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Design, synthesis and biological evaluation of novel ocotillol-type triterpenoid derivatives as antibacterial agents

Xiao Shao[a], Shengyu Zhang[a], Yunyun Zhou[b], Xianming Zeng[a] and Zhiwen Zhou*[a]

[a]Department of Pharmacy, Medical College, China Three Gorges University, Yichang, China

E-mail: zhiwen.zhou@hotmail.com

[b]Department of Quality Control, China Resources Sanjiu (Huangshi) Medical & Pharmaceutical Co., Ltd., Huangshi, China

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A new series of hydrophilic ocotillol-type triterpenoid derivatives have been synthesized and evaluated for their *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. Among which, compounds **9**, **10**, **11c, 12c, 13a-d** and **14a-d** displayed potent antibacterial activity against Gram-positive bacteria with MIC values of 1–16 μg/mL. Furthermore, additional testing against MRSA USA300 demonstrated that compounds **9**, **13b**, **13c** and **14c** also possess good antibacterial activity with MIC values of 2–8 μg/mL. The bactericidal effects revealed that compounds **9**, **13b** and **13c** displayed directly bactericidal activity against *B. subtilis* and MRSA USA300 with MBC values of 2–16 μg/mL. The subsequent synergistic activity assay showed that compounds **13b** and **13c** could enhance the susceptibility of MRSA USA300 and *B. subtilis* to kanamycin and chloramphenicol (FICI < 0.5). Compounds **13b** and **13c** were then evaluated for their cytotoxicity and displayed low toxicity at their antibacterial MICs. The optimized structure-activity relationship was also concluded.

Keywords: Ocotillol, triterpenoid, antibacterial activity, synergistic effect, cytotoxicity, structure-activity relationship.

Introduction

Since the introduction of the first sulfonamides and penicillins in 1935 and 1940, respectively, the once marked mortality rate associated with bacterial infections experienced a remarkable downturn[1,2]. Antibiotics and synthetic antibacterial agents such as nitrofuranes, cephalosporins, tetracyclines, macrolides and oxazolidinones have saved millions of lives and eased patients' suffering. However, despite a number of significant improvements in antibacterial therapy, many problems remain to be solved for most antimicrobial drugs available. For instance, appearance of multidrug resistant Gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) is a serious menace[3–5]. The use of amphotericin B known as the 'gold standard' is also limited by nephrotoxicity[6,7]. For these reasons, the development of novel antimicrobial drugs with new scaffolds is still necessary and very much in demand.

Natural products have been the mainstay in providing novel chemical scaffolds for many drugs as well as leads that were chemically modified and developed as antibacterial agents[8,9]. A number of natural products, e.g. berberine, cephalosporin and allicin, are still in use as antibacterial agents today[10]. The prevalence of natural product-derived antibacterial drugs may be due to the evolution of secondary metabolites as biologically active chemicals that conferred selectional advantages to the producing organisms[11]. In addition, natural products generally possess complex architectural scaffolds and densely deployed functional groups, affording the maximal number of interactions with molecular targets, often leading to exquisite selectivity for pathogens versus the host[12].

Triterpenoids are members of a larger family of structurally related compounds known as cyclosqualenoids that are widely distributed in the plant kingdom[13,14]. Active triterpenoids like squalamine, trodusquemine and petromyzanonamine disulfate (Fig. 1), which are well known steroid-polyamine conjugates have been reported to possess good antimicrobial activity[15,16]. Study showed that plant derived triperpenoid saponins are likely to have evolved to penetrate cell membranes by forming pore-like channels and interact with specific protein targets, leading a series of spe-

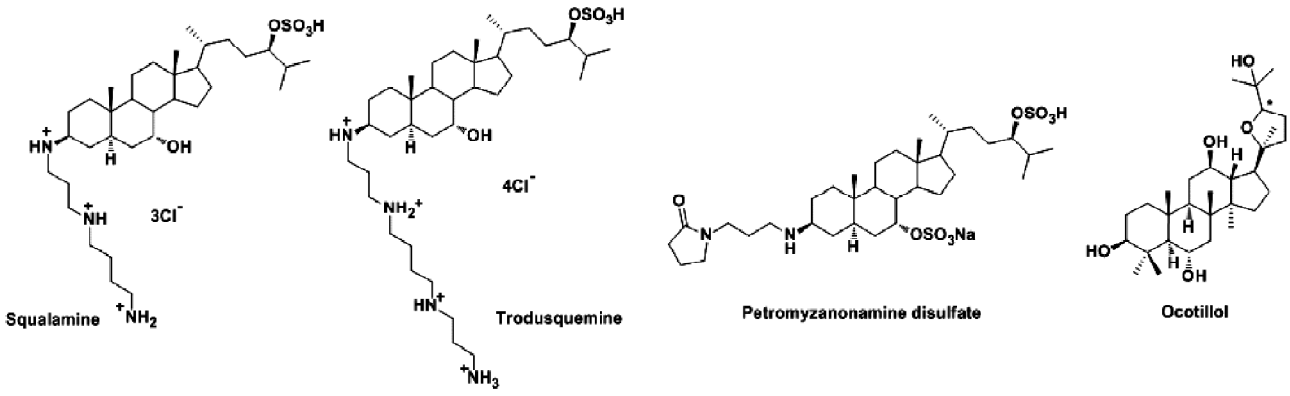

Fig. 1. Structures of squalamine, trodusquemine, petromyzanonamine disulfate and ocotillol.

cific biological effects such as secretion processes, ion channel activation/inhibition or change in the membrane structure[17,18]. However, the molecular mechanisms of interactions between triterpenoids and elements of cell membranes are widely unknown.

Ocotillol (Fig. 1), a triterpene isolated from *Fouquieria splendens* Engelm, is characterized by bearing a five-membered epoxy ring at C-20[19]. We previously reported a series of novel ocotillol-type triterpenoid derivatives and found that the compounds attached hydrophilic amino group or carboxyl group at C-3 side chain displayed potent antibacterial activity particularly against Gram-positive bacteria. Furthermore, these compounds showed strong synergistic effects when combined with kanamycin and chloramphenicol with MIC values of less than 0.0020 μg/mL against *S. aureus* and *B. subtilis* 168, respectively[20–22]. In order to further explore the antibacterial activity of ocotillol-type triterpenoid derivatives and establish their structure-activity relationships, herein, we described the synthesis and evaluation of series hydrophilic ocotillol-type triterpenoid derivatives bearing amino or carboxyl group at C-3 side chain as antibacterial agents. Although some compounds in the paper are common to those of a recently published paper[23] where main objective was to study the evaluation for their ability to reverse multidrug resistance in cancer. The present paper, in combination with previous publications[20–22], provides a more detailed explanation of the structure-activity relationships of this kind of compounds in the field of antimicrobial agents.

Results and discussion

*Structure determination of compounds **3** and **4**:*

Single crystals of **3** and **4** were obtained from ethyl acetate, and their X-ray crystallography clearly showed that 20,24-epoxy fraction had been formed and a pair of epimers had been synthesized. Crystal data, data collection parameters, and refinement statistics for **3** and **4** was illustrated in Table 1. ORTEP representations are shown in Figs. 2 and 3, respectively, together with the numbering scheme adopted. The X-ray single-crystal diffraction result confirms the configuration of C-24 of **3** and **4** are *S*-form and *R*-form, respectively.

