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Synthesis and Antibacterial Evaluation of Novel Hydrophilic Ocotillol-Type Triterpenoid Derivatives from 20(S)-Protopanaxadiol

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Abstract: Triterpenoid saponins are involved in plant defense systems to inhibit bacterial invasion. A new series of hydrophilic ocotillol-type triterpenoid derivatives **5-26** have been synthesized with antibacterial activity against Gram-positive bacteria, including a community associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA; strain USA300). From this series, compounds **6** and **15** were found to be the most active, both with MIC values of 2 μg/mL against *B. subtilis* 168 and 8 μg/mL against *S. aureus* USA300, respectively. Furthermore, subsequent assays showed that compounds **6** and **15** displayed strong synergistic effects at sub-MIC levels against both *S. aureus* USA300 and *B. subtilis* 168 when combined with two commercial antibiotics, kanamycin and chloramphenicol. Preliminary structure-activity relationship studies were also performed.

Keywords: Ocotillol; triterpenoid; antibacterial activity; synergistic effect.

1. Introduction

The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens.[1] Some of these strains, such as vancomycin-resistant *Enterococci* and multidrug resistant

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Staphylococcus aureus, are capable of surviving the effects of most, if not all, antibiotics currently in use.[2] Moreover, the widespread use and misuse of antibiotics has led to the rapid development of antibiotic resistance across a wide range of organisms, making the treatment of infectious diseases problematic.[3] These problems have highlighted the urgent need for designing and developing novel drug candidates endowed with antimicrobial activity, which are distinct from those of well-known classes of antimicrobial agents. [4]

Historically, plants have provided a rich source of anti-infective agents, contributing to human health and well-being. Their main benefits are that they are generally safer, offering powerful therapeutic benefits and more affordable treatment than synthetic compounds. One of the classes with the most active compounds is the triterpenoid, which comprises different types of compounds with diverse structures.[5] Data concerning their antibacterial activity published mainly in the last three decades have demonstrated the growth inhibition of various bacterial genera by medicinal plant extracts or isolated compounds,[6] including their effectiveness against multidrug resistant mycobacteria.[7]

Active triterpenoids like α-amyrin, betulinic acid, betulinaldehyde, and other related triterpenes such as imberbic acid, oleanolic acid (oleanic acid), ursolic acid, and squalamine have been reported to possess antimicrobial activity (Figure 1).[8-12] Plant derived triterpenoid saponins have been proven to interact with the membranes of living cells. The result of such interactions can be the destruction of the cell membrane resulting in cell death (non-reversible effect), or a transient change in the membrane structure followed by specific biological effects (e.g., secretion processes, ion channel activation/inhibition).[13] However, the molecular mechanisms of the interactions between saponins and elements of mammalian cell membranes are widely unknown.

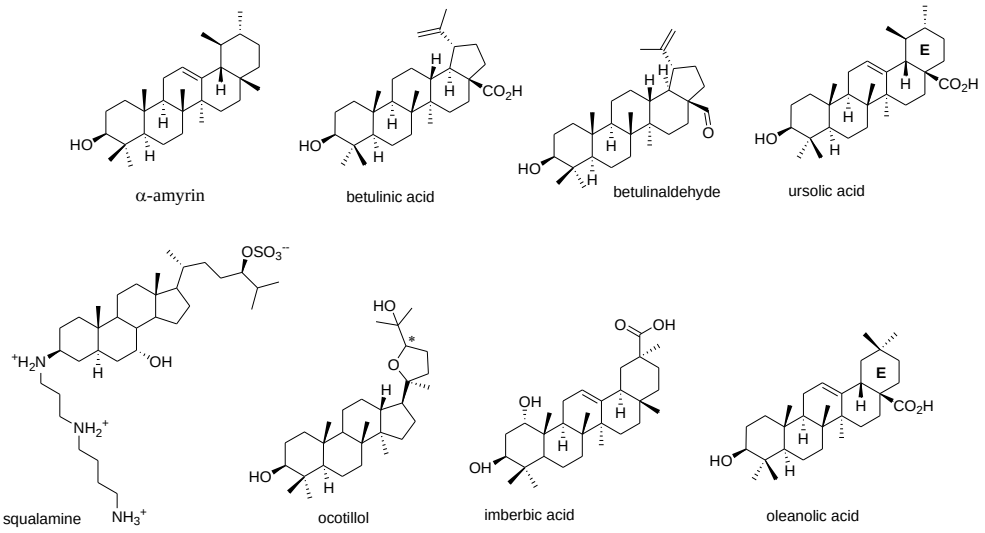


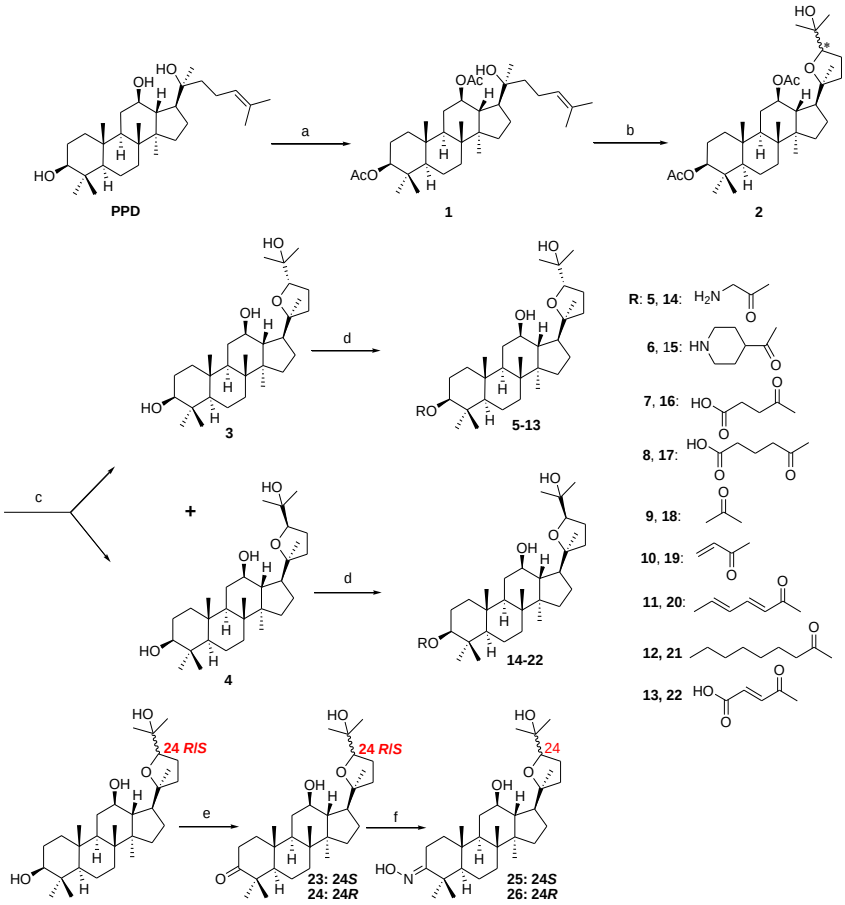
Figure 1. Active triterpenoids isolated and reported to possess antimicrobial activity

