C, C-9), 131.3 (C, C-13), 131.2 (CH, C-6'), 124.3 (CH, C-4), 124.0 (CH, C-8), 122.1 (CH, C-12), 117.4 (C, C-1'), 112.8 (CH, C-5'), 111.2 (CH, C-3'), 39.7 (CH_2, C-6), 39.6 (CH_2, C-10), 38.5 (CH_2, C-2), 26.7 (CH_2, C-7), 26.5 (CH_2, CH_2-11), 25.7 (CH_3, C-14), 22.8 (CH_2, C-3), 21.2 (CH_3, 4'-OAc), 17.7 (CH_3, 13-CH_3), 16.1 (CH_3, 5-CH_3),16.0 (CH_3, 9-CH_3); ESI *m/z* 399.1 $[M+1]^+$ ($C_{25}H_{34}O_4$).

Compound F-5

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.79 (1H, d, $J = 8.3$ Hz, H-6'), 7.08 (1H, dd, $J = 2.4$, 8.7 Hz, H-5'), 6.96 (1H, d, $J = 2.0$ Hz, H-3'), 5.10 (3H, overlapping, H-4, 8, 12), 2.89 (2H, t, $J = 7.5$ Hz, H-2), 2.38 (2H, q, $J = 7.3$ Hz, H-3), 2.32 (3H, s, 2'-OAc), 2.30 (3H, s, 4'-OAc), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 9-CH_3), 1.60 (3H, s, 13-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 198.7 (C, C-1), 169.1 (C, 4'-OAc), 168.4 (C, 2'-OAc), 153.7 (C, C-4'), 149.8 (C, C-2'), 136.7 (C, C-5), 135.1 (C, C-9), 131.3 (C, C-13), 130.8 (CH, C-6'), 128.2 (C, C-1'), 124.3 (CH, C-4), 124.2 (CH, C-8), 122.4 (CH, C-12), 119.1 (CH, C-5'), 117.4 (CH, C-3'), 41.4 (CH_2, C-2), 39.7 ($CH_2 \times 2$, C-6, 10), 26.7 (CH_2, C-7), 26.5 (CH_2, C-11), 25.7 (CH_3, C-14), 22.6 (CH_2, C-3), 21.1 ($CH_3 \times 2$, 2', 4'-OAc), 17.7 (CH_3, 13-CH_3), 16.0 ($CH_3 \times 2$, 5, 9-CH_3); ESI *m/z* 441.1 $[M+1]^+$ ($C_{27}H_{36}O_5$).

Procedure for the synthesis of compounds F-6 and F-7

Compound **F-1** (200 mg, 0.56 mmol) was dissolved in acetone (5 mL) and mixed with the potassium carbonate (0.77 g, 10 equiv.) suspended in acetone (20 mL) and stirred at room temperature for 2 h. Iodoethane (3 equiv.) was then added and the reaction was monitored over 10 h by TLC. The solution was filtered and concentrated under vacuum after the reaction was complete. The residue was dissolved in ethyl acetate (30 mL), washed with water and dried with anhydrous sodium sulfate. The products were purified by silica gel CC to give compounds **F-6** (132 mg, 61.2%) and **F-7** (75 mg, 32.4%).

Compound F-6

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65 (1H, d, $J = 8.6$ Hz, H-6'), 6.41 (1H, dd, $J = 2.5$, 8.6 Hz, H-5'), 6.44 (1H, d, $J = 2.3$ Hz, H-3'), 5.17 (1H, t, $J = 6.3$ Hz, H-4), 5.09 (2H, m, H-8, 12), 4.08 (2H, q, $J = 7.1$, 4'-OCH_2CH_3), 2.92 (2H, t, $J = 7.4$ Hz, H-2), 2.42 (2H, q, $J = 7.3$ Hz, H-3), 1.98 (4H, m, H-6, 10), 2.06 (4H, m, H-7, 11), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 13-CH_3), 1.59 (3H, s, 9-CH_3), 1.43 (3H, t, $J = 7.1$, 4'-OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 204.5 (C, C-1), 165.3 ($C \times 2$, C-2', 4'), 136.7 (C, C-5), 135.1 (C, C-9), 131.6 (C, C-13), 131.3 (CH, C-6'), 124.3 (CH, C-4), 124.0 (CH, C-8), 122.4 (CH, C-12), 113.4 (C, C-1'), 107.9 (CH, C-5'), 101.3 (CH, C-3'), 63.9 (CH_2, 4'-OCH_2CH_3), 39.7 (CH_2, C-6), 39.6 (CH_2, C-10), 38.0 (CH_2, C-2), 26.7 (CH_2, C-7), 26.5 (CH_2, C-11), 25.7 (CH_3, C-14), 23.2 (CH_2, C-3), 17.7 (CH_3, 13-CH_3), 16.0 ($CH_3 \times 2$, 5, 9-CH_3), 14.5 (CH_3, 4'-OCH_2CH_3); ESI *m/z* 385.1 $[M+1]^+$ ($C_{25}H_{36}O_3$).

