Tetrahedron 68 (2012) 3074–3085

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Total synthesis and structure–activity relationships of caspofungin-like macrocyclic antifungal lipopeptides

Jianzhong Yao [†], Hongming Liu [†], Ting Zhou [†], Hai Chen, Zhenyuan Miao, Guoqiang Dong, Shengzheng Wang, Chunquan Sheng [*], Wannian Zhang [*]

Department of Medicinal Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, PR China

ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 2 February 2012
Accepted 7 February 2012
Available online 13 February 2012

Keywords:
Echinocandin
Caspofungin
Macrocyclic lipopeptide
Synthesis
Antifungal
SAR

ABSTRACT

The echinocandins represent a well-known class of macrocyclic antifungal lipopeptides that can be used for treatment of invasive fungal infections. Due to their complex chemical structures and synthetic difficulties, the structure–activity relationships (SARs) of them are still limited. A total synthetic approach was developed to synthesize structurally diverse caspofungin-like antifungal cyclic lipopeptides, allowing for systemically investigating their SARs. Most of the designed cyclic lipopeptides showed potent antifungal activities with broad spectrum. In particular, several compounds (e.g., **30a**, **30e**–**h**, **31a**–**d**, **32c**, and **33a**,**b**) were more active in vitro against *Candida albicans* or *Aspergillus fumigatus* than caspofungin. The findings in this work indicated that the 'left' tripeptide segment of cyclic lipopeptide scaffold might be suitable for a hydrophilic structural motif, whereas the 'right' lipotripeptide segment was preferred as a hydrophobic core. The alkoxy-naphthoyl was found to be optimal side chain and alkyl length could affect the SARs of alkoxy-aroyl side chains.

1. Introduction

Invasive fungal infections have drastically increased over the past three decades due to the rising immunocompromised population as a result of organ transplantation, cancer chemotherapy, steroid therapy, and, in particular, HIV infection (AIDS).[1–3] *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* are the most common causes of invasive fungal infections.[4,5] It is generally difficult for them to be diagnosed and treated and they are associated with high mortality. In clinic, very few antifungal agents can be used for life-threatening fungal infections.[6] Although azole derivatives, such as fluconazole, itraconazole, and voriconazole are the most widely used antifungal agents because of their high therapeutic index, they are fungistatic and not fungicidal against pathogenic yeasts. The inability to kill yeasts leads to resistance to azoles in prolonged infections. So, the likelihood, which these azoles will lack efficacy in immunocompromised patients suffered from severe *Candida* infections, will be increased.[7–9] Amphotericin B (AmB) has also been commonly used to treat serious fungal infections. Although resistance to AmB is slowly developing in selected *Candida* species, there are significant side effects associated with its use, including nephrotoxicity.[10–12]

The development of echinocandin-like lipopeptides for the treatment of invasive fungal infections represents a breakthrough in antifungal chemotherapy.[13] The natural echinocandins, such as echinocandin B (**1**) and pneumocandin B_0 (**2**) (Fig. 1) are a well-known class of closely related lipopeptides characterized by their potent antifungal activity against *Candida* species, which were isolated from fermentation broths of Aspergillus cultures in the early 1970s.[14] Structurally, they are composed of a complex cyclic hexapeptide whose N-terminus is acylated by a long carboxylic acid chain. Up to now, three semi-synthetic echinocandins have been approved to enter clinical use by the Food and Drug Administration (FDA). The first licensed echinocandin was caspofungin (**3**) in 2001,[15–17] followed by micafungin (**4**) in 2005,[18] and anidulafungin (**5**) in 2006[19] (Fig. 1). Both micafungin and anidulafungin are derived from echinocandin B, the major component of the echinocandin family. Caspofungin is derived from pneumocandin B_0.[20] In addition, aminocandin (HMR-3270, **6**), a new candidate of the echinocandin class, is undergoing early clinical development.[21]

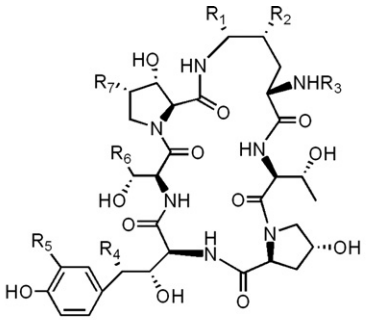
Being different from AmB and azoles, echinocandins are known non-competitive inhibitors of β-(1,3)-D-glucan synthase, an enzyme, that is, necessary for the synthesis of an essential component of fungal cell wall.[22–24] β-(1,3)-D-glucan is a carbohydrate polymer essential for the structural integrity of the cell wall of many of the medically important fungi. The inhibition of glucan biosynthesis

Abbreviations: Boc, *tert*-Butoxycarbonyl; Cbz, benyloxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Ms, methanesulfonyl.

* Corresponding authors. Tel./fax: +86 21 81871239 (C.S.); tel./fax: +86 21 81871243 (W.Z.); e-mail addresses: shengcq@hotmail.com (C. Sheng), zhangwnk@hotmail.com (W. Zhang).

† These three authors contributed equally to this work.

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2012.02.015



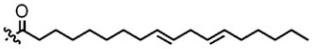
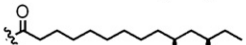
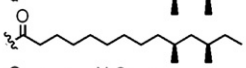
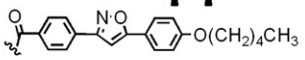
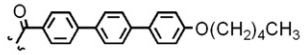
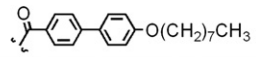
	R_1	R_2	R_4	R_5	R_6	R_7	R_3
Echinocandin B (**1**)	OH	OH	OH	H	CH_3	CH_3	
Pneumocandin B_0 (**2**)	OH	OH	OH	H	CH_2CONH_2	H	
Caspofungin (**3**)	$NH(CH_2)_2NH_2$	OH	OH	H	$(CH_2)_2NH_2$	H	
Micafungin (**4**)	OH	OH	OH	OSO_3H	CH_2CONH_2	CH_3	
Anidulafungin (**5**)	OH	OH	OH	H	CH_3	CH_3	
Aminocandin (**6**)	H	$NH(CH_2)_2NH_2$	H	H	H	CH_3	

Fig. 1. Structures of two natural echinocandins and four clinical available semi-synthetic echinocandins.

leads to weakened cell wall, which results in cell content leakage and cell death. However, the exact mechanism of action of the inhibitors remains unknown. Because glucan biosynthesis is totally absent in host cells, the echinocandins have good selectivity toward pathogenic fungi. Moreover, the echinocandins display fungicidal activity against most *Candida* spp., including strains that are resistant to fluconazole.[25] Clinical use of the echinocandins indicates that they are well tolerated with few drug–drug interactions and would not raise the problem of cross resistance.[25]

The excellent antifungal potency, unique mode of action, and low toxicity of echinocandins make them promising lead structures for optimization studies aimed at development of novel antifungal agents with an improved therapeutic index. Extensive semi-synthetic structure–activity relationship (SAR) studies had been carried out on natural echinocandin-like lipopeptides, such as echinocandin B,[26,27] mulundocandin,[28–31] FR131535,[32] and RO-09-3655.[33,34] Such studies provided a number of new analogues with improved antifungal activity and spectrum. However, these semi-synthetic modifications could not fully investigate the SAR of the echinocandins. On the other hand, total synthetic methods could offer an opportunity to rapidly obtain more SAR information that could not be accomplished by direct chemical modification of the natural product. Up to now, only a few studies were focused on the total synthesis of derivatives from echinocandin B[35] and A-192411.[36] In an attempt to extend this promising approach in a more comprehensive manner, a new route for the total synthesis of caspofungin derivatives was developed. Herein, we report design, total synthesis, and structure–activity relationships of novel caspofungin-like macrocyclic antifungal lipopeptides.

2. Results and discussion

2.1. Design rationale

Caspofungin (**3**), the first marketed antifungal lipopeptide, was used as a starting point for analogy design, whose chemical structure is composed of seven parts: 3-hydroxy-proline residue (A), 3-hydroxy-ornithine residue (B), 3,4-dihydroxy-homotyrosine residue (C), 4-hydroxy-proline residue (D), threonine residue (E), 4-hydroxy-5-ethylenediamino-ornithine residue (F), and fatty acid side chain (G). Previous SAR studies revealed that hydroxyl groups at the proline, ornithine, and homotyrosine residues were not necessary for antifungal activity and the ethylenediamine group at the ornithine residue was found to have little effect on the antifungal potency.[37] Therefore, these groups were removed in the present derivative design (Fig. 2) and such simplifications could also significantly facilitate the process of total synthesis. Klein et al. found that introduction of an amino group on the proline ring of echinocandin B (**1**) could enhance both antifungal activity and water solubility.[35] Hence, aminoproline were modified at the two proline residues (A and D) of caspofungin. Three kinds of amino acids, ornithine, lysrine, and threosine, were used to replace the 3-hydroxy-ornithine (B). L-Homotyrosine, an essential structural determinant of biological activity, was reserved in the present study.[37] For the threonine residue (E), it was mutated to serine, lysine or valine for SAR analysis. The hydrophobicity, length, and shape of the fatty acid side chains played an important role for the antifungal activity and toxicity of the lipopeptides.[27,35–37] Herein, the 10,12-dimethylmyristoyl side chain (G) of caspofungin was replaced by various alkoxy-aroyl side chains, whose chemotypes were derived from newer marketed cyclic lipopeptides, such as aminocandin and anidulafungin. As a result, target compounds **30**–**33** (Fig. 3) were subjected for chemical synthesis and antifungal activity evaluation.

2.2. Chemistry synthesis

Solid phase and solution phase synthesis of antifungal lipopeptides have been reported.[33–35] Because solution phase synthesis can produce larger quantities of compounds for biological testing and are more suitable for SAR investigations, it was used to prepare the target compounds. We have tested two possible synthetic routes: a linear coupling/deprotection strategy and a convergent coupling strategy between two tripeptide segments. The

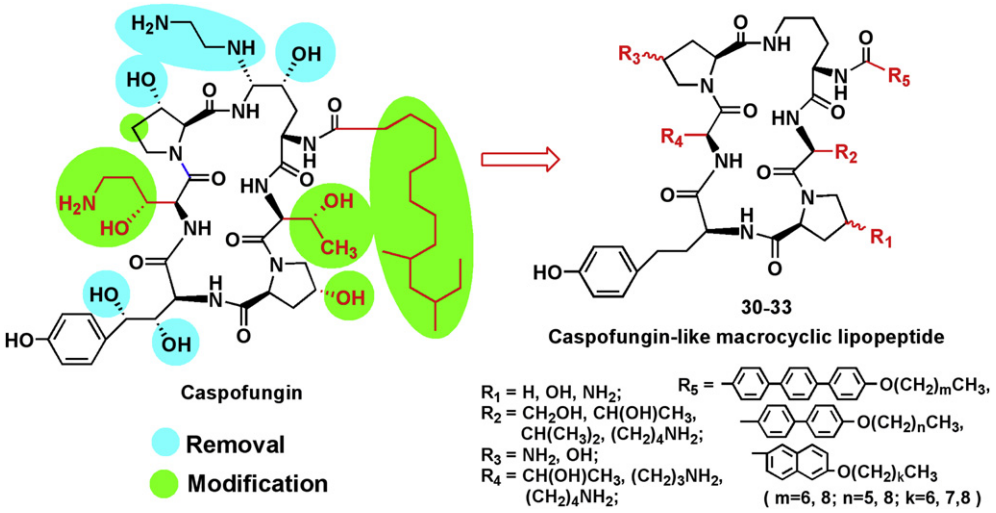


Fig. 2. Design rationale of the caspofungin-like macrocyclic antifungal lipopeptides.

latter was found to have several advantages, such as high synthetic efficiency and easy structure-diversity derivatization. As a result, a [3+3] coupling strategy for the preparation of the target compounds was depicted in Schemes 1–5. The chemical synthesis could be divided into four parts: (1) preparation of various alkoxy aromatic carboxylic acids side chains; (2) preparation of the 'right' lipotripeptides, namely Pro-Ser (or Thr/Val/Lys)-Orn tripeptides with lipophilic alkoxy aromatic carboxylic acid side chains; (3) preparation of the 'left' tripeptides, namely Pro-Orn (or Lys/Thr)-hTyr tripeptides; (4) coupling between the two tripeptides, followed reduction of the azide group or/and deprotection of *N*-Cbz group by hydrogenolysis.