Table 1. Selected crystal data for compounds **3** and **4**

Parameter	Compound **3**	Compound **4**
Empirical formula	$C_{30}H_{52}O_4$	$C_{30}H_{52}O_4$
Formula weight	476.72	476.72
CCDC	911853	841280
Crystal size (mm^3)	0.38×0.20×0.18	0.54×0.50×0.50
Crystal system	Orthorhombic	Orthorhombic
Space group	*P*2 (1) 2 (1) 2 (1)	*P*2 (1) 2 (1) 2 (1)
a (nm)	0.73882 (14)	0.76793 (14)
b (nm)	1.3919 (3)	1.3067 (3)
c (nm)	2.7256 (5)	2.8084 (5)
α (°)	90	90
β (°)	90	90
γ (°)	90	90
V (nm^3)	2.8029 (9)	2.8181 (9)
D_c ($g \cdot cm^{-1}$)	1.130	1.124
F (000)	1056	1056
Absorption (mm^{-1})	0.072	0.072
Θ for data collection (°)	1.64~25.50	1.72~25.50
Final *R* indices	0.0509	0.0416
wR_2	0.1009	0.1107
S	1.074	1.055

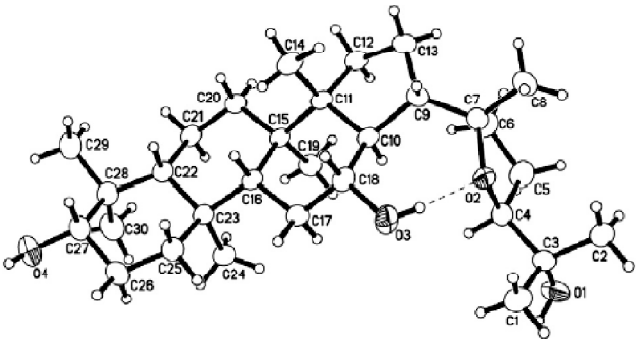

Fig. 2. The ORTEP figure of **3** with thermal ellipsoids shown at 30% probability.

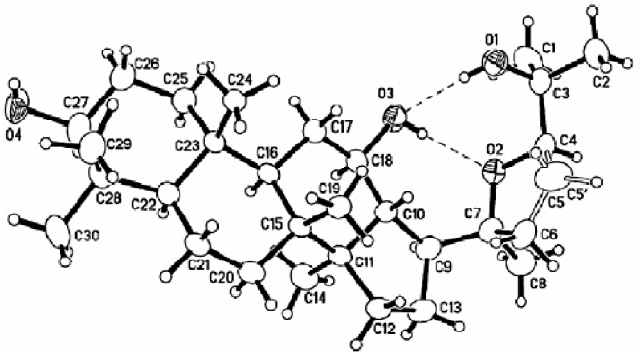

Fig. 3. The ORTEP figure of **4** with thermal ellipsoids shown at 30% probability.

Anbacterial and bactericidal activity:

The obtained novel ocotillol-type triterpenoid derivatives **9**, **10**, **11a-c, 12a-c, 13a-d** and **14a-d** were evaluated for their antimicrobial activities against different types of Gram-positive strains such as *Staphylococcus aureus* RN4220 and *Bacillus subtilis* 168, and Gram-negative strains such as *Escherichia coli* DH5, *Acinetobacter baumannii* ATCC19606 and *Pseudomonas aeruginosa* PAO1. Initial minimum inhibitory concentrations (MICs) screening results are presented in Table 2. The results showed that most of the ocotillol-type derivatives bearing amino or carboxyl group at C-3 side chain displayed good antibacterial activity against Gram-positive bacteria with MIC values of 1–16 μg/mL, probably due to their improved hydrophilicity. Compounds **9** and **10**, with amino group attached at C-3 position, displayed enhanced antibacterial activity (4–16 μg/mL) compared to that of **3** and **4** (8–128 μg/mL), which had a hydroxyl at the same position. These results suggest that substitutions at C-3 side chain of the steroid skeleton are critical determinants of antibacterial activity against Gram-positive bacteria. Compounds **13a-d** and **14a-d** showed excellent inhibitory activity with MIC values of 1–8 μg/mL against *S. aureus*, and with MIC values of 2–8 μg/mL against *B. subtilis*. Among which, **13c** and **14c** were found to be the most active with MIC values of 1 and 2 μg/mL against *S. aureus* and 2 μg/mL against *B. subtilis*, respectively. The structures of **13a-d** or **14a-d** are similar in all respects except for the length of C-3 side chain. A comparative study of **13a-d** or **14a-d** suggested that a specific chain length of C-3 can significantly influence the activity of the derivatives. Additionally, compared to **14a-d** (C-24*R*, 2–16 μg/mL), their stereoisomers **13a-d** (C-24*S*, 1–8 μg/mL) displayed generally better antibacterial activity against the Gram-positive bacteria, which indicated that the stereochemistry of the isopropanol group at C-24 may play a key role for the binding of the pharmacologically active compounds with the target receptor in bacteria. Meanwhile, compound **11c** possessing a carboxylic acid at C-3 side chain displayed good antibacterial activity against both Gram-positive bacterial strains with MIC values of 8 and 4 μg/mL, while its stereoisomer **12c** showed good antibacterial activity only against *B. subtilis* (4 μg/mL) and moderate antibacterial acti-

Table 2. *In vitro* antibacterial activity of ocotillol-type derivatives (MIC: μg/mL)

Strain	*S. aures*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. baumannii*
3	8	8	>128	>128	>128
4	64	128	128	>128	>128
9	4	4	>128	128	>128
10	8	16	64	>128	64
11a	128	128	>128	>128	>128
11b	128	>128	>128	>128	128
11c	8	4	>128	>128	>128
13a	4	4	>128	>128	>128
13b	2	4	>128	>128	>128
13c	1	2	64	64	32
13d	8	4	64	64	64
12a	128	>128	64	>128	>128
12b	>128	128	>128	128	>128
12c	16	4	>128	128	128
14a	4	8	64	128	128
14b	4	4	>128	>128	>128
14c	2	2	>128	64	128
14d	8	16	128	128	>128
KAN[a]	1	0.25	1	8	1

[a]KAN: kanamycin.

vity against *S. aureus* (16 μg/mL). However, when the amino group at C-3 was acetylated or benzoylated, the antibacterial activity of compounds **11a**, **11b**, **12a** and **12b** disappeared regardless of the stereochemistry at C-24. Furthermore, only one derivative, **13c** exhibited moderate activity against the Gram-negative bacteria with an MIC of 32 μg/mL observed for *A. baumannii*, which confirmed the capacity of these compounds to target Gram-negative bacteria, but further modification should be carried out. As shown in Table 3, the bioactive compounds against Gram-positive bacteria were chosen for testing against a significant highly pathogenic methicillin-resistant strains *S. aureus* USA300 (MRSA USA300), which were also demonstrated to be quinolone-resistant. The results revealed that compound **13c** showed excellent activity against MRSA USA300 with MIC of 2 μg/mL, while **9**, **13b** and **14c** displayed good activity with MIC of 8 μg/mL, respectively. However, compounds **10**, **11c**, **12c**, **13a**, **14a** and **14b** could only exhibit moderate to mild activity against this pathogen with MIC values of 32–64 μg/mL.

Table 3. Antibacterial activity of ocotillol-type derivatives against MRSA USA300 (MIC: μg/mL)

Compds.	MRSA USA300
9	8
10	64
11c	32
13a	32
13b	8
13c	2
12c	64
14a	32
14b	32
14c	8
KAN[a]	1

[a]KAN: kanamycin.

The testing of minimum bactericidal concentration (MBC) was also carried out against *B. subtilis* and MRSA USA300. The results are listed in Table 4. Compounds **9**, **13b**, **13c** and **14c** possessed good bactericidal activity against *B. subtilis* with MBC values of 2–16 μg/mL. In contrast, compounds **9**, **13b** and **13c** also displayed directly bactericidal activity against MRSA USA300 with MBC values of 16, 16 and 4 μg/mL, respectively, while compound **14c** only kept moderate bactericidal activity against this pathogen with MBC of 32 μg/mL, which, again, confirmed the significance of stereochemistry of C-24. The bactericidal effects revealed the ocotillol-type derivatives not only can affect bacterial cell viability, but also cause cell death, which could be as leads for further research.

Table 4. Bactericidal activity of **9**, **13b**, **13c** and **14c** against *B. subtilis* and MRSA USA300 (MBC: μg/mL)

Strain	*B. subtilis*	MRSA USA300
9	16	16
13b	8	16
13c	2	4
14c	4	32
KAN[a]	1	2

[a]KAN: kanamycin.

