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# Four New Cycloheximide Derivatives from *Streptomyces* sp. h-119

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**Abstract:** Four novel cycloheximide derivatives(1-4) and two known compounds—*l*-cycloheximide(5), and isocycloheximide(6) were obtained from marine-derived *Streptomyces* sp. h-119, which was isolated from the sediment samples collected in intertidal zone, Zhangzhou, Fujian Province. Their structures were elucidated by spectroscopic analyses, including 1D and 2D-NMR experiments, and by HR-Q-TOF mass spectrometry. Their antimicrobial activities were evaluated.

**Keywords:** Cycloheximide derivatives; *streptomyces* sp. h-119; spectroscopic analyses. 

## 1. Introduction

Currently, most antibiotics are from the secondary metabolites of actinomycetes, such as streptomycin[1], ansamitocins[2], rifamycin[3], mitomycin C[4], vancomycin[5], tetracycline[6]. Currently, antibiotics abuse has led to more and more obvious resistance of pathogens to antibiotics and significant decrease in efficacy of existing antibiotics. It is essential to focus research efforts on discovering new compounds with structural diversities and new activities. In our on-going course of looking for new antibiotics from microorganisms, a strain *Streptomyces* sp. was isolated from sediment samples of intertidal zone, Zhangzhou, Fujian Province, P. R. China. Study on the chemical constituents of the EtOAc extract yielded four new cycloheximide derivatives(**1**-**4**), along with two known compounds, *l*-cycloheximide(**5**)[7-8], and isocycloheximide(**6**)[9-10] (Figure 1). In this paper, the isolation, structure elucidation, and antimicrobial activities of four new cycloheximide derivatives were reported.

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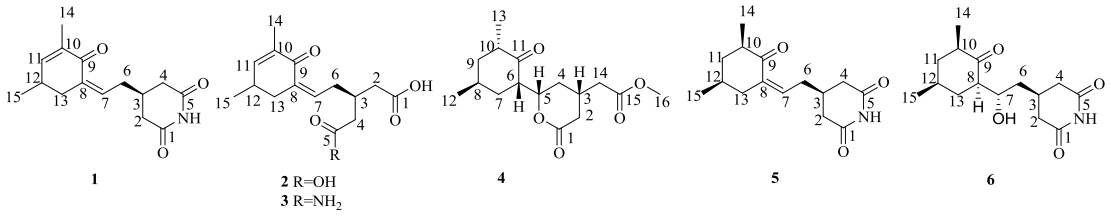



**Figure 1.** Structures of compounds **1-6**

## 2. Materials and Methods

### *2.1. Microorganism Material*

The actinomycete was isolated from the sediment samples collected in intertidal zone, Zhangzhou, Fujian Province, P. R. China. Both a traditional morphological assessment and 16S rDNA sequence analysis were performed to characterize it as *Streptomyces* sp.

### *2.2 Fermentation and Isolation*

A small spoon of spores growing on PDA' or reformed-$G_1$' agar slant was inoculated into a 250 mL Erlenmeyer flasks containing 50 mL of PDA or reformed-$G_1$ medium respectively. The flasks were incubated on a rotary shaker for 5 d at 28$^o$C with shaking at 220 rpm. Then, the cultures were used to inoculate 1000 plates of PDA and 150 slavers of reformed-$G_1$, and these were incubated for 18 days at 28$^o$C.

The culture media was cut into pieces and then extracted with EtOAc (4×). The ethyl acetate layer was concentrated in vacuum at 45$^o$C yielded brown oily residue (21.9 g/PDA, 3.9 g/ reformed-$G_1$).

Isolation:

a) For the fermentation with PDA medium , the extract (21.9 g) was separated into ten fractions (Fr. 1-10) by column chromatography (RP-18, 180 g), eluted with MeOH/$H_2O$ (0:100, 30:70, 50:50, 70:30, and 100:0). These fractions were further purified by repeated column chromatography over *Sephadex* LH-20, RP-18 silica gel and silica gel.

Fr. 4 (790.0 mg) was seperated by CC (RP-18, 80 g, MeOH/$H_2O$ 20:80, 30:70, 40:60) to give three fractions(Fr. 4A-4C). Fr. 4C was subjected to CC ($SiO_2$, PE/EtOAc 3:1) and then purified by preparative TLC (silica 60 F254, 0.25 mm) using $CHCl_3$/MeOH(10:1) as the developing solvent to give compound **1** (18.0 mg).

