SUPPLEMENTARY MATERIAL

A New Butenolide Derivative from the Deep-sea Fungus Aspergillus terreus SCSIO FZQ028

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Abstract: A new butenolide derivative (±)-asperteretal F (1) and related congener (2) recently reported containing an unusual 2-benzyl-3-phenyl substituted lactone core, together with five known compounds (3–7) were isolated and characterized from the fungus Aspergillus terreus. SCSIO FZQ028 derived from a deep-sea sediment of South China Sea. Their chemical structures were established on the basis of 1D- and 2D-NMR spectroscopic data, and HR-ESI-MS analysis. Additionally, all the compounds were evaluated for the antioxidative activities against DPPH, cytotoxic activities against two tumor cell lines (SF-268 and HepG-2), and antimicrobial activities. Compounds 2-4, and 7 showed significant activities against DPPH with IC\textsubscript{50}
ranging from 5.89 to 10.07 μg/mL. Compounds 2 and 4 showed moderate antimicrobial activities against all four tested bacteria.

**Keywords:** butenolide derivative; antioxidative activity; cytotoxic activity; antimicrobial activity; deep-sea fungus

Table S1. $^1$H and $^{13}$C NMR Data of 1 in CDCl$_3$ (700MHz, TMS, δ in ppm, J in Hz).

| Position | $\delta_H$ (mult, J in Hz) | $\delta_C$ | HMBC | COSY |
|----------|-----------------------------|-------------|-------|-------|
| 1        | 175.2                       |             |       |       |
| 2        | 125.7                       |             |       |       |
| 3        | 158.5                       |             |       |       |
| 4        | 6.46 (s, 1H)                | 99.2        | C-1, C-2 |       |
| 5        | 3.74 (s, 2H)                | 29.9        | C-1, C-2, C-3, C-2'' |       |
| 1'       | 123.3                       |             |       |       |
| 2'/6'    | 7.47 (d, 8.8 Hz, 2H)        | 131.7       | C-3, C-4', C-3'/5' | H-3'/5' |
| 3'/5'    | 6.82 (d, 8.8 Hz, 2H)        | 116.7       | C-1', C-4' | H-2'/6' |
| 4'       | 160.9                       |             |       |       |
| 1''      | 130.6                       |             |       |       |
| 2''      | 6.90 (s, 1H)                | 130.3       | C-5, C-4'', C-6'', C-1'' |       |
| 3''      | 121.4                       |             |       |       |
| 4''      | 152.9                       |             |       |       |
| 5''      | 6.64 (d, 8.3 Hz, 2H)        | 118.1       | C-1'', C-3'', C-4'' | H-6'' |
| 6''      | 6.93 (d, 8.3 Hz, 2H)        | 128.2       | C-2'', C-4'' | H-5'' |
| 1'''     | 2.64 (d, 16.7, 5.3 Hz, 1H)  | 32.1        | C-2'', C-3'', C-4'', | H-2''' |
| 2''''    | 2.92 (d, 16.6, 7.3 Hz, 1H)  |             | C-2'', C-3'' |       |
| 2'''     | 3.71 (m, 1H)                | 70.5        | C-3'', C-4'', C-5''' | H-1''' |
| 3'''     | 77.9                        |             |       |       |
| 4'''     | 1.28 (s, 3H)                | 25.8        | C-2'', C-3'', C-5''' |       |
| 5'''     | 1.21 (s, 3H)                | 21.2        | C-2'', C-3'', C-4''' |       |
Table S2. Antioxidative activities of compounds 1-7 (IC$_{50}$, μg/mL).

| Nos. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | Vc |
|------|----|----|----|----|----|----|----|----|
| IC$_{50}$ | 75.04 | 6.43 | 10.07 | 9.50 | 73.64 | 360.87 | 5.89 | 5.13 |

Table S3. Antibacterial activities of compounds 1-7.

| Nos./ zone of inhibition (mm) | S. aureus | B. thuringiensis | B. subtilis | E. coli |
|-----------------------------|-----------|------------------|-------------|---------|
| 1                           | 7.68      | 8.81             | 7.54        | NA      |
| 2                           | 8.94      | 9.77             | 7.98        | 7.53    |
| 3                           | 7.59      | 7.81             | 7.13        | NA      |
| 4                           | 8.16      | 9.13             | 7.49        | 7.64    |
| 5                           | NA        | 7.56             | NA          | NA      |
| 6                           | 7.83      | 7.42             | NA          | 7.16    |
| 7                           | 7.88      | NA               | NA          | 7.35    |
| positive control (Chl)      | 16.46     | 13.87            | 17.53       | 12.44   |

