SUPPLEMENTARY MATERIAL

Secondary metabolites from marine-derived *Streptomyce antibioticus* strain H74-21

Shuna Fu\(^a\), Fan Wang\(^a\), Hongyu Li\(^a\), Yixuan Bao\(^a\), Yu Yang\(^a\), Huifang Shen\(^b\), Birun Lin\(^b\)* and Guangxiong Zhou\(^a\)*

\(^a\)Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China;
\(^b\)Key Laboratory of New Technique for Plant Protection in Guangdong, Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou 510632, China

A new secondary metabolite, (2\(S\),3\(R\))-L-Threonine, \(N\)-[3-(formylamino)-2-hydroxybenzoyl]-ethyl ester (streptomyceamide C, 1), together with four known compounds 1, 4-dimethyl-3-isopropyl-2,5-piperidinedione (2), cyclo-((\(S\)))-Pro-8-hydroxy-(\(R\))-Ile (3), cyclo-((\(S\)))-Pro-(\(R\))-Leu (4), and seco-((\(S\)))-Pro-(\(R\))-Val (5), were isolated from the EtOH extract of the fermented mycelium of the marine-derived *streptomycete* strain H74-21, which was isolated from sea sediment in a mangrove district. The structure of the new compound was established on the basis of its spectroscopic data, including 1D NMR and 2D NMR, HR-TOF-MS. Their antifungal activities against *Candida albicans* and cytotoxicities against human breast adenocarcinoma cell line MCF-7, human glioblastoma cell line SF-268 and human lung cancer cell line NCI-H460 were tested. Compounds 1 only displayed cytotoxicity against human breast adenocarcinoma cell line MCF-7 with the IC\(_{50}\) value of 27.0 \(\mu\)g/mL. However, compounds 1-5 do not show antifungal activities at the test concentration of 1 mg/mL, and 2-5 have no cytotoxicities at the test concentration of 50\(\mu\)g/mL.

Keywords: *Streptomyce*; secondary metabolites; dipeptide; streptomyceamide C;
piperidinedione

**Compound 1**

![Figure S1. The IR spectrum of Compound 1](image1)

![Figure S2. HR-MS of compound 1](image2)
Figure S3. The $^1$H spectrum of compound 1 (300 MHz in CD$_3$OD)

Figure S4. The $^{13}$C spectrum of compound 1 (75 MHz in CD$_3$OD)
Figure S5. The DEPT spectrum of compound 1 (in CD$_3$OD)

Figure S6. The $^1$H-$^1$H COSY spectrum of compound 1 (in CD$_3$OD)
Figure S7. The HMQC spectrum of compound 1 (in CD$_3$OD)

Figure S8. The HMBC spectrum of compound 1 (in CD$_3$OD)
Figure S9. The NOE spectrum of compound 1 (300 MHz in CD$_3$OD)

Figure S10. The $^1$H spectrum of compound 1 (600 MHz in DMSO-$d_6$)
Figure S11. The NOE spectrum of compound 1 (600 MHz in DMSO-\textit{d}_6)

Figure S12. The rational or alternative configuration and their favorite conformers with the lowest energy of compound 1 in ChemDraw. a: A rational configuration for 1, in its favorite conformer, dihedral angle between H(8)-C(8)-C(13) and H(13)-C(13)-C(8) is about 120° (Karplus equation); b: An alternative and less possible configuration for 1, in its favorite conformer, dihedral angle between H(8)-C(8)-C(13) and H(13)-C(13)-C(8) is about 180°.
Figure S13. The observed NOE correlations of compound 1

Figure S14. The $^1$H-$^1$H COSY and key HMBC correlations of 1 and 2

Table S1. $^1$H (300 MHz) and $^{13}$C (75 MHz) NMR Data of 1 and 2 (δ in ppm J in Hz)

| Position | 1 (in CD$_3$OD) | 2 (in CDCl$_3$) |
|----------|-----------------|-----------------|
|          | δ (H)           | δ (C)           | δ (H)           | δ (C)           |
| 1        | 115.7           |                 |                 |                 |
| 2        | 152.6           |                 |                 |                 |
| 3        | 128.2           | 3.83 (1H, d, J = 4.3) | 69.4            |
| 4        | 8.30 (1H, d, J = 7.9) | 126.4          |                 |                 |
| 5        | 6.91 (1H, t, J = 7.9) | 119.6          | 166.8           |                 |
| 6        | 7.64 (1H, d, J = 7.9) | 123.5          | 4.18 (1H, d, J = 18.0) | 52.7            |
|          |                 | 3.90 (1H, d, J = 18.0) |                 |                 |
| 7        | 171.9           | 2.95 (3H, s)    | 33.7            |                 |
| 8        | 4.71 (1H, d, J = 6.9) | 59.7           | 2.30 (1H, m)    | 33.9            |
| 9        | 172.1           | 0.94 (3H, d, J = 6.9) | 18.2            |                 |
| 10       | 4.21 (2H, q, J = 7.1) | 62.8           | 1.06 (3H, d, J = 7.0) | 19.9            |
| 11       | 1.29 (3H, t, J = 7.1) | 14.6           | 3.00 (3H, s)    | 34.4            |
|    | Chemical Shift (ppm) |Multiplicity |   |  |  |
|----|----------------------|--------------|----|---|---|
| 12 | 8.37 (1H, s)         |              |   | 162.3 |   |
| 13 | 4.41 (1H, m)         |              |   | 68.6  |   |
| 14 | 1.25 (3H, d, J = 7.3)|              |   | 20.7  |   |