The synthesis of unusual amino acid 4-(*S*)-azido-L-proline methyl ester hydrochloride (**10**) was similar to the procedure by Klein et al. (Scheme 1).[35]

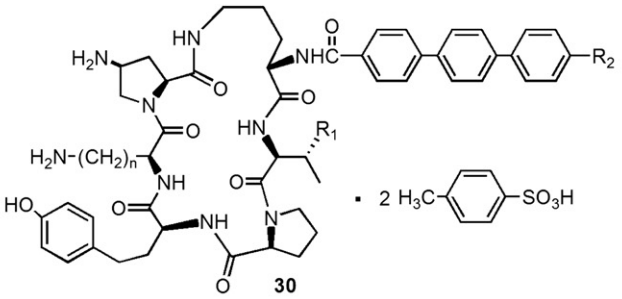
Scheme 2 outlines the synthesis of various alkoxy aromatic carboxylic acids side chains. Alkoxy naphthoic acids **12a**–**c** and alkoxy biphenyl carboxylic acids **14a**–**b** were prepared by direct alkylation of the phenolic hydroxyl group of 6-hydroxy-2-naphthoic acid (**11**) and 4′-hydroxy-1,1′-bisphenyl-4-carboxylic acid (**13**) using KOH as a base. The alkoxy terphenyl carboxylic acids **19a**–**b** were synthesized using the standard Suzuki reaction.[38] Initially, 4-bromo-4′-alkoxy-1,1′-biphenyl **16a**–**b** were prepared by alkylation of the phenolic hydroxyl group of 4-bromo-4′-hydroxy-1,1′-biphenyl (**15**) using K_2CO_3 as a base and then were treated with *n*-BuLi and the Br–Li exchange products were converted to bonic acid derivative **17a**–**b** by reacting with $(i\text{-PrO})_3B$ in methyl tertiary butyl ether (MTBE) followed by hydrolyzation with 2 M HCl. In contrast to *sec*-butyllithium,[27] the use of *n*-BuLi had several advantages, such as higher yields (93.0% and 94.1%) and less self-coupled side products. Alkoxy biphenyl boronic acids **17a**–**b** were coupled with methyl 4-iodobenzoate to give the alkoxy aromatic carboxylic acid esters **18a**–**b** in yields of 94.4–96.1%. The reaction conditions from Debono et al. were optimized in the present studies.[27] For the catalyst, the combination of $Pd(OAc)_2$ and Ph_3P was found to be better than tetrakis(tripheny1phosphine) palladium (0). Instead of using toluene as the solvent and K_2CO_3 as the base, the mixed solvent toluene/propanol (8:1) and Na_2CO_3 aqueous solution gave higher yields. Therefore, our optimal reaction conditions for the Suzuki reaction is as follows: $Pd(OAc)_2$ and Ph_3P as the catalyst, toluene/propanol (8:1) as the solvent, Na_2CO_3 aqueous solution as the base and carrying out the reaction at reflux for 4 h. The yields were improved from 33% to 94.4–96.1%. In the presence of KOH and hexadecyl trimethyl ammonium bromide, esters **18a**–**b** were hydrolyzed to acid **19a**–**b** by the phase transfer catalysis.

Scheme 3 and Scheme 4 illustrate the synthesis of the 'right' lipotripeptides **24** and the 'left' tripeptides **25**. In the presence of EDC hydrochloride and HOBt, proline methyl ester (**20a**) or its derivatives (**10** and **20b**) were coupled with *N*-Boc-(L)-threonine (**21a**) or *N*-Boc-(L)-serine (**21b**)/*N*-Boc-(L)-valine (**21c**)/*N*-Boc-(L)-lysine (**21d**)/*N*-Boc-(L)-ornithine (**21e**) to give dipeptides **22a**–**g** in yields of 73.9–95.8%. After removal of the *N*-Boc group by TFA, dipeptides **22a**–**e** was coupled with N^{α}-Fmoc-N^{δ}-Boc-ornithine by EDC, HOBt, and DIPEA to afford tripeptides **23a**–**e** in yields of 82.1–92.1%. Treatment of **23a**–**e** with Et_2NH provided *N*-Fmoc deprotection tripeptides at ornithine residue to allow for attachment to various alkoxy aromatic carboxylic acid side chains. Before various naphthyl, biphenyl, and terphenyl carboxylic acids **12**, **14**, and **19** could be coupled with above *N*-Fmoc deprotection tripeptides, they should be activated first. Instead of 2,4,5-trichloro phenol,[35] we found, HOBt could be used to prepare the the carboxylic acid 'activated ester' in a cheaper and safer way. Then, the 'right' lipotripeptides **24** were obtained by the coupling of 'activated esters' and above *N*-Fmoc deprotection tripeptides. In addition, after *N*-Boc deprotection, dipeptides **22d**–**g** were coupled with *N*-Boc-L-homotyrosine by EDC, HOBt, and DIPEA to give the 'left' tripeptides **25a**–**d**.

Scheme 5 depicts the final [3+3]-segment coupling approach for synthesis of target cyclolipohexapeptides **30**–**33**. Initially, treatment of of lipotripeptides **24** with TFA gave *N*-Boc deprotection lipotripeptides **26** and ester hydrolysis of tripeptides **25** by 1 M NaOH provided their C-terminal carboxyl deprotection tripeptides **27**. Then, in presence of PyBOP and DIPEA, the coupling of above lipotripeptides **26** and tripeptides **27** gave the linear lipohexapeptides **28**. After *N*-Boc deproetction and C-terminal ester hydrolysis, lipohexapeptides **28** were cyclized by HOBt, PyBOP, and DIPEA to produce cyclic lipohexapeptides **29**, which have one or two azide groups. Finally, reduction of the azide groups or/and deprotection of *N*-Cbz group by Pd–C catalyzed hydrogenolysis led to the target compounds **30**–**33** (Fig. 3).

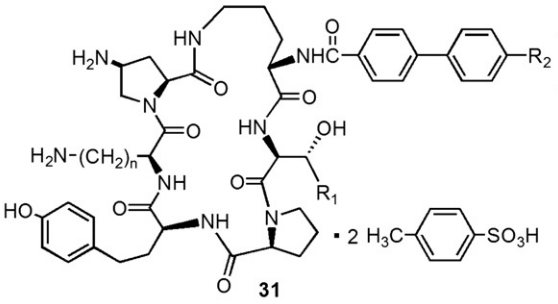
2.3. In vitro antifungal activities

The in vitro antifungal activities of the synthesized macrocyclic lipopeptides are listed in Table 1. The antifungal activity of each compound was expressed as the minimal inhibitory concentration (MIC) that achieved 80% inhibition of the tested fungi with caspofungin used as a reference drug. As shown in Table 1, most of the target compounds generally showed moderate to good inhibitory activities against all the tested fungal pathogens. They revealed the



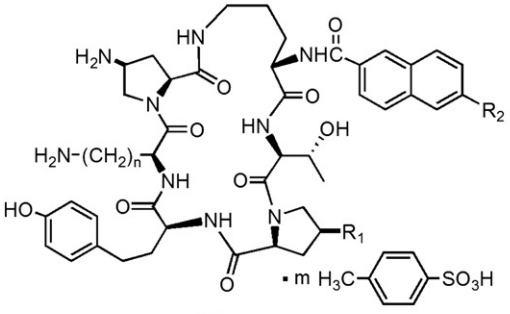
30

Compd.	R_1	R_2	n	Compd.	R_1	R_2	n
30a	-OH	$-O$-*n*-C_6H_{13}	3	**30e**	-OH	$-O$-*n*-C_6H_{13}	4
30b	-OH	$-O$-*n*-C_8H_{17}	3	**30f**	-OH	$-O$-*n*-C_8H_{17}	4
30c	$-CH_3$	$-O$-*n*-C_6H_{13}	3	**30g**	$-CH_3$	$-O$-*n*-C_6H_{13}	4
30d	$-CH_3$	$-O$-*n*-C_8H_{17}	3	**30h**	$-CH_3$	$-O$-*n*-C_8H_{17}	4

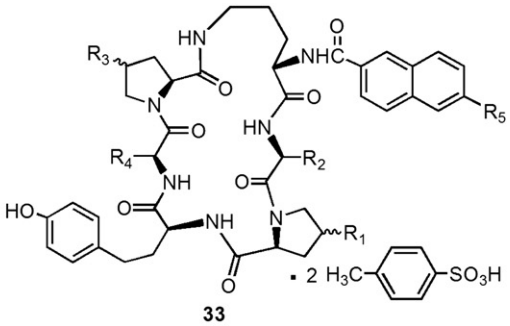


31

Compounds	R_1	R_2	n
31a	-H	$-O$-*n*-C_5H_{11}	3
31b	-H	$-O$-*n*-C_8H_{17}	3
31c	$-CH_3$	$-O$-*n*-C_5H_{11}	4
31d	$-CH_3$	$-O$-*n*-C_8H_{17}	4



32

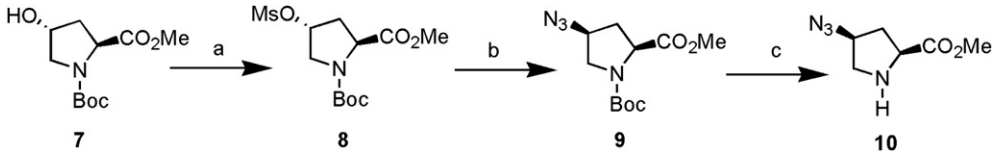


33

Compd.	R_1	R_2	n	m
32a	$-NH_2$	$-O$-*n*-C_7H_{15}	4	3
32b	$-NH_2$	$-O$-*n*-C_8H_{17}	3	3
32c	-H	$-O$-*n*-C_7H_{15}	4	2
32d	-H	$-O$-*n*-C_8H_{17}	3	2

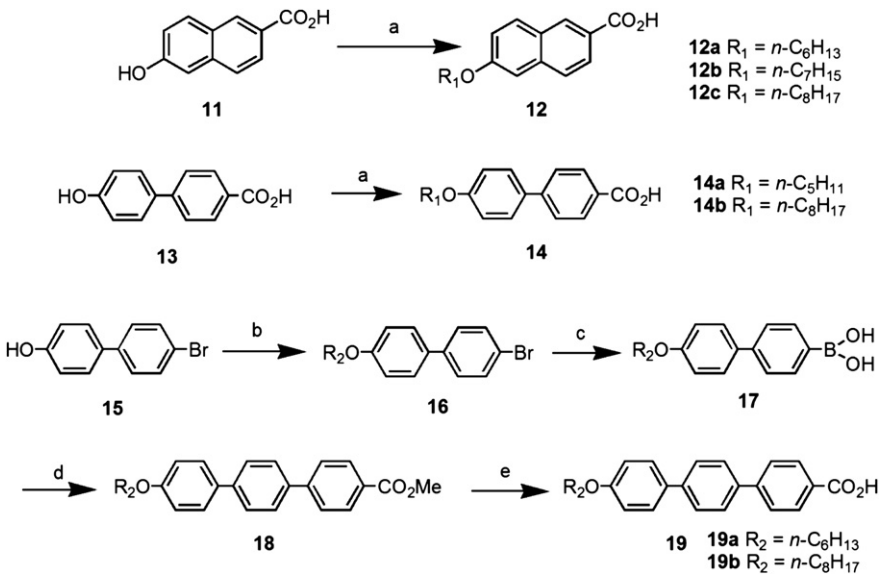
Compd.	R_1	R_2	R_3	R_4	R_5
33a	(S)-NH_2	$-CH(OH)CH_3$	(R)-OH	$-(CH_2)_4NH_2$	$-O$-*n*-C_7H_{15}
33b	(S)-NH_2	$-CH(OH)CH_3$	(R)-OH	$-(CH_2)_4NH_2$	$-O$-*n*-C_8H_{17}
33c	(R)-OH	$-(CH_2)_4NH_2$	(S)-NH_2	$-CH(OH)CH_3$	$-O$-*n*-C_6H_{13}
33d	(R)-OH	$-(CH_2)_4NH_2$	(R)-OH	$-(CH_2)_4NH_2$	$-O$-*n*-C_6H_{13}

Fig. 3. Chemical structures of the caspofungin-like macrocyclic lipopeptides.