Fr. 8(11.8 g) was subjected to CC (RP-18, 180 g, MeOH/$H_2O$ 30:70, 40:60, 50:50) to afford Fr. 8A(9.1 g ), part of which was then purified by passage over CC (*Sephadex* LH-20, MeOH), repeated CC (*Sephadex* LH-20, Acetone), and CC ($SiO_2$, PE/acetone 6:1) to afford compound **6** (27.1 mg).

Fr. 10 (789.0 mg) was subjected to CC (*Sephadex* LH-20, MeOH) and followed by CC (PE/EtOAc 4:1) to yield compound **5** (5.6 mg).

b) For the fermentation with reformed-$G_1$ medium, the extract (3.9 g) was separated into twelve fractions (Fr. 1-12) by CC (RP-18, 80 g), eluted with MeOH/$H_2O$ (0:100, 30:70, 50:50, 70:30, and 100:0). These fractions were further purified by repeated CC on *Sephadex* LH-20, RP-18 silica gel and silica gel.

Fr. 5 (974.0 mg) was purified by CC (*Sephadex* LH-20, MeOH) to give five fractions (Fr. 5A-5E). Fr. 5C was subjected to CC (*Sephadex* LH-20, acetone), CC (RP-18, 80 g MeOH/$H_2O$ 50:50) and CC($SiO_2$, PE/acetone 5:1) to afford compound **2** (8.0 mg).

Fr. 6 (878.0 mg) was subjected to CC (*Sephadex* LH-20, MeOH) and CC (*Sephadex* LH-20, acetone) gradually, affording two subfractions (Fr. 6A-6B). Both of them were purified by CC on silica gel (Fr. 6A: PE/acetone 4:1; Fr. 6B: PE/acetone 3:1) to give compound **4**(58.6 mg) and **3**(31.7 mg) respectively.

Compound **1**: white amorphous powder; $[\alpha]_{20\ D}$+27.1°(c 1.0, MeOH); UV(MeOH) $\lambda_{max}$(log ε) 201(3.47), 203(3.44), 204(3.43), 206(3.34), 207(3.30), 208(3.23), 212(2.82); IR(KBr)$\nu_{max}$ 3225, 2957, 2361, 2344, 1700, 1699, 1618, 1362, 1260, 1150 $cm^{-1}$. $^1H$ and $^{13}C$-NMR: Table 1. HR-Q-TOF-MS: 284.1257([M + Na]$^+$, $C_{15}H_{19}NNaO_3^+$.

Compound **2**: colorless oil; $[\alpha]_D^{20}$ -2.5°(c 1.0, MeOH); UV(MeOH) $\lambda_{max}$(log ε) 201(3.27), 202(3.20), 203(3.42), 204(3.27), 254(0.83), 262(0.83); IR(KBr)$\nu_{max}$ 2959, 2361, 2344, 1708, 1669, 1611, 1385, 1193 $cm^{-1}$. $^1H$ and $^{13}C$-NMR: Table 1. HR-Q-TOF-MS: 303.1203 ([M + Na]$^+$, $C_{15}H_{20}NaO_5^+$.

Compound **3**: colorless oil; $[\alpha]_D^{20}$ -97.7°(c 1.0, MeOH); UV(MeOH) $\lambda_{max}$(log ε) 201(3.13), 202(3.03), 203(3.12), 204(3.04), 206(2.93); IR(KBr)$\nu_{max}$ 3343, 3196, 2958, 2927, 1710, 1666, 1615, 1409, 1365, 1255, 1193$cm^{-1}$. $^1H$ and $^{13}C$-NMR: Table 1. HR-Q-TOF-MS: 302.1363 ([M + Na]$^+$, $C_{15}H_{21}NNaO_4^+$.

Compound **4**: colorless oil; $[\alpha]_D^{20}$ -162.4°(c 1.0, MeOH); UV(MeOH) $\lambda_{max}$(log ε) 201(3.03), 203(2.99), 204(2.91); IR(KBr)$\nu_{max}$ 2928, 1736, 1710, 1440, 1383, 1245, 1166 $cm^{-1}$. $^1H$ and $^{13}C$-NMR: Table 2. HR-Q-TOF-MS: 319.1516 ([M + Na]$^+$, $C_{16}H_{24}NaO_5^+$.

