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Terpeptin, A Novel Mammalian Cell Cycle Inhibitor, Produced by *Aspergillus terreus* 95F-1

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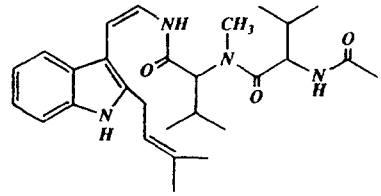
Abstract: Terpeptin, a novel peptide having cell cycle inhibitory activity, was isolated from the cultured broth of *Aspergillus terreus* 95F-1 and its structure was elucidated by spectral analyses. Terpeptin inhibited the cell cycle progression of mouse tsFT210 cells in the G2/M phase with a minimum inhibitory concentration of 62.5 μM.

The bioassay using mouse cdc2 mutant cell line, tsFT210 cell, is a useful and practical assay method for the screening of new mammalian cell cycle inhibitors of microbial origin[1]. Using this assay, we have found several new cell cycle inhibitors, tryprostatin A, B[2,3], spirotryprostatins A, B[4,5] and cyclotryprostatins A-D[6] in the fermentation broth of a fungus, *Aspergillus fumigatus* BM939.

In the course of continuous screening, we found a fungal strain 95F-1 that produced a new cell cycle inhibitor and we have now isolated a novel compound named terpeptin (**1**). Herein, the isolation, the structure elucidation and biological activity of **1** are described.



1

The producing strain 95F-1 was isolated from a soil sample collected at Naha City, Okinawa, Japan and identified as *Aspergillus terreus* 95F-1 through a taxonomic study.

The strain was cultured at 28 °C for 72 hours in a medium containing 2.5% glucose, 2% soybean, 0.5% polypeptone, 0.3% yeast extract, 1% soluble starch, 0.5% meat extract, 0.2% NaCl, 0.05% KH_2PO_4 and 0.05% $MgSO_4$ (pH 5.8).

The whole broth (7L) was extracted with 3.5 L acetone to give an aqueous acetone solution. The acetone solution was evaporated under reduced pressure to remove acetone and the obtained residue was extracted with EtOAc to afford an EtOAc extract (18.5 g). This extract was separated by successive column chromatographies on silica gel and Sephadex LH-20 (MeOH). The active fractions containing terpeptin were further purified by preparative HPLC on an ODS column with eluent CH_3CN-H_2O (75:25) to give a yellow amorphous solid of **1** (53.3 mg).

Terpeptin (**1**)[7] gave a [M+] at m/z 480 in EIMS and had the molecular formula $C_{28}H_{40}N_4O_3$ established by elemental analysis. The IR spectrum of **1** suggested the presence of amide groups (3312, 1627 cm^{-1}). The UV spectrum revealed the presence of an indole chromophore in **1** with the absorption maxima at 230 and 285 nm, like those of tryprostatins[2,3]. In the ^1H NMR spectrum[8], **1** showed signals due to a 1,2-disubstituted benzene ring (δ 7.30 d, J=8 Hz, 7-H; δ 7.24 d, J=8 Hz, 4-H; δ 7.03 td, J=8, 2 Hz, 6-H; δ 6.94 m, 5-H), an N-methyl (δ 3.06 s, 13-CH_3), three tertiary methyl groups (δ 1.65 s, 29-H_3; δ 1.68 s, 28-H_3; δ 1.79 s, 18-H_3), four secondary methyl groups (δ 0.67 d, J=6 Hz, 24-H_3; δ 0.70 d, J=6 Hz, 21-H_3; δ 0.82 d, J=7 Hz, 20-H_3; δ 0.85 d, J=6 Hz, 23-H_3), three exchangeable protons (δ 8.05 d, J=8 Hz, 16-H; δ 8.81 d, J=11 Hz, 10-H; δ 10.94 s, 1-H), two oxymethines (δ4.40 t, J=9 Hz, 15-H; δ 4.74 d, J=11 Hz, 12-H), and three olefinic protons (δ 5.31 m, 26-H; δ 5.79 d, J=7 Hz, 8-H; δ 6.76 t,J=10 Hz, 9-H) along with signals due to one methylene and two methines. The ^{13}C NMR spectrum of **1**[8], analyzed by the DEPT method, indicated the presence of three amide carbonyls (δ 167.6 s, C-11; δ 168.9 s, C-17; δ 172.6 s, C-14) and five quaternary sp^2 carbons (δ 136.6 s, C-2; δ 132.3 s, C-27; δ 126.9 s, C-3a; δ 105.1 s, C-3; δ 135.6 s, C-7a) .