Synergistically antibacterial activity:

Triterpenoid compounds have been proven to possess antibacterial effects by disruption of the cytoplasmic membrane which led to the leakage of intracellular constituents. As membrane perturbing agents, the ocotillol-type derivatives may be able to synergistically strengthen the antibacterial activity of antibiotics targeting intracellular process. The synergistic effects of compounds **13b** and **13c** were then investigated at their sub-MIC concentrations in combination with bacterial protein synthesis inhibitors kanamycin and chloramphenicol against MRSA USA300 and *B. subtilis*. The effects were evaluated by calculating the Fractional Inhibitory Concentration Index (FICI) using Fractional Inhibitory Concentration (FIC). As shown in Table 5, compounds **13b** and **13c** reduced the MICs of kanamycin from 1 μg/mL to 0.016 and 0.0020 μg/mL against MRSA USA300 (FICI = 0.018, 0.003), and from 0.25 μg/mL to 0.0078 and 0.0039 μg/mL against *B. subtilis* 168 (FICI = 0.033, 0.018), respectively. Strong synergistic activity was also observed by use of combinations of each of **13b** and **13c** with chloramphenicol. The FICIs of **13b** and **13c** and chloramphenicol were calculated to be between 0.0020 and 0.190, respectively, which was significant smaller than 0.5, suggesting that they acted synergistically to inhibit the growth of both strains. In contrast, when compounds **13b** and **13c** were combined with kanamycin, the MBC values of kanamycin significantly decreased from 4 μg/mL to 1 and 0.25 μg/mL against MRSA USA300, respectively. For *B. subtilis* 168, potent bactericidal activity was also observed during the combination of **13b**

Table 5. Synergistic effect of antibiotics with compounds **13b** and **13c** against MRSA USA300 and *B. subtilis* 168

Compd.	MIC (μg/mL)		MBC (μg/mL)		FICI (FIC index)[d]	
	MRSA USA300	*B. subtilis*168	MRSA USA300	*B. subtilis*168	MRSA USA300	*B. subtilis*168
KAN[a]	1	0.25	4	1	–	–
CHL[b]	4	2	N/A[c]	N/A	–	–
13b + KAN	0.016	0.0078	1	0.5	0.018	0.033
13c + KAN	0.0020	0.0039	0.25	0.25	0.003	0.018
13b + CHL	0.0078	0.25	N/A	N/A	0.0029	0.188
13c + CHL	0.0039	0.125	2	0.5	0.0029	0.125

[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.

and **13c** with kanamycin. Note that chloramphenicol alone was a bacteriostatic agent, but displayed promising bactericidal effect when combined with **13c**, with MBC values of 2 μg/mL against MRSA USA300 and 0.5 μg/mL against *B. subtilis* 168. These results suggested that ocotillol-type derivatives are suitable for combination with other antibiotics, specially the ones like kanamycin and chloramphenicol which are currently limited in use as a result of their toxicity.

Cytotoxicity assays:

In order to determine whether the antibacterial activity was caused by selective toxicity towards the bacterial cells, compounds **13b** and **13c** were chosen to test the cytotoxicity against human cervical (HeLa) and human epithelial kidney (HEK-293) cells by MTT assay. The results in Table 6 showed that both compounds **13b** and **13c** displayed low toxicity with IC_{50} values about 40 μg/mL against HeLa cells and about 130 μg/mL against HEK-293 cells, which suggested that ocotillol-type triterpenoid derivatives will not affect cell viability at their antibacterial MICs against Gram-positive bacteria.

Table 6. Cytotoxic activity of compounds **13b** and **13c** against HeLa and HEK-293 cells

Compds.	IC_{50}[a,b] (μg/mL)	
	HeLa	HEK-293
13b	42.31±4.32	135.23±6.18
13c	43.78±3.53	132.56±5.97
5-FU[c]	0.83±0.22	–

[a]IC_{50} is the concentrations required to inhibit 50% of cell growth. [b]Results are expressed as the mean ± S.D. of three independent experiments. [c]5-FU: 5-fluorouracil.

Structure-activity relationship of ocotillol-type derivatives as antimicrobial agents:

Based on the antibacterial activity of ocotillol-type triterpenoid derivatives and the literature we previously reported, an optimized structure-activity relationship could also be concluded. Hydrogen bond donors at C-3 and C-12 are required for activity against Gram-positive bacteria, while decreased activity was observed when the functional groups at C-3 and C-12 turned to ketone as hydrogen accepter. When the hydroxyl at C-3 position was replaced with amine group, the antibacterial activity against Gram-positive bacteria enhanced significantly. The length of 2–7 carbon atoms at C-3 side chain is preferred if there are amine groups attached on the side chain, and 5 carbon atoms were found to be the best. Furthermore, the antibacterial activity of C-24*S* isomer derivatives were generally better than that of their stereoisomers, which suggested C-24 configuration play a role in the interaction of compounds with cell membrane system.

Mechanism speculation:

The modes of action of triterpenoid compounds were mainly related to cell membrane of bacteria. According to the literature[24], spontaneous formation of complexes between triterpenoids and cholesterol in membranes is followed by association of these complexes into 'two-dimensional micellar-type structures' within the membrane. The hydrophilic chains of the triterpenoids, which are thought to be centrally orientated in the micellar-like complex, lead to formation of an aqueous pore. These pores would cause an increase in membrane permeability enabling ions and macromolecules up to proteins to pass the membrane bilayer. Compound **13c**

possessed good antibacterial activity, promising bactericidal effect and strong synergistic antibacterial activity which was suitable for the research of mode of action towards cell membrane system, and this part of the work is now in progress and will be reported in due course.

Experimental

General:

Most chemicals and solvents were analytical grade and, when necessary, were purified and dried with standard methods. Melting points were determined in open capillaries and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded respectively on Bruker AV-300 and Bruker AV-75 spectrometer using trimethylsilane (TMS) as an 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. Single crystal X-ray diffraction was analyzed by Bruker SMART APEX CCD-based diffractometer (Mo Kα radiation, λ = 0.71073 Å).

Synthetic chemistry:

Applied methodology to produce ocotillol-type triterpenoid derivatives **11a-c**, **12a-c**, **13a-d** and **14a-d** is as depicted in Fig. 4 and Fig. 5. The synthesis of two stereoisomeric triols **3** and **4** was done from 20(*S*)-protopanaxadiol (PPD) by the previously reported literature procedures[20,25]. Triol **3** was then oxidized by pyridinium chlorochromate (PCC) to provide ketone **5**, which was reacted with hydroxylamine hydrochloride in pyridine yielded oxime **7**. Then, the reduction of **7** with sodium cyanoborohydride in the presence of titanium chloride and ammonium acetate in methanol gave a mixture of 3β and 3α amine forms. Since it was difficult to purify the mixture using silica gel column chromatography, *t*-butoxycarbonylation was carried out by a general method, then the product was purified using silica gel column chromatography. Next, the fraction was subjected to de-*t*-butoxycarbonylation with trifluoroacetic acid (TFA) to give 3β-amine compound **9** (24*S*,3β)[26,27]. The same procedure applied to triol **4** afforded new compound **10** (24*R*, 3β). Compound **9** was then reacted with a series of carboxylic acids,