Ocotillol (Figure 1), a triterpene isolated from *Fouquieria splendens Engelm*, bears a tetrahydrofurane ring at C-20.[14] In previous work, we have reported a series of synthesised novel enantiopure ocotillol-type triterpenoid derivatives from 20(S)-protopanaxadiol and found that these compounds showed potent antibacterial activity against Gram-positive bacteria and mild activity against Gram-negative bacteria by substitution at C-12. Furthermore, these compounds displayed significant synergistic effects when combined with kanamycin and chloramphenicol with MIC values of less than 0.0020 μg/mL against *Staphylococcus aureus* USA300 and *Bacillus subtilis* 168.[15] In order to further explore the biological effects of ocotillol-type triterpenoid derivatives and establish their structure–activity relationships, a new series of hydrophilic ocotillol-type triterpenoid derivatives from 20(S)-protopanaxadiol were synthesised. The aims of this study were to evaluate their *in vitro* antibacterial, and synergistic antibacterial activities, and to examine the structure-activity relationship of ocotillol-type triterpenoids.

2. Materials and Methods

2.1. General

Most chemicals and solvents were analytical grade and, when necessary, were purified and dried by standard methods. Melting points were taken on an XT-4 micro melting point apparatus. 1H NMR spectra were recorded with a Bruker AV-300 or ACF 500 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using an Agilent QTOF 6520.



Reagents and conditions: (a)Ac_2O, DMAP, CH_2Cl_2, rt. (b)m-CPBA, CH_2Cl_2, rt. (c)KOH, MeOH, ref. (d) an acid or an anhydride, CH_2Cl_2, rt. (e) PCC, CH_2Cl_2, rt. (f) anhydrous pyridine, $NH_2OH{\cdot}HCl$, 80°C

Figure 2. Synthesis of ocotillol-type derivatives **5-22**, **25**, **26**

2.2. Chemistry

*2.2.1. General procedure for synthesis of **3** and **4***

*(20S,24S)-Epoxy-dammarane-3β,12β,25-triol (**3**) and (20S, 24R)-Epoxy-dammarane-3β,12β,25-triol (**4**)* were synthesized from 20(S)-protopanaxadiol as previously described [15,16].

2.2.2. *General procedure for synthesis of **5-22***

To a solution of **3** or **4** (70 mg, 0.15 mmol) and DMAP (25 mg, 0.18 mmol) in dry dichloromethane (4 mL), an acid or an anhydride (0.30 mmol), EDCI (57.6 mg, 0.30 mmol) if necessary was added. The reaction mixture was stirred at room temperature for 5 h, then the organic solution was washed with saturated aqueous $NaHCO_3$ solution, water and brine, dried over anhydrous

sodium sulfate and concentrated. The organic mixture was purified by silica gel column chromatography (dichloromethane / methanol, 60:1) to afford the desired product.

*(20S, 24S)-Epoxy-3β-O-(2-amino acetyl)-dammarane-12β, 25-diol (**5**)*

3 (70mg, 0.15mmol), EDCI (57.6 mg, 0.30 mmol) and boc-glycine (53mg, 0.30mmol) and then trifluoroacetic acid (1mL) in CH_2Cl_2 (10mL) to remove the boc substituent. White solid (yield 86%); mp. 215–218°C; ESI-MS *m/z* 534.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{32}H_{55}NNaO_5$ $[M+Na]^+$: 556.3978, found: 556.3985; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.56 (dd, *J*=10.5Hz, 6.0Hz, 1H), 3.88 (dd, *J*=11.0Hz, 5.5Hz, 1H), 3.53 (td, *J*=10.0Hz, 4.5Hz, 1H), 3.48(m, 2H), 2.23-2.28 (m, 1H), 1.90-2.08 (m, 5 H), 1.28 (s, 3H), 1.26 (s, 3H), 1.23 (s, 3H),1.11 (s, 3H), 1.02(s, 3H) , 0.92 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 87.3, 87.0, 81.4, 70.4, 70.0, 56.0, 52.1, 50.1, 48.9, 48.8, 44.0, 39.7, 38.5, 37.9, 37.0, 34.6, 32.1, 31.6, 31.6, 28.8, 28.5, 28.0, 27.8, 25.0, 24.2, 23.7, 18.1, 17.7, 16.4, 16.3, 15.4.

*(20S, 24S)-Epoxy-3β-O-(4-piperidine formyl)-dammarane-12β, 25-diol (**6**)*

3 (70mg, 0.15mmol), EDCI (57.6 mg, 0.30 mmol) and 1-boc-isonipecotic acid (87.8mg, 0.30mmol) and then trifluoroacetic acid (1mL) in CH_2Cl_2 (10mL) to remove the boc substituent. White solid (yield 70%); mp. 205–208°C; ESI-MS *m/z* 588.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{36}H_{62}NO_5$ $[M+H]^+$: 588.4628, found: 588.4634; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.49 (t, *J*=9.9Hz, 6.2Hz, 1H), 3.87 (dd, *J*=10.3Hz, 5.0Hz, 1H), 3.53 (td, *J*=9.8Hz, 4.1Hz, 1H), 3.14 (d, *J*=12.3Hz, 2H), 2.72 (t, *J*=10.9 Hz, 11.4 Hz, 2H), 2.18-2.51 (m, 6H), 1.80-2.03 (m, 7 H), 1.62-1.78 (m, 6 H), 1.27 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H), 1.01(s, 3H) , 0.91 (s, 3H), 0.86 (s, 3H) , 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.3, 87.7, 87.4, 81.0, 70.7, 70.3, 56.3, 52.4, 50.4, 49.2, 49.1, 45.3, 45.2, 41.3, 40.0, 38.8, 38.3, 38.2, 37.3, 34.9, 32.5, 31.9, 31.9, 31.4, 29.1, 28.8, 28.5, 28.2, 25.3, 24.5, 24.0, 18.4, 18.0, 16.8, 16.6, 15.7.