**Compound 2**

![IR Spectrum](image1)

*Figure S15. The IR spectrum of compound 2*

![HR-MS Spectrum](image2)

*Figure S16. The HR-MS spectrum of compound 2*
Figure S17. The $^1$H spectrum of compound 2 (300 MHz in CDCl$_3$)

Figure S18. The $^{13}$C spectrum of compound 2 (75 MHz in CDCl$_3$)
Figure S19. The DEPT spectrum of compound 2 (in CDCl₃)

Figure S20. The ¹H-¹H COSY spectrum of compound 2 (in CDCl₃)
Figure S21. The HMQC spectrum of compound 2 (in CDCl₃)

Figure S22. The HMBC spectrum of compound 2 (in CDCl₃)
Figure S23. The NOE spectrum of compound 2 (in CDCl₃)
Table S2. $^1$H (300 MHz) and $^{13}$C (75 MHz) NMR data of 3-5 ($\delta$ in ppm $J$ in Hz)

| Position | 3 (in CD$_3$OD) | 4 (in CD$_3$OD) | 5 (in CD$_3$OD) |
|----------|----------------|----------------|----------------|
|          | $\delta$ (H)   | $\delta$ (C)   | $\delta$ (H)   | $\delta$ (C)   | $\delta$ (H)   | $\delta$ (C)   |
| 1        | 172.6          | 172.9          | 172.8          |
| 2        |                |                |                |
| 3        | 3.56-3.52 (1H, m) | 3.54-3.51 (2H, m) | 3.59-3.55 (1H, m) | 46.3          | 46.6          | 46.3          |
|          | 3.49-3.46 (1H, m) |                |                |                | 3.54-3.50      |
| 4        | 2.03-1.98 (1H, m) | 2.06-1.99 (1H, m) | 2.05-2.01 (1H, m) | 23.4          | 23.8          | 23.4          |
|          | 1.96-1.88 (1H, m) |                |                |                | 1.98-1.94      |
| 5        | 2.33-2.28 (1H, m) | 2.35-2.31 (1H, m) | 2.35-2.33 (1H, m) | 29.7          | 29.2          | 29.7          |
|          | 1.98-1.90 (1H, m) |                |                |                | 1.98-1.94      |
| 6        | 4.06 (1H, br. s) | 61.5           | 4.15 (1H, br. s) | 60.4          | 4.05 (1H, br. s) | 61.7          |
| 7        | 167.8          | 169.1          | 167.7          |
| 8        |                |                |                |
| 9        | 4.48 (1H, t, $J = 6.9$) | 4.29 (1H, t, $J = 7.1$) | 54.8          | 4.23 (1H, t, $J = 7.1$) | 60.2          |
| 10       | 2.18-2.12 (1H, m) | 1.99-1.94 (1H, m) | 39.5          | 2.50 (1H, dq, $J = 7.3$) | 30            |
|          | 1.57-1.53 (1H, m) |                |                |                |                |
| 11       | 1.47-1.41 (1H, m) | 37.2           | 2.06-2.01 (1H, m) | 25.9          | 1.11 (3H, d, $J = 7.3$) | 19            |
|          | 1.35-1.29 (1H, m) |                |                |                |                |
| 12       | 0.95 (3H, t, $J = 6.9$) | 1.01 (3H, d, $J = 6.5$) | 23.5          | 0.95 (3H, d, $J = 6.9$) | 16.8          |
| 13       | 1.07 (3H, d, $J = 6.9$) | 0.97 (3H, d, $J = 6.5$) | 22.3          |                |                |
Table S3. Antifungal activities of isolated compounds 1–5 (MIC: µg/mL)

| Compounds | 1    | 2    | 3    | 4    | 5    | Antimycin A<sub>2α</sub> | Antimycin A<sub>7α</sub> |
|-----------|------|------|------|------|------|--------------------------|--------------------------|
| MIC       | >1000| >1000| >1000| >1000| >1000| 31.25                    | 31.25                    |

Table S4. Cytotoxic activities of isolated compounds 1–5 (IC<sub>50</sub>: µg/mL)

| Compounds | MCF-7 | SF-268 | NCI-H468 |
|-----------|-------|--------|----------|
| 1         | 27    | >50    | >50      |
| 2         | >50   | >50    | >50      |
| 3         | >50   | >50    | >50      |
| 4         | >50   | >50    | >50      |
| 5         | >50   | >50    | >50      |

