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Total synthesis and antibacterial study of cyclohexapeptides Desotamide B, Wollamide B and their analogs

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Abstract As natural-product-derived antibiotics, Desotamides A-D and wollamides exhibit growth inhibitory activity against Gram-posivite bacteria (IC_{50} 0.6-7 μM) and are noncytotoxic to mammalian cells (IC_{50}> 30 μM). Herein we firstly report the total synthesis of above two cyclohexapeptides as well as a series of structural variants through solid phase

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peptide synthesis (SPPS), of which DE-3 displayed a 2-fold increase of anti-bacterial activity when compared with the original peptide DE-1. This strategy may offer good improvements for the synthesis of other cyclic peptides.

Keywords Desotamides • Wollamides • Total synthesis • SPPS • Antibacterial

Introduction

In light of emerging drug-resistance among human pathogenic bacteria, bacterial infection has been proved a critical problem calling for the development of new antibacterial molecules.[1-3] It is well known that natural products serve as a valuable treasure in drug discovery and are broadly used in much clinical therapeutics such as treatment of cancer, inflammation, and hyper cholesterolemia.[4-7] Among them, natural-product-derived antibiotics, especially the naturally occurring cyclopeptides, play a pivotal role and capture high attention owing to their intriguing chemical structures and potent biological activities, such as daptomycin[8], vancomycin[9], tyrocidine-A[10].

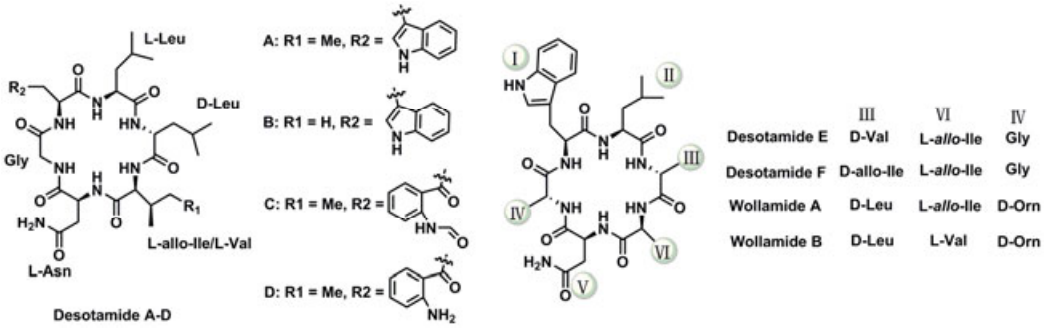
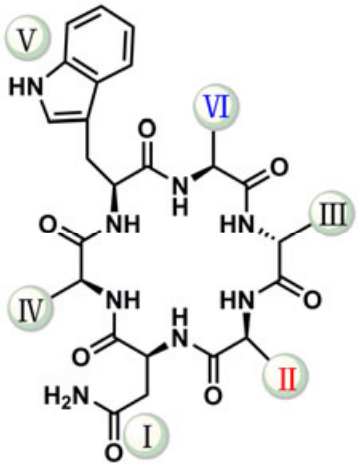


Figure1. Structure of natural cyclopeptides Desotamides A-F and Wollamides A-B.

The cyclohexapeptide desotamides A-D have been isolated from the deep-sea-derived *Streptomyces scopuliridis* SCSIO ZJ46, among which desotamides A and B exhibit notable antibacterial activities against pathogenic Gram-positive strains of *Streptococcus pneumonia* NCTA 7466, *Staphylococcus aureus* ATCC 29213 and methicillin-resistant *Staphylococcus*

epidermidis shhs-E1 (MRSE, a clinical isolate).[11] Another two new homologues desotamides E-F and wollamides A-B have been isolated from soil-derived *Streptomycesnov. sp.* (MST-115088) near Wollogorang Station, Queensland, Australia, which exhibit growth inhibitory activity against Gram-posivite bacteria (IC_{50} 0.6-7 μM) and are noncytotoxic to mammalian cells (IC_{50}> 30 μM).[12] All these natural desotamides and wollamides contain L-*allo*-Ile (Figure 1) other than desotamide B and wollamide B, which show significant growth inhibitory activity against *Staphylococcus aureus* (ATCC9144 and ATCC25923 with IC_{50} 0.9 μM and 0.6 μM).[13]