Detailed analyses of ^1H-^1H COSY , HMQC and NOESY spectra of **1**, coupled with the structural information from the UV and IR spectra, led us to postulate the presence of the partial structures A-E in **1**(Fig.1). The *cis*-relation of Δ^8 olefin, in the partial structure B, for example, was established by the $J_{8,9}$ value (7 Hz) and the NOE observed between 8-H and 9-H in the NOESY spectrum.

HMBC experiment on **1** allowed the attribution of all quaternary carbons and supported these partial structures. For instance, C-2, C-3, C-3a and C-7a in the partial structure A were assigned on the basis of the HMBC correlations (5-H, 7-H/C-3a; 4-H, 6-H/C-7a; 1-H/C-2,C-3; 4-H/C-3). The assignments of the quaternary sp^2 carbons and three amide carbonyls in the partial structures C, D and E were also performed according to the long-range couplings shown by solid line arrows on those partial structures in Fig.1. Accordingly, partial structures D and E were identified as N- methyl valine and N-acetyl valine residues, respectively.

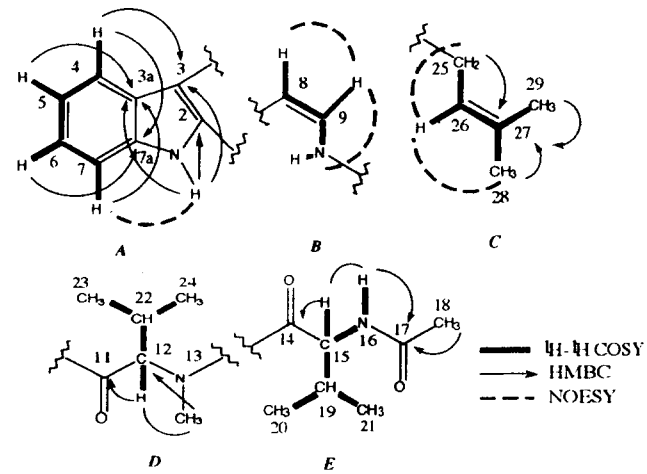


Fig.1 Partial structures for *1*

A further extensive inspection of the HMBC spectrum of **1** revealed the connections of the partial structures. Namely, the correlation between 25-H_2 and C-2 indicated that the isoprenyl moiety (partial structure C) attached to C-2 in the partial structure A. Similarly, the partial structure B was connected to partial structure A at the C-3 position according to the cross peaks between H-8 and C-2, C-3a and between H-9 and C-3 in the HMBC spectrum. Furthermore, the connectivities of the amino acid moieties, partial structures D and E, were shown by the long-range correlations; 9-H, 10-H→ C-11, 13-CH_3 → C-14 as shown in Fig. 2. This was also supported by the NOEs observed between 10-H and 12-H and between 13-CH_3 and 15-H, respectively, in the NOESY spectrum. Thus the planar structure of **1** was deduced.