*(20S, 24S)-Epoxy-3β-O-(3-carboxy propionyl)-dammarane-12β, 25-diol (**7**)*

3 (70mg, 0.15mmol) and succinic anhydride (30mg, 0.30mmol); white solid (yield 80%); mp. 213–216°C; ESI-MS *m/z* 577.4 $[M + H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{34}H_{57}O_7$ $[M+H]^+$: 577.4104, found: 577.4108; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.50 (t, *J*=8.9 Hz, 6.9 Hz, 1H), 3.85 (t, *J*=8.0 Hz, 7.3 Hz, 1H), 3.52 (dd, *J*=10.1 Hz, 6.3 Hz, 1H), 2.63-2.67 (m, 4H), 2.15-2.22 (m, 1H), 1.92-2.10 (m, 2 H), 1.29 (s, 3H), 1.26 (s, 6H), 1.00 (s, 3H), 0.98(s, 3H) , 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.6, 172.1, 87.7, 87.4, 81.5, 70.8, 70.5, 56.3, 52.4, 50.8, 49.1, 48.9, 40.0, 38.8, 38.2, 37.3, 34.9, 32.5, 31.9, 31.7, 29.7, 29.5, 29.3, 29.1, 28.2, 28.0, 25.4, 24.3, 23.9, 18.4, 18.0, 16.7, 16.6, 15.7.

*(20S, 24S)-Epoxy-3β-O-(4-carboxy-butyryl)-dammarane-12β, 25-diol (**8**)*

3 (70mg, 0.15mmol) and glutaric anhydride (34.2mg, 0.30mmol); white solid (yield 71%); mp. 200–203°C; ESI-MS *m/z* 591.4 [M + H]+; HR-MS (ESI) *m/z*: calculated for $C_{35}H_{59}O_7$ $[M+H]^+$: 591.4261, found: 591.4268; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.48 (dd, *J*=9.7Hz, 6.3Hz, 1H), 3.89 (dd, *J*=9.8Hz, 4.7Hz, 1H), 3.53 (td, *J*=10.0Hz, 5.7Hz, 1H), 2.36-2.42 (m, 4H), 2.20-2.28 (m, 1H), 1.82-2.05 (m, 7 H), 1.27 (s, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.11 (s, 3H), 1.01 (s, 3H), 0.91(s, 3H) , 0.84 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.9, 87.7, 87.4, 81.1, 70.9, 70.5, 56.3, 52.4, 50.8, 49.1, 48.9, 40.0, 38.8, 38.2, 37.3, 34.9, 34.0, 33.4, 32.5, 31.9, 31.7, 29.6, 29.1, 28.8, 28.3, 28.1, 25.5, 24.2, 22.9, 20.4, 18.4, 18.0, 16.7, 16.6, 15.7.

*(20S, 24S)-Epoxy-3β-O-acetyl-dammarane-12β, 25-diol (**9**)* [15]

3 (70mg, 0.15mmol) and acetic anhydride (30.6mg, 0.30mmol); white solid (yield 65%); mp. 198–202°C; ESI-MS *m/z* 519.4 [M + H]+; HR-MS (ESI) *m/z*: calculated for $C_{32}H_{55}O_5$ $[M+H]^+$: 519.4049, found: 519.4055; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.47 (dd, *J*=9.7Hz, 6.4 Hz, 1H), 3.88 (dd, *J*=10.1Hz, 5.3 Hz, 1H), 3.50 (dd, *J*=10.3Hz, 4.2Hz, 1H), 2.20-2.33 (m, 1H), 2.05 (s, 3H), 1.67-2.02 (m, 5 H),1.28 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H), 1.10 (s, 3H), 1.01 (s, 3H), 0.91 (s, 3H), 0.85 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9, 85.8, 84.8, 79.0, 75.8, 70.7, 56.0, 52.7, 50.4, 50.0, 46.7, 40.1, 39.8, 39.2, 39.1, 37.4, 34.8, 31.2, 28.6, 28.3, 27.9, 27.5, 27.2, 26.0, 24.3, 23.0, 22.1, 18.5, 17.7, 16.3, 15.8, 15.6.

*(20S, 24S)-Epoxy-3β-O-acryloyl-dammarane-12β, 25-diol (**10**)*

3 (70mg, 0.15mmol) and acrylic anhydride (37.8mg, 0.30mmol); white solid (yield 63%); mp. 193–195°C; ESI-MS *m/z* 531.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{33}H_{55}O_5$ $[M+H]^+$: 531.4049, found: 531.4057; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30 (d, *J*=17.3Hz, 1H), 6.05 (dd, *J*=17.2Hz, 10.3Hz,), 5.73 (d, *J*=10.2Hz, 1H), 4.50 (dd, *J*=9.1Hz, 5.9Hz, 1H), 3.79 (t, *J*=8.0Hz, 7.0Hz, 1H), 3.52 (td, *J*=10.4Hz, 5.9Hz, 1H), 2.11-2.23 (m, 1H), 1.78-1.99 (m, 8 H), 1.27 (s, 9H), 1.12 (s, 3H), 1.03 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H) , 0.88(s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.3, 130.3, 129.4, 87.6, 87.3, 81.2, 70.7, 70.3, 56.3, 52.4, 50.4, 49.2, 49.1, 40.0, 38.9, 38.3, 37.4, 35.0, 32.5, 32.0, 31.9, 29.1, 28.8, 28.2, 25.9, 25.2, 24.5, 23.9, 18.5, 18.0, 16.7, 16.6, 15.7.

*(20S,24S)-Epoxy-3β-O-(2E,4E-diene adipoyl)-dammarane-12β,25-diol (**11**)*

3 (70mg, 0.15mmol), EDCI (57.6 mg, 0.30 mmol) and sorbic acid (33.6mg, 0.30mmol); white solid (yield 52%); mp. 210–213°C; ESI-MS *m/z* 571.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{36}H_{59}O_5$ $[M+H]^+$: 571.4363, found: 571.4367; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (dd, *J*=15.4Hz, 9.9Hz, 1H), 6.08-6.24 (m, 2H), 5.77 (d, *J*=15.4Hz, 1H), 4.55 (dd, *J*=10.2Hz, 5.4Hz, 1H), 3.89 (dd, *J*=10.4Hz, 5.1Hz, 1H), 3.54 (td, *J*=10.7Hz, 5.6Hz, 1H), 2.21-2.31 (m, 1H), 1.94-2.15 (m, 2 H), 1.77-1.93 (m, 3 H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 (s, 3H),1.10 (s, 3H), 0.99 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H) , 0.89 (s, 3H) , 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.3, 144.8, 139.2, 130.1, 119.9, 87.6, 87.4, 80.7, 70.7, 70.3, 56.3, 52.4, 50.4, 49.2, 49.1, 40.0, 38.9, 38.3, 37.4, 35.0, 32.4, 31.9, 31.9, 29.1, 28.8, 28.2, 25.9, 25.2, 24.5, 24.0, 18.8, 18.5, 18.0, 16.8, 16.6, 15.7.