Compound F-7

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.78 (1H, d, $J = 8.6$ Hz, H-6'), 6.48 (1H, dd, $J = 2.0$, 8.6 Hz, H-5'), 6.41 (1H, d, $J = 2.0$ Hz, H-3'), 5.17 (1H, t, $J = 6.9$ Hz, H-4), 5.09 (2H, t, $J = 6.9$ Hz, H-8, 12), 4.08 (4H, q, $J = 7.0$, 2', 4'-OCH_2CH_3), 2.98 (2H, t, $J = 7.6$ Hz, H-2), 2.36 (2H, q, $J = 7.4$ Hz, H-3), 1.98 (4H, m, H-6, 10), 2.06 (4H, m, H-7, 11), 1.68 (3H, s, H-14), 1.62 (3H, s, 5-CH_3), 1.59 (3H, s, 9-CH_3), 1.60 (3H, s, 13-CH_3), 1.44 (3H, t, $J = 7.0$, 4'-OCH_2CH_3), 1.43 (3H, t, $J = 7.0$, 2'-OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 200.6 (C, C-1), 163.5 (C, C-4'), 160.0 (C, C-2'), 135.6 (C, C-5), 134.9 (C, C-9), 132.6 (C, C-13), 131.3 (CH, C-6'), 124.3 (CH, C-4), 124.2 (CH, C-8), 123.5 (CH, C-12), 121.2 (C, C-1'), 105.3 (CH, C-5'), 99.3(CH, C-3'), 64.0 (CH_2, 4'-OCH_2CH_3), 63.7 (CH_2, 2'-OCH_2CH_3), 43.9 (CH_2, C-2), 39.7 ($CH_2 \times 2$, C-6, 10), 26.7 (CH_2, C-7), 26.6 (CH_2, C-11), 25.7 (CH_3, C-14), 23.3 (CH_2, C-3), 17.7 (CH_3, 13-CH_3), 16.0 ($CH_3 \times 2$, 5, 9-CH_3), 14.7 ($CH_3 \times 2$, 2', 4'-OCH_2CH_3); ESI *m/z* 413.2 $[M+1]^+$ ($C_{27}H_{40}O_3$).

Procedure for the synthesis of compound F-8 and F-9

Compound **F-1** (200 mg, 0.56 mmol) was dissolved in 10 mL acetonitrile and mixed with dry potassium carbonate (0.15 g, 1.12 mmol) and stirred for 1 h at room temperature. Benzyl bromide (133 µL, 1.12 mmol) was then added to the mixture, which was then refluxed for 3 h. The solution was

filtered and concentrated after the reaction was complete. The residue was dissolved in ethyl acetate (30 mL), washed with water and dried with anhydrous sodium sulfate. The products were purified by silica gel CC to give compounds **F-8** (220 mg, 87.8%) and **F-9** (30 mg, 10%).

Compound F-8

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65 (1H, d, $J = 9.5$ Hz, H-6'), 7.2–7.5 (5H, H-3"-7"), 6.48 (2H, m, H-3', 5'), 5.17 (1H, t, $J = 7.3$ Hz, H-4), 5.09 (2H, m, H-8, 12), 5.02 (2H, s, H-1"), 2.95 (2H, t, $J = 7.5$ Hz, H-2), 2.42 (2H, t, $J = 7.5$ Hz, H-3), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.60 (3H, s, 13-CH_3), 1.59 (3H, s, 9-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 204.6 (C, C-1), 165.2 (C, C-4'), 164.9 (C, C-2'), 136.8 (C, C-2"), 135.8 (C, C-5), 135.1 (C, C-9), 131.7 (C, C-13), 131.3 (C, C-6'), 128.7 (C × 2, C-4", 6"), 128.3 (C, C-5"), 127.5 (C × 2, C-3", 7"), 124.3 (C, C-4), 124.0 (C, C-8), 122.3 (C, C-12), 113.7 (C, C-1'), 108.0 (C, C-5'), 101.9 (C, C-3'), 70.2 (C, C-1"), 39.7 (C, C-6), 39.6 (C, C-10), 38.1 (C, C-2), 26.7 (C, C-7), 26.5 (C, C-11), 25.7 (C, C-14), 23.2 (C, C-3), 18.0 (CH_3, 13-CH_3), 16.1 (CH_3, 5-CH_3), 16.0 (CH_3, 9-CH_3); ESI *m/z* 447.1 $[M+1]^+$ ($C_{30}H_{38}O_3$).

Compound F-9

White solid; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65(1H, d, $J =$ 8.4 Hz, H-6'), 7.2–7.5 (10H, H-3"-7", 3"'-7"'), 6.59 (1H, d, $J = 2.2$ Hz, H-3'), 6.57 (1H, dd, $J = 2.2$, 8.6 Hz, H-5'), 5.09 (4H, m, H-8, 12, 1"'), 5.02 (2H, s, H-1"), 5.01 (1H, t, $J =$ 7.2 Hz, H-4), 2.95 (2H, t, $J = 7.6$ Hz, H-2), 2.42 (2H, t, $J =$ 7.3 Hz, H-3), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.63 (3H, s, 5-CH_3), 1.59 (3H, s, 9-CH_3), 1.60 (3H, s, 13-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 200.4 (C, C-1), 163.2 (C, C-4'), 159.6 (C, C-2'), 136.1 (C, C-4"), 136.0 (C, C-2"), 135.7 (C, C-5), 134.9 (C, C-9), 132.7 (C, C-13), 131.3 (C, C-6'), 128.7 (C × 2, C-4", 4"'), 128.7 (C × 2, C-6", 6"'), 128.3 (C, C-5"), 128.2 (C, C-5"'), 127.6 (C × 4, C-3", 7", 3"', 7"'), 124.3 (C, C-4), 123.2 (C, C-8), 121.7 (C, C-12), 110.0 (C, C-1'), 106.2 (C, C-5'), 100.3 (C, C-3'), 70.7 (C, C-1"), 70.2 (C, C-1"'), 44.0 (C, C-6), 39.7 (C × 2, C-2, 10), 26.7 (C, C-7), 26.6 (C, C-11), 25.7 (C, C-14), 23.1 (C, C-3), 17.7 (CH_3, 13-CH_3), 16.0 (CH_3 × 2, 5, 9-CH_3); ESI *m/z* 537.1 $[M+1]^+$ ($C_{37}H_{44}O_3$).