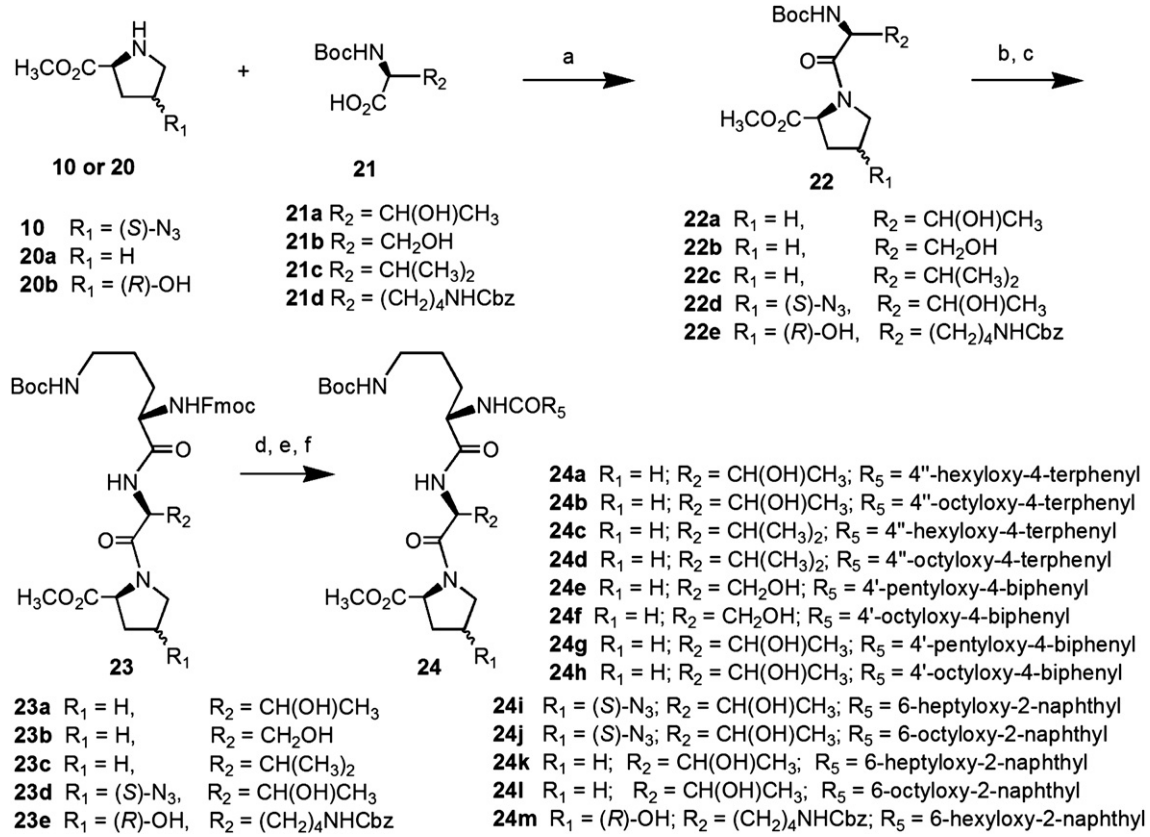


7 8 9 10

Scheme 1. The synthesis of methyl 4-(*S*)-azido-prolinate. Reaction conditions: a. methanesulfonyl chloride, pyridine, 0 °C to rt, 12 h; b. NaN_3, DMF, 60 °C, 24 h; c. TFA, CH_2Cl_2, rt, 1 h, yield 91.9% (a→c, three steps).

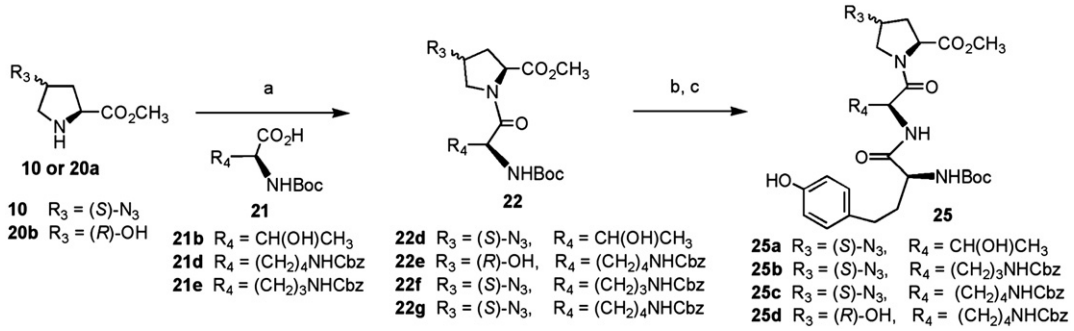


Scheme 2. The synthesis of alkoxy aromatic carboxylic acids side chains. Reaction conditions: a. R_1Br, KOH, EtOH/H_2O (10:1), reflux, 24 h, yields 73.5–78.2%; b. R_2-Br, K_2CO_3, 2-butanone, reflux, 8 h, yields 94.6–96.1%; c. (i-PrO)$_3$B, N_2, n-BuLi, 2 M HCl, methyl tertiary butyl ether (MTBE), −60 °C to rt, 12 h, yields 93.0–94.1%; d. methyl 4-iodobenzoate, $Pd(OAc)_2$, Ph_3P, Na_2CO_3, toluene/propanol (8:1), reflux, 4 h, 94.4–96.1%; e. 5 M KOH, xylene, hexadecyl trimethyl ammonium bromide, reflux, 30 min, yields 97.5–97.4%.

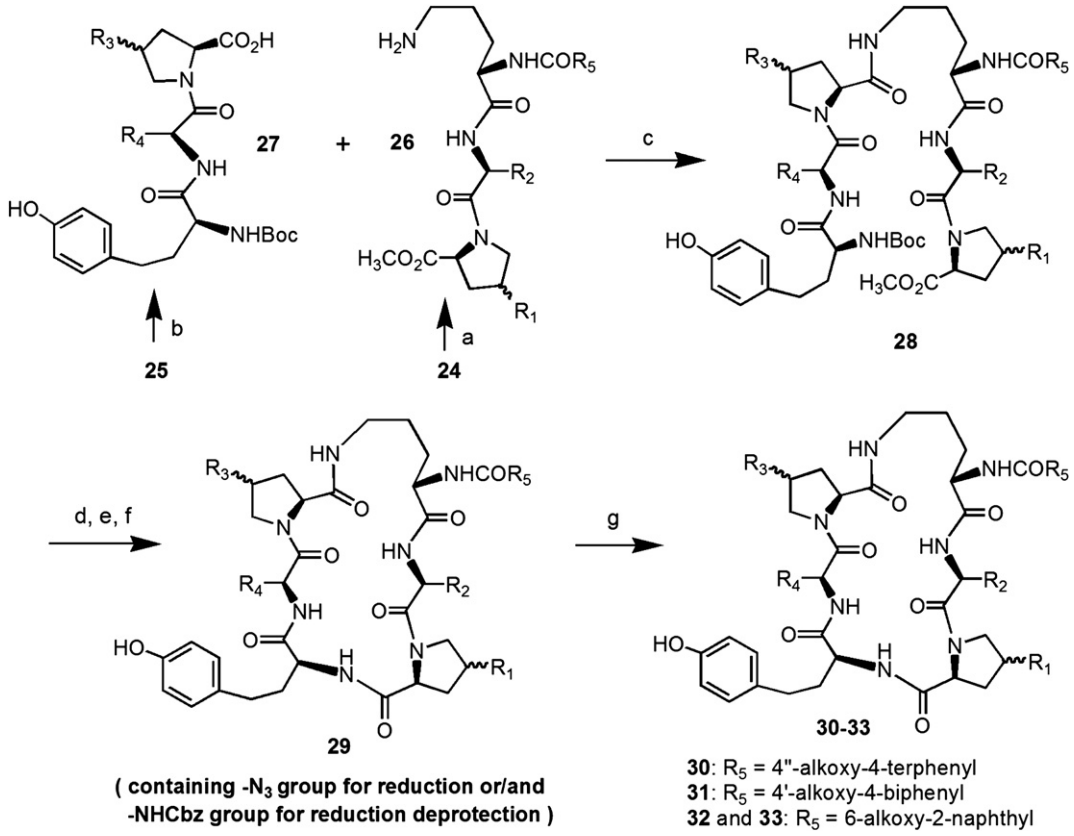


Scheme 3. The synthesis of 'right' lipotripeptide segments of the target macrocyclic lipopeptides. Reaction conditions: a. EDC, HOBt, DIPEA, CH_2Cl_2, 0 °C to rt, 24 h, yields 73.9–95.8%; b. TFA, CH_2Cl_2, rt, 1 h; c. N^{α}-Fmoc-N^{δ}-Boc-ornithine, EDC, DIPEA, CH_2Cl_2, 0 °C to rt, 24 h, yields 81.1–92.1% (b→c, two steps); d. Et_2NH, acetonitrile, 0 °C, 2 h; e. **12**, **14** or **19**, DCC, HOBt, DMAP, rt, CH_2Cl_2, 48 h; f. products from step d and e, rt, DMF, 48 h, yields 74.6–92.9% (d→f, three steps).

highest activity against *C. albicans* with their MIC values in the range of 0.125–8 μg/mL. In particular, eight compounds were found to be more active than caspofungin. The MIC values of compound **32c** was 0.125 μg/mL, which was 4-fold more potent than caspofungin. Reduced inhibitory activities against other *Candida* spp. (such as *Candida parasilosis*, *Candida tropicalis*, and *Candida krusei*) were observed for most of the compounds. This trend was also evident for caspofungin. Moreover, several compounds were more potent than caspofungin. For example, compounds **31c** and **31d** showed the highest activity against *C. tropicalis* (MIC=1 μg/mL),



Scheme 4. The synthesis of 'left' tripeptide segments of the target macrocyclic lipopeptides. Reaction conditions: a. EDC, HOBt, DIPEA, CH_2Cl_2, 0 °C to rt, 24 h, yields 91.1–93.1%; b. TFA, CH_2Cl_2, rt, 1 h; c. *N*-Boc-L-homotyrosine, EDC, DIPEA, CH_2Cl_2, HOBt, 0 °C to rt, 24 h, yields 80.8–86.0% (b→c, two steps).



Scheme 5. The synthesis of the caspofungin-like macrocyclic lipopeptides via a [3+3]-segment coupling strategy. Reaction conditions: a. TFA, CH_2Cl_2, rt, 45 min; b. 1 M NaOH, EtOH, rt, overnight; c. PyBOP, DIPEA, CH_2Cl_2, rt, 10 h; d. TFA, CH_2Cl_2, rt, 1 h; e. 1 M NaOH, EtOH, rt, overnight; f. HOBt, PyBOP, DIPEA, rt, DMF, 48 h; yields 31.6–80.1% (a→f, six steps); g. Pd–C, H_2, EtOH, rt, 24 h, yields 74.2–99.2%.

whereas the MIC value of caspofungin was 4 μg/mL. On the *A. fumigatus* strain, most of the compounds from series **30**, **32**, and **33** also revealed good activity (MIC range: 0.5–1 μg/mL). Particularly, five compounds (e.g., **30f**–**h** and **33a**–**b**) were more active than caspofungin. However, there were also eight compounds (e.g., **30b**, **31a**–**d**, **32a**, and **33c**–**d**) to be totally inactive against *A. fumigatus*. Most of the compounds only showed moderate activity against *Candida neoformans* with their MIC values in the range of 16–64 μg/mL. The activity of several compounds (e.g., **30e**–**f**, **31a**–**d**, and **32b**) were comparable to that of caspofungin (MIC=16 μg/mL). For the dermatophyte (i.e., *Trichophyton rubrum*), moderate activities were observed for the majority of the compounds (MIC range: 16–64 μg/mL) and several of them (e.g., **30e**, **30h**, **31a**–**b**, **31d**, and **32a**) were as potent as caspofungin, whereas one compound (e.g., **31c**) showed more potent than caspofungin.

2.4. Structure–activity relationships

To clearly analyze the SAR results, compound **30a** was used as a template, whose chemical structure could be divided into seven parts like caspofungin: aminoproline residue (A), N^{δ}-amino-disengaged ornithine residue (B), homotyrosine residue (C), proline residue (D), threonine residue (E), N^{δ}-amino-cyclized ornithine residue (F), and alkoxy-aroyl side chains (G). For the part A, the replacement of its (*S*)-NH_2 by the (*R*)-OH had little effect on the antifungal activity. For example, good activity was observed for compounds **33a**–**b**. For the part B, the Orn/Lys exchange (e.g., compounds **30a**–**d** vs **30e**–**h**) was tolerable and resulted in a slight improvement of antifungal activity. However, the Orn/Thr exchange (e.g., compound **33c**) was found to be unfavorable for the antifungal activity. For the part D, proline was sensitive for

Table 1
In vitro antifungal activities of target compounds (MIC_{80}, μg/mL)

Compounds	*C. albicans*	*A. fumigatus*	*C. neoformans*	*C. parapsilosis*	*C. tropicalis*	*C. krusei*	*T. rubrum*
30a	0.25	1	64	8	8	2	64
30b	2	>64	>64	32	16	8	>64
30c	1	1	32	4	16	2	32
30d	1	1	32	4	16	2	32
30e	0.25	1	16	2	8	1	16
30f	0.5	0.5	16	2	16	1	32
30g	0.5	0.5	32	4	2	2	32
30h	1	0.5	32	4	16	2	16
31a	0.25	>64	16	nd	4	nd	16
31b	0.25	>64	16	nd	16	nd	16
31c	0.25	>64	16	nd	1	nd	1
31d	0.25	>64	16	nd	1	nd	16
32a	8	>64	64	4	64	1	16
32b	8	64	16	2	16	1	32
32c	0.125	1	64	4	16	0.5	32
32d	0.5	1	32	4	4	1	32
33a	0.25	0.5	>64	32	>64	1	32
33b	0.5	0.5	32	32	>64	2	64
33c	>64	>64	>64	32	>64	4	64
33d	>64	>64	>64	32	>64	8	>64
Caspofungin	0.5	1	16	1	4	0.25	16

Abbreviations: C. albicans, Candida albicans; C. tropicalis, Candida tropicalis; C. parapsilosis, Candida parapsilosis; C. krusei, Candida krusei; C. neoformans, Cryptococcus neoformans; A. fumigatus, Aspergillus fumigatus; T. rubrum, Trichophyton rubrum.

structural modification. The introduction of amino group on the proline ring (e.g., compounds **32a**–**b** vs **32c**–**d**) led to the decreased antifungal activity and narrowed spectrum. Interestingly, compounds **33a**–**b**, which contain amino group on proline ring still showed good activity. So, we could draw a conclusion that the antifungal activity was decreased to a great degree if two prolines (A and D) were both substituted with aminoproline. The importance of threonine (part E) was investigated by mutation with other amino acid residues. When threonine was replaced by the more hydrophobic valine (e.g., compounds **30c**–**d** and **30g**–**h**), the antifungal activities were slightly decreased (especially for *C. albicans* and *A. fumigatus*). The replacement of the threonine (e.g., compounds **31a**–**b**) with the serine had little effect on the activity against *A. fumigatus*, *C. albicans*, and *C. neoformans*. However, compounds bearing a hydrophilic lysine on the part E showed very weak antifungal activity. Therefore, the hydrophobicity of the amino acid at part E might be important for the antifungal activity and threonine was preferred at this position.