	I	II	III	IV	V	VI
DE-1	Asn	Val	D-Leu	Leu	Trp	Gly
DE-2	Asn	Val	Leu	Leu	Trp	Gly
DE-3	Asn	Ile	D-Leu	Leu	Trp	Gly
DE-4	Asn	Ile	D-Ile	Leu	Trp	Gly
WO-1	Asn	Val	D-Leu	Leu	Trp	Orn
WO-2	Asn	Val	D-Leu	Leu	Trp	D-Orn

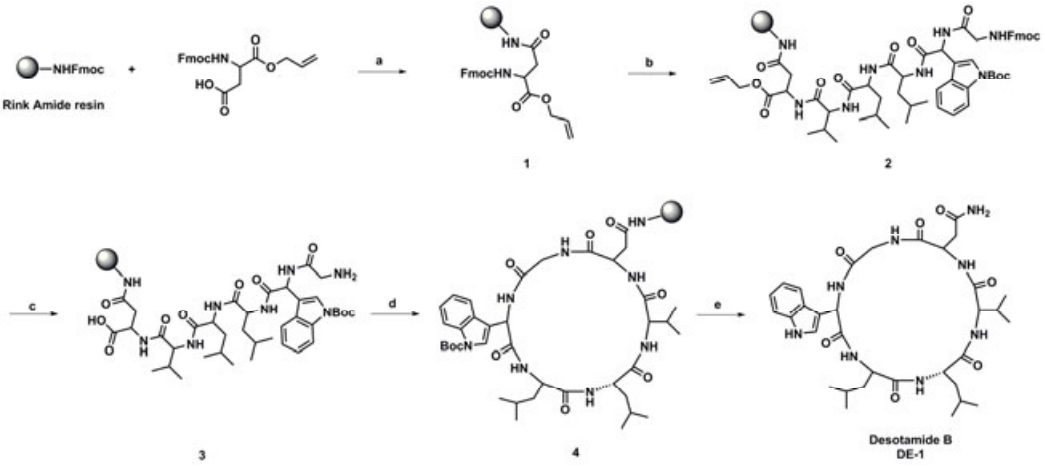
Figure. 2 Structure of target cyclopeptides.

To fully explore the potential of desotamide B (**DE-1**) and wollamide B (**WO-2**) as candidates of antibacterial drug development and to further investigate their structure-activity relationship (SAR), the present work directly targeted at the total synthesis of **DE-1** and **WO-2** as well as their corresponding analogues (Figure 2), and biological studies of the antibacterial and cytotoxic properties.

Results and Discussion

Chemistry

As shown in Scheme 1, we firstly anchored Fmoc-Asp-OAllyl to the Rink Amide AM resin using HCTU/DIPEA as coupling reagent to obtain resin-bond amino acid **1**. Then, the linear peptide **2** was readily assembled *via* SPPS with different Fmoc protected amino acids. Uncapping of Allyl group in the presence of $Pd(PPh_3)_4$ and phenylsilane, followed by the treatment of 20% piperidine/DMF to release the N-terminal amino group, we can afford linear peptide precursor **3**. The subsequent on-resin cyclization step was achieved under the condition of PyAOP/HOAt/NMM for 12 hours to obtain protected cyclopeptide (**4**) on resin. The final cleavage and global deprotection using TFA/Phenol/Water/TIPS (88:5:5:2, *v/v/v/v*) cocktail successfully produced target cyclopeptides. The structure of the synthesized cyclopeptide was confirmed by HRMS and NMR.



Scheme 1. Synthesis route of the desotamide B (**DE-1**). Conditions and reagents: a) HCTU, DIPEA, DMF, 1 h, rt; b) i) 20% piperidine/DMF, 5+10 min, rt; ii) Fmoc-AA-OH, HCTU, DIPEA, DMF, 1 h, rt; c) i) $Pd(PPh_3)_4$, phenylsilane, DCM; ii) 20%

piperidine/DMF, 5+10 min, rt; d) PyAOP, HOAt, NMM, 12 h, rt; e) TFA/H_2O/phenol/TIPs (88:5:5:2, *v/v/v/v*), 2 h, rt.