Procedure for the synthesis of compound F-10

Compound **F-1** (200 mg, 0.56 mmol) was dissolved in methanol (5 mL), and then palladium carbon (1.1 equiv.) was added and hydrogen was piped in to the reaction vessel. The reaction was carried out at room temperature for 6 h. The solution was filtered and vacuum concentrated after the reaction was complete. The products were purified by silica gel CC to give compound **F-10** (196 mg, 96.6%).

Compound F-10

Colorless resin; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65 (1H, d, $J = 9.4$ Hz, H-6'), 6.38–6.42 (2H, overlapping, H-3', 5'), 2.89 (2H, t, $J = 7.4$ Hz, H-2), 2.20 (3H, H-5, 9, 13), 1.00–1.80 (16H, H-3, 4, 6, 7, 8, 10, 11, 12), 0.80–0.90 (12H, CH3 × 4); ^{13}C NMR ($CDCl_3$, 100 MHz): 205.3 (C, C-1), 165.2 (C, C-2'), 162.3 (C, C-4'), 132.4 (C, C-6'), 113.9 (C, C-1'), 107.6 (C, C-5'), 103.6 (C, C-3'), 39.4 (C, C-2), 36.9–37.6 (C × 4, C-4, 6, 8, 10), 31.2 (C, C-12), 28.2 (C, C-9), 25.0 (C, C-5), 24.7 (C, C-13), 23.0 (C, C-7), 22.9 (C, C-11), 22.7 (C, C-3), 20.0 (C, C-14), 19.9 (CH_3, 13-CH_3), 19.8 (CH_3 × 2, 5, 9-CH_3); ESI *m/z* 363.3 $[M+1]^+$ ($C_{23}H_{38}O_3$).

Procedure for the synthesis of compound F-11

Compound **11** (80 mg, 0.2 mmol) was dissolved in methanol (5 mL) and then palladium carbon (1.1 equiv.) was added and hydrogen was piped in to the reaction vessel. The reaction was carried out at room temperature for 6 h. The solution was filtered and vacuum concentrated after the reaction was complete. The products were purified by silica gel CC to give compound **F-11** (76 mg, 94.1%).

Compound F-11

White solid; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.65 (1H, d, $J =$ 8.6 Hz, H-6'), 6.30–6.42 (2H, overlapping, H-3', 5'), 4.26 (1H, d, $J = 11.7$ Hz, H-3), 3.18 (1H, m, H-4), 2.20 (2H, H-4", 8"), 1.00-1.80 (12H, H-1", 2", 3", 5", 6", 7"), 1.36 (3H, s, 5-CH_3), 1.09 (3H, d, 6.0, 4-CH_3), 0.80–0.90 (9H, 4"-CH_3, 8"-CH_3 × 2); ^{13}C NMR ($CDCl_3$, 100 MHz): 195.9 (C, C-7'), 172.0 (C, C-2), 166.2 (C, C-2'), 164.0 (C, C-4'), 133.7 (C, C-6'), 114.3 (C, C-1'), 108.7 (C, C-5'), 103.7(C, C-3'), 88.8 (C, C-5), 54.8 (C, C-3), 41.5 (C × 2, C-4, 7"), 40.4 (C, C-1"), 39.5 (C, C-3"), 37.4 (C, C-5"), 32.8 (C, C-4"), 28.1 (C, C-8"), 24.9 (C, C-6"), 22.9 (CH_3, 8-CH_3), 22.8 (C, C-9"), 21.3 (CH_3, 8"-CH_3), 20.7 (C, C-2"), 19.8 (CH_3, 4"-CH_3), 13.7 (CH_3, 4-CH_3); ESI *m/z* 405.2 $[M+1]^+$ ($C_{24}H_{36}O_5$).

Procedure for the synthesis of compound F-12

Hydroxylamine hydrochloride (27.6 mg, 2 equiv.) and sodium acetate (32.8 mg, 2 equiv.) dissolved in 1 mL of water were added to an **F-1** solution in methanol (5 mL) (71.2 mg, 0.2 mmol). The mixture was heated and refluxed for 48 h and the reaction was monitored by TLC. Following concentration, the product was washed with water and

extracted with dichloromethane. The solution was filtered, vacuum concentrated and purified by silica gel CC to give compound **F-12** (70 mg, 94.3%).