**NOTE:** NA=no activity (<7 mm)

Figure S1. Key $^1$H-$^1$H COSY and HMBC correlations of compound 1.
Figure S2. $^1$H NMR spectrum of (±)-asperteretal F (1) (CDCl$_3$)

Figure S3. $^{13}$C NMR and DEPT spectrum of (±)-asperteretal F (1) (CDCl$_3$)
Figure S4. HSQC spectrum of (±)-asperteretal F (1) (CDCl₃)

Figure S5. HMBC spectrum of (±)-asperteretal F (1) (CDCl₃)
Figure S6. $^1$H-$^1$H COSY spectrum of (±)-asperteretal F (I) (CDCl$_3$)

Figure S7. HRESIMS spectrum of (±)-asperteretal F (I)
Figure S8. UV spectrum of (±)-asperteretal F (1)

Figure S9. IR spectrum of (±)-asperteretal F (1)
Figure S10. UV and CD spectrum of (±)-asperteretal F (I)
Figure S11. $^1$H NMR spectrum of asperteretal E (2) (CDCl$_3$)

Figure S12. $^{13}$C NMR and DEPT spectrum of asperteretal E (2) (CDCl$_3$)
Figure S13. HSQC spectrum of asperteretal E (2)

Figure S14. HMBC spectrum of asperteretal E (2) (CDCl₃)
Figure S15. $^1$H $^1$H COSY spectrum of asperteretal E (2) (CDCl$_3$)

Figure S16. HRESIMS spectrum of asperteretal E (2)
Figure S17. UV and CD spectrum of asperteretal E (2)
The physicochemical data of the known compounds

Asperterat E (2): Pale yellow oil; HRESIMS ([M-H]⁻ m/z 365.1387, calcd for C_{22}H_{32}O_{3}⁻, 365.1394). \(^1H\) NMR (700 MHz, CDCl₃): δ_H 7.48 (2H, d, J=8.8 Hz, H-2'/6'), δ_H 7.00 (1H, d, J=8.3 Hz, H-6'), δ_H 6.90 (2H, d, J=8.8 Hz, H-3'/5'), δ_H 6.82 (H, s, H-2'), δ_H 6.43 (H, brs, H-4), δ_H 3.79 (2H, m, H-5), δ_H 2.72 (2H, t, J=6.7 Hz, H-1'), δ_H 1.78 (2H, t, J=6.7 Hz, H-2''), δ_H 1.35 (6H, s, H-4''/5''). \(^13C\) NMR (700 MHz, CDCl₃): δ_C 172.3 (qC, C-1), δ_C 126.1 (qC, C-2), δ_C 155.7 (qC, C-3), δ_C 97.2 (CH, C-4), δ_C 29.2(CH₂, C-5), δ_C 122.9 (qC, C-1'), δ_C 130.6 (CH, C-2'/6'), δ_C 116.2 (CH, C-3'/5'), δ_C 157.9 (qC, C-4'), δ_C 122.1 (qC, C-1''), δ_C 127.6 (CH, C-2''), δ_C 123.1 (qC, C-3'), δ_C 148.8 (qC, C-4'), δ_C 116.2 (CH, C-5''), δ_C 127.6 (CH, C-6''), δ_C 22.9 (CH₂, C-1''), δ_C 32.7 (CH₂, C-2''), δ_C 75.7(qC, C-3''), δ_C 26.9 (CH₃, C-4''''), δ_C 26.9 (CH₃, C-5''').