The cyclopeptide could be split into single amino acids units and readily assembled to linear form through standard Fmoc/tBu SPPS strategy. However, the cyclization step is critical and may depend on the peptide sequence, structure constraints and the resulting ring size.[14] In previous studies, classical methods used to be cyclized in solution phase after cleavage of the on-resin linear precursors. Tedious operations and repeated purification were inevitable for this synthetic method, therefore, leading in some cases to a considerable loss of product.[15] Thus, on-resin head-to-tail cyclization strategy was regarded as an attractive alternative in which the final cyclization step was achieved while the peptide still remained anchoring to the resin. We chose the Allyl as the capping group for the starting amino acid Asp which was fully orthogonal to Fmoc/tBu SPPS and could be readily released by employing $Pd(PPh_3)_4$ treatment in high chemical yield after the linear peptide was assembled.[16] [17] The cyclization reagent, PyAOP/HOAt/NMM, was proved to be an effective condition for the on-resin cyclization of the linear peptide unit with the remarkable chemical yield of 76.9-88.5% (based on the loading amount of the first coupling amino acid).

Pharmacological Activity Studies

The synthesized cyclopeptides were subjected to the in vitro cytotoxicity study against the human tumor cell lines MCF-7 and HepG-2. The cytotoxicity assay was described by measuring IC_{50} of the cell lines. The results of the cytotoxic assay were presented in **Table 1**.

Table 1. Cytotoxic Activity (IC_{50} μM) for the cyclopeptide

Compound No.	MCF-7	HepG-2
De-1	268.5	329.0
De-2	766.9	405.0
De-3	158.0	606.8
De-4	289.2	318.0
Wo-1	372.5	137.6
Wo-2	115.4	79.2
Nutlin-3*	8.9	5.2

*Nutlin-3 was measured as a positive control.

Next, antibacterial activity was evaluated against the Gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA)*, Staphylococcus aureus* ATCC 25923, and Gram-negative bacteria, *Salmonella typhimurium* CMCC 50097, *Klebsiella peneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, using tetracycline and vancomycin as antibacterial agent control. The results of the antibacterial studies are exhibited in **Table 2**.

Table 2. Antibacterial Activity (MIC ug/mL) for the cyclopeptides

	DE-1	DE-2	DE-3	DE-4	WO-1	WO-2	VAN	TET
MRSA2	>128	>128	128	>128	>128	32	64	---
MRSA4	>128	>128	32	>128	>128	32	<8	---
MRSA5	128	>128	64	>128	>128	32	<8	---
S. aur[a)]	>128	>128	64	>128	>128	64	<8	---
S. typ	>128	>128	>128	>128	>128	128	---	<8
K. pen	>128	>128	>128	>128	>128	128	---	<8
E. coli	>128	>128	>128	>128	>128	64	---	<8

Abbreviations: *S. aur: Staphylococcus aureus* ATCC 25923; *S. typ: Salmonella typhimurium* CMCC 50097; *K. pen: Klebsiella peneumoniae* ATCC 13883; *E. coli: Escherichia coli* ATCC 25922. VAN: vancomycin; TET: tetracycline.

To our delight, DE-3 displayed an inhibitory potency for MRSA2, MRSA4, MRSA5, and *Staphylococcus aureus* with an increase (at least 2 fold) compared to DE-1, suggesting that the replacement of Val^{II} with Ile resulted in an improved bioactivity of DE-1. Furthermore, the loss activity of DE-2 and DE-4 indicated that the $^{D}Leu^{III}$could be indispensable for antibacterial activity in DE-1. On the other hand, it is worth of noting that the total deprivation of activity of WO-1 may suggest that the $^{D}Orn^{VI}$ could be a determining factor for the antibacterial activity of WO-2. In addition, almost all the target cyclopeptides lack cytotoxicities (IC_{50}>100 μM） against two human tumor cell line MCF-7 and HepG-2. WO-1 showed lower cytotoxicity than WO-2, indicating that D-$Orn^{VI}$is critical for antibacterial activity but may increase cytotoxicity.