Compound F-12

White solid; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.31 (1H, d, J = 8.3 Hz, H-6'), 6.44 (1H, d, J = 2.5 Hz, H-3'), 6.41 (1H, dd, J = 2.6, 8.0 Hz, H-5'), 5.21 (1H, t, J = 7.3 Hz, H-4), 5.09 (2H, t, overlapping, H-8, 12), 2.92 (2H, t, J = 8.0 Hz, H-2), 2.32 (2H, q, J = 7.8 Hz, H-3), 2.06 (4H, m, H-7, 11), 1.98 (4H, m, H-6, 10), 1.68 (3H, s, H-14), 1.60 (6H, s, 5, 13-CH_3), 1.59 (3H, s, 9-CH_3); ^{13}C NMR ($CDCl_3$, 1 00 MHz): 162.9 (C, C-2'), 159.8 (C, C-4'), 157.9 (C, C-1), 136.9 (C, C-5), 135.1 (C, C-9). 131.3 (C, C-13), 129.2 (C, C-6'), 124.3 (C, C-4), 124.0 (C, C-8), 122.5 (C, C-12), 111.0 (C, C-1'), 107.0 (C, C-5'), 103.9 (C, C-3'), 39.7 (C, C-6), 39.6 (C, C-10), 32.0 (C, C-2), 26.7 (C, C-7), 26.5 (C, C-11), 25.7 (C, C-14), 25.0 (C, C-3), 17.7 (CH_3, 13-CH_3), 16.0 (CH_3, 5-CH_3), 16.0 (CH_3, 9-CH_3); ESI m/z 372.2 $[M+1]^+$ ($C_{23}H_{33}NO_3$).

Antibacterial minimum inhibitory concentration (MIC) assay

All of the natural acetophenones derivatives isolated from *F. ferulioides* were tested first for their ability to directly inhibit the growth a panel of bacteria including effluxing and a methicillin-resistant *S. aureus* strain. Of the six *S. aureus* strains employed, five were antibiotic resistant (SA1199B, XU212, RN4220, EMRSA-15, EMRSA-16) and one laboratory standard strain (ATCC25923). Among the tested strains, SA1199B possesses the NorA efflux protein, which confers resistance to certain fluoroquinolones and quaternary ammonium antiseptics (Kaatz et al. 1993). Strain XU212 possesses the TetK efflux transporter and is resistant to both tetracycline and methicillin (Gibbons and Udo 2000). Strain RN4220, encodes for the MsrA macrolide efflux protein and is resistant to the macrolide antibiotic erythromycin (Ross et al. 1989). EMRSA-15 and EMRSA-16, commonly occurring epidemic methicillin-resistant *S. aureus* (MRSA) strains, were also used and were the generous gift of Dr Paul Stapleton (Richardson and Reith 1993).

MICs of the compounds were evaluated according to Clinical Laboratory Standards Institute guidelines (CLSI 2015) with minor modifications. Mueller–Hinton broth (MHB; Oxoid) was cation adjusted to contain 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively. The bacterial suspensions were adjusted to 5×10^5 cfu/mL. Dimethyl sulfoxide 2% in broth served as the negative control and vancomycin (Sigma Chemical Co. Ltd) served as the positive control. Twenty microlitres of 5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Biosharp) solution was added into each well after incubation at 37 °C for 18 h. The minimum concentration that completely inhibited the growth of bacteria was recorded as the MIC value of the compound against the strains. Both controls and treatments were tested in duplicate.