*(20S,24S)-Epoxy-3β-O-octanoyl-dammarane-12β,25-diol (**12**)*

3 (70mg, 0.15mmol) and octanoic anhydride (81.0mg, 0.30mmol); white solid (yield 82%). mp. 219–223°C; ESI-MS *m/z* 603.5 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{38}H_{67}O_5$ $[M+H]^+$: 603.4989, found: 603.4994; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.42 (dd, *J*=10.2Hz, 6.4Hz, 1H), 3.80 (dd, *J*=10.2Hz, 5.0Hz, 1H), 3.46 (td, *J*=10.0Hz, 4.5Hz, 1H), 2.12-2.31 (m, 3H), 1.69-2.05 (m, 5 H), 1.29 (s, 3H), 1.26 (s, 6H), 1.05 (s, 3H), 0.98 (s, 3H) , 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 87.6, 87.4, 80.7, 70.7, 70.3, 56.3, 52.4, 50.4, 49.2, 49.1, 40.0, 38.9, 38.2, 37.4, 35.1, 35.0, 32.4, 31.9, 31.9, 31.3, 29.4, 29.2, 28.8, 28.2, 28.1, 27.8, 26.4, 25.4, 25.3, 24.0, 22.8, 18.5, 18.0, 16.7, 16.6, 15.7, 14.3.

*(20S,24S)-Epoxy-3β-O-(2E-ene butyryl)-dammarane-12β,25-diol (**13**)*

3 (70mg, 0.15mmol) and maleic anhydride (29.9mg, 0.30mmol); white solid (yield 45%); mp. 215–218°C; ESI-MS *m/z* 575.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{34}H_{55}O_7$ $[M+H]^+$: 575.3948, found: 575.3953; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.41 (d, *J*=12.8 Hz, 1H), 6.30 (d, *J*=12.8 Hz, 1H), 4.59 (t, *J*=8.3 Hz, 6.9 Hz, 1H), 3.78 (t, *J*=7.5 Hz, 7.5 Hz, 1H), 3.45 (td, *J*=10.8 Hz, 5.6Hz, 1H), 2.14-2.26 (m, 1H), 1.87-2.12 (m, 5 H), 1.29 (s, 6H), 1.26 (s, 6H), 1.10 (s, 3H), 0.98 (s, 3H) ,0.92 (s, 3H), 0.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.0, 160.2, 126.5, 123.1, 86.8, 85.6, 71.3, 70.6, 56.4, 52.5, 50.7, 49.8, 48.4, 40.2, 38.9, 38.4, 37.5, 35.1, 33.0, 31.7, 31.6, 29.6, 29.0, 28.5, 28.3, 28.0, 26.5, 25.4, 23.9, 23.1, 18.6, 16.8; 15.8, 14.5.

*(20S, 24R)-Epoxy-3β-O-(2-amino acetyl)-dammarane-12β, 25-diol (**14**)*

4 (70mg, 0.15mmol), EDCI (57.6 mg, 0.30 mmol) and boc-glycine (53mg, 0.30mmol) and then trifluoroacetic acid (1mL) in CH_2Cl_2 (10mL) to remove the boc substituent. White solid (yield 80%); mp. 223–228°C; ESI-MS *m/z* 534.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{32}H_{55}NNaO_5$ $[M+Na]^+$: 556.3978, found: 556.3983; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.54 (dd, *J*=10.0Hz, 6.9Hz, 1H), 3.85 (dd, *J*=8.5Hz, 6.5Hz, 1H), 3.52 (td, *J*=10.5Hz, 4.5Hz, 1H), 3.48(m, 2H), 2.18-2.23 (m, 1H), 1.97-2.10 (m, 3 H), 1.82-1.94 (m, 3 H), 1.28 (s, 3H), 1.27 (s, 3H), 1.10 (s, 3H), 0.99 (s, 3H), 0.91(s, 3H) , 0.89 (s, 3H), 0.85 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 86.4, 85.4, 81.4, 70.9, 70.0, 56.0, 52.0, 50.4, 49.3, 47.9, 44.0, 39.7, 38.6, 37.9, 37.0, 34.7, 32.5, 31.3, 31.1, 28.5, 27.9, 27.8, 27.5, 26.1, 24.9, 23.7, 18.1, 18.1, 16.3, 16.3, 15.3.

*(20S, 24R)-Epoxy-3β-O-(4-piperidine formyl)-dammarane-12β, 25-diol (**15**)*

4 (70mg, 0.15mmol), EDCI (57.6 mg, 0.30 mmol) and 1-boc-isonipecotic acid (87.8mg, 0.30mmol) and then trifluoroacetic acid (1mL) in CH_2Cl_2 (10mL) to remove the boc substituent. White solid (yield 86%); mp. 213–217°C; ESI-MS *m/z* 588.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{36}H_{62}NO_5$ $[M+H]^+$: 588.4628, found: 588.4631; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.51 (t, *J*=8.6Hz, 7.3Hz, 1H), 3.85 (dd, *J*=10.2Hz, 5.1Hz, 1H), 3.56 (td, *J*=9.4Hz, 6.1Hz, 1H), 3.25 (d, *J*=11.9Hz, 2H), 2.99 (t, *J*=9.2Hz, 8.8 Hz, 2H), 2.01-2.21 (m, 6H), 1.81-1.92 (m, 3 H), 1.26 (s, 6H), 1.10 (s, 3H), 0.99 (s, 3H), 0.91(s, 3H) , 0.89 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4, 86.7, 85.6, 81.9, 71.1, 70.3, 56.2, 52.2, 50.6, 49.6; 48.2, 43.0, 42.9, 41.3, 40.0, 38.8; 38.6, 38.2, 37.3; 34.9; 32.8, 32.1, 31.6, 31.4; 28.8, 28.3, 28.1, 27.8, 26.3, 25.2; 23.9, 18.8, 18.3, 16.7, 16.6, 15.6.

*(20S, 24R)-Epoxy-3β-O-(3-carboxypropionyl)-dammarane-12β,25-diol (**16**)*

4 (70mg, 0.15mmol) and succinic anhydride (30mg, 0.30mmol); white solid (yield 90%); mp. 214–216°C; ESI-MS *m/z* 577.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{34}H_{57}O_7$ $[M+H]^+$: 577.4106, found: 577.4109; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.50 (t, *J*=8.9Hz, 6.9Hz, 1H), 3.85 (t, *J*=8.0Hz, 7.3Hz, 1H), 3.52 (dd, *J*=10.1Hz, 6.3Hz, 1H), 2.63-2.67 (m, 4H), 2.15-2.22 (m, 1H), 1.90-2.07 (m, 2 H), 1.29 (s, 3H), 1.26 (s, 6H), 1.00 (s, 3H), 0.98(s, 3H) , 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.1, 172.2, 86.9, 85.7, 81.7, 71.3, 70.7, 56.4, 52.4, 50.8, 49.6, 48.3, 40.1, 39.0, 38.3, 37.4, 35.1, 32.9, 31.6, 31.5, 29.8, 29.4, 28.9, 28.3, 28.2, 27.9, 26.4, 25.4, 24.0, 18.5 (overlapping signal), 16.8, 16.7, 15.7.