In summary, a series of desotamide and wollamide analogues were designed and successfully synthesized, with reasonable yield via the on-resin head-to-tail cyclization strategy. This strategy may be potentially applicable to the synthesis of other cyclic peptides. The biological evaluation suggested that DE-3 displayed a 2-fold increase of antibacterial activity when compared with the original peptide DE-1. Of note, we have demonstrated that the $^{D}Orn^{VI}$ could be a determining factor for the antibacterial activity of WO-2. Further synthetic and SAR studies are currently in progress in our laboratory.

Materials and methods

All reagents were purchased from J&K Chemical Ltd., Adamas and Innochem Chemical Reagent. Amino acids were commercial available from GL Biochem Shanghai Co. Ltd. All solvents used were bought from Sinopharm Chemical Reagent Co. Ltd. Dichloromethane (DCM) and *N*, *N*-Dimethylformamide (DMF) were distilled over calcium hydride (CaH_2) under argon atmosphere and stored in flask containing 4 Å molecular sieves. All reaction vessels were oven-dried before use. High resolution mass spectra were measured on a Waters Xevo G2 QTOF mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 600 MHz instrument. Chemical shifts (δ) were reported relative to TMS (0 ppm) for ^{1}H-NMR and ^{13}C-NMR spectra. Reactions were monitored by visualized by ninhydrin and/or phosphomolybdic acid. The crude peptides were dissolved with CH_3CN/H_2O and analyzed or purified by analytical or semi-preparative RP-HPLC, respectively. A Vydac C4 or C18 column (5 μm, 4.6 mm×250 mm) with a 1 mL/min flow rate was used for analytical RP-HPLC, and a Vydac C4 column (10 μm, 10mm×250 mm or 22 mm×150 mm) with a 3-6 mL/min flow rate was used for semi-preparative RP-HPLC. The solvents systems were buffer

A (0.1% TFA in CH_3CN), buffer B (0.1% TFA in water). Data were recorded and analyzed using the software system LC Solution.

General method for the cyclohexapeptides synthesis

Synthesis was performed in a peptide synthesizer under anhydrous conditions.[18] Rink Amide AM resin 1 g was swelled with DCM (5 mL) for 20 min. The resin was treated with 20% piperidine/DMF twice (5 min and 10 min) to deprotect the Fmoc group, followed by washing with DCM and DMF 5 times respectively. The first amino acid (Fmoc-Asp-OAllyl, 3 equiv, 1 mmol) was loaded on the Rink Amide resin by treatment with HCTU (0.9 mmol), DIPEA (2 mmol) and DMF (6 mL). After 1 h, the resin was washed with DCM and DMF 5 times respectively. The deprotection, washing, coupling and washing steps were repeated until all the amino acid residues were assembled. Then the Allyl group was deprotected in the presence of $Pd(PPh_3)_4$, phenylsilane in DCM, followed by the deprotection of Fmoc. The cyclization was carried out on resin by treatment with 5 equiv PyAOP, 5 equiv HOAt, 10 equiv NMM in pure NMP for 12 h. The cyclopeptide was cleaved from the resin by treating with regent B (88% TFA, 5% H_2O, 5% phenol, 2% TIPS) for 4 h at room temperature. After completion of the cleavage reaction, TFA was evaporated by blowing with Ar. The crude cyclopeptides were obtained by precipitation with 40 mL of cold diethyl ether and centrifugation at 3500 r/min for 3 min (3 times). The supernatant diethyl ether was decanted from the centrifuge tube and the crude peptides were allowed to air dry. The crude peptides were purified by semi-preparative RP-HPLC.