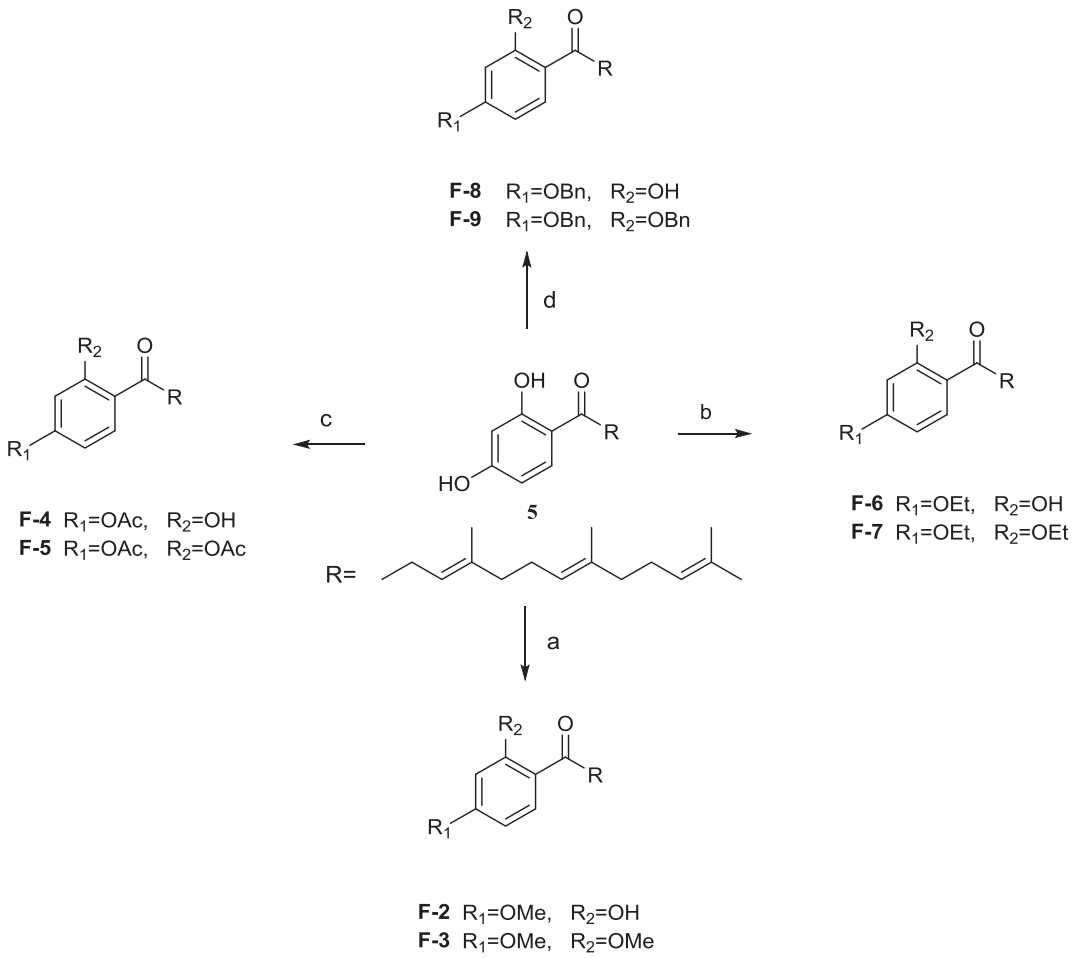
EtBr efflux assay

An EtBr efflux assay was carried out for 20 compounds to explore if they had indirect effects on antibiotic-resistant *S. aureus* strains (Lechner et al. 2008). Efflux activity of five strains (SA119B, XU212, RN4220, EMRSA-15 and EMRSA-16) was determined by measuring accumulation of the fluorescent dye EtBr (Sigma; 25 µmol/L) in the absence or presence of known efflux inhibitors CCCP (100 µmol/L) and in the absence or presence of those compounds (MIC > 128 mg/L: 100 µmol/L; MIC < 128 mg/L: 1/4 MIC) revealed to have activity in the bioassay and MIC tests. Fluorescence of the suspension was monitored continuously (excitation and emission wavelengths were 530 and 600 nm, respectively; slit width was 5 nm) every 5 min for 1 h. All tested compounds and the control were measured in triplicate.

Results and discussion

Chemistry

We previously reported the isolation of two new antibacterial acetophenone sesquiterpene derivatives that inhibited efflux of antibiotic-resistant *S. aureus* strains. In the present study, 11 synthetic acetophenone sesquiterpene analogues (**F-2**–**F-12**) together with 16 related natural products (**1**–**16**) isolated from *F. ferulioides* were obtained to explore the SARs of this class of compounds. In order to disclose the function of 2'-OH and 4'-OH moieties (Scheme 1), eight acetophenone derivatives (**F-2**–**F-9**) were obtained by alkylation or acylation of the natural product **5** (Peggy et al. 2008; Babu et al. 2005; Gabriel et al. 2011). To reveal the effect of the presence of unsaturation of the side chain on the antibacterial activities, compounds **F-10** and **F-11** were provided by catalytic hydrogenation of natural compounds **5** and **11**, respectively (Scheme 2). The oxime **F-12** was furnished by condensation of compound **5** with hydroxylamine hydrochloride to study the effect of the ketone on activity (Scheme 2). All structures were confirmed using spectroscopic methods (^{1}H-NMR, ^{13}C-NMR and MS). The structures of all isolated and synthesized compounds are depicted in Fig. 1.



(a) Acetone, K_2CO_3, Dimethyl sulfate, rt, 4 h; (b) Acetone, K_2CO_3, C_2H_5I, rt, 10 h; (c) Acetic anhydride, NaAc, reflux, 6 h; (d) acetonitrile, K_2CO_3, BnBr, reflux, 3 h;

Scheme 1 Syntheses of the compounds (**F-2**–**F-9**)