*(20S, 24R)-Epoxy-3β-O-(4-carboxy-butyryl)-dammarane-12β, 25-diol (**17**)*

4 (70mg, 0.15mmol) and glutaric anhydride (34.2mg, 0.30mmol); white solid (yield 71%); mp. 201–204°C; ESI-MS *m/z* 591.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{35}H_{59}O_7$ $[M+H]^+$: 591.4261, found: 591.4266; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.49 (t, *J*=8.9Hz, 6.6Hz, 1H), 3.85 (t, *J*=7.9Hz, 7.2Hz, 1H), 3.52 (dd, *J*=10.9Hz, 4.9Hz, 1H), 2.37-2.44 (m, 4H), 2.15-2.22 (m, 1H), 1.80-2.01 (m, 4 H), 1.26 (s, 6H), 1.10 (s, 3H), 1.05 (s, 3H), 0.98(s, 3H) , 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.2, 172.5, 86.4, 85.3, 80.8, 70.9, 70.2, 56.0, 51.9, 50.3, 49.2, 47.9; 39.7, 38.5; 37.8, 37.0, 34.7, 33.6, 32.9, 32.5, 31.2; 31.1, 29.6; 29.2, 28.5, 27.9, 27.7, 27.4, 26.0, 24.9, 18.1, 18.0, 16.4, 16.3, 15.3.

*(20S, 24R)-Epoxy-3β-O-acetyl-dammarane-12β, 25-diol (**18**)*

4 (70mg, 0.15mmol) and acetic anhydride (30.6mg, 0.30mmol); white solid (yield 81%); mp. 189–193°C; ESI-MS *m/z* 519.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{32}H_{55}O_5$ $[M+H]^+$: 519.4049, found: 519.4055; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.48 (dd, *J*=9.7Hz, 6.4 Hz, 1H), 3.88 (dd, *J*=10.0Hz, 5.2 Hz, 1H), 3.51 (dd, *J*=10.2Hz, 4.3Hz, 1H), 2.09-2.30 (m, 1H), 2.04 (s, 3H), 1.65-1.99 (m, 5 H), 1.27 (s, 6H), 1.10 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8, 85.9, 83.5, 78.8, 75.8, 71.2, 56.2, 56.0, 52.4, 50.7, 50.0, 46.5, 39.8, 39.1, 39.0, 37.3, 34.7, 31.4, 28.6, 28.2, 27.7, 27.4, 27.0, 26.3, 24.4, 22.4, 22.0, 18.4, 17.8, 16.2, 15.7, 15.6.

*(20S, 24R)-Epoxy-3β-O-acryloyl-dammarane-12β, 25-diol (**19**)*

4 (70mg, 0.15mmol) and acrylic anhydride (37.8mg, 0.30mmol); white solid (yield 72%); mp. 194–197°C; ESI-MS *m/z* 531.4 [M+H]+; HR-MS (ESI) *m/z*: calculated for $C_{33}H_{55}O_5$ $[M+H]^+$: 531.4049, found: 531.4056; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30 (d, *J*=17.3 Hz, 1H), 6.05 (dd, *J*=17.2 Hz, 10.3 Hz,), 5.73 (d, *J*=10.2 Hz, 1H), 4.50 (dd, *J*=9.1 Hz, 5.9 Hz, 1H), 3.79 (t, *J*=8.0 Hz, 7.0 Hz, 1H), 3.52 (td, *J*=10.4 Hz, 5.9 Hz, 1H), 2.11-2.23 (m, 1H), 1.90-2.09 (m, 6 H), 1.75-2.08 (m, 7 H), 1.27 (s, 9H), 1.12 (s, 3H), 1.03 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H) , 0.88(s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.2, 130.0, 129.0, 86.7, 85.6, 81.1, 71.1, 70.3, 56.3, 52.2, 50.6, 49.6, 48.2, 40.0, 38.8, 38.2, 37.3, 35.0, 32.8, 31.5, 31.4, 28.8, 28.2, 28.1, 27.8, 26.3, 25.2, 23.9, 18.3, 18.4, 16.7, 16.6, 15.6.

*(20S,24R)-Epoxy-3β-O-(2E,4E-diene adipoyl)-dammarane-12β,25-diol (**20**)*

4 (70mg, 0.15mmol) and sorbic acid (33.6mg, 0.30mmol); white solid (yield 60%); mp. 212–215°C; ESI-MS *m/z* 571.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{36}H_{59}O_5$ $[M+H]^+$: 571.4363, found: 571.4368; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23 (dd, *J*=15.4Hz, 9.9Hz, 1H), 6.08-6.24 (m, 2H), 5.77 (d, *J*=15.4Hz, 1H), 4.55 (dd, *J*=10.1Hz, 5.0Hz, 1H), 3.85 (dd, *J*=8.5Hz, 6.8Hz, 1H), 3.53 (td,

J=10.5Hz, 5.8Hz, 1H), 2.13-2.23 (m, 1H), 1.88-2.02 (m, 2 H), 1.52-1.88 (m, 6 H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 (s, 3H),1.10 (s, 3H), 0.99 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H) , 0.89 (s, 3H) , 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.3, 144.7, 139.1, 130.0, 119.8, 86.7, 85.6, 80.6, 71.2, 70.3, 56.3, 52.2, 50.6, 49.6, 48.2, 40.0, 38.9, 38.3, 37.3, 35.0, 32.8, 31.5, 31.4, 29.9, 28.8, 28.2; 28.1; 27.8; 26.3; 25.2; 24.0; 18.4; 18.4; 16.7; 16.6; 15.6.

*(20S,24R)-Epoxy-3β-O-octanoyl-dammarane-12β,25-diol (**21**)*

4 (70mg, 0.15mmol) and octanoic anhydride (81.0mg, 0.30mmol); white solid (yield 83%); mp. 209–213°C; ESI-MS *m/z* 603.5 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{38}H_{67}O_5$ $[M+H]^+$: 603.4989, found: 603.4996 ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.40 (dd, *J*=10.1Hz, 5.7Hz, 1H), 3.77 (dd, *J*=8.6Hz, 6.8Hz, 1H), 3.45 (td, *J*=10.4Hz, 4.6Hz, 1H), 2.21 (t, *J*=7.4Hz, 2H), 2.17-2.19 (m, 1H), 1.89-2.08 (m, 2 H), 1.71-2.08 (m, 3 H), 1.29 (s, 3H), 1.26 (s, 6H), 1.05 (s, 3H), 0.98 (s, 3H) , 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 86.7, 85.6, 80.6, 71.2, 70.3, 56.3, 52.2, 50.6, 49.6, 48.2, 40.0, 38.9, 38.1, 37.3, 35.0, 35.0, 32.8, 31.9, 31.5, 31.4, 29.3, 29.1, 28.8, 28.2, 28.1, 27.8, 26.3, 25.4, 25.2, 23.9, 22.8, 18.4, 18.3, 16.7, 16.6, 15.6, 14.2.