Cyclo-(Asn-Val-^{D}Leu-Leu-Trp-Gly) (DE-1): ^{1}H-NMR (600 MHz, *d*-DMSO) δ: 10.85 (1H, s), 8.32-8.31 (1H, d, J=6 Hz), 8.28-8.27 (1H,d, J=6 Hz), 8.24-8.23 (1H, d, J=6 Hz), 7.92-7.90 (1H, t), 7.67-7.66 (2H, t), 7.61 (1H,s), 7.54-7.53 (1H,d, J=6 Hz), 7.35-7.34 (1H, d, J=6 Hz), 7.16(1H, s), 7.10-7.07(2H, s), 7.01-6.99 (1H, t), 4.55-4.51 (1H, dd, J=6 Hz), 4.39-4.37 (3H, t), 4.04-4.02 (1H, t), 3.94-3.90 (1H, dd, J=6 Hz), 3.37-3.34 (1H, dd, J=6 Hz), 3.18-3.15 (1H, dd, J=6 Hz), 3.02-2.98 (1H, dd, J=6 Hz), 2.83-2.80 (1H, dd, J=6 Hz), 2.69-2.65 (1H, dd, J=6 Hz), 2.25-2.19 (1H, m), 1.59-1.55 (2H, t), 1.50-1.45 (4H,m), 0.93-0.85 (18H, m). ^{13}C-NMR (600 MHz, *d*-DMSO) δ: 173.48, 172.49, 172.10, 171.42, 171.38, 171.25, 169.60, 136.59, 127.51, 124.01, 121.39, 118.84, 118.57, 111.83, 110.47, 59.27, 55.81, 52.10, 51.19, 49.88, 43.67, 41.86, 37.13, 24.89, 24.60, 23.08, 22.99, 22.85, 22.62, 19.58, 17.87. White lyophilized powder; Yield 79.8%; Retention time17.749 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{34}H_{50}N_8O_7$, Calc. for 683.3875, found $(M+H)^+$: 683.3885.

Cyclo-(Asn-Val-Leu-Leu-Trp-Gly) (DE-2): ^{1}H-NMR (600MHz, *d*-DMSO) δ: 10.83 (1H, s), 8.22-8.19 (2H, t), 7.94-7.89 (2H, dd, J=6 Hz), 7.59-7.56 (2H, dd, J=6 Hz), 7.41 (1H, s), 7.31-7.29 (1H, d, J=12 Hz), 7.16 (1H, s), 7.05-7.03 (1H, t), 6.97-6.95 (2H, t), 6.91 (1H, s), 4.47-4.43 (1H, dd, J=6 Hz), 4.24-4.18 (2H, m), 4.11-4.07 (1H, dd, J=6 Hz), 4.01-3.99 (1H, t) ,3.75-3.71 (1H, dd, J=6 Hz), 3.22-3.19 (1H, dd, J=6 Hz), 2.98-2.94 (1H, dd, J=6 Hz), 2.69-2.65 (1H, dd, J=6 Hz), 2.59-2.55 (1H, dd, J=6 Hz), 2.04-2.01 (1H, dd, J=6 Hz), 0.88-0.87 (4H, d, J=6 Hz), 0.86-0.81 (15H, m), 0.78-0.77 (5H, d, J=6 Hz). ^{13}C-NMR (600MHz, *d*-DMSO) δ: 172.33, 171.70, 171.44, 170.80, 170.64, 170.61, 168.47, 136.01, 134.29, 127.14, 123.57, 120.81, 119.25, 118.21, 118.14, 111.27, 109.84, 58.07, 54.30, 52.92, 51.64, 50.87, 42.81, 40.91, 35.74, 30.17, 26.77, 24.23, 23.16, 22.69, 21.50, 21.08, 18.98,

17.92. White lyophilized powder; Yield 76.9%; Retention time18.127 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{34}H_{50}N_8O_7$, Calc. for 683.3875, found $(M+H)^+$: 683.3879.

Cyclo-(Asn-Ile-^{D}Leu-Leu-Trp-Gly) (DE-3): ^{1}H-NMR (600 MHz, *d*-DMSO) δ: 10.82 (1H, s), 8.29-8.28 (1H, d, J=6 Hz), 8.26-8.24 (1H, d, J=12 Hz), 8.17-8.16 (1H, d, J=6 Hz), 7.86-7.84 (1H, t), 7.65-7.64 (2H, d, J=6 Hz), 7.59 (1H, s), 7.49-7.48 (1H, d, J=6 Hz), 7.31-7.29 (1H, d, J=6 Hz), 7.12 (1H, s), 7.06-7.03 (2H, t), 6.97-6.95 (1H, t), 4.48-4.45 (1H, dd, J=6 Hz),4.35-4.30 (3H, m), 4.02-4.00 (1H, t), 3.88-3.85 (1H, dd, J=6 Hz), 3.13-3.09 (1H, dd, J=6 Hz), 2.97-2.93 (1H, t), 2.79-2.75 (1H, dd, J=6 Hz), 2.64-2.61 (1H, dd, J=6 Hz), 1.45-1.39 (4H, m), 1.21(6H, s), 0.88-0.86 (6H, s), 0.83-0.79 (12H, m). ^{13}C-NMR (600MHz, *d*-DMSO) δ: 173.00, 171.98, 171.53, 170.95, 170.87, 170.68, 169.06, 136.06, 129.60, 126.98, 123.50, 120.8 6, 118.31, 118.04, 111.31, 109.93, 58.12, 55.32, 51.50, 50.67, 49.39, 43.14, 41.24, 36.48,35.28, 35.06, 29.03, 28.97, 28.76, 28.67, 28.63, 28.52, 27.21,26.55, 26.51, 25.05, 24.38, 24.08, 24.05, 22.56, 22.51, 22.30, 22.05, 15.52, 11.46. White lyophilized powder; Yield 78.5%; Retention time17.026 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{35}H_{52}N_8O_7$, Calc.for 697.4032, found $(M+H)^+$: 697.4039.