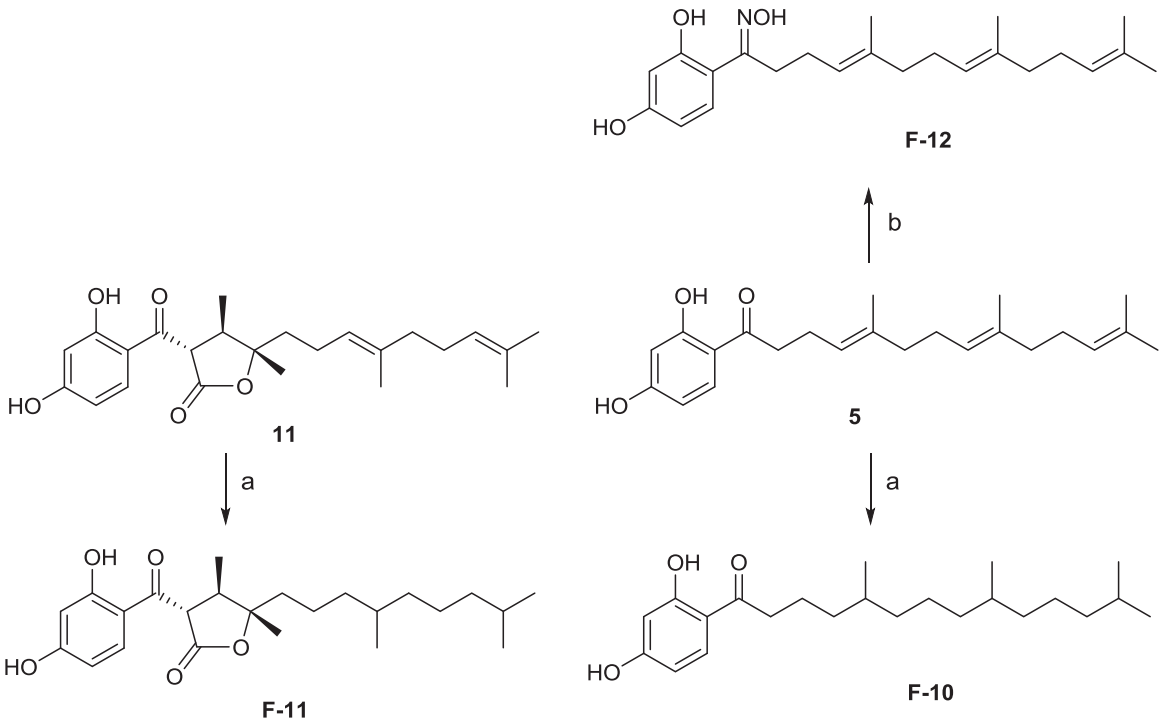
Antibacterial MIC assay

The results of the MIC assay are shown in Table 1. Against strain SA1199B, compounds **5**, **7**, **8**, **11**, **12** and **13** were more active than the control antibiotic norfloxacin. For strain XU212, compounds **5**, **6**, **7**, **8**, **9**, **10**, **11** and **12** showed better inhibitory activity than the control antibiotic tetracycline. For erythromycin-resistant strain RN4220, compounds **7**, **8**, **11** and **12** showed better inhibitory activity than the control antibiotic erythromycin. For standard *S. aureus* strain ATCC 25923, compound **7** showed the best inhibitory activity. For the epidemic MRSA strains, compounds **7**, **11** and **12** showed the best inhibitory activity against strain EMRSA-15 while compound **5**, **7** and **10** showed the best inhibitory activity against strain EMRSA-16.

For natural product **5**, when its 4'-OH moiety was replaced by an ether group (**F-2** and **F-6**), an ester (**F-4**) or with a group with large steric hindrance (**F-8**), its antibacterial activity was greatly decreased. If the 4'-OH and 2'-OH groups of **5** were both replaced (**F-3**, **F-5**, **F-7** and **F-9**), their antibacterial activity was almost totally ablated.

In comparing compound **5** with **8** and **11**, if only the first double bond of side chain was replaced, there was no significant improvement in activity. Comparing compound **5** with **12**, there was no significant improvement of the activity even if both the first and the third double bonds of side chain were saturated. Comparing compound **5** with **9**, **10**, **13** and **14**, if the second double bond of the side chain was replaced, their antibacterial activity was decreased. The antibacterial activity was greatly decreased if both the second and third double bonds of side chain were replaced (**15** and **16**). If the double bonds were all reduced, then the activity disappeared (**F-10** and **F-11**).

By comparing compound **5** with **1** and **3**, we can see that the activity disappeared if the side chain was absent and this



(a) CH_3OH, H_2-Pd/C, rt, 6 h; (b) CH_3OH, Hydroxylamine hydrochloride, NaOAc, reflux, 48 h;

Scheme 2 Syntheses of compounds (**F-10**–**F-12**)

may be due to the side chain being important for cellular uptake. When the α-H of a ketone was replaced by an hydroxyl (as in the case of **7** and **5**), the activity was enhanced, and for the replacement of the ketone with oximido (**F-12**), antibacterial activity was slightly improved.

EtBr efflux assay

As shown in Table 1, 20 compounds exhibited weak direct activity on five drug-resistant strains. To explore whether these compounds had indirect effects, an EtBr efflux assay was carried out. Among the tested compounds, compound **14** showed strong efflux inhibitory effects against strain SA1199B (Fig. 2). Comparison of **14** with **13**, where the 4'-OH moiety was replaced by an ether, the efflux inhibitory effect was enhanced. For **14** with **13** and **9**, 4'-OH and 8-OAc are the efficacy groups of efflux inhibitory effect against strain SA1199B.

Compound **13** had moderate efflux pump inhibitory effects against strain XU212. By comparing compound **13** with **9**, it would appear that the 8-OAc functional group is a requirement for inhibition of EtBr efflux in XU212 (Fig. 3).

Compounds **5**, **14** and **F-3** exhibited moderate efflux inhibitory effects against RN4220 (Figs. 4–6). By comparing **F-2** with **F-3** and **5**, when the 2'-OH and 4'-OH groups were intact or were replaced by ether at the same time, the activity was enhanced.

Conclusion

In conclusion, a SAR study of acetophenone natural products from *F. ferulioides* and their structural modification products showed that the 2',4'-dihydroxyphenyl, the length of the side chain and the double bonds of side chain, the ketone group and the group alpha to this moiety played important roles in their activity against drug-resistant *S. aureus*. These findings are encouraging, and further in vivo investigation, for example, clearance of an effluxing strain (SA1199B) in a mouse model by an efflux substrate antibiotic (such as norfloxacin) and an inhibitor (e.g. **14**), is required. Some of the synthetic compounds with no or weak direct antibacterial activity had efflux inhibitory effects, which may show synergism activity when combined with antibiotics. Synergy would be a promising strategy to resolve problems caused by drug-resistant strains, and under this strategy, some of the classical antibiotics could be reused in clinical therapy of infections, resulting in the use of lower antibiotic doses and an improved safety profile.