When alkoxy-aroyl side chains were attached to the cyclohexapeptide core of caspofungin, good antifungal activity was also observed for them. Three types of aryl groups, namely terphenyl, biphenyl, and naphthyl, were investigated in the present study. In vitro antifungal assay revealed that the naphthyl group was better than the triphenyl group. For example, as compared with terphenyl derivatives **30b**, the corresponding naphthyl analogue **32d** showed better antifungal activity and broader spectrum. Compounds with biphenyl side chains (e.g., **31c**–**d**) were generally more potent than the corresponding tripehnyl analogues (e.g., **30e**–**f**). However, this trend was not found for the activity against *A. fumigatus* because compounds **31c** and **31d** were inactive. Moreover, we investigated the effect of the alkyl length on the antifungal activity. All the findings demonstrated that the antifungal activities were slightly decreased when the alkyl length prolonged (e.g., **30a** vs **30b**; **30e** vs **30f**; **30g** vs **30h**; **31a** vs **31b**; **31c** vs **31d**; **33a** vs **33b**).

3. Conclusions

In summary, a total synthetic route was developed for the preparation of caspofungin-like antifungal macrocyclic lipopeptidyl amine derivatives. The present [3+3]-segment coupling strategy has several advantages, such as high synthetic efficiency, mild reaction conditions, and easy structure-diversity derivatization, which make it possible for systemically investigating their SAR. A series of structurally diverse caspofungin analogues were rationally designed and synthesized. Most of the target compounds showed potent in vitro antifungal activities with broad spectrum. In particular, several compounds (e.g., **30a**, **30e**–**h**, **31a**–**d**, **32c**, and **33a**–**b**) were more active against *C. albicans* or *A. fumigatus* than caspofungin and were promising leads for further structural optimization. All the findings suggested that the complex chemical structure of caspofungin could be simplified and optimized. More importantly, new SAR information was obtained for the antifungal echinocandins. Major difference of hydrophobicity was observed for the amino acid component of the cyclohexapeptide scaffold of caspofungin-like macrocyclic lipopeptides. Hydrophilic amino acids were favored for the 'left' tripeptide segment (A–C) of the cyclohexapeptide scaffold, whereas the 'right' lipotripeptide fragment (D–G) was preferred as a hydrophobic core. The replacement of the alkyl-acyl side chain of caspofungin by alkoxy-aroyl generally led to the increase of antifungal activity against either *C. albicans* or *A. fumigatus*. The alkoxy-naphthoyl group was found to be optimal side chain. Moreover, the alkyl length could affect the SARs of alkoxy-aroyl side chains. Further biological evaluations and structural optimization studies are in progress.

4. Experimental section

4.1. Chemistry synthesis

1-Hydroxybenzotrizole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), methyl tertiary butyl ether (MTBE), (benzotriazol-1-yl-oxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), trifluoroactic acid (TFA), 4-dimethylamiopryidine (DMAP), *N*,*N*-diisopropylethylamine (DIPEA), *N*,*N*′-dicyclohexylcarbodiimide (DCC), *p*-toluenesulfonic acid (PTSA), *N*,*N*-dimethylformamide (DMF), methanesulfonyl chloride (MsCl) and all other reagents solvents were commercially available.

^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$, MeOD or DMSO-d_6 as solvents. Chemical shift are given in parts per million (δ). Elemental analyses

were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4%. The mass spectra were recorded on an Esquire 3000 LC–MS mass spectrometer. Silica gel thin-layer chromatography was performed on precoated plates GF_{254} (Qingdao Haiyang Chemical, China). The purities of final compounds were assessed on the basis of RP HPLC, and the results were greater than 95%. All solvents were analytical pure and no further purification is needed.

*4.1.1. 6-Hexyloxy-2-naphthoic acid (**12a**).* A solution of 6-hydroxy-2-naphthoic acid (**11**) (5.0 g, 26.6 mmol) and KOH (6.0 g, 107.1 mmol) in EtOH/H_2O (10:1, 100 mL) was refluxed under N_2 for 30 min. Then, 1-bromohexane (8.77 g, 53.2 mmol) was added and the resulting mixture was refluxed for 24 h. After cooling, the pH value of the solution was adjusted to 6.0 by dilute HCl. The solid was filtered and recrystalized in MeOH and CH_2Cl_2 to afford **12a** (5.66 g, yield 78.2%) as white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 12.87 (s, 1H), 8.51 (s, 1H), 8.01 (d, 1H, 9.05 Hz), 7.92 (dd, 1H, J=1.65 Hz), 7.86 (d, 1H, J=8.65 Hz), 7.39 (d, 1H, J=2.35 Hz), 7.23 (dd, 1H, J=2.45 Hz), 4.11 (t, 2H, J=6.55 Hz), 1.78 (m, 2H), 1.34 (m, 2H), 1.20 (m, 4H), 0.89 (m, 3H). Anal. calcd for $C_{17}H_{20}O_3$: C, 75.00; H, 7.35; found: C, 75.21; H, 7.33. The synthetic procedure for compounds **12b–c** was similar to the preparation of compound **12a** except using other 1-bromoalkanes instead of 1-bromopentane.[39]

*4.1.2. 4′-Pentyloxy-biphenyl-4-carboxylic acid (**14a**).* A solution of 4′-hydroxy-biphenyl-4-carboxylic acid (**13**) (5.0 g, 23.4 mmol) and KOH (5.3 g, 94.6 mmol) in EtOH/H_2O (10:1, 100 mL) was refluxed under N_2 for 30 min. Then, 1-bromopentane (7.06 g, 46.8 mmol) was added and the resulting mixture was refluxed for 24 h. After cooling, the pH value of the solution was adjusted to 6.0 by dilute HCl. The solid was filtered and recrystalized in MeOH and CH_2Cl_2 to afford **14a** (5.08 g, yield 76.6%) as white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 12.7 (s, 1H), 7.98 (d, 2H), 7.75 (d, 2H), 7.68 (d, 2H), 7.05 (d, 2H), 4.02 (t, 2H, J=6.5 Hz), 1.74 (m, 2H), 1.39 (m, 4H), 0.91 (t, 3H, J=7.2 Hz). MS (ESI$^+$) m/z: 307.35 [M+Na$^+$, 100%]. Anal. calcd for $C_{18}H_{20}O_3$: C, 76.06; H, 7.04; found: C, 75.25; H, 7.02. The synthetic procedure for compounds **14b** was similar to the preparation of compound **14a** except using 1-bromooctane instead of 1-bromopentane.[39]

*4.1.3. 4-Bromo-4′-hexyloxy-biphenyl (**16a**).* A suspension of **15** (3.5 g, 14.06 mmol), 1-bromohexane (3.5 g, 21.21 mmol), and K_2CO_3 (4.9 g, 35.28 mmol) in 2-butanone (25 mL) was refluxed for 8 h. After cooling, the reaction mixture was diluted by CH_2Cl_2 (50 mL). After filtration, the mixture was washed by H_2O (50 mL) and saturated brine (50 mL), dried over anhydrous Na_2SO_4, and filtrated. The solvent was condensed to 15 mL under reduced pressure, and the solid was collected and washed by cool hexane (0 °C) to afford **16a** (4.5 g, yield 96.1%) as white solid. Mp: 124–125 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): 7.52 (d, 2H, J=8.55 Hz), 7.46 (d, 2H, J=8.80 Hz), 7.40 (d, 2H, J=8.55 Hz), 6.96 (d, 2H, J=8.80 Hz), 3.99 (t, 2H, J=6.55 Hz), 1.80 (m, 2H), 1.47 (m, 2H), 1.35 (m, 4H), 0.91 (t, 3H, J=7.05 Hz). Anal. calcd for $C_{18}H_{21}BrO$: C, 64.86; H, 6.31; found: C, 65.04; H, 6.29. The synthetic procedure for compounds **16b** was similar to the preparation of compound **16a** except using 1-bromooctane instead of 1-bromohexane.[39]

*4.1.4. 4-Bronic acid-4′-hexyloxy-biphenyl (**17a**).* n-BuLi (2.5 M, 7.4 mL, 18 mmol) was added dropwise to a solution of **16a** (4.3 g, 12.9 mmol) in MTBE (50 mL) at −20 °C under nitrogen atmosphere. After stirring for 2 h, the reaction mixture was cooled to −60 °C and was added THF (6 mL). Then, a solution of (i-PrO)$_3$B (6.1 mL, 26 mmol) in MTBE (8 mL) was added dropwise to the resulting mixture and was stirred for 1 h. The reaction mixture was allowed to warm to room temperature, stirred overnight and was treated with 2 M HCl (50 mL) for 10 min. After the separation of the organic layer, the solvent was evaporated under reduced pressure. The residue was added n-hexane (40 mL) and stirred for 10 min. After filtration, the solid was washed by n-hexane/MTBE (8:1) to give **17a** (3.6 g, yield 93.6%) as white solid. Mp: 169–170 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.01 (s, 2H), 7.84 (d, 2H, J=8.20 Hz), 7.61 (d, 2H, J=6.80 Hz), 7.59 (d, 2H, J=8.20 Hz), 7.01 (d, 2H, J=6.80 Hz), 4.00 (t, 2H, J=6.50 Hz), 1.72 (m, 2H), 1.43 (m, 2H), 1.32 (m, 4H), 0.88 (t, 3H, J=7.0 Hz). Anal. calcd for $C_{18}H_{23}BO_3$: C, 72.53; H, 7.72; found: C, 72.71; H, 7.70. The synthetic procedure for compounds **17b** was similar to the preparation of compound **17a** except using **16b** instead of **16a**.[39]

*4.1.5. 4-Methoxycarbonyl-4″-hexyloxy-terphenyl (**18a**).* A solution of **17a** (3.08 g, 10.34 mmol) and methyl 4-iodobenzoate (2.78 g, 10.56 mmol) in mixed solvent containing toluene/propanol (8:1, 25 mL) and 2 M Na_2CO_3 aqueous solution (6 mL) was added $Pd(OAc)_2$ (0.24 g, 1.1 mmol) and Ph_3P (0.84 g, 3.2 mmol). Then, the reaction mixture was refluxed for 4 h under nitrogen atmosphere. After filtration, the filter cake was washed by toluene, MTBE/EtOAc (2:1), and H_2O. The residue was dried over P_2O_5 to give **18a** (3.79 g, yield 94.4%) as white solid. Mp: 264–265 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): 8.05 (d, 2H, J=8.25 Hz), 7.89 (d, 2H, J=8.50 Hz), 7.82 (d, 2H, J=8.50 Hz), 7.76 (d, 2H, J=8.25 Hz), 7.67 (d, 2H, J=8.70 Hz), 7.03 (d, 2H, J=8.70 Hz), 4.02 (t, 2H, J=6.50 Hz), 3.88 (s, 3H), 1.72 (m, 2H), 1.43 (m, 2H), 1.30 (m, 4H), 0.89 (t, 3H, J=7.0 Hz). Anal. calcd for $C_{26}H_{28}O_3$: C, 80.41; H, 7.22; found: C, 80.59; H, 7.20. The synthetic procedure for compounds **18b** was similar to the preparation of compound **18a** except using **17b** instead of **17a**.[39]

*4.1.6. 4-Carboxylic acid-4″-hexyloxy-terphenyl (**19a**).* A suspension of **18a** (3.88 g, 10.0 mmol), hexadecyl trimethyl ammonium bromide (0.23 g, 0.62 mmol), and 5 M KOH aqueous solution (8 mL, 40.0 mmol) in xylene (40 mL) was refluxed for 3 h. After cooling to 10 °C, the reaction mixture was filtrated and the filter cake was washed by H_2O. The solid was suspended in 1,2-dimethoxyethane (50 mL) and the pH value of the solution was adjusted to 1 by 6 M HCl. Then, the resulting mixture was refluxed for 30 min and cooled to room temperature. After filtration, the solid was washed by MTBE and H_2O to give **19a** (3.65 g, yield 97.6%) as white power. Mp: >280 °C (decomposed). ^{1}H NMR (DMSO-d_6, 500 MHz): 12.91 (s, 1H), 8.03 (d, 2H, J=8.15 Hz), 7.85 (d, 2H, J=8.25 Hz), 7.81 (d, 2H, J=8.15 Hz), 7.75 (d, 2H, J=8.25 Hz), 7.67 (d, 2H, J=8.60 Hz), 7.04 (d, 2H, J=8.60 Hz), 4.02 (t, 2H, J=6.55 Hz), 1.73 (m, 2H), 1.43 (m, 2H), 1.32 (m, 4H), 0.90 (t, 3H, J=7.10 Hz). Anal. calcd for $C_{25}H_{26}O_3$: C, 80.21; H, 6.95; found: C, 80.37; H, 6.93. The synthetic procedure for compounds **19b** was similar to the preparation of compound **19a** except using **18b** instead of **18a**.[39]