*(20S,24R)-Epoxy-3β-O-(2E-ene butyryl)-dammarane-12β,25-diol (**22**)*

4 (70mg, 0.15mmol) and maleic anhydride (29.9mg, 0.30mmol); white solid (yield 42%); mp. 198-204°C; ESI-MS *m/z* 575.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{34}H_{55}O_7$ $[M+H]^+$: 575.3948, found: 575.3954; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.41 (d, *J*=12.8Hz, 1H), 6.30 (d, *J*=12.8Hz, 1H), 4.59 (t, *J*=8.3Hz, 6.9Hz, 1H), 3.78 (t, *J*=7.5Hz, 7.5Hz, 1H), 3.45 (td, *J*=10.8Hz, 5.6Hz, 1H), 2.14-2.26 (m, 1H), 1.89-2.00 (m, 3 H), 1.76-1.88 (m, 3 H), 1.29 (s, 6H), 1.26 (s, 6H), 1.10 (s, 3H), 0.98 (s, 3H) ,0.92 (s, 3H), 0.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.9, 160.1, 127.6, 122.3, 86.9, 85.7, 71.2, 70.5, 56.3, 52.4, 50.7, 49.7, 48.3, 40.1, 38.9, 38.3, 37.4, 35.1, 33.0, 31.7, 31.5, 29.6, 28.9, 28.4, 28.2, 27.9, 26.5, 25.3, 23.8, 23.1, 18.5, 16.7, 15.7, 14.5.

2.2.3. *General procedure for synthesis of **25-26***

*(20S, 24S)-Epoxy-dammarane-12β,25-diol-3-one (**23**) and (20S, 24R)-Epoxy-dammarane-12β,25-diol-3-one (**24**)* were synthesized from **3** and **4** as previously described [15].

To a solution of **23** or **24** (180 mg, 0.38 mmol) in anhydrous pyridine (8 mL), Hydroxylamine hydrochloride (53 mg, 0.76 mmol) was added. The reaction mixture was stirred at 80℃ for 2 h, then diluted by ethyl acetate. The organic solution was washed with 10% HCl, water and brine, dried over anhydrous sodium sulfate and concentrated. The organic mixture was purified by silica gel column chromatography to afford the desired product.

*(20S,24S)-Epoxy-3-oxime-dammarane-12β,25-diol (**25**)*

23 (180 mg, 0.38 mmol); white solid (yield 82%); mp. 208–212°C; ESI-MS *m/z* 490.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{30}H_{52}NO_4$ $[M+H]^+$: 490.3896, found: 490.3899; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.88 (dd, *J*=10.2Hz, 4.9Hz, 1H), 3.52 (td, *J*=10.4Hz, 5.5Hz, 1H), 2.93-3.03 (m, 1H), 2.21-3.35 (m, 2H), 1.96-2.09 (m, 2H), 1.58-1.95 (m, 6 H), 1.28 (s, 3H), 1.24 (s, 3H), 1.14 (s, 3H), 1.10 (s, 3H), 1.06 (s, 3H), 1.04 (s, 3H), 0.98 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.5, 87.3, 87.0, 70.2, 69.9, 55.2, 52.0, 49.4, 48.8, 48.8, 47.2, 39.6, 39.5, 36.7, 33.9, 33.9, 32.1, 32.0, 31.5, 28.7, 28.4, 27.8, 26.6, 24.9, 24.2, 20.8, 19.6, 17.6, 15.9, 15.0.

*(20S,24R)-Epoxy-3-oxime-dammarane-12β,25-diol (**26**)*

24 (180 mg, 0.38 mmol); white solid (yield 86%); mp. 206–210°C; ESI-MS *m/z* 490.4 $[M+H]^+$; HR-MS (ESI) *m/z*: calculated for $C_{30}H_{52}NO_4$ $[M+H]^+$: 490.3896, found: 490.3901; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.82-3.87 (m, 2H), 3.50 (td, *J*=10.3Hz, 4.3Hz, 1H), 2.94-3.04 (m, 1H), 2.15-3.33 (m, 2H), 1.95-2.07 (m, 2H), 1.75-1.94 (m, 4 H), 1.59-1.73 (m, 2 H), 1.34 (s, 3H), 1.28 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.9, 86.4, 85.3, 70.7, 70.0, 55.3, 51.9, 49.7, 49.4, 47.8, 47.2, 39.7, 39.5, 36.7, 34.0, 33.9, 32.5, 31.6, 31.1, 28.5, 27.8, 27.4, 26.6, 26.0, 24.9, 20.8, 19.6, 17.9, 15.9, 15.0.

2.3. Pharmacology

The antibacterial activity and the synergistic antibacterial activity were performed as described [15-17].

3. Results and Discussion

We chose 20(S)-protopanaxadiol (PPD), one of the main ginsengenin components of *Panax ginseng*, as our starting material (Figure 2). Protection of the 3, 12-diol of 20(S)-protopanaxadiol as diacetate in the presence of DMAP and acetic anhydride afforded compound **1**, followed by epoxidation and cyclisation *in situ* to generate tetrahydrofuran **2** as a mixture of diastereomers. After base treatment, two stereoisomeric triols, **3** and **4**, were obtained at the desired yields. Triol **3** was then allowed to react with a series of amino acids, anhydrides and fatty acids to form 3-*O*-derivatives **5-13**. The same procedure was applied to triol **4** to generate compounds **14**-**22**. Additionally, oxidation of triol **3** or **4** as mono ketones **23** and **24**, followed by reaction with hydroxylamine chloride gave target compounds **25** and **26**, respectively.

The novel ocotillol-type derivatives **5-22**, **25** and **26** were examined for their antimicrobial activities against different representative Gram-positive strains such as *Staphylococcus aureus* RN4220 and *Bacillus subtilis* 168, and Gram-negative strains such as *Escherichia coli* DH5α, *Acinetobacter baumannii* ATCC19606 and *Pseudomonas aeruginosa* PAO1. Initial minimum inhibitory concentration (MIC) screening results are shown in Table 1. The data demonstrated that compounds **3**, **5**, **6**, **7**, **8**, **14**, **15**, **16** and **21** displayed excellent antibacterial activity against Gram-positive bacteria with MIC values of 2-16 μg/mL. Compounds **6** and **15** that have a secondary amine group at the C-3 position were found to be the most active with MIC values of 16 and 8 μg/mL against *S. aureus* RN4220 and 2 μg/mL against *Bacillus subtilis* 168, respectively. Compared to the parent molecule **4**, compounds **16** and **17** with aliphatic carboxylic acid groups also showed enhanced activities against Gram-positive bacteria with MICs ranging from 8 to 32 μg/mL, while the inhibitory effects of **7** and **8** were comparable to that of **3**.