Butyro lactone III (3): Pale yellow oil; HRESIMS ([M-H]⁻ m/z 439.1399, calcd for C_{24}H_{35}O_{4}⁻, 439.1398). [α]_D^{25} = +73.2 (c 0.2 CH₂CH₂OH); UV (CH₃CH₂OH) λ_{max} nm:306.60, 226.80, 205.40. \(^1H\) NMR (700 MHz, MeOH): δ_H 7.58 (2H, d, J=7.5 Hz, H-2'/6'), δ_H 6.56 (1H, d, J=8.4 Hz, H-6'), δ_H 6.86 (2H, d, J=7.8 Hz, H-3'/5'), δ_H 6.46 (H, s, H-2'), δ_H 6.47 (H, brs, H-5), δ_H 3.44 (2H, m, H-5), δ_H 2.77 (2H, m, H-1''), δ_H 3.66 (1H, dd, J=5.5, 7.4 Hz, H-2''), δ_H 1.26 (3H, s, H-4''), δ_H 1.16 (3H, s, H-5'''). \(^13C\) NMR (700 MHz, MeOH): δ_C 171.5 (qC, C-1), δ_C 141.5 (qC, C-2), δ_C 127.7 (qC, C-3), δ_C 86.8 (qC, C-4), δ_C 39.5 (CH₂, C-5), δ_C 171.9 (qC, C-6), δ_C 53.8 (OCH₂, C-7), δ_C 123.8 (qC, C-1'), δ_C 130.1 (CH, C-2'/6'), δ_C 116.5 (CH, C-3'/5'), δ_C 158.5 (qC, C-4'), δ_C 120.5 (qC, C-1''), δ_C 132.9 (CH, C-2''), δ_C 126.3 (qC, C-3''), δ_C 153.3 (qC, C-4'''), δ_C 117.2 (CH, C-5''), δ_C 130.5 (CH, C-6''), δ_C 32.0 (CH₂, C-1''), δ_C 70.5 (CH, C-2''), δ_C 77.9 (qC, C-3''), δ_C 25.9 (CH₃, C-4''), δ_C 20.8 (CH₃, C-5''').

Aspernolides A (4): Pale yellow oil; HRESIMS ([M-H]⁻ m/z 423.1467, calcd for C_{24}H_{34}O_{4}⁻, 423.1449). [α]_D^{25} = +80.76 (c 2.58 CHCl₃). \(^1H\) NMR (700 MHz, CDCl₃): δ_H 7.61 (2H, d, J=8.9 Hz, H-2'/6'), δ_H 6.53 (1H, d, J=8.4 Hz, H-6'), δ_H 6.93 (2H, d, J=8.9 Hz, H-3'/5'), δ_H 6.90 (H, s, H-2'), δ_H 6.49 (H, brs, H-5''), δ_H 3.75 (3H, s, H-7'), δ_H 3.54 (2H, m, H-5), δ_H 2.56 (2H, m, H-1''), δ_H 1.69 (2H, t, J=6.7 Hz, H-2''), δ_H 1.25 (3H, s, H-4'''), δ_H 1.24 (3H, s, H-5'''). \(^13C\) NMR (700 MHz, CDCl₃): δ_C 169.5 (qC, C-1), δ_C 137.4 (qC, C-2), δ_C 128.6 (qC, C-3), δ_C 86.2 (qC, C-4), δ_C 38.7(CH₂, C-5), δ_C 169.9(qC, C-6), δ_C 53.6 (OCH₂, C-7), δ_C 121.9 (qC, C-1'), δ_C 129.6 (CH, C-2'/6'), δ_C 116.1 (CH, C-3'/5'), δ_C 157.2 (qC, C-4'), δ_C 123.8 (qC, C-1''), δ_C 131.7 (CH, C-2''), δ_C 120.4 (qC, C-3''),δ_C 153.0 (qC, C-4''), δ_C 116.7 (CH, C-5''), δ_C 129.2 (CH, C-6''), δ_C 22.2 (CH₂, C-1''), δ_C 32.5 (CH₂, C-2''), δ_C 74.4 (qC, C-3''), δ_C 26.8 (CH₃, C-4''), δ_C 26.8 (CH₃, C-5''').

Butyro lactone-IV (5): Pale yellow oil; HRESIMS ([M-H]⁻ m/z 439.1399, calcd for C_{24}H_{35}O_{4}⁻, 439.1398). [α]_D^{25} = +20.11 (c 0.09 CH₂CH₂OH); UV (CH₃CH₂OH) λ_{max} nm:305.60, 294.80, 228.80, 207.20. \(^1H\) NMR (700 MHz, CDCl₃): δ_H 7.61 (2H, d, J=8.7 Hz, H-2'/6'), δ_H 6.50 (1H, d, J=8.2 Hz, H-6'), δ_H 6.91 (2H, d, J=8.6 Hz, H-3'/5'), δ_H 6.65 (H, s, H-2'), δ_H 6.56 (H, brs, H-5'), δ_H 3.77 (3H, s, H-7), δ_H 3.56 (2H, m, H-5), δ_H 3.03 (2H, m, H-1''), δ_H 4.52 (1H, t, J=8.9 Hz, H-2''), δ_H 1.66 (3H, s, H-4''), δ_H 1.57 (3H, s, H-5'''). \(^13C\) NMR (700 MHz, CDCl₃): δ_C 169.4 (qC, C-1), δ_C
137.5 (qC, C-2), δC 126.9 (qC, C-3), δC 86.3 (qC, C-4), δC 39.0 (CH2, C-5), δC 169.9(qC, C-6), δC 53.7 (OCH3, C-7), δC 122.4 (qC, C-1’), δC 129.7 (CH, C-2’/6’), δC 116.2 (CH, C-3’/5’), δC 158.4 (qC, C-4’), δC 124.9 (qC, C-1’’), δC 130.2 (CH, C-2’’), δC 128.2 (qC, C-3’’), δC 158.9 (qC, C-4’’), δC 108.6 (CH, C-5’’), δC 127.1 (CH, C-6’’), δC 30.6 (CH2, C-1”), δC 89.3 (CH, C-2”), δC 72.5 (qC, C-3”), δC 26.0 (CH3, C-4”), δC 23.9 (CH3, C-5”).