Cyclo-(Asn-Ile-^{D}Ile-Leu-Trp-Gly) (DE-4): ^{1}H-NMR (600 MHz, *d*-DMSO) δ: 10.80 (1H, s), 8.29-8.27 (3H, dd, J=6 Hz), 7.88-7.87 (1H, t), 7.66-7.62 (3H, m), 7.49-7.47 (1H, d, J=12 Hz), 7.30-7.29 (1H, d, J=6 Hz), 7.10 (2H, s), 7.05-7.02 (1H, t), 6.97-6.94 (1H, t), 4.51-4.46 (1H, m), 4.40-4.39 (1H, m), 4.13-4.10 (1H, dd, J=6 Hz), 4.07-4.06 (1H, dd, J=6 Hz), 3.85-3.82 (1H, dd, J=6 Hz), 3.30-3.27 (1H, dd, J=6 Hz), 3.11-3.08 (1H, dd, J=6 Hz), 2.96-2.92 (1H, dd, J=6 Hz), 2.84-2.80 (1H, dd, J=6 Hz), 2.64-2.61 (1H, dd, J=6 Hz), 1.96-1.92 (1H, m), 1.72-1.67 (1H, m), 1.56-1.54 (1H, t), 1.45-1.39 (3H, m), 1.34-1.28 (1H, m), 1.25-1.18 (1H,

m), 1.16-1.11 (1H, m), 0.88-0.87 (3H, d, J=6 Hz), 0.83-0.78 (16H, m). ^{13}C-NMR (600 MHz, *d*-DMSO) δ: 172.72, 172.12, 171.36, 170.90, 170.87, 170.79, 168.77, 136.05, 126.99, 123.43, 120.84, 118.30, 118.05, 111.30, 110.03, 58.21, 57.34, 55.24, 50.67, 49.17, 43.21, 41.55, 36.62, 35.34, 34.70, 27.40, 24.66, 24.43, 23.87, 22.68, 22.36, 15.76, 14.98, 11.56, 10.14. White lyophilized powder; Yield 81.3%; Retention time 18.277 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{35}H_{52}N_8O_7$, Calc. for 697.4032, found $(M+H)^+$: 697.4030.

Cyclo-(Asn-Val-^{D}Leu-Leu-Trp-Orn) (WO-1): ^{1}H-NMR (600 MHz, *d*-DMSO) δ: 10.81 (1H, s), 8.38-8.33 (2H, dd, J=6Hz), 8.26-8.24 (1H, d, J=12Hz), 7.66-7.65 (1H, d, J=6Hz), 7.57-7.54 (3H, d), 7.51-7.47 (2H, dd, J=6Hz), 7.36-7.35 (1H, d, J=6Hz), 7.31-7.30 (1H, d, J=6Hz), 7.11 (1H, s), 7.05-7.03 (1H, t), 6.99-6.95 (2H,m), 4.60-4.56 (1H, dd, J=6Hz), 4.43-4.39 (1H, dd, J=6Hz), 4.28-4.25 (2H, m), 4.17-4.13 (1H, dd, J=6Hz), 4.01-3.99 (1J, dd, J=6Hz), 3.18-3.15 (1H, dd, J=6Hz), 2.95-2.91 (1H, dd, J=6Hz), 2.64-2.61 (3H, dd, J=6Hz), 2.26-2.23 (1H, m), 1.56-1.36 (10H, m), 0.90-0.87 (6H, t), 0.84-0.82 (13H, t). ^{13}C-NMR (600 MHz, *d*-DMSO) δ: 173.96, 172.23, 172.07, 171.57, 171.14, 171.10, 136.57, 127.46, 124.07, 121.37, 118.82, 118.62, 111.80, 110.47, 59.01, 55.68, 52.37, 52.25, 50.86, 49.98, 42.42, 38.72, 37.51, 29.19, 27.60, 27.00, 24.88, 24.54, 23.62, 23.01, 22.97, 22.85, 22.56, 19.56, 17.43. White lyophilized powder; Yield 86.3%; Retention time18.216 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{37}H_{57}N_9O_7$, Calc. for 740.4454, found $(M+H)^+$:740.4456.