The structures of **3**, **5-8** and **4**, **14-17** are similar in all respects except for the stereochemistry at the C-24 position. Structural comparison of the derivatives between **5-8** and **14-17** showed that the presence of a secondary amine at C-3 enhanced the antimicrobial activity significantly against *Bacillus subtilis* 168, while primary amine and aliphatic carboxylic acid groups at the same position were able to maintain activity regardless of the stereochemistry at C-24. These results suggest that substitutions at the C-3 hydroxyl group of the steroid skeleton are critical determinants of antibacterial activity against Gram positive bacteria, although the stereochemistry of C-24 also played a dramatic effect on the activity of triols **3** and **4**. Our results showed that the compounds bearing a carboxylic acid or an amine at C-3 position possess superior antibacterial activities to other analogs because of their lower pKa/pKb characteristics. Additionally, compared to carboxylic acids **7** and **16**, compounds **13** and **22** bearing an α,β-unsaturated carboxylic acid at C-3 were only effective against *Bacillus subtilis* 168 with MICs at 32 μg/mL, probably due to an increased pKa caused by Π-electron conjugation. Furthermore, compound **21** possessing an octanoate and **25** with oxime at C-3 showed inhibitory effects only against *S. aureus* at 16 and 64 μg/mL, respectively. Note that compound **21** which doesn't contain hydrogen bond donors at C-3, showed specific activity against one single bacterial strain (*S. aureus*; Table 1). This result demonstrated that hydrogen bond donors at C-3 may not be absolutely required for antibacterial activity although we previously demonstrated oxidation of a hydroxyl group to a ketone at C-3 led to a loss of activity,[15] and this ocotillol-type structure has good potential for the development of drug candidates selective against single bacterial species. Moreover, only one derivative, **26** exhibited mild activity against the Gram-negative bacteria with an MIC of 64 μg/mL observed for *P. aeruginosa* PAO1, which confirmed the capacity of these compounds to target Gram-negative bacteria, but further improvement should be undertaken through substitution at C-12, as shown previously.[15]

Table 1. *In vitro antibacterial activity of ocotillol-type derivatives. (MIC: μg/mL)*

Strain	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. baumannii*
3	8	8	>128	>128	>128
4	64	128	>128	>128	128
5	32	8	>128	>128	128
6	16	2	>128	>128	>128
7	16	8	>128	>128	>128
8	16	16	>128	>128	>128
9	>128	>128	>128	>128	>128
10	>128	128	>128	>128	>128
11	>128	>128	>128	>128	>128
12	128	>128	>128	>128	128
13	128	32	>128	>128	128
14	16	16	>128	>128	128
15	8	2	128	>128	128
16	16	8	>128	128	>128
17	32	32	>128	>128	>128
18	>128	>128	>128	>128	128
19	>128	>128	128	>128	>128
20	>128	>128	>128	>128	>128
21	16	>128	128	>128	128
22	>128	32	>128	>128	>128
25	64	128	>128	>128	>128
26	>128	>128	>128	64	>128
KAN[a]	2	0.5	1	8	1

[a] KAN: kanamycin. *S. aureus: Staphylococcus aureus RN4220; B. subtilis: Bacillus subtilis 168; E. Coli: Escherichia coli DH5; P. aeruginosa: Pseudomonas aeruginosa PAO1; A. baumannii: Acinetobacter baumannii ATCC 19606.*

The bioactive compounds against Gram-positive bacteria were chosen for testing against a significant highly pathogenic community-associated methicillin resistant strain *S. aureus* USA300 (Table 2). The results revealed that besides triol **3**, compounds **5**, **6**, and **15** with amino groups displayed excellent antibacterial activity with MICs of 8-16 μg/mL, while **7**, **8** and **16** with a carboxylic group retained their mild activity against this pathogen with MICs of 32-64 μg/mL.

Table 2. Antibacterial activity of ocotillol-type derivatives against MRSA[a]. (MIC: μg/mL)

Compound	*S. aureus* USA 300
3	8
5	16
6	8
7	32
8	64
14	32
15	8
16	64
17	>128
21	>128
KAN[b]	1

[a] MRSA: community acquired methicillin-resistant *S. aureus* USA300; [b] KAN: kanamycin.

Compounds **5-8** and **14-16,** which displayed promising antibacterial activity against Gram-positive bacteria, were then further investigated for their minimum bactericidal concentrations (MBC) and the data were listed in Table 3. Amines **5** and **6** displayed bactericidal activity with MBC values of 32 μg/mL against *S. aureus* RN4220, 16, and 8 μg/mL against *B. subtilis* and 32, and 8 μg/mL against *S. aureus* USA300, respectively. In contrast, their (*R*)-epimers **14** and **15** showed moderate

bactericidal activity of 32 μg/mL against *S. aureus* RN4220, 128, and 8 μg/mL against *B. subtilis* and 32, and 16 μg/mL against *S. aureus* USA 300, respectively. This result showed that the secondary amine group at C-3 provided enhanced activity compared to the primary amine, and stereochemistry at C-24 had an impact on activity. As a result, (24*S*)-epimers were demonstrated to be more efficient (**5** vs **14** against *B. subtilis*; **6** vs **15** against *S. aureus* USA300).

In contrast, compound **7** with carboxylic acid at C-3 caused cell death with MBCs of 64 μg/mL against both *S. aureus* RN4220 and *B. subtilis* 168 while its epimer **16** can specifically kill *B. subtilis* 168 with an MBC value of 32 μg/mL.

Triterpenoids from natural products have been shown to possess antibacterial activity by increasing cell membrane permeability. As membrane perturbing agents, these compounds may be able to synergistically strengthen the antibacterial effects of antibiotics targeting intracellular process. As a result, the synergistic effects of compounds **6** and **15** were then investigated at sub-MIC concentrations against *S. aureus* USA300 and *B. subtilis* 168 when combined with kanamycin and chloramphenicol, as both of these two antibiotics target the intracellular sites of protein synthesis. Synergistic effects were evaluated by calculating the Fractional Inhibitory Concentration Index (FICI) using the Fractional Inhibitory Concentration (FIC).[18] As illustrated in Table 4, compound **6** could reduce the MIC of kanamycin against *S. aureus* USA300 from 1 μg/mL to 0.25 μg/mL (FICI= 0.28). Strong synergistic antibacterial activity against *B. subtilis* 168 was also observed when a combination of **6** and/or **15** with chloramphenicol was used, with FICI values of 0.13 and 0.25, respectively. However, only an additive effect was observed when **6** was combined with kanamycin against *B. subtilis* 168 (FICI=0.56) and other combinational activities gave indifferent interactions (1 < FICI < 2). Additionally, when **6** was combined with kanamycin, the MBC of kanamycin against *S. aureus* USA300 was also significantly enhanced from 4 to 1 μg/mL, and from 1 to 0.25 μg/mL against *B. subtilis* 168.