**Butyrolactone I (6):** Pale yellow oil; LRESIMS [M-H]⁻ m/z 423.1; [2M-H]⁻ m/z 847.1. [α]D25 =+43.43 (c 0.07 MeOH); UV (MeOH) λmax nm:287.20, 209.40. ¹H NMR (700 MHz, MeOH): δH 7.62 (2H, d, J=7.5 Hz, H-2’/6’), δH 6.54 (1H, d, J=8.4 Hz, H-6”), δH 6.85 (2H, d, J=7.8 Hz, H-3’/5’), δH 6.46 (H, s, H-2”), δH 6.42 (H, brs, H-5”), δH 3.75 (3H, s, H-7), δH 3.46 (2H, m, H-5), δH 3.06 (2H, d, J=7.4 Hz, H-1”), δH 5.07 (1H, m, H-2”), δH 1.66 (3H, s, H-4”), δH 1.57 (3H, s, H-5”). ¹C NMR (700 MHz, MeOH): δC 172.4 (qC, C-1), δC 138.1 (qC, C-2), δC 127.6 (qC, C-3), δC 86.8 (qC, C-4), δC 39.8 (CH2, C-5), δC 172.4(qC, C-6), δC 53.7 (OCH3, C-7), δC 123.6 (qC, C-1”), δC 129.8 (CH, C-2’/6’), δC 116.4 (CH, C-3’/5’), δC 158.4 (qC, C-4”), δC 125.5 (qC, C-1”), δC 132.5 (CH, C-2”), δC 128.3 (qC, C-3”), δC 154.9 (qC, C-4”), δC 115.0 (CH, C-5”), δC 129.8 (CH, C-6”), δC 28.7 (CH2, C-1”), δC 123.6 (CH, C-2”), δC 132.9 (qC, C-3”), δC 25.9 (CH3, C-4”), δC 17.8 (CH3, C-5”).

**Aspernolides B (7):** Pale yellow oil; LRESIMS [M-H]⁻ m/z 441.1; [2M-H]⁻ m/z 883.1. [α]D25 =+98.1 (c 0.2 MeOH);UV (MeOH) λmax nm:306.80, 207.00. ¹H NMR (700 MHz, MeOH): δH 7.59 (2H, d, J=7.4 Hz, H-2’/6’), δH 6.50 (1H, d, J=8.0 Hz, H-6”), δH 6.86 (2H, d, J=7.8 Hz, H-3’/5’), δH 6.45 (H, s, H-2”), δH 6.47 (H, d, J=8.0 Hz, H-5”), δH 3.78 (3H, s, H-7), δH 3.46 (2H, m, H-5), δH 2.43 (2H, m, H-1”), δH 1.52 (2H, t, H-2”), δH 1.19 (3H, s, H-4”), δH 1.18 (3H, s, H-5”). ¹C NMR (700 MHz, MeOH): δC 171.1 (qC, C-1), δC 141.5 (qC, C-2), δC 127.7 (qC, C-3), δC 86.9 (qC, C-4), δC 39.6 (CH2, C-5), δC 171.9 (qC, C-6), δC 53.8 (OCH3, C-7), δC 123.6 (qC, C-1”), δC 130.3 (CH, C-2’/6’), δC 116.6 (CH, C-3’/5’), δC 159.1 (qC, C-4”), δC 129.6 (qC, C-1”), δC 132.9 (CH, C-2”), δC 125.3 (qC, C-3”), δC 155.2 (qC, C-4”), δC 115.2 (CH, C-5”), δC 129.7 (CH, C-6”), δC 25.8 (CH2, C-1”), δC 44.7 (CH2, C-2”), δC 71.5 (qC, C-3”), δC 29.3 (CH3, C-4”), δC 29.1 (CH3, C-5”).