SUPPLEMENTARY MATERIAL

Isolation and identification of two new compounds from the

*Penicillium* sp. SYPF7381

Qing-Mei Feng a, Xing-Yu Li a, Bing-Xin Li a, Tian-Yuan Zhang a, Hai-Feng Wang a, Meng-Yue Zhang a, Ying-Ying Wu a, Gang Chen a, Yi-Xuan Zhang a, Yue-Hu Pei a

a Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China;

fengqingmei1991@163.com (Q.M. Feng); livingyu_0102@163.com (X.Y. Li);
libingxin23@126.com (B.X. Li); zty19840115@163.com (T.Y. Zhang);
wanghai70310@163.com (H.F. Wang); iamzmy@126.com (M.Y. Zhang);
wyy280884549@163.com (Y.Y. Wu); chengang1152001@163.com (G. Chen).

* Address for Correspondence:
Yue-Hu Pei, Yi-Xuan Zhang, Tel: +86-24-23986485, Fax: +86-24-23986479,
E-mail address: peiyueh@vip.163.com (Yue-Hu Pei); zhangyxzzh@163.com (Yi-Xuan Zhang).

Abstract

Two new compounds, (R, 2E, 5E)-3,5,7-trimethyl-2,5-octadienedioic-8-methyl ester (1) and neovasipyridone G (3), together with a new natural product compound (R,2E,5E)-3,5,7-trimethyl-2,5-octadienedioic acid (2), and six known compounds (4-9) were isolated from *Penicillium* sp. SYPF7381. Their structures were elucidated on the base of extensive spectroscopic analysis, and the absolute configuration of compounds 1 and 2 were determined by optical rotation. In addition, the anti-inflammatory activities of all compounds were assayed in RAW 264.7 cells by assessing LPS-induced NO production. Furthermore, the structure-antiinflammation activity relationships for these isolated compounds were summarized based on the experimental as well as the docking results.

Keywords: anti-inflammatory activity, structure elucidation, *Penicillium* sp., molecular docking.
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Anti-inflammatory activity in vitro

Figure S36. Anti-inflammatory activity assay in RAW-264.7 cells. (Each bar represents mean ±SE of three independent experiments. ***P < 0.001 compared with control group, ** P < 0.01, *P < 0.05 compared with LPS group; NO: nitric oxide; LPS: lipopolysaccharide).

Molecular Docking

Table S2. Experimental and Calculated Data Related to the Anti-inflammatory activity of compounds 1-3, 6-7

Figure S37. Molecular docking model of compounds 1-3, 6, 7 with iNOS (PDB ID: 3NQS) and its binding modes of compounds with the key residues of iNOS.
Table S1. $^1$H and $^{13}$C NMR data for compound 1 and 3 (CDCl$_3$) $^a$.

| NO. | $\delta_C$ 1 $^b$ | $\delta_H$ 1 ($J$ in Hz) $^c$ | NO. | $\delta_C$ 3 $^b$ | $\delta_H$ 3 ($J$ in Hz) $^c$ | NO. | $\delta_C$ 3 $^b$ | $\delta_H$ 3 ($J$ in Hz) $^c$ |
|-----|------------------|--------------------------|-----|------------------|--------------------------|-----|------------------|--------------------------|
| 1   | 160.1            |                          | 14  | 28.6             | 1.34 (3H, s)             | 2   | 76.1            | 3.80 (1H, s)             |
| 2   | 117.0            | 5.71 (1H, s)             | 3   | 70.9             |                          | 3   | 191.7           |                          |
| 3   | 130.3            |                          | 4   | 191.7            |                          | 4   | 51.2            | 2.82 (2H, s)             |
| 5   | 133.7            |                          | 5   | 104.8            |                          | 6   | 128.1           | 5.30 (1H, d, $J$ = 9.15 Hz) |
| 7   | 39.0             | 3.40, m                  | 8   | 140.1            | 5.13 (1H, d, $J$ = 9.5 Hz) | 9   | 18.0            | 1.24 (3H, d, $J$ = 7.0 Hz) |
| 8   | 175.6            |                          | 9   | 34.0             | 2.26 (1H, m)             | 10  | 16.1            | 1.60 (3H, s)             |
| 9   | 130.3            |                          | 10  | 30.1             | 1.20 (1H, m)             | 11  | 18.5            | 2.09 (3H, s)             |
| 10  | 16.1             | 1.60 (3H, s)             | 11  | 11.9             | 0.81 (3H, t, $J$ = 7.35 Hz) | 12  | 13.1            | 1.41 (3H, s)             |
| OCH$_3$ | 52.0           | 3.67 (3H, s)             | 13  | 20.7             | 0.91 (3H, d, $J$ = 6.59 Hz) |

$^a$ TMS was used as an internal standard; chemical shifts ($\delta$) are expressed in ppm; $J$ values in Hz.