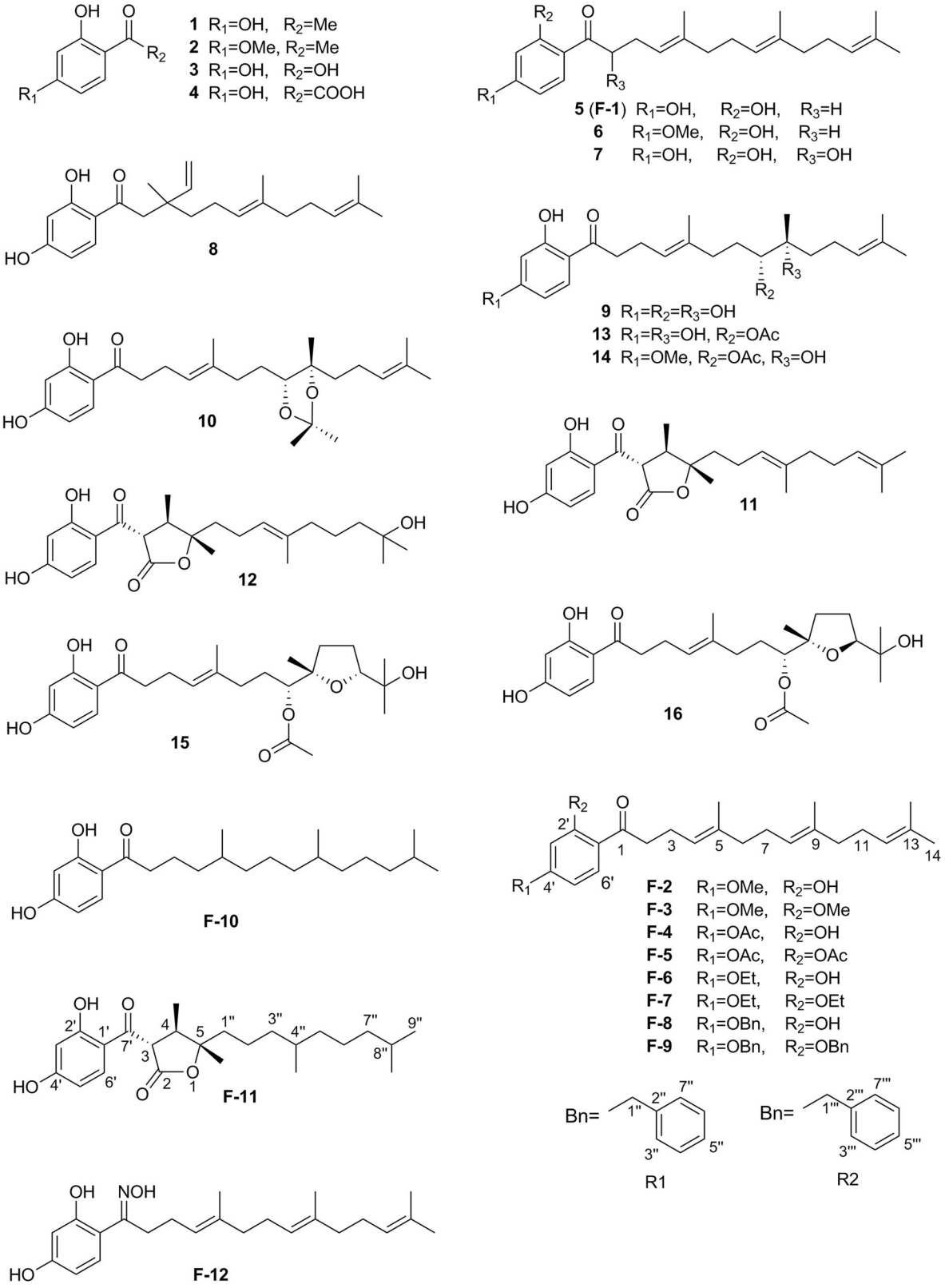


Fig. 1 Structures of compounds isolated from *F. ferulioides* (**1**–**16**) and obtained via chemical synthesis or modification (**F-2**–**F-12**)

Table 1 Antibacterial activities of the compounds (MIC in mg/L)

Compounds	SA1199B	XU212	ATCC25923	RN4220	EMRSA-15	EMRSA-16
1	>128	>128	>128	>128	>128	>128
2	>128	>128	>128	>128	>128	>128
3	>128	>128	>128	>128	>128	>128
4	>128	>128	>128	>128	>128	>128
5	16	1	16	32	16	1
6	>128	1	>128	>128	>128	>128
7	2	1	2	2	4	2
8	16	4	16	4	16	32
9	64	64	64	64	64	64
10	>128	0.5	>128	16	>128	0.5
11	8	2	8	4	2	128
12	8	2	8	16	2	16
13	16	32	128	64	64	32
14	>128	>128	>128	>128	>128	>128
15	>128	>128	>128	>128	>128	>128
16	128	>128	>128	128	>128	>128
F-2	>128	1	>128	>128	>128	>128
F-3	>128	>128	>128	>128	>128	>128
F-4	64	32	64	128	>128	64
F-5	64	32	32	128	>128	>128
F-6	>128	>128	>128	>128	>128	>128
F-7	>128	32	64	>128	>128	>128
F-8	>128	>128	>128	>128	>128	>128
F-9	>128	>128	>128	>128	>128	>128
F-10	>128	>128	>128	>128	>128	>128
F-11	128	32	128	>128	128	>128
F-12	8	2	4	2	2	32
Nor	32	8	0.5	0.5	0.5	128
Tet	0.25	128	0.25	0.5	0.25	0.25
Ery	0.25	>128	0.25	64	>128	>128
Oxa	0.25	128	0.25	0.25	>128	>128
Van	0.25	0.5	0.25	0.5	0.25	0.25