Table 3. Bactericidal activity of compounds **5-8** and **14-16** against Gram-positive bacteria. (MBC:μg/mL)

Compound	*S. aureus* RN4220	*B. subtilis* 168	*S. aureus* USA 300
5	32	16	32
6	32	8	8
7	64	64	>128
8	>128	128	>128
14	32	128	32
15	32	8	16
16	>128	32	>128
KAN[a]	2	1	4

[a] KAN: kanamycin

Table 4. Synergistic effect of different antibiotics with compounds 6 and 15 against *S. aureus* USA 300 and *B. subtilis* 168.

Compound	MIC (μg/mL)		MBC (μg/mL)		FICI (FIC index)[d]	
	S. aureus USA300	*B.subtilis* 168	*S. aureus* USA300	*B.subtilis* 168	*S. aureus* USA300	*B.subtilis* 168
KAN[a]	1	0.25	4	1	-	-
CHL[b]	4	2	N/A[c]	N/A	-	-
6+KAN	0.25	0.125	1	0.25	0.28	0.56
15+KAN	1	0.25	4	1	1.13	1.13
6+CHL	4	0.125	N/A	N/A	1.5	0.13
15+CHL	4	0.25	N/A	N/A	1.5	0.25

[a] KAN: kanamycin; [b] CHL: chloramphenicol; [c] N/A: not applicable; [d] FICI: according to the literature: FIC of drug A (FIC A)=MIC of drug A in combination/MIC of drug A alone; FIC of drug B (FIC B)=MIC of drug B in combination/MIC of drug B alone; hence FICI=FIC A+ FIC B. "Synergy" was defined when FICI was less than or equal to 0.5; while "additive" in which the FICI was greater than 0.5 and less than or equal to 1.0; whereas "indifferent" when the FICI was greater than 1.0 and less than or equal to 2.0; and "antagonistic" in cases which the FICI was greater than 2.0.

As kanamycin was transported into the cell cytoplasm by a lipid-dependent pathway,[19] whilst chloramphenicol can utilize both lipid and porin transport processes,[20] the synergistic effect of the ocotillol derivatives with both of the two antibiotics suggested our compounds may affect lipid transport pathways to increase the susceptibility of bacteria to antibiotics. The ineffectiveness of these compounds against Gram-negative bacteria (data not shown) indicated that lipopolysaccharides (LPS) present in the outer membrane of Gram negative bacteria may protect the ocotillol-sensitive cytoplasmic membrane. These results illustrated that some old drugs like kanamycin or second line antibiotics (chloramphenicol) may have potential use at lower doses and thus lowered cytotoxicity when used in combination with these ocotillol-type triterpenoid derivatives.

Based on the results presented above, and our recently reported data,[15, 16] a pharmacophore model of compound 3 was calculated using Accelrys DS2.5 showing possible hydrogen bond (Green: acceptor; Pink: donors) and hydrophobic (Grey) interaction sites.[17] The energy minimized structure showed a "V" form composed by the core backbone and the tetrahydrofuran sidechain, while the three hydrogen donor groups and one hydrogen acceptor group presented nearly at the same plane, suggesting which may the binding surface of the molecule.

Compared to our previous work,[15, 16] an optimized structure-activity relationship could also be concluded and is shown in Figure 3: a) C-3 and C-12 requirements: regardless aromaticity of functional groups, hydrogen bond donors rather than acidic substitutions at C-3 and C-12 are required for maintaining or improving activity against Gram positive bacteria;[16] the functional groups at C-3 and C-12 converted to ketones showed decreased activity;[15] a non-hydrogen bond donor ester substitution at C-12 showed mild activity against Gram negative bacteria; b) C-3 and the activity: at the 3-OH, aliphatic chain substituted derivatives may also display, only mild activity against Gram positive bacteria, including community-acquired methicillin resistant *S. aureus* USA300. c) C-24 and C-3 relationship: the (24S)-configuration is preferred for antibacterial activity of compounds without substitution at the 3-OH; substitution at 3-OH may cause conformational changes resulting in bioactive (24R)-compounds, suggesting this minor change of conformation by substitution at the 3-OH may be preferred for compound activity. [16] Based on the computational modeling and the SAR study, the functional groups and their positionings required for activity are known, which may help in the future synthetic work to replace the complex ocotillol core by simplified structure and facilitate the derivatisation of these bioactive compounds.

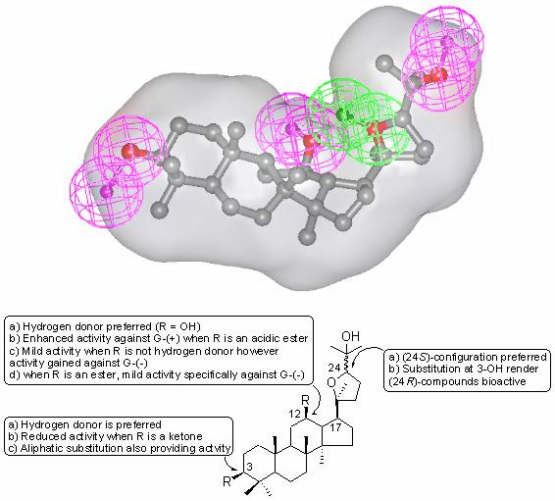


Figure 3. Binding surface of compound 3 (top) and Preliminary SARs of ocotillol-type derivatives (bottom)

4. Conclusion

A new series of hydrophilic ocotillol-type triterpenoid derivatives were synthesised and assayed for their antibacterial activity against Gram-positive and Gram-negative bacteria. Compounds **3**, **5**, **6**, **7**, **8**, **14**, **15** and **16** displayed good antibacterial activity against Gram-positive bacteria including the significant community acquired methicillin resistant pathogen *S. aureus* USA300 with MIC values of 2-16 μg/mL, suggesting that substitutions at the C-3 hydroxyl group of the steroid skeleton are critical

determinants of activity, while stereochemistry of C-24 may also be important when the C-3 hydroxyl group was free of substitution. However, none of the derivatives exhibited antibacterial activity against Gram-negative organisms, suggesting that modification at C-12, as previously reported, will be a suitable target for derivatisation when targeting this group.[15] The subsequent synergistic activity assay of these derivatives was also carried out with results showing that compounds **6** and **15** could reduce the MICs of kanamycin and chloramphenicol against *S. aureus* USA300 and *B. subtilis* 168 significantly. Unlike cholesterol, these compounds targeting the cell membrane are highly hydrophilic despite having similar steroid structures, which may provide new insights into the development of therapeutic agents distinct from those of well-known classes of antimicrobial agents. Further investigations on the mechanism of antibacterial action of these ocotillol-type triterpenoid derivatives are currently under way and the results will be reported in due course.

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