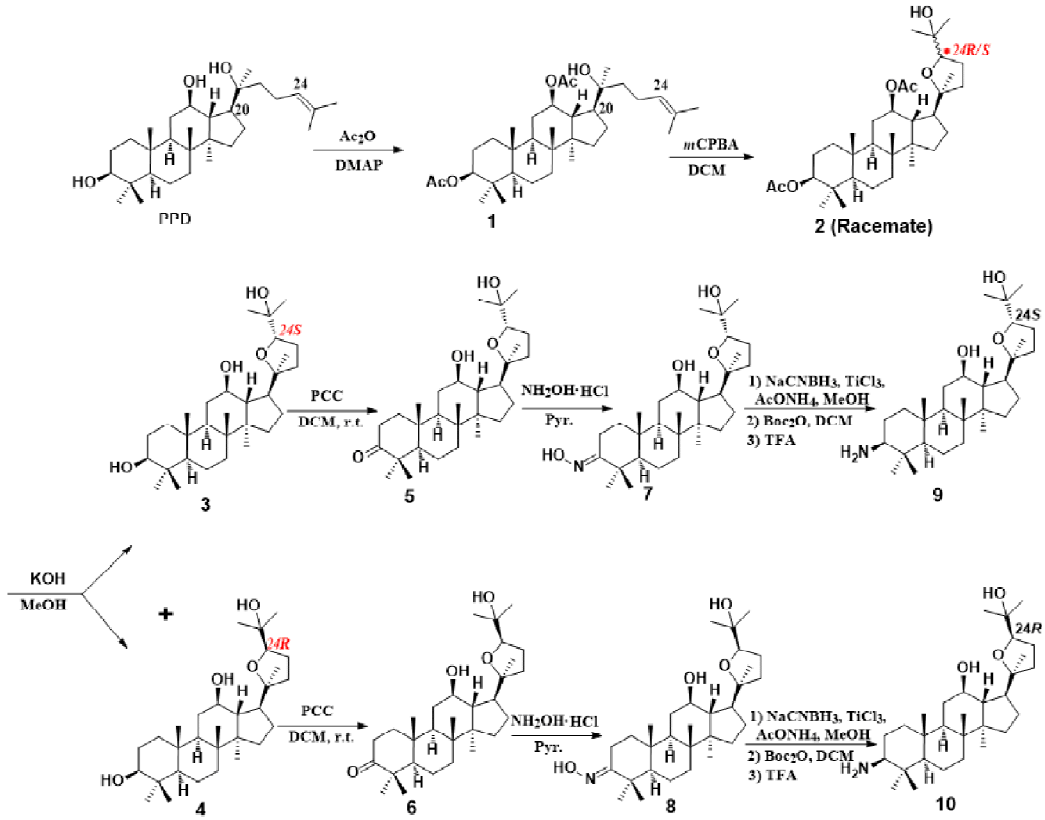

Fig. 4. Synthesis of compounds **9** and **10**.

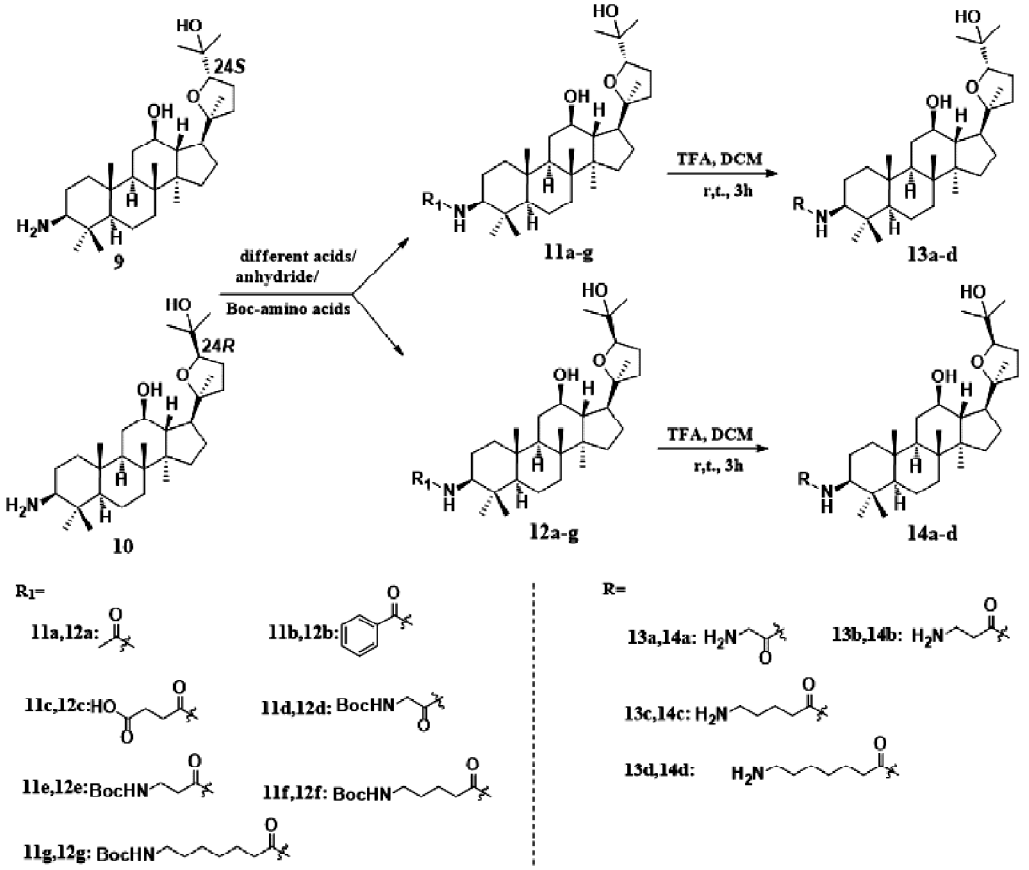

Fig. 5. Synthesis of target compounds **11a-c**, **12a-c**, **13a-d** and **14a-d**.

anhydrides and protected amino acids to give **11a-g**, and amino compounds **13a-d** were obtained by deprotection using TFA. The same procedure applied to **10** produced compounds **12a-g**, and amino compounds **14a-d** were also gained by deprotection in TFA.

*General procedure for the preparation of 20(S)-protopanaxadiol (PPD), **3** and **4**:*

PPD were prepared from total ginsenosides by alkaline hydrolysis according to the literature[25]. (20*S*,24*S*)-Epoxy-dammarane-3β,12β,25-triol (**3**) and (20*S*,24*R*)-epoxy-dammarane-3β,12β,25-triol (**4**) were synthesized from **PPD** as previously described[20].

*(20S,24S)-Epoxy-dammarane-3β,12β,25-triol (**3**):*

M.p. 199–203°C; ESI-MS *m/z* 477.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.87 (dd, *J* 10.2, 5.1 Hz, 1H), 3.52 (td, *J* 10.2, 4.8 Hz, 1H), 3.19 (dd, *J* 10.8, 5.4 Hz, 1H), 2.25 (td, *J* 10.5, 4.2 Hz, 1H), 1.27 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 87.3, 87.0, 78.7, 70.4, 69.9, 55.8, 52.0, 50.1, 48.8, 48.7, 39.6, 38.8 (overlapping signal), 37.0, 34.6, 32.1, 31.5, 31.5, 28.7, 28.4, 27.9, 27.8, 27.3, 24.9, 24.1, 18.2, 17.6, 16.1, 15.3, 15.2; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{53}O_4$ $[M+H]^+$: 477.3938, Found: 477.3946.

*(20S,24R)-Epoxy-dammarane-3β,12β,25-triol (**4**):*

M.p. 193–195°C; ESI-MS *m/z* 477.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.84 (dd, *J* 8.4, 6.6 Hz, 1H), 3.52 (td, *J* 10.5, 4.8 Hz, 1H), 3.19 (dd, *J* 10.8, 5.1 Hz, 1H), 2.19 (td, *J* 10.8, 3.6 Hz, 1H), 1.28 (s, 3H), 1.27 (s, 3H), 1.10 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 86.4, 85.3, 78.7, 70.9, 70.0, 55.9, 51.9, 50.4, 49.3, 47.9, 39.7, 38.9, 38.8, 37.1, 34.7, 32.5, 31.2, 31.1, 28.5, 27.9, 27.8, 27.5, 27.39, 26.0, 24.9, 18.2, 18.1, 16.2, 15.3, 15.2; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{53}O_4$ $[M+H]^+$: 477.3938, Found: 477.3934.

*General procedure for the synthesis of (20S,24S)-epoxy-dammarane-3β-amine-12β, 25-diol (**9**) and (20S, 24R)-epoxy-dammarane-3β-amine-12β,25-diol (**10**):*

To a solution of **3** (or **4**) (100 mg, 0.21 mmol) in dry dichloromethane (8 mL) was added pyridinium chlorochromate (88 mg, 0.40 mmol), the mixture was stirred at room temperature for 3 h, then filtered, concentrated *in vacuo* and the residue was purified over silica gel with petroleum ether-ethyl acetate to give ketone **5** (or **6**) as a white solid.