Cyclo-(Asn-Val-^{D}Leu-Leu-Trp-^{D}Orn) (WO-2): ^{1}H-NMR (600 MHz, *d*-DMSO) δ: 10.81 (1H, s), 8.38-8.33 (2H, dd, J=6 Hz), 8.26-8.24 (1H, d, J=12 Hz), 7.66-7.64 (1H, d, J=12 Hz), 7.54 (3H, s), 7.51-7.48 (2H, t), 7.36-7.35 (1H, d, J=6 Hz), 7.31-7.30 (1H, d, J=6 Hz), 7.11 (1H, s), 7.05-7.03 (1H, t), 7.00-6.95 (2H, t), 4.60-4.56 (1H, dd, J=6 Hz), 4.43-4.40 (1H, dd, J=6 Hz),

4.30-4.25 (2H, m), 4.17-4.14 (1H, dd, J=6 Hz), 4.01-3.99 (1H, dd, J=6 Hz), 3.18-3.15 (1H, dd, J=6 Hz), 2.96-2.92 (1H, dd, J=6 Hz), 2.65-2.58 (4H, m), 2.26-2.23 (1H, m), 1.45-1.37 (5H, m), 1.21 (3H, s), 0.99-0.88 (6H, t), 0.85-0.82 (14H, t). ^{13}C-NMR (600 MHz, *d*-DMSO) δ: 173.48, 171.75, 171.59, 171.08, 170.65, 136.09, 129.61, 126.98, 123.58, 120.89, 118.34, 118.14, 111.32, 109.99, 58.54, 55.21, 51.89, 51.77, 50.38, 49.50, 41.92, 38.26, 37.03, 24.41, 24.06, 22.53, 22.49, 22.37, 22.10, 19.09, 16.96, 13.90. White lyophilized powder; Yield 88.5%; Retention time 18.312 min (Gradient: 20-80% of buffer B in 30 min); HRMS: $C_{37}H_{57}N_9O_7$, Calc. for 740.4454, found $(M+H)^+$: 740.4465.

Biological Evaluation

Cytotoxic Screening

The cell proliferation inhibitory activities of target cyclopeptides against human cell lines MCF-7 and HepG-2 were tested using the previously published methods.[13]

Antibacterial assay

The antibacterial activities of compounds DE-1-DE-4, WO-1 and WO-2 were assessed using sequential 2-fold serial dilutions of each agent in MH broth according to the previously reported and a standard method (Broth Microdilution Procedure) provided by clinical and laboratory Standards Institute (CLSI).[14] Vancomycin and tetracycline were used as control. Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA, Clinical

isolated strain), *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Salmonella typhimurium* CMCC 50097, *Klebsiella peneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922) were provided by Huashan Hospital and China State Institute of Pharmaceutical Industry. These strains were grown in Mueller Hinton (MH) broth media at 37 ºC overnight. The cultures were diluted to 1:20 in the same broth, cultured at 37 ºC until absorbance 0.4-0.5 at 600 nm and diluted in MH broth to 1:200. 10 μL of the serially 2-fold diluted peptides (80 μg to 128 μg) in MH broth were mixed with 90 μL of the above bacterial suspension, then incubated 24 h at 37 ºC in an incubator. The MIC was defined as the lowest concentration of the peptide at which there was no growth of bacterial.

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Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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