MIC minimum inhibitory concentration, *Nor* norfloxacin, *Tet* tetracycline, *Ery* erythromycin, *Oxa* oxacillin, *Van* vancomycin

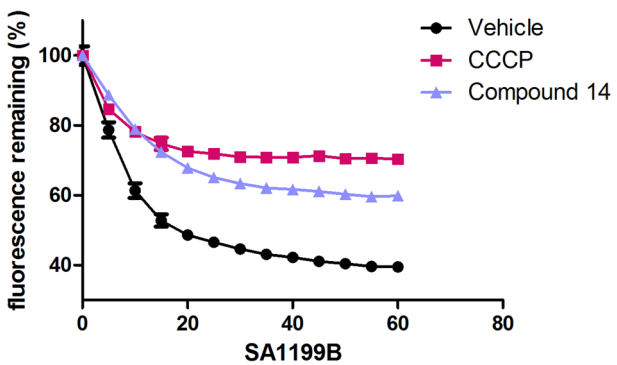


Fig. 2 Efflux inhibitory effects of compound **14** for strain SA1199B

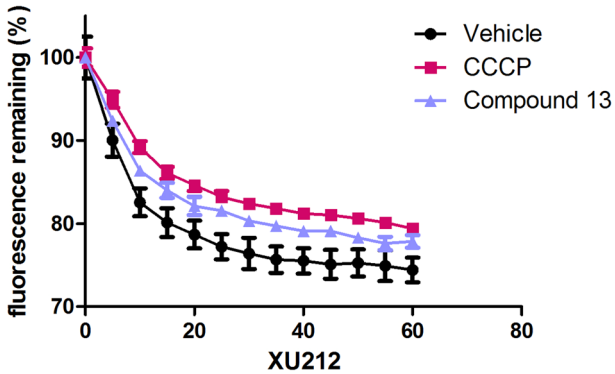


Fig. 3 Efflux inhibitory effects of compound **13** for strain XU212

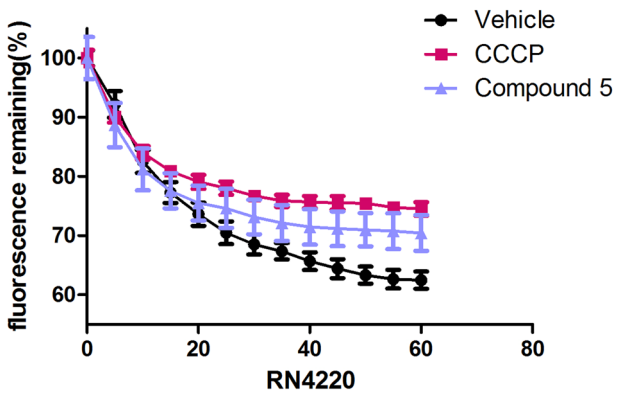


Fig. 4 Efflux inhibitory effects of compound **5** for strain RN4220

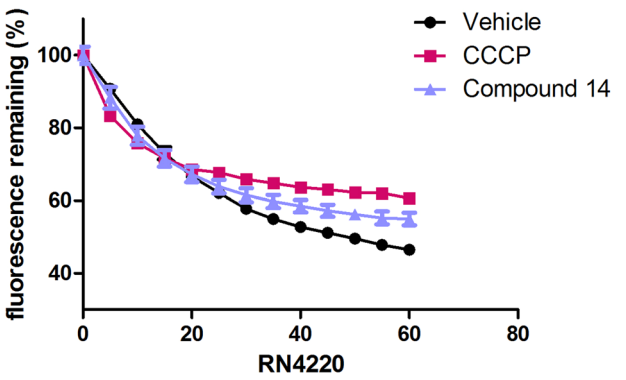


Fig. 5 Efflux inhibitory effects of compound **14** for strain RN4220

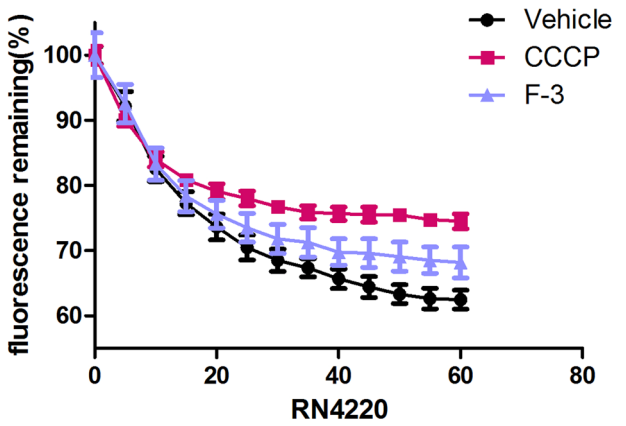


Fig. 6 Efflux inhibitory effects of compound **F-3** for strain RN4220

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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