To a solution of **5** (or **6**) (100 mg, 0.21 mmol) in anhydrous pyridine (8 mL), hydroxylamine hydrochloride (29 mg, 0.42 mmol) was added. The reaction mixture was stirred at 70°C for 3 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 residue was purified by silica gel column chromatography to afford the desired oxime **7** (or **8**) as a white solid.

To a solution of **7** (1.0 g, 2.04 mmol) in methanol (25 mL) was added sodium cyanoborohydride (0.7 g, 11 mmol) and ammonium acetate (0.9 g, 11.5 mmol) under nitrogen atmosphere, the mixture was chilled in ice water, and 15% aqueous titanium trichloride (2.9 mL) was added dropwise over 20 min. After stirring at room temperature for 15 h, the resulting mixture was adjusted with 2 *N* sodium hydroxide to pH 10. The aqueous solution was extracted with chloroform, and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure to give a mixture of 3α- and 3β-amine (805 mg).

To the mixture in dichloromethane (20 mL) was added Boc_2O (452 mg, 2.1 mmol). The reaction mixture was stirred at room temperature for 5 h and then diluted with dichloromethane. The solution was washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified over silica gel with petroleum ether-ethyl acetate to give (20*S*,24*S*)-epoxy-dammarane-3β-*N*-(*tert*-butoxycarbonyl)-12β,25-diol (461 mg, 41%).

Trifluoroacetic acid (0.6 mL) was added to a solution of (20*S*,24*S*)-epoxy-dammarane-3β-*N*-(*tert*-butoxycarbonyl)-*1*2β,25-diol (461 mg, 0.8 mmol) in dichloromethane, the mixture was stirred at room temperature for 3 h and then adjusted with 1 *N* sodium hydroxide to pH 10. The aqueous solution was extracted with dichloromethane, and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give **9** (323 mg, 80%) as a white solid. The same procedure applied to oxime **8** (1.0 g, 2.04 mmol) afforded 3β-amine **10** (310 mg, 31%, from **8** to **10**) as a white solid.

*(20S,24S)-Epoxy-12β,25-dihydroxy-dammarane-3-one (**5**):*

White solid (67 mg, 65%); m.p. 167–169°C; ESI-MS *m/z* 475.3 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.87 (dd, *J* 10.3, 4.8 Hz, 1H), 3.52 (td, *J* 9.9, 4.3 Hz, 1H), 2.42–2.49 (m, 1H), 2.39 (dd, *J* 10.9, 7.8 Hz, 1H), 2.25 (td, *J* 10.3, 4.2 Hz, 1H), 1.25 (s, 3H), 1.22 (s, 3H), 1.13 (s, 3H), 1.06 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 217.5, 87.3, 87.1, 70.2, 69.9, 54.8, 51.9, 49.3, 48.8, 48.6, 47.1, 39.9, 39.5, 36.8, 34.1, 33.7, 32.5, 32.2, 31.6, 28.9, 28.5, 27.5, 26.9, 24.8, 24.3, 19.9, 19.6, 17.5, 15.9, 15.3; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{51}O_4$ $[M+H]^+$: 475.3787, Found: 475.3780.

*(20S,24R)-Epoxy-12β,25-dihydroxy-dammarane-3-one (**6**):*

White solid (62 mg, 62%); m.p. 161–163°C; ESI-MS *m/z* 475.3 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.86 (dd, *J* 8.5, 6.2 Hz, 1H), 3.49 (td, *J* 10.3, 4.8 Hz, 1H), 2.47–2.53 (m, 1H), 2.39 (dd, *J* 11.5, 7.5 Hz, 1H), 2.18 (td, *J* 10.9, 3.2 Hz, 1H), 1.25 (s, 6H), 1.13 (s, 3H),1.09 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 217.4, 86.4, 85.4, 70.7, 69.8, 55.1, 52.3, 50.0, 49.6, 48.1, 47.5, 39.8, 39.2, 37.1, 34.2, 33.6, 32.9, 31.3, 31.1, 28.8, 28.1, 27.6, 26.8, 25.9, 24.6, 20.4, 19.9, 18.0, 16.1, 15.3; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{51}O_4$ $[M+H]^+$: 475.3787, Found: 475.3790.

*(20S,24S)-Epoxy-3-oxime-dammarane-12β,25-diol (**7**):*

White solid (77 mg, 75%); m.p. 206–211°C; ESI-MS *m/z* 490.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.86 (dd, *J* 9.8, 5.0 Hz, 1H), 3.49 (td, *J* 10.0, 4.8 Hz, 1H), 2.89–2.98 (m, 1H), 2.23–3.31 (m, 2H), 2.00–2.10 (m, 2H), 1.60–1.89 (m, 6H), 1.29 (s, 3H), 1.23 (s, 3H), 1.18 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 168.7, 87.5, 86.9, 71.1, 70.0, 54.9, 52.1, 49.8, 49.0, 48.7, 47.1, 40.0, 39.7, 36.8, 34.0, 34.0, 32.3, 32.1, 31.8, 28.9, 28.4, 28.1, 26.3, 25.0, 23.9, 21.0, 19.7, 17.8, 16.0, 15.5; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{52}NO_4$ $[M+H]^+$: 490.3896, Found: 490.3902.

*(20S,24R)-Epoxy-3-oxime-dammarane-12β,25-diol (**8**):*

White solid (80 mg, 78%); m.p. 206–210°C; ESI-MS *m/z*

490.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.89 (dd, *J* 10.2, 4.9 Hz, 1H), 3.49 (td, *J* 10.1, 5.8 Hz, 1H), 2.91–2.95 (m, 1H), 2.19–3.17 (m, 2H), 2.01–2.12 (m, 2H), 1.27 (s, 3H), 1.21 (s, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 1.06 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 167.2, 85.9, 85.1, 71.0, 70.1, 55.2, 52.0, 49.9, 49.3, 48.0, 47.1, 40.0, 39.8, 36.5, 34.2, 34.0, 32.6, 31.4, 30.9, 28.4, 28.0, 27.4, 26.3, 26.0, 25.0, 21.1, 19.7, 18.0, 16.1, 15.3; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{52}NO_4$ $[M+H]^+$: 490.3896, Found: 490.3902.

*(20S,24S)-Epoxy-3β-amine-dammarane-12β,25-diol (**9**):*

White solid (323 mg, 33%, from **7** to **9**); m.p. 178–186°C; ESI-MS *m/z* 476.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.90 (dd, *J* 10.6, 5.7 Hz, 1H), 3.55 (dd, *J* 11.0, 5.1 Hz, 1H), 2.62 (dd, *J* 9.5, 3.7 Hz, 1H), 2.19–2.23 (m, 1H), 1.25 (s, 3H), 1.24 (s, 3H), 1.08 (s, 3H), 1.03 (s, 3H), 1.00 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 89.3, 88.2, 78.6, 70.0, 64.6, 55.4, 51.3, 49.5, 48.8, 48.6, 42.1, 38.9, 38.4, 37.0, 33.6, 31.9, 31.6, 31.5, 28.8, 28.4, 28.0, 27.7, 27.4, 25.0, 24.1, 17.9, 17.6, 15.9, 15.6, 15.1; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{54}NO_3$ $[M+H]^+$: 476.4104, Found: 476.4108.

*(20S,24R)-Epoxy-3β-amine-dammarane-12β,25-diol (**10**):*

White solid (310 mg, 31%, from **8** to **10**); m.p. 165–171°C; ESI-MS *m/z* 476.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.86 (dd, *J* 10.4, 5.5 Hz, 1H), 3.50 (dd, *J* 10.9, 5.0 Hz, 1H), 2.65 (td, *J* 9.8, 3.9 Hz, 1H), 2.18–2.25 (m, 1H), 1.27 (s, 3H), 1.24 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 85.9, 84.8, 78.2, 70.4, 64.5, 55.4, 51.5, 49.3, 48.8, 48.6, 41.9, 38.6, 38.6, 37.2, 33.5, 31.8, 31.6, 31.3, 28.9, 28.3, 28.1, 27.6, 27.4, 25.2, 24.3, 17.8, 17.6, 16.2, 15.8, 15.5; HR-MS (ESI) *m/z*: Calcd. for $C_{30}H_{54}NO_3$ $[M+H]^+$: 476.4104, Found: 476.4110.