*4.1.7. N-Boc-Thr-Pro-OMe (**22a**).* DIPEA (35.8 mL, 205.2 mmol) was added dropwise to a solution of methyl L-prolinate hydrochloride (**20a**) (8.50 g, 51.3 mmol) in CH_2Cl_2 (200 mL) at 0 °C and the solution was stirred for 20 min. Then, *N*-Boc-threonine (**21a**) (11.25 g, 51.3 mmol) in CH_2Cl_2 (50 mL) was added dropwise to the reaction mixture. After the addition of HOBt (9.7 g, 71.8 mmol), the solution was stirred for 20 min at 0 °C and was added EDC hydrochloride (13.77 g, 71.8 mmol) in portion. The resulting mixture was stirred at 0 °C for 0.5 h and stirred at room temperature for 24 h. Then, citrate solution (10%, 200 mL) was added. The organic layer was separated, washed by saturated $NaHCO_3$ solution, H_2O and saturated brine, dried over anhydrous Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure to afford yellow oil, which was recrystallized in EtOAc to give **22a** (15.2 g, yield 89.7%) as white solid. Mp: 59–61 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 6.62 (d, 1H, J=7.25 Hz), 4.30 (m, 1H), 4.08 (m, 1H), 3.80 (m, 1H), 3.72 (m, 1H), 3.65 (m, 1H), 3.60 (s, 3H), 2.18 (m, 1H), 1.91 (m, 2H), 1.81 (m, 1H),

1.37 (s, 9H), 1.11 (d, 3H, *J*=6.30 Hz). MS (ESI^+) *m/z*: 353.83 ($M+Na^+$, 100%). Anal. calcd for $C_{15}H_{26}N_2O_6$: C, 54.55; H, 7.88; N, 8.48; found: C, 54.76; H, 7.85; N, 8.45. The synthetic procedure for dipeptides **22b**–**g** was similar to the preparation of dipeptide **22a**.[39]

*4.1.8. N^{δ}-Boc-Orn(N^{α}-Fmoc)-Thr-Pro-OMe (**23a**).* A solution of **22a** (8.50 g, 25.74 mmol) and TFA (16 mL) in CH_2Cl_2 (50 mL) was stirred for 1 h at room temperature and the solvent was evaporated under reduced pressure. The residue was dried over P_2O_5 to give the deprotected product, which was solved in 50 mL of CH_2Cl_2 and was added N^{α}-Fmoc-N^{δ}-Boc-ornithine (11.24 g, 24.7 mmol), HOBt (5.18 g, 38.6 mmol), EDC hydrochloride (6.88 g, 36.1 mmol), and DIPEA (20.0 mL, 77.22 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure and the residue was added citrate solution (10%, 250 mL) and EtOAc (300 mL). The organic layer was separated, washed by saturated $NaHCO_3$ solution, H_2O, and saturated brine, dried over anhydrous Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure to afford yellow oil, which purified by Sephadex column chromatography (eluent: MeOH) to give **23a** (14.6 g, yield 88.7%) as white solid. Mp: 94–95 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.76 (d, 2H, *J*=7.50 Hz), 7.59 (m, 2H), 7.39 (m, 2H), 7.31 (m, 2H), 6.97 (br, 1H), 5.61 (br, 1H), 5.30 (s, 1H), 4.75 (m, 1H), 4.69 (m, 1H), 4.53 (m, 1H), 4.40 (m, 2H), 4.31 (m, 1H), 4.21 (m, 2H), 3.80 (s, 3H), 3.75 (m, 1H), 3.12 (m, 2H), 2.26 (m, 1H), 2.03 (m, 1H), 2.02 (m, 2H), 1.88 (m, 1H), 1.67 (m, 2H), 1.52 (m, 1H), 1.44 (s, 9H), 1.21 (d, 3H, *J*=6.40 Hz). MS (ESI^+) *m/z*: 690.04 ($M+Na^+$, 100%), 1355.62 ($2M+Na^+$, 55%). Anal. calcd for $C_{35}H_{46}N_4O_9$: C, 63.06; H, 6.91; N, 8.41; found: C, 63.28; H, 6.89; N,8.38. The synthetic procedure for tripeptides **23b**–**e** was similar to the preparation of tripeptide **23a**.[39]

*4.1.9. N^{δ}-Boc-Orn(N^{α}-4″-hexyloxy-terphenyl-4-formyl)-Thr-Pro-OMe (**24a**).* A solution of **19a** (0.52 g, 1.39 mmol), HOBt (0.24 g, 1.78 mmol), DCC (0.37 g, 1.80 mmol), and DMAP (25 mg, 0.20 mmol) in CH_2Cl_2 (50 mL) was stirred for 48 h. After filtration, the solvent was evaporated under reduced pressure to afford the 'activated ester', which could be directly used in the next step without further purification. Et_2NH (1.3 mL) was added to a solution of tripeptide **23a** (0.55 g, 0.83 mmol) in CH_2Cl_2 (50 mL) under ice bath and the reaction mixture was stirred for 2 h. The solvent was evaporated under reduced pressure and the residue was dried over anhydrous Na_2SO_4 for 24 h to give the *N*-Fmoc deprotected product. Then, it was solved in DMF (20 mL) and was added fresh 'activated ester' prepared above. Then the reaction mixture was stirred at room temperature for 48 h. The resulting mixture was added CH_2Cl_2 (100 mL), washed by H_2O and saturated brine, dried over anhydrous Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH=30:1, v/v) to give **24a** (0.51 g, yield 91.4%) as white solid. Mp: 183–184 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.91 (d, 2H, *J*=8.25 Hz), 7.64 (m, 6H), 7.55 (d, 2H, *J*=8.70 Hz), 6.98 (d, 2H, *J*=8.70 Hz), 4.81 (m, 1H), 4.71 (m, 1H), 4.54 (m, 1H), 4.22 (m, 1H), 4.01 (t, 2H, *J*=6.58 Hz), 3.84 (m, 1H), 3.76 (m, 1H), 3.73 (s, 3H), 3.18 (m, 2H), 2.25 (m, 1H), 2.01 (m, 4H), 1.81 (m, 3H), 1.63 (m, 2H), 1.49 (m, 2H), 1.43 (s, 9H), 1.36 (m, 4H), 1.24 (d, 3H, *J*=6.35 Hz), 0.92 (t, 3H, *J*=7.08 Hz). MS (ESI^+) *m/z*: 823.83 ($M+Na^+$, 40%), 1624.11 ($2M+Na^+$, 100%). MS (ESI^-) *m/z*: 799.92 (M−H, 100%). Anal. calcd for $C_{45}H_{60}N_4O_9$: C, 67.50; H,7.50; N, 7.00; found: C, 67.68; H, 7.49; N, 6.98. The synthetic procedure for lipotripeptides **24b**–**m** was similar to the preparation of lipotripeptide **24a**.[39]

*4.1.10. N-Boc-hTyr-Thr-Pro(4S-N_3)-OMe (**25a**).* A solution of dipeptide **22d** (2.23 g, 6.01 mmol) and TFA (6 mL) in CH_2Cl_2 (50 mL) was stirred at room temperature for 40 min. The solvent was evaporated under reduced pressure to give the *N*-Boc deprotected product, which was dried in vacuum and used directly in the next step without further purification. The obtained product was dissolved in CH_2Cl_2 (35 mL), and was added *N*-Boc-L-homotyrsoine (1.77 g, 6.01 mmol), HOBt (1.29 g, 9.60 mmol), EDC hydrochloride (1.73 g, 9.02 mmol) and DIPEA (2.12 mL, 12.24 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 40 min, warmed to room temperature and stirred for 24 h. The solvent was evaporated under reduced pressure and the residue was added citrate solution (10%, 250 mL) and EtOAc (300 mL). The organic layer was separated, washed by saturated $NaHCO_3$ solution, H_2O and saturated brine, dried over anhydrous Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure to afford yellow oil, which purified by silica gel column chromatography (hexane/ethyl acetate=1:1, v/v) to give **25a** (2.72 g, yield 82.6%) as white solid. Mp: 94–96 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ: 9.11 (s, 1H), 7.79 (d, 1H, *J*=6.81 Hz), 7.06 (d, 1H, *J*=8.28 Hz), 6.94 (d, 2H, *J*=8.40 Hz), 6.65 (d, 2H, *J*=8.40 Hz), 4.40–4.50 (m, 2H), 4.25–4.35(m, 1H), 4.01–4.10(m, 1H), 3.85–3.95 (m, 1H), 3.75–3.83 (m, 1H), 3.62–3.66 (m, 2H), 3.61 (s, 3H), 2.30–2.55 (m, 3H), 1.90–2.00(m, 1H), 1.75–1.1.85 (m, 1H), 1.65–1.1.75 (m, 1H), 1.40(s, 9H), 1.15 (d, 3H, *J*=6.23 Hz). MS (ESI^+) *m/z*: 571.81 ($M+Na^+$, 100%). Anal. calcd for $C_{25}H_{36}N_6O_8$: C, 54.74; H, 6.57; N, 15.33; found: C, 54.95; H, 6.55; N, 15.27. The synthetic procedure for tripeptides **25b**–**d** was similar to the preparation of tripeptide **25a**.[39]

*4.1.11. cyclo-[Orn(N^{α}-4′-hexyloxy-terphenyl-4-formyl)-Thr-Pro-hTyr-Orn(N^{δ}-Cbz)-Pro(4S-N_3)] (**29A$_1$**).* A solution of tripeptide **24a** (0.36 g, 0.45 mmol) and TFA (1.5 mL) in CH_2Cl_2 (12 mL) was stirred at room temperature for 45 min. The solvent was evaporated under reduced pressure, and the residue was dried over P_2O_5 to give the *N*-Boc deprotected product **26a**, which was used directly in the next step. A solution of tripeptide **25b** (2.88 g, 4.14 mmol) in EtOH (50 mL) and 1 M NaOH aqueous solution (20.7 mL, 20.7 mmol) was stirred overnight at room temperature. The pH value of the reaction mixture was adjusted to 4.5 by 0.5 M HCl. The solvent was removed under reduced pressure and the residue was distributed in EtOH (100 mL) and H_2O (50 mL). The organic layer was separated the water layer was extracted by EtOAc (50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtrated. The solvent was evaporated under reduced pressure and the residue was dried over P_2O_5 to give **27b** as white solid, which used directly in the next step without further purification. To a solution of **26a** (0.263 g, 0.38 mmol) in CH_2Cl_2 (15 mL) were added **27b** (0.256 g, 0.38 mmol), PyBOP (0.196 g, 0.38 mmol), and DIPEA (0.25 mL, 1.44 mmol). The reaction mixture was stirred at room temperature for 10 h. The solvent was removed under reduced pressure and the residue was added EtOAc (100 mL), which was washed with saturated $NaHCO_3$ solution, H_2O and saturated brine, dried over anhydrous Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure to afford crude hexalipopeptide **28a**, which used directly in the next step without further purification. A solution of above crude hexalipopeptide **28a** in CH_2Cl_2 (15 mL) was added TFA (1.5 mL), and was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was added EtOH (12 mL). The pH value of the solution was adjusted to 7.0 by 1 M NaOH aqueous solution and 1 M NaOH aqueous solution (2.0 mL) was added. The resulting mixture was stirred overnight and the pH value was adjusted to 7.0 by 1 M HCl. The solvent was removed under reduced pressure and the residue was dried over P_2O_5. The obtained yellow solid was dissolved in dry DMF (420 mL), and were added PyBOP (550 mg, 1.06 mmol), HOBt (143 mg, 1.06 mmol), and DIPEA (0.13 mL, 0.75 mmol). The reaction mixture was stirred at room temperature for 48 h and was concentrated under vacuum. The residue was treated with EtOAc (60 mL) and saturated Na_2HCO_3 solution (60 mL). After shaking, the organic layer was separated, washed with H_2O and brine, dried over anhydrous Na_2SO_4 and filtrated. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH=25:1, v/v) to give

cyclic lipopeptide **29A$_1$** (340 mg, yield 73.5%, six steps) as white solid. MS (ESI$^+$) *m/z*: 1254.95 (M+Na$^+$, 100%). Anal. calcd for $C_{67}H_{81}N_{11}O_{12}$: C, 65.31; H, 6.58; N, 12.51; found: C, 65.47; H, 6.57; N, 12.46. The synthetic procedure for cyclic lipopeptides **29A$_{2-8}$**, **29B$_{1-4}$**, **29C$_{1-4}$**, and **29D$_{1-4}$** was similar to the preparation of **29A$_1$**.[39]