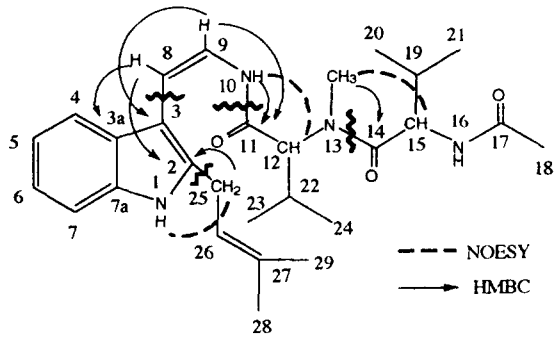


Fig.2 Planar structure of *1*

Recently, new diketopiperadines named tryprostatins[2,3] have been isolated from *Aspergillus fumigatus* as novel cell cycle inhibitors, but no peptide similar to **1** has been reported. The structure of **1** consists of an isoprenyl group, an indole moiety which was derived from a tryptophan by decarboxylation and further dehydrogenation at the C-8 and C-9 positions, and two unusual amino acids, N-methyl valine and N-acetyl valine. The compounds with an indole and amino acid moieties connecting straightly, such as in hemiasterlins[9] or chondriamides[10], are rare among natural products. It is of great biogenetic and biological interest that **1**, a novel peptide, has been isolated for the first time as a new G2/M inhibitor of the mammalian cell cycle. In the randomly cultured assay[1], terpeptin inhibited the cell cycle progression of mouse tsFT210 cells in the G2/M phase with a minimum inhibitory concentration of 62.5 μM. Detailed studies on the biological activity and stereochemistry of **1**will be reported elsewhere.

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References and notes:

1. Osada, H.; Cui, C.-B.; Onose, R.; Hanaoka, F. *Bioorg. Med. Chem.* **1996**, in press.
2. Cui, C.-B.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, 49, 527-533.
3. Cui, C.-B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, 49, 534-540.
4. Cui, C.-B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, 49, 832-835.
5. Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, 52, 12651-12666 .
6. Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, in press.
7. terpeptin: mp 92-95 °C.$[\alpha]_D$ -135.2 (C 0.1, $CHCl_3$). UVλ_{max} nm (ε): 230 (87000), 285 (6700) IR (KBr) υ_{max}cm^{-1} 3312, 2966, 2932, 1627, 1494, 1460, 1093, 744. Elemental analysis: found C:63.09 H:8.58 N:9.38, calcd. C:62.92 H:8.61 N:10.49 ($C_{28}H_{40}N_4O_3 \cdot 3H_2O$).
8. NMR experiments were performed on Varian Unity 400 spectrometers using TMS as internal standard and the chemical shifts were recorded in δ values.
   ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.67 (3H, d, J=6 Hz, 24-H_3), 0.70 (3H, d, J=6 Hz, 20-H_3), 0.82 (3H, d, J=7 Hz, 21-H_3), 0.85 (3H, d, J=6 Hz, 23-H_3), 1.65 (3H, s, 29-H_3), 1.68 (3H, s, 28-H_3), 1.79 (3H, s, 18-H_3), 1.85 (1H, m, 19-H), 2.15 (1H, m, 22-H), 3.06 (3H, s, 13-CH_3), 3.35 (2H, m, 25-H_2), 4.40 (1H, t, J=9 Hz, 15-H), 4.74 (1H, d, J=11 Hz, 12-H), 5.31 (1H, m, 26-H), 5.79 (1H, d, J=7 Hz, 8-H), 6.76 (1H, t, J=10 Hz, 9-H), 6.94 (1H, m, 5-H), 7.03 (1H, td, J=8, 2 Hz, 6-H), 7.24 (1H, d, J=8 Hz, 4-H), 7.30 (1H, d, J=8 Hz, 7-H), 8.05 (1H, d, J=8 Hz, 16-H), 8.81 (1H, d, J=11 Hz, 10-H), 10.94 (1H, s, 1-H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 17.6 (q, C-29), 18.3 (q, C-24), 18.4 (q, C-21), 18.5 (q, C-20), 18.9 (q, C-23), 22.0 (q, C-18), 25.4 (q, c-28), 25.6 (t, C-25), 26.1 (d, C-22), 29.9 (d, C-19), 30.3 (q, 13-CH_3), 54.0 (d, C-15), 60.4 (d, C-12), 104.0 (d, C-8), 105.1 (s, C-3), 110.7 (d, C-7), 118.3 (d, C-4), 118.6 (d, C-5), 120.3 (d, C-6), 120.6 (d, C-9), 120.8 (d, C-26), 126.9 (s, C-3a), 132.3 (s, C-27), 135.6 (s, C-7a),136.6 (s, C-2), 167.6 (s, C-11), 168.9 (s, C-17), 172.6 (s, C-14)
9. Coleman, J. E.; Dilip de Silva, E.; Kong, F.-M.; Andersen, R. J.; Allen, T. M. *Tetrahedron* **1995**, 51, 10653-10662.
10. Palermo, J. A.; Flower, P. B.; Seldes, A. M. *Tetrahedron Lett.* **1992**, 33, 3097-3100.

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