*General procedure for the synthesis of compounds **11a-c**, **12a-c**, **13a-d** and **14a-d**:*

To a solution of **9** (or **10**) (60 mg, 0.126 mmol, 1.0 eq.) in dry dichloromethane was added EDCI (24 mg, 0.126 mmol, 1.0 eq.), DMAP (15 mg, 0.126 mmol, 1.0 eq.) and corresponding carboxylic acids, anhydrides or Boc-amino acids (1.5 eq.). After stirring at room temperature for 12–15 h, the reaction mixture was removed *in vacuo* and diluted by ethyl acetate, washed with water and brine successively, dried over anhydrous sodium sulfate, filtered, concentrated, and purified by column chromatography over silica gel with petroleum ether-ethyl acetate or dichloromethane-methanol to give **11a-g** (or **12a-g**).

To a solution of intermediates **11d-g** (or **12d-g**) (1.0 eq.), trifluoroacetic acid (2.0 eq.) was added under an ice bath. After stirring at room temperature for 3–5 h, the solvent present in the reaction mixture was removed *in vacuo* and the residue was purified by column chromatography over silica gel (40:1–10:1 dichloromethane:methanol) to give **13a-d** (or **14a-d**).

*(20S,24S)-Epoxy-3β-N-acetyl-dammarane-12β,25-diol (**11a**):*

White solid (45 mg, 69%); m.p. 185–193°C; ESI-MS *m/z* 518.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.81 (dd, *J* 9.9, 5.1 Hz, 1H), 3.52 (dd, *J* 10.1, 6.1 Hz, 1H), 3.16 (dd, *J* 9.8, 3.9 Hz, 1H), 2.18–2.29 (m, 1H), 2.11 (s, 3H), 1.65–1.99 (m, 5H), 1.27 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.09 (s, 3H), 1.01 (s, 3H), 0.93 (s, 3H), 0.88 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.9, 85.8, 84.8, 77.6, 75.8, 62.3, 56.7, 51.9, 50.4, 49.8, 45.2, 40.1, 39.7, 39.3, 39.2, 36.7, 34.2, 30.9, 28.8, 28.5, 28.0, 27.7, 27.1, 25.9, 24.2, 22.9, 22.1, 18.8, 17.6, 16.3, 15.9, 15.4; HR-MS (ESI) *m/z*: Calcd. for $C_{32}H_{56}NO_4$ $[M+H]^+$: 518.4209, Found: 518.4213.

*(20S,24R)-Epoxy-3β-N-acetyl-dammarane-12β,25-diol (**12a**):*

White solid (41 mg, 63%); m.p. 162–168°C; ESI-MS *m/z* 518.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.79 (dd, *J* 9.8, 4.9 Hz, 1H), 3.51 (dd, *J* 10.3, 5.8 Hz, 1H), 3.11 (dd, *J* 9.9, 3.8 Hz, 1H), 2.15–2.23 (m, 1H), 2.10 (s, 3H), 1.63–1.91 (m, 5H), 1.25 (s, 3H), 1.23 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H), 0.90 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.1, 85.9, 84.3, 76.8, 75.1, 62.4, 55.9, 52.1, 50.6, 49.9, 44.9, 40.5, 39.7, 39.4, 39.2, 37.0, 34.1, 30.8, 28.9, 28.4, 28.1, 27.8, 26.9, 25.8, 24.3, 23.1, 22.5, 18.9, 17.6, 16.1, 15.8, 15.5; HR-MS (ESI) *m/z*: Calcd. for $C_{32}H_{56}NO_4$ $[M+H]^+$: 518.4209, Found: 518.4211.

*(20S,24S)-Epoxy-3β-N-benzoyl-dammarane-12β,25-diol (**11b**):*

White solid (43 mg, 59%); m.p. 180–187°C; ESI-MS *m/z* 580.4 $[M+H]^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.02 (d, *J* 7.8 Hz, 2H), 7.57 (t, *J* 7.4, 7.0 Hz, 1H), 7.41 (t, *J* 7.6, 7.5 Hz, 2H), 4.72 (dd, *J* 10.5, 5.6 Hz, 1H), 3.89 (t, *J* 7.2, 7.3 Hz, 1H), 3.21 (dd, *J* 9.9, 3.9 Hz, 1H), 2.14–2.24 (m, 1H), 1.26 (s, 6H), 1.09

(s, 3H), 1.03 (s, 6H), 0.95 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 165.6, 132.8, 131.2, 129.5, 128.5, 85.8, 84.9, 78.3, 71.4, 63.3, 56.2, 52.2, 50.9, 49.8, 47.9, 40.2, 38.9, 38.7, 37.5, 35.6, 33.2, 31.7, 31.2, 28.9, 28.7, 27.8, 27.3, 26.5, 25.1, 24.0, 18.6, 18.3, 16.9, 16.6, 15.8; HR-MS (ESI) *m/z*: Calcd. for $C_{37}H_{58}NO_4$ $[M+H]^+$: 580.4366, Found: 580.4369.

*(20S,24R)-Epoxy-3β-N-benzoyl-dammarane-12β,25-diol (**12b**):*

White solid (40 mg, 55%); m.p. 168–172°C; ESI-MS *m/z* 580.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.03 (t, *J* 7.1, 1.5 Hz, 2H), 7.51 (t, *J* 7.6, 7.6 Hz, 1H), 7.43 (t, *J* 7.5, 7.1 Hz, 2H), 4.72 (dd, *J* 10.3, 4.5 Hz, 1H), 3.84 (dd, *J* 9.9, 4.7 Hz, 1H), 3.18 (td, *J* 10.1, 5.2 Hz, 1H), 2.15–2.23 (m, 1H), 1.27 (s, 3H), 1.25 (s, 3H), 1.10 (s, 3H), 1.06 (s, 3H), 1.03 (s, 3H), 0.98 (s, 3H), 0.95 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 166.5, 132.9, 131.3, 130.0, 128.7, 85.8, 84.20, 81.3, 71.2, 63.8, 56.5, 52.1, 50.6, 49.2, 49.4, 40.2, 40.0, 38.7, 37.1, 35.0, 32.8, 31.9, 31.3, 29.1, 28.9, 28.3, 28.2, 25.5, 24.6, 24.1, 18.5, 18.0, 16.9, 16.2, 15.9; HR-MS (ESI) *m/z*: Calcd. for $C_{37}H_{58}NO_4$ $[M+H]^+$: 580.4366, Found: 580.4370.

*(20S,24S)-Epoxy-3β-N-(3-carboxy propionyl)-dammarane-12β,25-diol (**11c**):*

White solid (43 mg, 59%); m.p. 190–197°C; ESI-MS *m/z* 576.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.50 (t, *J* 8.1, 7.5 Hz, 1H), 3.82 (t, *J* 8.6, 6.4 Hz, 1H), 3.38 (dd, *J* 9.8, 6.0 Hz, 1H), 2.61–2.76 (m, 4H), 2.13–2.38 (m, 1H), 1.29 (s, 3H), 1.27 (s, 6H), 1.06 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 176.7, 172.1, 87.7, 87.4, 81.5, 70.8, 70.5, 55.6, 52.5, 51.1, 50.0, 48.1, 39.9, 39.1, 38.5, 37.7, 35.1, 33.0, 31.8, 31.2, 30.0, 29.3, 28.9, 28.1, 28.2, 28.0, 26.8, 25.9, 24.1; 18.9, 18.2, 16.5, 16.1, 15.6; HR-MS (ESI) *m/z*: Calcd. for $C_{34}H_{58}NO_6$ $[M+H]^+$: 576.4264, Found: 576.4268.