*4.1.12. Macrocyclic lipopeptide amine p-tosylate (**30a**).* A solution of cyclic lipopeptide **29A$_1$** (160 mg, 0.13 mmol) in EtOH (25 mL) was added PTSA·H$_2$O (60 mg, 0.32 mmol) and 10% Pd–C (90 mg). The reaction mixture was stirred at room temperature under H$_2$ for 24 h. After filtration, the solvent was removed under reduced pressure. The residue was purified by Sephadex column chromatography (MeOH) to afford the target compound **30a** (176 mg, yield 95.7%) as white solid. Mp: 228–231 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.96 (d, 2H, *J*=8.41 Hz), 7.78 (d, 2H, *J*=8.41 Hz), 7.72 (m, 8H), 7.61 (d, 2H, *J*=8.76 Hz), 7.23 (d, 4H, *J*=7.93 Hz), 7.01 (m, 4H), 6.70 (d, 2H, *J*=8.46 Hz), 4.96 (m, 1H), 4.75 (m, 1H), 4.66 (m, 1H), 4.55 (m, 1H), 4.27 (m, 2H), 4.02 (t, 2H, *J*=6.39 Hz), 4.01 (m, 2H), 3.95 (m, 1H), 3.82 (m, 1H), 3.66 (m, 2H), 3.03 (m, 2H), 2.91 (m, 1H), 2.60 (m, 3H), 2.20–2.12 (m, 10H), 2.11–1.90 (m, 7H), 1.90–1.70 (m, 7H), 1.35–1.48 (m, 6H), 1.29 (d, 3H, *J*=6.06 Hz), 0.94 (t, 3H, *J*=7.19 Hz). ^{13}C NMR (CD$_3$OD, 500 MHz): 174.97, 174.74, 174.01, 173.73, 172.02, 171.06, 170.35 (7× C=O); 160.49, 156.80, 145.43, 141.96, 141.72, 139.22, 133.90, 132.87, 130.55, 129.83, 129.19, 128.91, 128.45, 128.03, 127.79, 126.97, 116.28, 116.03 (36× Ar–C); 69.20, 68.83, 64.35, 60.94, 58.17, 54.90, 54.21, 52.32, 51.90, 51.37, 40.77, 38.47, 34.75, 33.59, 32.75, 30.40, 30.30, 27.31, 27.24, 26.84, 25.11, 24.45, 24.41, 23.64, 21.28, 20.20, 14.32 (30× Alkyl-C). MS (ESI$^+$) *m/z*: 1072.84 (M+H$^+$, 100%). Anal. calcd for $C_{73}H_{93}N_9O_{16}S_2$: C, 61.91; H, 6.57; N, 8.90; found: C, 62.08; H, 6.55; N, 8.87.The synthetic procedure for *p*-tosylate cyclic lipopeptide amines **30b–h**, **31a–d**, **32a–d**, and **33a–d** was similar to the preparation of the compound **30a** except using **29A$_{2-8}$**, **29B$_{1-4}$**, **29C$_{1-4}$**, and **27D$_{1-4}$** instead of **29A$_1$**, respectively.

*4.1.13. Macrocyclic lipopeptide amine p-tosylate (**30b**).* White solid (Yield 93.4%), Mp: 205–208 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.96 (d, 2H, *J*=8.48 Hz), 7.77 (d, 2H, *J*=8.48 Hz), 7.71 (m, 8H), 7.60 (d, 2H, *J*=8.80 Hz), 7.23 (d, 4H, *J*=7.95 Hz), 7.00 (m, 4H), 6.70 (d, 2H, *J*=8.49 Hz), 4.95 (m, 1H), 4.75 (m, 1H), 4.66 (m, 1H), 4.55 (m, 1H), 4.27 (m, 2H), 4.02 (t, 2H, *J*=6.47 Hz), 4.01 (m, 1H), 3.98 (m, 1H), 3.96 (m, 1H), 3.82 (m, 1H), 3.66 (m, 2H), 3.02 (m, 2H), 2.91 (m, 1H), 2.60 (m, 3H), 2.20–2.12 (m, 10H), 2.11–1.90 (m, 7H), 1.90–1.70 (m, 7H), 1.48–1.35 (m, 10H), 1.27 (d, 3H, *J*=6.10 Hz), 0.92 (t, 3H, *J*=7.14 Hz). ^{13}C NMR (CD$_3$OD, 500 MHz): 174.97, 174.74, 174.01, 173.73, 172.06, 171.08, 170.34 (7× C=O); 160.48, 156.80, 145.38, 141.96, 141.78, 139.21, 133.88, 132.87, 130.56, 129.86, 129.19, 128.91, 128.45, 128.03, 127.79, 126.97, 116.28, 116.04 (36× Ar–C); 69.20, 68.82, 64.36, 60.93, 58.18, 54.92, 54.16, 52.33, 51.88, 51.37, 40.77, 38.44, 40.74, 38.45, 34.75, 34.44, 32.95, 30.46, 30.42, 30.35, 27.31, 27.24, 27.17, 26.60, 25.99, 24.51, 24.38, 23.67, 21.29, 20.24, 14.34 (32× Alkyl-C). MS (ESI$^+$) *m/z*: 1072.84 (M+H$^+$, 100%). MS (ESI$^+$) *m/z*: 1100.85 (M+H$^+$, 100%). Anal. calcd for $C_{75}H_{97}N_9O_{16}S_2$: C,62.37; H, 6.72; N, 8.73; found: C, 62.52; H, 6.70; N, 8.70.

*4.1.14. Macrocyclic lipopeptide amine p-tosylate (**30c**).* White solid (Yield 95.5%), Mp: 193–196 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.95 (d, 2H, *J*=8.42 Hz), 7.76 (d, 2H, *J*=8.42 Hz), 7.69 (m, 8H), 7.59 (d, 2H, *J*=8.73 Hz), 7.23 (d, 4H, *J*=7.93 Hz), 6.99 (m, 4H), 6.70 (d, 2H, *J*=8.46 Hz), 4.82 (m, 2H), 4.65–4.40 (m, 3H), 4.22 (m, 1H), 4.03 (m, 1H), 4.00 (t, 2H, *J*=6.49 Hz), 3.95–3.55 (m, 5H), 3.03 (m, 1H), 2.91 (m, 2H), 2.58 (m, 2H), 2.49 (m, 1H), 2.36–2.25 (m, 10H), 2.25–1.90 (m, 7H), 1.87–1.70 (m, 7H), 1.50–1.30 (m, 6H), 1.08 (d, 3H, *J*=6.80 Hz), 1.00 (d, 3H, *J*=6.59 Hz), 0.93 (t, 3H, *J*=6.97 Hz). ^{13}C NMR (CD$_3$OD, 500 MHz): 174.76, 174.39, 173.38, 171.91, 171.45, 170.12 (7× C=O); 160.44, 156.72, 145.38, 141.86, 139.21, 133.89, 133.15, 130.43, 129.91, 129.14, 128.90, 128.44, 128.37, 127.77, 126.96, 116.27, 116.00 (36×Ar–C); 69.17, 63.96, 61.07, 58.05, 56.98, 53.92, 52.48, 52.36, 51.23, 40.09, 38.60, 34.20, 33.00, 32.74, 31.91, 31.52, 30.39, 30.05, 29.37, 26.83, 26.75, 26.47, 25.94, 25.02, 23.66, 21.30, 20.15, 18.78, 14.34 (31× Alkyl-C). MS (ESI$^+$) *m/z*: 1070.81 (M+H$^+$, 100%). Anal. calcd for $C_{74}H_{95}N_9O_{15}S_2$: C, 62.85; H, 6.72; N, 8.92; found: C, 63.02; H, 6.70; N, 8.89.

*4.1.15. Macrocyclic lipopeptide amine p-tosylate (**30d**).* White solid (Yield 99.2%), Mp: 230–233 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.96 (d, 2H, *J*=8.38 Hz), 7.77 (d, 2H, *J*=8.38 Hz), 7.71 (m, 8H), 7.60 (d, 2H, *J*=8.69 Hz), 7.23 (d, 4H, *J*=7.94 Hz), 7.00 (m, 4H), 6.70 (d, 2H, *J*=8.47 Hz), 4.81 (m, 2H), 4.55 (m, 1H), 4.49 (m, 2H), 4.22 (m, 1H), 4.03 (m, 1H), 4.01 (t, 2H, *J*=6.45 Hz), 3.95–3.60 (m, 5H), 3.03 (m, 1H), 2.91 (m, 2H), 2.58 (m, 2H), 2.49 (m, 1H), 2.36–2.25 (m, 10H), 2.25–1.90 (m, 7H), 1.87–1.70 (m, 7H), 1.50–1.25 (m, 8H), 1.08 (d, 3H, *J*=6.81 Hz), 1.00 (d, 3H, *J*=6.62 Hz), 0.91 (t, 3H, *J*=6.89 Hz). ^{13}C NMR (CD$_3$OD, 500 MHz): 174.78, 174.42, 173.40, 171.93, 171.46, 170.11 (7× C=O); 160.46, 156.74, 145.38, 141.92, 141.84, 139.23, 133.92, 133.12, 130.44, 129.89, 129.14, 128.91, 128.45, 128.37, 127.78, 126.97, 116.27, 116.00 (36× Ar–C); 69.16, 63.98, 61.07, 58.05, 56.97, 53.92, 52.48, 52.36, 51.29, 40.09, 38.60, 33.45, 33.01, 31.91, 31.52, 30.47, 30.42, 30.37, 27.30, 27.24, 27.17, 26.11, 25.65, 25.01, 23.68, 21.29, 20.15, 18.80, 14.39 (33× Alkyl-C). MS (ESI$^+$) *m/z*: 1098.98 (M+H$^+$, 100%). Anal. calcd for $C_{76}H_{99}N_9O_{15}S_2$: C, 63.29; H, 6.87; N, 8.74; found: C, 63.47; H, 6.85; N, 8.71.

*4.1.16. Macrocyclic lipopeptide amine p-tosylate (**30e**).* White solid (Yield 91.7%), Mp: 221–223 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.97 (d, 2H, *J*=8.37 Hz), 7.78 (d, 2H, *J*=8.37 Hz), 7.71 (m, 8H), 7.60 (d, 2H, *J*=8.74 Hz), 7.22 (d, 4H, *J*=7.96 Hz), 7.00 (m, 4H), 6.70 (d, 2H, *J*=8.45 Hz), 5.00 (m, 1H), 4.81 (m, 1H), 4.75 (m, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 4.36 (m, 1H), 4.25 (m, 1H), 4.02 (t, 2H, *J*=6.40 Hz), 4.00 (m, 2H), 3.92 (m, 1H), 3.69 (m, 2H), 3.03 (m, 2H), 2.89 (m, 1H), 2.60 (m, 3H), 2.40–2.30 (m, 9H), 2.21 (m, 1H), 2.11 (m, 2H), 2.00–1.60 (m, 12H), 1.51 (m, 3H), 1.380 (m, 5H), 1.29 (d, 3H, *J*=6.14 Hz), 0.94 (t, 3H, *J*=7.08 Hz). ^{13}C NMR (CD$_3$OD, 500 MHz): 174.78, 174.65, 173.87, 173.52, 172.08, 171.39, 170.35 (7× C=O); 160.48, 156.81, 145.45, 141.97, 141.78, 139.20, 133.92, 132.83, 130.53, 129.87, 129.18, 128.91, 128.44, 128.03, 127.81, 126.97, 116.29, 116.03 (36× Ar–C); 69.19, 68.72, 64.26, 61.03, 58.11, 54.56, 54.22, 52.34, 51.71, 51.60, 40.70, 38.45, 35.07, 33.65, 33.16, 32.75, 30.48, 30.39, 28.38, 26.97, 26.84, 25.08, 23.64, 23.02, 21.29, 20.15, 14.32 (31× Alkyl-C). MS (ESI$^+$) *m/z*: 1086.87 (M+H$^+$, 50%), 1108.95 (M+Na$^+$, 100%). Anal. calcd for $C_{74}H_{95}N_9O_{16}S_2$: C, 62.14; H, 6.65; N,8.82; found: C, 62.31; H, 6.63; N, 8.79.

*4.1.17. Macrocyclic lipopeptide amine p-tosylate (**30f**).* White solid (Yield 96.5%), Mp: 206–210 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.97 (d, 2H, *J*=8.27 Hz), 7.79 (d, 2H, *J*=8.27 Hz), 7.71 (m, 8H), 7.61 (d, 2H, *J*=8.60 Hz), 7.23 (d, 4H, *J*=7.77 Hz), 7.01 (m, 4H), 6.70 (d, 2H, *J*=8.40 Hz), 5.00 (m, 1H), 4.81 (m, 1H), 4.75 (m, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 4.36 (m, 1H), 4.25 (m, 1H), 4.01 (t, 2H, *J*=6.45 Hz), 3.99 (m, 2H), 3.92 (m, 1H), 3.69 (m, 2H), 3.03 (m, 2H), 2.89 (m, 1H), 2.60 (m, 3H), 2.40–2.30 (m, 9H), 2.21 (m, 1H), 2.11 (m, 2H), 2.00–1.60 (m, 12H), 1.51 (m, 3H), 1.45–1.30 (m, 9H), 1.28 (d, 3H, *J*=6.12 Hz), 0.92 (t, 3H, *J*=6.95 Hz). ^{13}C NMR(CD$_3$OD, 500 MHz): 174.79, 174.65, 173.89, 173.54, 172.12, 171.37, 171.25 (7× C=O); 160.49, 156.84, 145.46, 141.99, 141.80, 139.20, 133.92, 132.82, 130.54, 129.88, 129.21, 128.92, 128.46, 128.05, 127.82, 126.98, 116.29, 116.02 (36× Ar–C); 69.17, 68.65, 64.26, 62.88, 58.11, 54.50, 54.23, 52.34, 51.72, 51.60, 40.70, 38.45, 35.05, 33.64, 33.16, 32.98, 32.85, 32.78, 30.44, 30.38, 28.41, 27.18, 27.01, 25.10, 23.69, 23.03, 21.30, 20.15, 14.37 (33× Alkyl-C). MS (ESI$^+$) *m/z*: 1114.89 (M+H$^+$, 80%), 1137.08 (M+Na$^+$, 100%). Anal. calcd for $C_{76}H_{99}N_9O_{16}S_2$: C, 62.59; H, 6.79; N, 8.65; found: C, 62.76; H, 6.77; N, 8.62.