$^b$ Data were measured in CDCl$_3$ at 150MHz.

$^c$ Data were measured in CDCl$_3$ at 600MHz.

1 The spectra of compound 1

![Figure S1. The IR spectrum of compound 1](image-url)
Figure S2. The HR-ESI-MS spectrum of compound 1

Figure S3. The $^1$H-NMR spectrum (CDCl$_3$, 600MHz) of compound 1
Figure S4. The $^{13}$C-NMR spectrum (CDCl$_3$, 150MHz) of compound 1

Figure S5. The HSQC spectrum (CDCl$_3$, 600MHz) of compound 1
Figure S6. The HMBC spectrum (CDCl$_3$, 600MHz) of compound 1

![HMBC spectrum of compound 1](image.png)

Figure S7. Key correlations observed in the HMBC spectra of 1.
2 The spectra of compound 2

Figure S8. The IR spectrum of compound 2

Figure S9. The HR-ESI-MS spectrum of compound 2
Figure S10. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 2

Figure S11. The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 2
Figure S12. The HSQC spectrum (CDCl$_3$, 600MHz) of compound 2

Figure S13. The HMBC spectrum (CDCl$_3$, 600MHz) of compound 2
3 The spectra of compound 3

Figure S14. The IR spectrum of compound 3

Figure S15. The HR-ESI-MS spectrum of compound 3
Figure S16. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 3

Figure S17. The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 3
Figure S18. The HSQC spectrum (CDCl$_3$, 600MHz) of compound 3

Figure S19. The HMBC spectrum (CDCl$_3$, 600MHz) of compound 3
Figure S20. Key correlations observed in the HMBC spectra of 3.

Figure S21. The NOESY spectrum (CDCl₃, 600MHz) of compound 3.
In order to further verified the absolute configuration of compound 3, the ECD spectra of ((2R, 3S, 9R)-3, (2R, 3S, 9S)-3 and it’s enantiomer were calculated at B3LYP/6-31G (d) level in methanol on the base of TDDFT method. Compound 3 was drawn via SpecDic software with sigma = 0.3 and UV shift = 4 nm. The ECD spectrum of 3 (2R, 3S, 9R) and 3 (2R, 3S, 9S) both showed a positive Cotton effect at 320 nm and 255 nm and showed a negative Cotton effect at 289 nm.
4 The spectra of compound 4

Figure S24. The $^1$H-NMR spectrum (DMSO-$d_6$, 400MHz) of compound 4

Figure S25. The $^{13}$C-NMR spectrum (DMSO-$d_6$, 100MHz) of compound 4
5 The spectra of compound 5

Figure S26. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 5

Figure S27. The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 5
6 The spectra of compound 6

Figure S28. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 6

Figure S29. The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 6
The spectra of compound 7

**Figure S30.** The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 7

**Figure S31.** The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 7
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Figure S32. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 8

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9 The spectra of compound 9

Figure S34. The $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 9

Figure S35. The $^{13}$C-NMR spectrum (CDCl$_3$, 100MHz) of compound 9
Anti-inflammatory activity *in vitro*

**Figure S36.** Anti-inflammatory activity assay in RAW-264.7 cells. (Each bar represents mean ±SE of three independent experiments. ### $P < 0.001$ compared with control group, ** $P < 0.01$, *** $P < 0.001$ compared with LPS group; NO: nitricoxide; LPS: lipopolysaccharide).
Molecular Docking

Table S2. Experimental and Calculated Data Related to the Anti-inflammationary of compounds 1-3, 6-7.

| Compound | glide gscore\(^a\) | glide energy\(^b\) | Inhibition rate of NO (%) |
|----------|---------------------|--------------------|---------------------------|
| 1        | -3.512              | -37.338            | 0.4 22.4                  |
| 2        | -4.115              | -38.322            | 28 29.2                   |
| 3        | -4.741              | -48.062            | 37.6 40.6                 |
| 6        | -4.016              | -34.503            | 30.8 28.8                 |
| 7        | -5.197              | -38.543            | 31.2 33.4                 |

\(^a\)The glide gsore of compounds 1-3, 6-7 with this receptor iNOS (PDB ID: 3NQS).
\(^b\)The glide energy of compounds 1-3, 6-7 with this receptor iNOS (PDB ID: 3NQS).

Compound 1

Compound 2

Compound 3
Figure S37. Molecular docking model of compounds 1-3, 6, 7 with iNOS (PDB ID: 3NQS) and its binding modes of compounds with the key residues of iNOS.