*(20S,24R)-Epoxy-3β-N-(3-carboxy propionyl)-dammarane-12β,25-diol (**12c**):*

White solid (45 mg, 62%); m.p. 156–162°C; ESI-MS *m/z* 576.4 $[M+H]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.50 (t, *J* 7.9, 7.4 Hz, 1H), 3.85 (t, *J* 8.3, 6.1 Hz, 1H), 3.30 (t, *J* 10.1 Hz, 1H), 2.62–2.69 (m, 4H), 2.12–2.23 (m, 1H), 1.28 (s, 3H), 1.25 (s, 6H), 1.09 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 177.1, 172.3, 86.9, 85.7, 81.7, 71.3, 70.7, 55.9, 52.8, 51.3, 50.6, 48.3, 40.6, 39.1, 38.1, 37.8, 35.4, 33.3, 31.7, 31.0, 30.4, 29.3, 28.7, 28.4, 28.2, 27.5, 26.8, 25.7, 24.3, 18.8, 18.3, 16.7, 16.2, 15.9; HR-MS (ESI) *m/z*: Calcd. for $C_{34}H_{58}NO_6$ $[M+H]^+$: 576.4264, Found: 576.4260.

*(20S,24S)-Epoxy-3β-N-(2-aminoacetyl)-dammarane-12β,25-diol (**13a**):*

Yellow oily matter (32 mg, 48%). ESI-MS: *m/z* 533.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.55 (dd, *J* 10.1, 4.9 Hz, 1H), 3.88 (td, *J* 8.9, 3.2 Hz, 1H), 3.40 (s, 2H), 3.20 (t, *J* 10.0 Hz, 1H), 1.28 (s, 3H), 1.25 (s, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 1.01 (s, 6H), 0.89 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 87.3, 87.0, 81.4, 70.3, 69.9, 55.9, 52.0, 50.2, 48.6, 39.7, 38.6, 38.2, 37.4, 34.5, 32.2, 31.8, 29.5, 29.2, 28.5, 28.2, 27.8, 27.6, 26.3, 25.1, 24.5, 23.4, 18.2, 17.9, 16.7, 16.3, 15.6.

*(20S,24R)-Epoxy-3β-N-(2-aminoacetyl)-dammarane-12β,25-diol (**14a**):*

Yellow oily matter (35 mg, 52%). ESI-MS: *m/z* 533.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.54 (dd, *J* 10.5, 5.8 Hz, 1H), 3.85 (td, *J* 9.2, 4.1 Hz, 1H), 3.41 (s, 2H), 3.15 (t, *J* 9.6 Hz, 1H), 1.29 (s, 3H), 1.26 (s, 3H), 1.17 (s, 3H), 1.11 (s, 3H), 1.08 (s, 6H), 0.90 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.9, 86.4, 85.3, 81.4, 70.8, 70.0, 56.1, 51.9, 49.9, 48.7, 39.8, 38.7, 38.2, 37.3, 34.3, 32.7, 31.9, 29.6, 29.2, 28.7, 28.2, 27.9, 27.6, 26.1, 25.6, 24.7, 23.1, 18.7, 17.4, 16.7, 16.7, 15.3.

*(20S,24S)-Epoxy-3β-N-(3-aminopropionyl)-dammarane-12β,25-diol (**13b**):*

Yellow oily matter (32 mg, 46%). ESI-MS: *m/z* 547.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.51 (dd, *J* 10.2, 4.8 Hz, 1H), 3.88 (td, *J* 9.8, 5.1 Hz, 1H), 3.20 (dd, *J* 9.9, 5.1 Hz, 1H), 2.37 (t, *J* 5.8 Hz, 2H), 2.28 (t, *J* 5.8 Hz, 2H), 1.29 (s, 3H), 1.25 (s, 3H), 1.13 (s, 3H), 1.08 (s, 3H), 0.95 (s, 6H), 0.90 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.6, 87.1, 86.7, 81.9, 70.6, 68.8, 56.2, 52.3, 50.6, 48.7, 48.1, 42.6, 39.5, 38.8, 38.2, 37.4, 34.3, 32.1, 31.9, 29.5, 28.6, 28.2, 28.0, 27.7, 27.4, 26.8, 25.4, 24.9, 23.5, 18.8, 17.9, 16.5, 16.1, 15.4.

*(20S,24R)-Epoxy-3β-N-(3-aminopropionyl)-dammarane-12β,25-diol (**14b**):*

Yellow oily matter (36 mg, 53%). ESI-MS: *m/z* 547.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.55 (dd, *J* 10.1, 5.0 Hz, 1H), 3.80 (td, *J* 9.5, 4.8 Hz, 1H), 3.23 (dd, *J* 10.0, 4.9 Hz, 1H), 2.42 (t, 2H, *J* 6.0 Hz), 2.30 (t, *J* 6.0 Hz, 2H), 1.28 (s, 3H), 1.21 (s, 3H), 1.15 (s, 3H), 1.11 (s, 3H), 0.98 (s, 6H), 0.91 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.2, 87.5, 86.3, 81.4, 71.0, 68.1, 56.1, 52.7, 50.8, 48.9, 48.4, 42.8, 39.6, 38.7, 38.2,

37.5, 34.6, 32.3, 31.9, 29.6, 28.8, 28.4, 28.1, 27.8, 27.2, 26.8, 25.2, 24.9, 23.6, 18.9, 17.6, 16.8, 16.2, 15.4.

*(20S,24S)-Epoxy-3β-N-(5-aminovalanyl)-dammarane-12β,25-diol (**13c**):*

Yellow oily matter (35 mg, 48%). ESI-MS: *m/z* 575.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.48 (dd, *J* 10.1, 4.6 Hz, 1H), 3.85 (td, *J* 9.9, 5.2 Hz, 1H), 3.20 (td, *J* 10.0, 4.9 Hz, 1H), 2.30–2.49 (m, 4H), 2.12–2.23 (m, 1H), 1.27 (s, 3H), 1.24 (s, 3H), 1.14 (s, 3H), 1.09 (s, 3H), 0.99 (s, 3H), 0.92 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.5, 86.4, 85.3, 80.8, 70.9, 70.2, 56.6, 51.9, 50.3, 49.4, 48.9, 39.3, 38.3, 37.6, 37.3, 35.1, 34.6, 32.1, 31.8, 31.3, 29.2, 28.6, 28.3, 27.9, 27.7, 27.4, 26.6, 25.8, 24.2, 23.8, 18.9, 17.9, 16.8, 16.3, 15.2.

*(20S,24R)-Epoxy-3β-N-(5-aminovalanyl)-dammarane-12β,25-diol (**14c**):*

Yellow oily matter (34 mg, 47%). ESI-MS: *m/z* 575.4 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.55 (dd, *J* 10.3, 4.8 Hz, 1H), 3.85 (td, *J* 10.0, 5.3 Hz, 1H), 3.20 (td, *J* 10.2, 4.7 Hz, 1H), 2.50 (t, *J* 7.1 Hz, 2H), 2.20–2.30 (m, 3H), 2.12 (s, 2H), 1.26 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H), 1.11 (s, 3H), 0.99 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.9, 87.7, 87.4, 81.1, 70.8, 70.5, 56.1, 52.8, 50.9, 49.7, 48.7, 39.5, 38.4, 37.8, 37.1, 35.3, 34.8, 32.8, 31.6, 31.1, 28.9, 28.5, 28.1, 27.8, 27.6, 27.1, 26.7, 25.7, 24.8, 23.2, 18.7, 17.6, 16.9, 16.5, 15.7.

*(20S,24S)-Epoxy-3β-N-(7-aminoheptyl)-dammarane-12β,25-diol (**13d**):*

Yellow oily matter (35 mg, 46%). ESI-MS: *m/z* 603.5 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.51 (m, 1H), 3.81 (td, *J* 10.1, 3.9 Hz, 1H), 3.17 (dd, *J* 8.1, 7.0 Hz, 1H), 2.48 (t, *J* 7.5 Hz, 2H), 2.28 (t, *J* 7.9 Hz, 2H), 1.76–1.98 (m, 6H), 1.29 (s, 3H), 1.27 (s, 3H), 1.15 (s, 3H), 1.11 (s, 3H), 0.98 (s, 6H), 0.89 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.8, 87.4, 86.9, 80.8, 70.9, 69.5, 56.0, 52.3, 50.8, 49.0, 48.2, 40.1, 39.4, 38.7, 38.1, 37.5, 34.9, 32.6, 31.9, 31.1, 29.1, 28.8, 28.4, 28.0, 27.8, 27.4, 26.9, 26.5, 25.9, 25.2, 24.6, 23.9, 18.8, 17.9, 16.8, 16.5, 15.4.