## 3. Results and Discussion

### *3.1. Structure elucidation*

Compound **1** was obtained as white amorphous powder. The HR-Q-TOF-MS showed the quasi-molecular-ion peak ([M + Na]$^+$) at *m/z* 284.1257, establishing the molecular formula $C_{15}H_{19}NO_3$. The IR absorption at 1699 $cm^{-1}$ and 1700 $cm^{-1}$ indicated the presence of carbodiimide and carbonyl groups. The $^{13}C$-NMR(DEPT) spectra of **1**(Table 1) displayed signals for five quaternary C-atoms (three CO, and two olefinic), and four CH(two olefinic), four $CH_2$, and two Me groups. Analysis of the HMBC spectra, we observed the correlations of the H-atoms of Me(14) with C(9), C(10), C(11), Me(15) with C(11), C(12), C(13), and $CH_2$(13) with C(7), C(8), C(9). In combination with the $^1H$,$^1H$-COSY H-C(11)↔H-C(12)↔H-C(13), Fragment **1a** (Figure 2) was established. Meanwhile, the HMBCs from CH(3) to C(2), C(4), and those of NH to C(1), C(2), C(4), C(5) indicated the presence of 3-glutarimidyl moiety characteristic of the cycloheximide(Fragment **1b** of Figure 2). Finally, the HMBC correlations of the $CH_2$ (6) to C(3) and C(7) indicated that Fragment **1a** and Fragment **1b** were connected via C(6). From the NOE spectra, the correlation H(7)↔H (13) revealed that the double bond of compound **1** was Z configuration (Figure 3). From the point of biosynthesis[10], the absolute configuration of C(3) was assigned. Therefore, compound **1** was determined to be (Z)-4-(2-(3,5-dimethyl–2-oxocyclohex-3-enylidene) ethyl) piperidine-2, 6-dione.

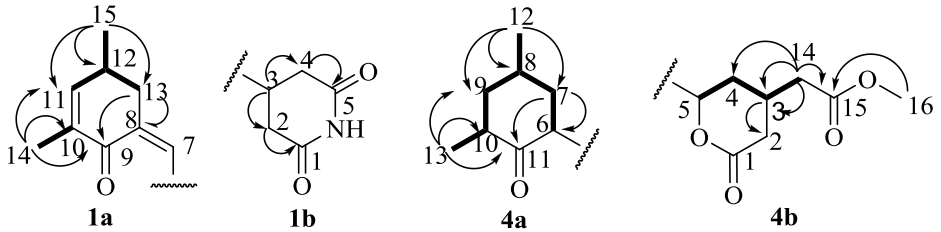
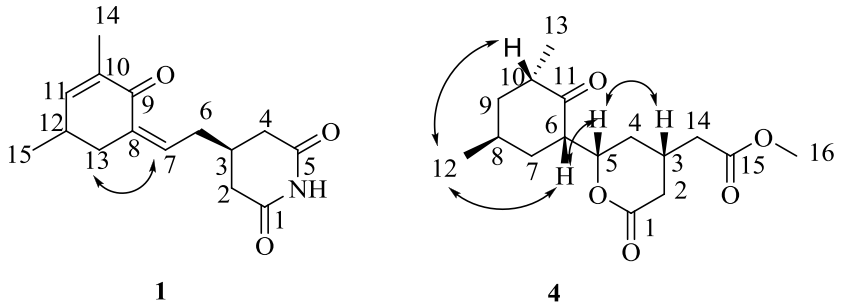



**Figure 2.** The structure of fragments **1a** and **1b** of compound **1**, fragments **4a** and **4b** of compound **4**, and selected HMBCs(H-C) and $^1H$,$^1H$-COSY correlation(bold line)