*4.1.18. Macrocyclic lipopeptide amine p-tosylate (**30g**).* White solid (Yield 92.9%), Mp: 188–190 °C. ^{1}H NMR (CD$_3$OD, 500 MHz) δ: 7.96 (d, 2H, *J*=8.35 Hz), 7.78 (d, 2H, *J*=8.35 Hz), 7.70 (m, 8H), 7.60 (d, 2H,

J=8.62 Hz), 7.23 (d, 4H, *J*=7.96 Hz), 7.00 (m, 4H), 6.70 (d, 2H, *J*=8.44 Hz), 4.80 (m, 2H), 4.55 (m, 1H), 4.50 (m, 2H), 4.23 (m, 1H), 4.02 (t, 2H, *J*=6.44 Hz), 3.99 (m, 1H), 3.95–3.60 (m, 5H), 3.05–2.80 (m, 3H), 2.60–2.40 (m, 3H), 2.38–2.20 (m, 10H), 2.20–1.90 (m, 7H), 1.85–1.60 (m, 7H), 1.50–1.35 (m, 8H), 1.08 (d, 3H, *J*=6.78 Hz), 1.00 (d, 3H, *J*=6.24 Hz), 0.92 (t, 3H, *J*=6.82 Hz). ^{13}C NMR (CD_3OD, 500 MHz): 174.82, 174.75, 174.43, 173.40, 171.93, 171.77, 170.14 (7× C=O); 160.48, 156.75, 145.42, 141.95, 141.84, 139.27, 133.91, 133.15, 130.44, 129.90, 129.14, 128.91, 128.45, 128.37, 127.80, 126.99, 116.29, 116.02 (36× Ar–C); 69.19, 64.05, 61.83, 58.04, 57.02, 54.02, 51.99, 51.86, 51.39, 40.88, 38.71, 34.48, 33.45, 33.01, 32.72, 32.30, 31.87, 30.40, 29.38, 27.62, 26.85, 26.61, 26.00, 23.65, 21.29, 20.27, 18.74, 14.33 (32× Alkyl-C). MS (ESI$^+$) *m*/*z*: 1084.99 (M+H$^+$, 50%), 1106.91 (M+Na$^+$, 100%). Anal. calcd for $C_{75}H_{97}N_9O_{15}S_2$: C, 63.07; H, 6.80; N, 8.83; found: C, 63.24; H, 6.78; N, 8.80.

*4.1.19. Macrocyclic lipopeptide amine p-tosylate (**30h**).* White solid (Yield 97.7%), Mp: 205–209 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ: 7.95 (d, 2H, *J*=8.36 Hz), 7.78 (d, 2H, *J*=8.36 Hz), 7.70 (m, 8H), 7.60 (d, 2H, *J*=8.70 Hz), 7.24 (d, 4H, *J*=7.99 Hz), 7.00 (m, 4H), 6.70 (d, 2H, *J*=8.43 Hz), 4.80 (m, 2H), 4.55 (m, 1H), 4.50 (m, 2H), 4.23 (m, 1H), 4.02 (t, 2H, *J*=6.45 Hz), 3.99 (m, 1H), 3.95–3.60 (m, 5H), 3.05–2.80 (m, 3H), 2.60–2.40 (m, 3H), 2.38–2.20 (m, 10H), 2.20–1.90 (m, 7H), 1.85–1.60 (m, 7H), 1.50–1.30 (m, 12H), 1.08 (d, 3H, *J*=6.78 Hz), 1.00 (d, 3H, *J*=6.24 Hz), 0.91 (t, 3H, *J*=6.85 Hz). ^{13}C NMR (CD_3OD, 500 MHz): 174.76, 174.74, 174.43, 173.40, 171.70, 171.65, 170.14 (7× C=O); 160.47, 156.75, 145.36, 141.94, 141.77, 139.23, 133.91, 133.15, 130.44, 129.86, 129.14, 128.91, 128.46, 128.37, 127.80, 126.98, 116.28, 116.03 (36× Ar–C); 69.17, 64.04, 61.06, 58.05, 57.02, 54.01, 51.99, 51.86, 51.39, 40.89, 38.71, 34.42, 33.45, 32.96, 32.72, 32.30, 31.87, 30.47, 30.43, 30.36, 27.62, 27.31, 27.25, 27.18, 26.59, 25.99, 23.67, 23.49, 21.29, 20.25, 18.74, 14.36 (34× Alkyl-C). MS (ESI$^+$) *m*/*z*: 1112.93 (M+H$^+$, 100%). Anal. calcd for $C_{77}H_{101}N_9O_{15}S_2$: C, 63.51; H, 6.94; N, 8.66; found: C, 63.70; H, 6.92; N, 8.63.

*4.1.20. Macrocyclic lipopeptide amine p-tosylate (**31a**).* White solid (Yield 81.60%). MS (ESI$^+$) *m*/*z*: 968.59 (M+H$^+$, 100%). Anal. calcd for $C_{65}H_{85}N_9O_{16}S_2$: C,59.50; H, 6.48; N, 9.61; found: C, 59.65; H, 6.46; N, 9.58.

*4.1.21. Macrocyclic lipopeptide amine p-tosylate (**31b**).* White solid (Yield 79.20%). MS (ESI$^+$) *m*/*z*: 1010.90 (M+H$^+$, 100%), 1032.93 (M+Na$^+$, 40%). Anal. calcd for $C_{68}H_{91}N_9O_{16}S_2$: C,60.31; H, 6.73; N, 9.31; found: C, 60.48; H, 6.71; N, 9.28.

*4.1.22. Macrocyclic lipopeptide amine p-tosylate (**31c**).* White solid (Yield 82.40%). MS (ESI$^+$) *m*/*z*: 996.89 (M+H$^+$, 53%), 1018.96 (M+Na$^+$, 100%). Anal. calcd for $C_{67}H_{89}N_9O_{16}S_2$: C,60.04; H, 6.65; N, 9.41; found: C, 60.19; H, 6.63; N, 9.38.

*4.1.23. Macrocyclic lipopeptide amine p-tosylate (**31d**).* White solid (Yield 80.80%). MS (ESI$^+$) *m*/*z*: 1038.86 (M+H$^+$, 53%), 1060.76 (M+Na$^+$, 100%). Anal. calcd for $C_{70}H_{95}N_9O_{16}S_2$: C,60.83; H, 6.88; N, 9.12; found: C, 60.99; H, 6.86; N, 9.09.

*4.1.24. Macrocyclic lipopeptide amine p-tosylate (**32a**).* White solid (Yield 95.4%), Mp: 242–245 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.36 (s, 1H), 7.85 (m, 3H), 7.70 (d, 6H, *J*=8.21 Hz), 7.24 (m, 8H), 6.94 (d, 2H, *J*=8.53 Hz), 6.65 (d, 2H, *J*=8.53 Hz), 5.00 (m, 1H), 4.82 (m, 1H), 4.75 (m, 1H), 4.67 (m, 1H), 4.52 (m, 1H), 4.40 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 4.11 (t, 2H, *J*=6.44 Hz), 4.09–3.85 (m, 5H), 3.65 (m, 1H), 3.02 (m, 2H), 2.89 (m, 1H), 2.77 (m, 1H), 2.68 (m, 2H), 2.42 (m, 1H), 2.37–2.25 (m, 10H), 2.24–1.95 (m, 5H), 1.90–1.70 (m, 8H), 1.52 (m, 3H), 1.47–1.30 (m, 8H), 1.26 (d, 3H, *J*=6.14 Hz), 0.92 (t, 3H, *J*=7.01 Hz). ^{13}C NMR (CD_3OD, 500 MHz): 174.88, 174.63, 173.42, 172.67, 172.27, 171.63, 170.75 (7× C=O); 160.25, 156.61, 143.34, 141.90, 138.06, 132.85, 131.54, 130.65, 129.92, 129.29, 128.93, 128.14, 126.94, 125.44, 121.00, 117.00, 107.56 (36× Ar–H); 69.27, 68.42, 62.74, 60.97, 58.25, 54.22, 53.95, 52.30, 51.76, 51.53, 40.68, 35.62, 35.05, 33.63, 32.93, 32.78, 32.55, 30.30, 30.15, 28.28, 27.15, 23.62, 23.07, 21.29, 20.38, 14.36 (31× Alkyl-C). MS (ESI$^+$) *m*/*z*: 1013.83 (M+H, 80%), 1035.72 (M+Na$^+$, 100%). Anal. calcd for $C_{74}H_{100}N_{10}O_{19}S_3$: C, 58.12; H, 6.54; N, 9.16; found: C, 58.29; H, 6.52; N, 9.13.

*4.1.25. Macrocyclic lipopeptide amine p-tosylate (**32b**).* White solid (Yield 96.3%), Mp: 243–246 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.34 (s, 1H), 7.82 (m, 3H), 7.70 (d, 6H, *J*=8.20 Hz), 7.22 (m, 8H), 6.95 (d, 2H, *J*=8.50 Hz), 6.66 (d, 2H, *J*=8.50 Hz), 4.97 (m, 1H), 4.87 (m, 1H), 4.76 (m, 1H), 4.65 (m, 1H), 4.54 (m, 1H), 4.40 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 4.11 (t, 2H, *J*=6.45 Hz), 4.09–3.80 (m, 5H), 3.62 (m, 1H), 3.02 (m, 2H), 2.89 (m, 1H), 2.77 (m, 1H), 2.68 (m, 2H), 2.42 (m, 1H), 2.37–2.23 (m, 10H), 2.23–2.00 (m, 5H), 2.00–1.60 (m, 9H), 1.52 (m, 2H), 1.48–1.30 (m, 9H), 1.25 (d, 3H, *J*=6.14 Hz), 0.91 (t, 3H, *J*=6.94 Hz). ^{13}C NMR (CD_3OD, 500 MHz): 175.08, 174.69, 173.52, 172.75, 172.28, 171.29, 170.70 (7× C=O); 160.22, 156.59, 143.32, 141.92, 138.06, 132.85, 131.54, 130.65, 129.94, 129.26, 128.93, 128.12, 126.93, 125.44, 120.97, 116.21, 107.55 (36× Ar–C); 69.25, 68.53, 62.76, 60.88, 58.28, 54.26, 53.99, 52.45, 51.89, 51.59, 40.70, 35.60, 35.08, 33.63, 32.93, 32.58, 30.43, 30.33, 30.10, 28.32, 27.18, 24.45, 23.65, 21.29, 20.43, 14.37 (31× Alkyl-C). MS (ESI$^+$) *m*/*z*: 1013.81 (M+H, 100%). Anal. calcd for $C_{74}H_{100}N_{10}O_{19}S_3$: C, 58.12; H, 6.54; N, 9.16; found: C, 58.27; H, 6.53; N, 9.14.

*4.1.26. Macrocyclic lipopeptide amine p-tosylate (**32c**).* White solid (Yield 98.20%), Mp: 193–196 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.36 (s, 1H), 7.84 (m, 3H), 7.72 (d, 4H, *J*=7.38 Hz), 7.22 (m, 6H), 6.99 (d, 2H, *J*=8.47 Hz), 6.70 (d, 2H, *J*=8.47 Hz), 5.00 (m, 1H), 4.75 (m, 1H), 4.66 (m, 1H), 4.54 (m, 1H), 4.36 (m, 1H), 4.25 (m, 1H), 4.10 (t, 2H, *J*=6.42 Hz), 4.05 (m, 2H), 3.90 (m, 1H), 3.85 (m, 1H), 3.63 (m, 2H), 3.05 (m, 2H), 2.90 (m, 1H), 2.70–2.47 (m, 3H), 2.40–2.25 (m, 8H), 2.21 (m, 1H), 2.07 (m, 2H), 2.00–1.65 (m, 14H), 1.50 (m, 3H), 1.43 (m, 3H), 1.33 (m, 4H), 1.28 (d, 3H, *J*=6.05 Hz), 0.91 (t, 3H, *J*=6.96 Hz). ^{13}C NMR(CD_3OD, 500 MHz): 174.84, 174.59, 173.83, 173.47, 171.99, 171.39, 170.66 (7× C=O); 160.20, 156.74, 143.50, 141.80, 138.04, 132.79, 131.50, 130.49, 129.90, 129.24, 128.89, 128.11, 127.11, 125.40, 120.96, 116.24, 107.52 (28× Ar–C); 69.22, 68.61, 64.21, 60.94, 58.07, 54.53, 54.10, 52.73, 51.71, 51.45, 40.64, 38.72, 35.15, 33.67, 33.18, 32.89, 32.72, 30.43, 30.26, 30.11, 28.29, 27.22, 27.10, 24.96, 23.57, 22.91, 21.24, 20.13, 14.33 (32× Alkyl-C). MS (ESI$^+$) *m*/*z*: 999.06 (M+H$^+$, 100%), 1021.06 (M+Na$^+$, 48%). Anal. calcd for $C_{67}H_{91}N_9O_{16}S_2$: C, 59.96; H, 6.79; N, 9.40; found: C, 60.15; H, 6.77; N, 9.37.