*(20S,24R)-Epoxy-3β-N-(7-aminoheptyl)-dammarane-12β,25-diol (**14d**):*

Yellow oily matter (38 mg, 51%). ESI-MS: *m/z* 603.5 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.48 (m, 1H), 3.79 (td, *J* 9.8, 4.2 Hz, 1H), 3.25 (dd, *J* 7.7, 6.8 Hz, 1H), 2.50 (t, *J* 7.1 Hz, 2H), 2.28 (t, *J* 7.6 Hz, 2H), 1.88–2.07 (m, 6H), 1.27 (s, 3H), 1.23 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 0.96 (s, 6H), 0.90 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.2, 87.8, 86.2, 80.6, 71.3, 63.3, 56.5, 52.7, 50.9, 49.3, 48.6, 40.8, 39.6, 38.7, 38.2, 37.7, 34.4, 32.8, 32.3, 31.6, 29.5, 28.7, 28.2, 27.8, 27.6, 27.3, 26.9, 26.1, 25.6, 25.2, 24.8, 23.5, 18.8, 17.5, 16.7, 16.4, 15.6.

Single-crystal structure determination:

Suitable single crystals of **3** and **4** were selected and mounted in air onto thin glass fibers. X-Ray intensity data of **3** and **4** were measured at 293 K on a Bruker SMART APEX CCD-based diffractometer (Mo Kα radiation, λ = 0.71073 Å). None of the crystals showed evidence of crystal decay during data collection. All structures were solved by a combination of direct methods and difference Fourier syntheses and refined against F^2 by the full-matrix least squares technique. Crystal data, data collection parameters, and refinement statistics for **3** and **4** are listed in Table 1.

Pharmacology:

The antibacterial activity, synergistic antibacterial activity and cytotoxicity assays were performed as descried previously[20]. The minimum inhibitory concentrations (MICs) were determined against Gram-positive (*Staphylococcus aureus* RN4220, *Bacillus subtilis* 168 and MRSA USA300) and Gram-negative strains (*Escherichia coli* DH5, *Acinetobacter baumannii* ATCC19606 and *Pseudomonas aeruginosa* PAO1) using a standard LB medium dilution technique. The compounds possessing good antibacterial activity against *Bacillus subtilis* 168 and MRSA USA300 were then selected to determine their bactericidal activity against the same two pathogens, and kanamycin was used as a positive control. The cytotoxicity test of the synthesized compounds *in vitro* against human cervical (HeLa) and human epithelial kidney (HEK-293) cells were performed by MTT assay, and 5-fluorouracil was used as a positive control.

Conclusion

A novel series of hydrophilic ocotillol-type triterpenoid derivatives were synthesized and evaluated for their antibacterial activity against several pathogens. Among which, compounds **9**, **10**, **11c, 12c, 13a-d** and **14a-d** displayed potent antibacterial activity against Gram-positive bacteria with MIC values of 1–16 μg/mL. Furthermore, additional testing against MRSA USA300 demonstrated that compounds **9**,

13b, **13c** and **14c** also possess good antibacterial activity with MIC values of 2–8 μg/mL. The subsequent synergistic activity assay of these derivatives was also carried out with results showing that compounds **13b** and **13c** could enhance the susceptibility of MRSA USA300 and *B. subtilis* 168 to kanamycin and chloramphenicol (FICI < 0.5). Compounds **13b** and **13c** were then evaluated for their cytotoxicity and displayed low toxicity with IC_{50} values about 40 μg/mL against HeLa cells and about 130 μg/mL against HEK-293 cells, respectively. As a result of the good antibacterial activity, low toxicity and novel structures of ocotillol-type triterpenoid derivatives, the present findings may provide new insights into the development of therapeutic agents for the molecules which were distinct from those of well-known classes of antimicrobial agents. Further investigations on 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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References

1. M. S. Butler and A. D. Buss, *Biochem. Pharmacol.*, 2006, **71**, 919.
2. F. Von Nussbaum, M. Brands, B. Hinzen, S. Weigan and D. Häbich, *Angew. Chem. Int. Ed.*, 2006, **45**, 5072.
3. L. B. Rice, *Am. J. Infect. Control*, 2006, **34**, S11.
4. E. D. Brown and G. D. Wright, *Nature*, 2016, **529**, 336.
5. C. T. Walsh and G. Wright, *Chem Rev.*, 2005, **105**, 391.
6. V. Fanos and L. Cataldi, *J. Chemotherapy*, 2000, **12**, 463.
7. L. Ostrosky-Zeichner, K. A. Marr, J. H. Rex and S. H. Cohen, *Clin. Infect. Dis.*, 2003, **37**, 415.
8. M. Lahlou, *Pharmacol. Pharm.*, 2013, **4**, 17.
9. J. Y. Ortholand and A. Ganesan, *Curr. Opin. Chem. Biol.*, 2004, **8**, 271.
10. K. S. Lam, *Trends Microbiol.*, 2007, **15**, 279.
11. S. B. Singh and J. F. Barrett, *Biochem. Pharmacol.*, 2006, **71**, 1006.
12. F. E. Koehn and G. T. Carter, *Nat. Rev. Drug Discov.*, 2005, **4**, 206.
13. X. H. Gao, D. Deeb, H. Jiang, Y. B. Liu and S. A. Dulchavsky, *J. Neuro-oncol.*, 2007, **84**, 147.
14. M. B. Sporn and N. Suh, *Carcinogenesis*, 2000, **21**, 525.
15. H. S. Kim, S. N. Khan, J. R. Jadhav, J. W. Jeong, K. G. Jung and J. H. Kwak, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3861.
16. H. S. Kim, B. S. Choi, K. C. Kwon, S. O. Lee, H. J. Kwak and C. H. Lee, *Bioorg. Med. Chem.*, 2000, **8**, 2059.
17. M. F. Melzig, G. Bader and R. Loose, *Planta Med.*, 2001, **67**, 43.
18. Y. Bi, C. Ma, Z. W. Zhou, T. T. Zhang and H. Y. Zhang, *Rec. Nat. Prod.*, 2015, **9**, 356.
19. E. W. Warnhoff and C. M. M. Halls, *Can. J. Chem.*, 1965, **43**, 3311.
20. Z. W. Zhou, C. Ma, H. Y. Zhang, Y. Bi, X. Chen and Q. G. Meng, *Eur. J. Med. Chem.*, 2013, **68**, 444.
21. Y. Bi, C. Ma, H. Y. Zhang, Z. W. Zhou, J. Yang, Z. L. Zhang, Q. G. Meng and J. Y. Xu, *Chem. Boil. Drug Des.*, 2014, **84**, 489.
22. Y. Bi, X. X. Liu, H. Y. Zhang, X. Yang, Z. Y. Liu, J. Lu and Q. G. Meng, *Molecules*, 2017, **22**, 590.
23. Q. Ren, G. Yang, M. Guo, J. Guo, Y. Li and J. Lu, *Eur. J. Med. Chem.*, 2019, **161**, 118.
24. J. M. Augustin, V. Kuzina, S. B. Andersen and S. Bak, *Phytochemistry*, 2011, **72**, 435.
25. G. J. Du, Q. Dai, S. Williams, C. Z. Wang and C. S. Yuan, *Anti-cancer drug*, 2011, **22**, 35.
26. M. Tsukahara, T. Nishino and I. Furuhashi, *Chem. Pharm. Bull.*, 2005, **53**, 1103.
27. C. M. Ma, S. Q. Cai, J. R. Cui, R. Q. Wang and P. F. Tu, *Eur. J. Med. Chem.*, 2005, **40**, 582.