**1** **4**

**Figure 3**. Selected NOE correlations for compounds **1** and **4**(H↔H)

Compound **2** was obtained as colorless oil. The molecular formula was determined as $C_{15}H_{20}O_5$ by its HR-Q-TOF-MS and NMR data.The IR absorption at 1708cm$^{-1}$ and 1669 cm$^{-1}$ indicated the presence of carboxyl and carbonyl groups. The $^{13}$C-NMR (DEPT) spectra of **2**(Table 1) displayed signals for five quaternary C-atoms (two olefinic and three CO), and four CH (two olefinic), four $CH_2$, and two Me groups. The structure of cyclohexenone, a $C_8$ moiety composed of 2 × Me, 1 × $CH_2$, 2 × CH, and three quaternary C-at at atoms, was determined on the basis of the HMBCs from the H-atoms of Me(14) to C(9), C(10), C(11), Me(15) to C(11), C(12), C(13), $CH_2$(13) to C(8), C(9) , and CH(7) to C(8), C(9), C(13), along with the $^1$H,$^1$H-COSY correlations H-C(11)↔H-C(12)↔H-C(13). Further more, the HMBCs from $CH_2$(6) to C(2), C(3),and from $CH_2$(2) to C(1), C(4), in combination with the $^1$H,$^1$H-COSY correlation H(1)↔H-C(2)↔H-C(3)↔H-C(4), led to the establishment of the side chain(Figure 2). The $^1$H, $^1$H-COSY correlation from H(6) to H(7) revealed that the side chain was attached to olefinic carbon C(7).Analysis the $^1$H, $^{13}$C-NMR data of **1** and **2**(Table 1), we found that **2** was the hydrolyzate of **1**. Therefore, compound **2** was determined to be (Z)-3-(2-(3,5-dimethyl-2-oxocyclohex-3-enylidene) ethyl) pentanedioic acid.

**Table 1.** $^1$H and $^{13}$C-NMR data of **1**, **2** and **3** (at 600 MHZ in $CDCl_3$(compound **1** and **3**), and Acetone(compound **2**) $\delta$ in ppm, $J$ in Hz)

| Position(H) | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | / | 172.0s | / | 172.9s | / | 176.1s |
| 2 | 2.35(m) | 37.4t | 2.46(m) | 37.1t | 2.43(m) | 38.4t |
| 3 | 2.36(m) | 30.3d | 2.50(m) | 32.0d | 2.54(m) | 32.8d |
| 4 | 2.73(m) | 37.3t | 2.43(m) | 37.0t | 2.35(m) | 39.7t |
| 5 | / | 172.0s | / | 172.9s | / | 176.1s |
| 6 | 2.29(m) | 32.5t | 2.39(m) | 31.1t | 2.33(m) | 32.3t |
| 7 | 6.58(t, 7.5) | 131.8d | 6.61(t, 7.9) | 133.6d | 6.64(br s) | 134.3d |
| 8 | / | 136.9s | / | 136.1s | / | 136.1s |
| 9 | / | 188.5s | / | 187.4s | / | 189.3s |
| 10 | / | 135.5s | / | 134.8s | / | 135.2s |
| 11 | 6.66(br s) | 151.0d | 6.73(br s) | 150.4d | 6.67(br s) | 151.6d |
| 12 | 2.57(br s) | 30.8d | 2.59(m) | 30.7d | 2.57(m) | 30.8d |
| 13 | 2.77(m) | 33.9t | 2.90(dd,14.3,5.7) | 33.5t | 2.83(dd,14.5,5.0) | 33.8t |
| | 2.25(dd,14.4,8.0) | | 2.29(dd,14.4,8.2) | | 2.25(dd,14.8,7.4) | |
| 14 | 1.77(brs) | 16.4q | 1.78(br s) | 15.7q | 1.83(s) | 16.4q |
| 15 | 1.20(d, 7.2) | 20.9q | 1.14(d, 7.0) | 20.3q | 1.15(d, 7.8) | 20.9q |

Compound **3** was obtained as colorless oil and determined to have the molecular $C_{15}H_{21}NO_4$ by HR-Q-TOF-MS and NMR data. The IR spectrum exihibited the absorption at 1709 cm$^{-1}$, 1665 cm$^{-1}$ and 1614 cm$^{-1}$for carboxyl, carbonyl and acylamino groups. The $^1$H and $^{13}$C-NMR data of **3**(Table 1) were similar to those of **2**. The difference was the molecular formula that the N-atom was replaced by O-atom. Therefore, Compound **3** was detdemined to be (3R)-5-amino-3-((Z)-2-(3, 5-dimethyl-2-oxocyclohex-3-enylidene) ethyl)-5- oxopentanoic acid.