*4.1.27. Macrocyclic lipopeptide amine p-tosylate (**32d**).* White solid (Yield 93.0%), Mp: 162–165 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.35 (s, 1H), 7.84 (m, 3H), 7.71 (d, 4H, *J*=8.10 Hz), 7.22 (m, 6H), 7.00 (d, 2H, *J*=8.43 Hz), 6.69 (d, 2H, *J*=8.43 Hz), 4.95 (m, 1H), 4.78 (m, 1H), 4.66 (m, 1H), 4.56 (m, 1H), 4.33 (m, 1H), 4.25 (m, 1H), 4.11 (t, 2H, *J*=6.43 Hz), 4.05 (m, 2H), 3.90 (m, 1H), 3.85 (m, 1H), 3.63 (m, 2H), 3.05 (m, 2H), 2.93 (m, 1H), 2.70–2.47 (m, 3H), 2.40–2.25 (m, 10H), 2.21 (m, 1H), 2.10–1.65 (m, 14H), 1.50 (m, 2H), 1.30–1.45 (m, 8H), 1.28 (d, 3H, *J*=6.03 Hz), 0.91 (t, 3H, *J*=6.96 Hz). ^{13}C NMR(CD_3OD, 500 MHz): 175.07, 174.75, 173.97, 173.72, 172.03, 171.08, 170.70 (7× C=O); 160.23, 156.76, 143.54, 141.75, 138.07, 132.87, 131.55, 130.56, 129.85, 129.28, 128.92, 128.13, 126.95, 125.45, 120.99, 116.26, 107.57 (28× Ar–C); 69.26, 68.77, 64.33, 60.86, 58.17, 54.36, 54.10, 52.41, 51.89, 51.23, 40.80, 38.68, 35.09, 33.67, 32.94, 32.84, 30.44, 30.40, 30.34, 30.30, 27.29, 27.23, 24.54, 24.43, 23.66, 21.29, 20.23, 14.38 (32× Alkyl-C). MS (ESI$^+$) *m*/*z*: 999.07 (M+H$^+$, 100%). Anal. calcd for $C_{67}H_{91}N_9O_{16}S_2$: C, 59.96; H, 6.79; N, 9.40; found: C, 60.13; H, 6.78; N, 9.38.

*4.1.28. Macrocyclic lipopeptide amine p-tosylate (**33a**).* White solid (Yield 82.7%), Mp: 22–227 °C. MS (ESI$^+$) *m*/*z*: 1036.93 (M+Na$^+$,

100%). Anal. calcd for $C_{67}H_{91}N_9O_{17}S_2$: C, 59.25; H, 6.71; N, 9.29; found: C, 59.42; H, 6.69; N, 9.26.

*4.1.29. Macrocyclic lipopeptide amine p-tosylate (**33b**).* White solid (Yield 75.0%), Mp: 217–219 °C. MS (ESI$^+$) *m/z*: 1028.91 (M+H$^+$, 100%), 1050.88 (M+Na$^+$, 75%). Anal. calcd for $C_{68}H_{93}N_9O_{17}S_2$: C, 59.52; H, 6.78; N, 9.19; found: C, 59.70; H, 6.76; N, 9.16.

*4.1.30. Macrocyclic lipopeptide amine p-tosylate (**33c**).* White solid (Yield 74.2%), Mp: 184–187 °C. MS (ESI$^+$) *m/z*: 1000.79 (M+H$^+$, 100%). Anal. calcd for $C_{66}H_{89}N_9O_{17}S_2$: C, 58.97; H, 6.63; N, 9.38; found: C, 59.13; H, 6.61; N, 9.35.

*4.1.31. Macrocyclic lipopeptide amine p-tosylate (**33d**).* White solid (Yield 79.5%), Mp: 191–194 °C. MS (ESI$^+$) *m/z*: 1028.87 (M+H$^+$, 100%). Anal. calcd for $C_{68}H_{93}N_9O_{17}S_2$: C, 59.52; H, 6.78; N, 9.19; found: C, 59.68; H, 6.77; N, 9.16.

4.2. Microbiological study

In vitro antifungal activity was measured according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. The minimum inhibitory concentration (MIC) determination was performed by means of the serial dilution method in 96-well microtest plates with RPMI 1640 (Sigma) buffered with 0.165 M MOPS (Sigma) as the test medium. Caspofungin was used as the reference drug. Tested fungal strains were obtained from the ATCC or were clinical isolates. The MIC value was defined as the lowest concentration of test compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared to the the fungi growth of the control. Moreover, the experiment for each tested compound was carried out in parallel for two or three times. The MIC value was determined only if it could be accurately repeated. Tested compounds were dissolved in DMSO serially diluted in growth medium. The yeasts were incubated at 35 °C, and the growth MIC was determined at 24 h for *Candida* species, at 72 h for *Cryptococcus neoformans*, and at 7 days for filamentous fungi.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No. 30772674, No. 81172950 and No. 30930107), 863 Hi-Tech Program of China (Grant No. 2012AA020302), Shanghai Municipal Health Bureau (Grant No. XYQ2011038) and Key Laboratory of Drug Research for Special Environments, PLA.

Supplementary data

The spectral data of the intermediates are available. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2012.02.015.

References and notes

1. Caston-Osorio, J. J.; Rivero, A.; Torre-Cisneros, J. *Int. J. Antimicrob. Agents* **2008**, *32*, S103.
2. Cuenca-Estrella, M.; Bernal-Martinez, L.; Buitrago, M. J.; Castelli, M. V.; Gomez-Lopez, A.; Zaragoza, O.; Rodriguez-Tudela, J. L. *Int. J. Antimicrob. Agents* **2008**, *32*, S143.
3. Patterson, T. F. *Lancet* **2005**, *366*, 1013.
4. Latge, J. P. *Clin. Microbiol. Rev.* **1999**, *12*, 310.
5. Steenbergen, J. N.; Casadevall, A. *J. Clin. Microbiol.* **2000**, *38*, 1974.
6. Sheng, C.; Zhang, W. *Curr. Med. Chem.* **2011**, *18*, 733.
7. Sheehan, D. J.; Hitchcock, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999**, *12*, 40.
8. Holleman, S.; Dy, F.; Mirels, L. F.; Stevens, D. A.; White, T. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 1704.
9. Casalinuovo, I. A.; Di Francesco, P.; Garaci, E. *Eur. Rev. Med. Pharmacol. Sci.* **2004**, *8*, 69.
10. Laniado-Laborin, R.; Cabrales-Vargas, M. N. *Rev. Iberoam. Micol.* **2009**, *26*, 223.
11. Fanos, V.; Cataldi, L. *J. Chemother.* **2000**, *12*, 463.
12. Ostrosky-Zeichner, L.; Marr, K. A.; Rex, J. H.; Cohen, S. H. *Clin. Infect. Dis.* **2003**, *37*, 415.
13. Denning, D. W. *J. Antimicrob. Chemother.* **2002**, *49*, 889.
14. Benz, F.; Knusel, F.; Nuesch, J.; Treichler, H.; Voser, W.; Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459.
15. Hope, W. W.; Shoham, S.; Walsh, T. J. *Expert Opin. Drug Metab. Toxicol.* **2007**, *3*, 263.
16. Keating, G. M.; Figgitt, D. P. *Drugs* **2003**, *63*, 2235–2263.
17. Morrison, V. A. *Curr. Opin. Invest. Drugs* **2002**, *3*, 1432.
18. Temesgen, Z.; Barreto, J.; Vento, S. *Drugs Today (Barc)* **2009**, *45*, 469.
19. de la Torre, P.; Meyer, D. K.; Reboli, A. C. *Future Microbiol.* **2008**, *3*, 593.
20. Schmatz, D. M.; Abruzzo, G.; Powles, M. A.; McFadden, D. C.; Balkovec, J. M.; Black, R. M.; Nollstadt, K.; Bartizal, K. *J. Antibiot.* **1992**, *45*, 1886.
21. Warn, P. A.; Sharp, A.; Morrissey, G.; Denning, D. W. *Int. J. Antimicrob. Agents* **2010**, *35*, 146.
22. Denning, D. W. *Lancet* **2003**, *362*, 1142.
23. Wagner, C.; Graninger, W.; Presterl, E.; Joukhadar, C. *Pharmacology* **2006**, *78*, 161.
24. Zaas, A. K.; Alexander, B. D. *Expert Opin. Pharmacother.* **2005**, *6*, 1657.
25. Sucher, A. J.; Chahine, E. B.; Balcer, H. E. *Ann. Pharmacother.* **2009**, *43*, 1647.
26. Debono, M.; Abbott, B. J.; Fukuda, D. S.; Barnhart, M.; Willard, K. E.; Molloy, R. M.; Michel, K. H.; Turner, J. R.; Butler, T. F.; Hunt, A. H. *J. Antibiot.* **1989**, *42*, 389.
27. Debono, M.; Turner, W. W.; LaGrandeur, L.; Burkhardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zeckner, D. J.; Gordee, R. S. *J. Med. Chem.* **1995**, *38*, 3271.
28. Lal, B.; Gund, V. G.; Bhise, N. B.; Gangopadhyay, A. K. *Bioorg. Med. Chem.* **2004**, *12*, 1751.
29. Lal, B.; Gund, V. G.; Gangopadhyay, A. K.; Nadkarni, S. R.; Dikshit, V.; Chatterjee, D. K.; Shirvaikar, R. *Bioorg. Med. Chem.* **2003**, *11*, 5189.
30. Bouffard, F. A.; Zambias, R. A.; Dropinski, J. F.; Balkovec, J. M.; Hammond, M. L.; Abruzzo, G. K.; Bartizal, K. F.; Marrinan, J. A.; Kurtz, M. B.; McFadden, D. C.; Nollstadt, K. H.; Powles, M. A.; Schmatz, D. M. *J. Med. Chem.* **1994**, *37*, 222.
31. Black, R. M.; Balkovec, J. M.; Nollstadt, K. M.; Dreikorn, S.; Bartizal, K. F.; Abruzzo, G. K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2879.
32. Fujie, A.; Iwamoto, T.; Sato, B.; Muramatsu, H.; Kasahara, C.; Furuta, T.; Hori, Y.; Hino, M.; Hashimoto, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 399.
33. Masubuchi, K.; Okada, T.; Kohchi, M.; Murata, T.; Tsukazaki, M.; Kondoh, O.; Yamazaki, T.; Satoh, Y.; Ono, Y.; Tsukaguchi, T.; Kobayashi, K.; Ono, N.; Inoue, T.; Horii, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1273.
34. Masubuchi, K.; Okada, T.; Kohchi, M.; Sakaitani, M.; Mizuguchi, E.; Shirai, H.; Aoki, M.; Watanabe, T.; Kondoh, O.; Yamazaki, T.; Satoh, Y.; Kobayashi, K.; Inoue, T.; Horii, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 395.
35. Klein, L. L.; Li, L.; Chen, H. J.; Curty, C. B.; DeGoey, D. A.; Grampovnik, D. J.; Leone, C. L.; Thomas, S. A.; Yeung, C. M.; Funk, K. W.; Kishore, V.; Lundell, E. O.; Wodka, D.; Meulbroek, J. A.; Alder, J. D.; Nilius, A. M.; Lartey, P. A.; Plattner, J. J. *Bioorg. Med. Chem.* **2000**, *8*, 1677.
36. Wang, W.; Li, Q.; Hasvold, L.; Steiner, B.; Dickman, D. A.; Ding, H.; Clairborne, A.; Chen, H. J.; Frost, D.; Goldman, R. C.; Marsh, K.; Hui, Y. H.; Cox, B.; Nilius, A.; Balli, D.; Lartey, P.; Plattner, J. J.; Bennani, Y. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 489.
37. Zambias, R. A.; Hammond, M. L.; Heck, J. V.; Bartizal, K.; Trainor, C.; Abruzzo, G.; Schmatz, D. M.; Nollstadt, K. M. *J. Med. Chem.* **1992**, *35*, 2843.
38. Suzuki, A.; Miyaura, N.; Yanagi, T. *Synth. Commun.* **1981**, *11*, 513.
39. See Supplementary data.