Positive control

4 41 25.1

Figure S24. 16S rDNA of H74-21

ATCTGCCCTGCACTCTGGGACACAGCCCTGGAAACGGGGTCTAATACCCGATATCACTC
TTGCAGGCATCTGTGAGGTTGCAAGCTCCCGCGGTGTCAGGATGACGCCTGGCCCTTA
TCAGCTTGTGTTGAGGTAAATTGCTCAACCAGGGCAGACGGGTTAGCCCGGCTAGA
GGGCGACCGCCACACTGGGACTGAGACACCGGCCACTCTAGCGGGAGCACAGA
GTGGGGAATATTGCAACAATGGGGGAAAGCCTGTAGTCAGGCAGCCGCGGTGAGG
ACCGCTTCTGGGTGTTAACCCTTTACGACGGGGAGAAGCAGACTGGAATGACCTT
GCAGAAAGACGGCGCCTGCTAATACCTGAGCCCGAGCCGCTAGTACGAGGAGCACAG
AGCGTTGTCCGGAAATTAGTTGGGGTAAAGGCTCTTGAGGCGGCTTGTCGTCG
GTGAAAGCCCGCCCTTAACCCCGGGTCTGCATTCGATACGGGCTACTGAGGAGCTG
GTAGGGGAGATCAGGAAATTCCTGTTGTAGCGGGTAACGGCGAAGTCAGTACG
ACCGCTTCTGGGGATCTCTGGGGCAATTACTGACGCTGAGGGGACACGCGAAGAGCTG
GGAGCGAAGAGGATAGATACCTTGTGATTCACGCGTAAACGGTGAGGAAAGTGAGT
GGTGGCGACATTACCTGACTGCTGACGCGTAAACCGATAGGAGGAGCTG
GAGTACGCGCAGCCAGCTAAACAGGCTGAAACGGTGAGGAAAGTGAGT
GGAGCATGTTGGCTTAACTTCGAGCGCAGCGGAAGAACCTTAAACCTTACGCTG
CCGAAACCGCCAGATGGTGCAGGCCCTTGTGGTACAGGGTACGGGAATGTGC
TGTCGTCGCTGCTGCTGAGTAGTGTGGGTAAAGTCGCCACAGAGGCAAAAAATCGG
TTTGTGTTTGGCAGCATGGCTCTTCTGGGGATGTTGGGGACTGACGAGGACACCGGC
CAAGGGGACGACGTTCAAGTGTCCACTGACGAGATGTTGGGGGATGTTGGGGGATG
GCACACGTGCTACAATGGGGCGCTAACAAAGACGACGATACGGTGAGGTTGGG

GTGACGAAAGAGGATAGATACCTTGTGATTCACGCGTAAACGGTGAGGAAAGTGAGT
GGTGGCGACATTACCTGACTGCTGACGCGTAAACCGATAGGAGGAGCTG
GAGTACGCGCAGCCAGCTAAACAGGCTGAAACGGTGAGGAAAGTGAGT
GGAGCATGTTGGCTTAACTTCGAGCGCAGCGGAAGAACCTTAAACCTTACGCTG
CCGAAACCGCCAGATGGTGCAGGCCCTTGTGGTACAGGGTACGGGAATGTGC
TGTCGTCGCTGCTGCTGAGTAGTGTGGGTAAAGTCGCCACAGAGGCAAAAAATCGG
TTTGTGTTTGGCAGCATGGCTCTTCTGGGGATGTTGGGGGACTGACGAGGACACCGGC
CAAGGGGACGACGTTCAAGTGTCCACTGACGAGATGTTGGGGGATGTTGGGGGATG
GCACACGTGCTACAATGGGGCGCTAACAAAGACGACGATACGGTGAGGTTGGG


| Strain No  | Names of identified species                                      |
|-----------|------------------------------------------------------------------|
| cfcc3080  | *Streptomyces griseoruber* strain cfcc3080                       |
| NBRC 12838| *Streptomyces antibioticus* strain NBRC 12838                    |
| CSSP528   | *Streptomyces antibioticus* strain CSSP528                       |
| 1022-257  | *Streptomyces antibioticus* strain 1022-257                      |
| cfcc3075  | *Streptomyces antibioticus* strain cfcc3075                      |
| cfcc3085  | *Streptomyces antibioticus* strain cfcc3085                      |
| EAAG90    | *Streptomyces antibioticus* strain EAAG90                        |
| NBRC 12873| *Streptomyces griseoruber* strain NBRC 12873                    |
| CSSP408   | *Streptomyces griseoruber* strain CSSP408                       |
| S5        | *Actinobacterium* S5                                            |
| 15721     | *Streptomyces bungoensis* strain 15721                           |
| A316      | *Streptomyces griseoruber* strain A316                           |
| HBUM174899| *Streptomyces longwoodensis* strain HBUM174899                  |
| DSM 40089 | *Streptomyces galbus* strain DSM 40089                           |
| JM-R35    | *Streptomyces caeruleatus* strain JM-R35                         |
| JCM 3373  | *Streptomyces lasaliensis* strain JCM 3373                      |
| NBRC 12849| *Streptomyces cellostaticus* strain NBRC 12849                   |
| BCCO 10_1548| *Streptomyces curacoi* strain BCCO 10_1548                     |
| 1043      | *Streptomyces panayensis* strain 1043                            |
| NBRC 15711| *Streptomyces bungoensis* strain NBRC 15711                     |