Compound **4** was obtained as white amorphous powder. The HR-Q-TOF-MS showed the quasi-molecular-ion peak ($[M + Na]^+$) at *m/z* 319.1516, which was consistent with the molecular formula $C_{16}H_{24}O_5$.The IR absorption at 1736 $cm^{-1}$ and 1710 $cm^{-1}$ indicated the presence of ester groups. The $^{13}$C-NMR(DEPT) spectra of **4**(Table 2) displayed signals for three quaternary C-atoms (three CO), and five CH(one O-bearing), five $CH_2$, and three Me groups(one O-bearing). Comparison the $^1$H-NMR and $^{13}$C-NMR data of **4** with those of SPRI-70014[11] in literature revealed that **4** had the same ring C-atom skeleton. The HMBCs from the H-atoms of Me(12) to C(7), C(8), C(9), Me(13) to C(9), C(10), C(11), and $CH_2$(7) to C(6), C(11), along with the $^1$H,$^1$H-COSY correlations H-C(6)↔H-C(7)↔H-C(8)↔H-C(9)↔H-C(10) established the structure of fragment **4a** (Figure 2). Additionally, the HMBCs from O-bearing Me(16) to C(15) indicated that the Me group(16) was attached to carbon C(15). What's more, the HMBCs from $CH_2$(14) to C(2), C(3), C(4), C(15), CH(5) to C(3), C(4), and from $CH_2$(2) to C(1), C(3),C(4), C(14) led to the establishment of fragment **4b**(Figure 2). Finally, the $^1$H,$^1$H-COSY correlations H-C(5)↔H-C(6) connected the fragment **4a** and **4b**. The relative configuration of **4** was assigned on the basis of NOE spectrum(Figure 2). The presence of NOE correlations H(3)↔H-C(5)↔H-C(6)↔Ha-C(7)↔H-C(10)↔Me(12) indicated that compound **4** had the same relative configuration as that of SPRI-70014. Indeed, compound **4** was the C(16) carboxylic derivative of SPRI-70014. Thus, from the above data, the structure of compound **4** was established to be methyl 2-((2S,4R)-2-((1R,3R,5R)-3,5-dimethyl-2-oxocyclohexyl)-6-oxotetrahydro-2H-pyran-4-yl)acetate.

**Table 2.** $^1$H and $^{13}$C-NMR data of **4** (at 600 MHZ in Acetone, $\delta$ in ppm, *J* in Hz)

| **Position(H)** | $\delta_H$ | $\delta_C$ | **Position(H)** | $\delta_H$ | $\delta_C$ |
|---|---|---|---|---|---|
| 1 | / | 170.5s | 9 | 1.89(m)<br>1.63(td, 13.1, 4.2) | 42.8t |
| 2 | 2.78(dd, 17.7, 6.4)<br>2.22(m) | 35.8t | 10 | 2.60(m) | 40.7d |
| 3 | 2.49(m) | 28.3d | 11 | / | 211.8s |
| 4 | 2.22(m)<br>1.32(dd, 24.6, 12.1) | 32.7t | 12 | 1.24(d, 7.3, 3H) | 18.1q |
| 5 | 4.58(ddd, 11.6, 6.8, 2.3) | 78.4d | 13 | 0.98(d, 6.4, 3H) | 14.2q |
| 6 | 2.73(m) | 49.8d | 14 | 2.34(t, 7.3) | 40.2t |
| 7 | 2.19(m)<br>1.78(td, 13.5, 4.9) | 35.9t | 15 | / | 171.6s |
| 8 | 2.19(m) | 26.8d | 16-OMe | 3.68(d, 1.4, 3H) | 51.8q |

The known compounds *l*- cycloheximide(**5**)[7-8] and isocycloheximide(**6**)[9-10] were identified through direct comparison with published data.

### *3.2 Cytotoxicity and Antimicrobial activity*

Compound **6** showed a moderate antifungal activity to *Colletotrichum gleosporioides* Penz,(inhibition zone at a concentration 20 μg/6 mm disk: 10 mm). Compounds **1**-**5** had no effects on the tested microbes.

## Acknowledgments

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## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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