Lipid-Lowering Polyketides from the Fungus *Penicillium steckii* HDN13-279

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**Abstract:** Seven new polyketides, named tanzawaic acids R–X (1–6, 11), along with seven known analogues (7–10 and 12–14), were isolated from *Penicillium steckii* HDN13-279. Their structures, including the absolute configurations, were elucidated by NMR, MS, X-ray diffraction, circular dichroism (CD) analyses and chemical derivatization. Five compounds (2, 3, 6, 10 and 12) significantly decreased the oleic acid (OA)-elicited lipid accumulation in HepG2 liver cells at the concentration of 10 µM, among which, four compounds (3, 6, 10 and 12) significantly decreased intracellular total cholesterol (TC) levels and three compounds (3, 6, and 10) significantly decreased intracellular triglyceride (TG) levels. Moreover, the TG-lowering capacities of Compounds 6 and 10 were comparable with those of simvastatin, with the TG levels being nearly equal to blank control. This is the first report on the lipid-lowering activity of tanzawaic acid derivatives.

**Keywords:** *Penicillium steckii*; tanzawaic acid derivatives; lipid-lowering activity

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1. Introduction

Metabolic syndrome (MetS), including obesity, insulin resistance (IR), dyslipidemia and hypertension, have long been a worldwide problem [1–3]. Dyslipidemia, as one of the most common causes of MetS, can further result in atherosclerosis, myocardial infarction and cerebrovascular diseases, seriously threatening human life [4–6]. Due to the current undesirable side-effects of lipid-regulating drugs, it is urgent to find new classes of bioactive compounds with lipid-lowering capacity and a safer profile [7–10]. Famous drugs for the treatment of dyslipidemia are lovastatin analogues, reported to be produced by various fungal species, including *Aspergillus* spp., *Penicillium citrinum*, *Pleurotus* spp., and *Monascus ruber*, thus, suggesting fungi as promising sources for the discovery of new drug leads against MetS [11,12].

In our ongoing search for new bioactive metabolites from natural sources [13,14], *Penicillium steckii* HDN13-279 was selected for investigation due to the interesting HPLC–UV profile of its ethyl acetate extract. This species, widely distributed through marine and terrestrial environments, has been reported as an important producer of tanzawaic acids [15–18]. Tanzawaic acid derivatives are a class of polyketides characterized by a core structure with a poly-hydrogenated naphthalene ring and a penta-2,4-dienoic acid side chain. Several bioactivities have been reported for these compounds,
namely anticoccidial, cytotoxic, anti-inflammatory, and capacity to inhibit protein tyrosine phosphatase 1B (PTP1B) and superoxide anion production [18–24]. In the present study, we report the isolation and structure elucidation of seven new polyketides and seven analogues isolated from P. steckii HDN13-279. The capacities of the isolated compounds to decrease oleic acid (OA)-elicited lipid accumulation in HepG2 liver cells were also reported.

2. Results and Discussion

The fungal strain P. steckii HDN13-279 was fermented (45 L) under shaking conditions at 28 °C for 9 days. The EtOAc extract (40 g) was fractionated by silica gel vacuum liquid chromatography (VLC), C-18 ODS column chromatography, Sephadex LH-20 column chromatography, ODS MPLC, and finally HPLC to yield Compounds 1–14 (Figure 1).

Tanzawaic acid R (1) was obtained as a pale yellow oil with the molecular formula C_{18}H_{26}O_{4} analyzed by HRESIMS. The 1D NMR data (Table 1) indicated the presence of two methyls, three methylenes (with one oxygenized), 11 methines (including five sp^{2} methines and one oxymethine), and two non-protonated carbons (including one carbonyl). The planar structure of Compound 1 was proved to be the same as tanzawaic acid H [25], supported by the similar 1D NMR and 2D NMR data (Figure 2). The clear differences in 1D NMR spectra (see Supplementary Materials), especially those at CH-2-11 (δC 32.6; δH 1.66, 1.13 in 1 vs. δC 33.7; δH 2.32, 0.65 in tanzawaic acid H), CH-12 (δC 42.6, δH 1.29 in 1 vs. δC 47.3, δH 1.13 in tanzawaic acid H), CH-13 (δC 67.0, δH 3.83 in 1 vs. δC 72.4, δH 3.70 in tanzawaic acid H) and CH-14 (δC 125.5, δH 5.83 in 1 vs. δC 130.9, δH 5.55 in tanzawaic acid H) [25], suggested they have different stereochemistry.

The relative configuration of 1 was assigned by NOESY spectroscopic data. The NOESY correlations from H-5 to H-7/H_{3}-18, H-6 to H-8/H-12 indicated a trans fusion of the rings and
placed H-7, Me-18 and the penta-2,4-dienoic acid moiety on the same side of the decalin ring, while H-6, H-8, H-12 on the other side. In addition, the NOESY correlations between H-9a (δ_H 1.69) and H-8/H-11a (δ_H 1.66), H-9b (δ_H 0.74) and H-7, H-10 and H-8/H-12, H-11a and H-12/H-13, H-12 and H-13 indicate that the hydroxymethyl at C-10 and the hydroxyl at C-13 were located at the same side as the penta-2,4-dienoic acid moiety (Figure 3). Thus, the NOESY data suggested that Compound 1 was a C-13 epimer of tanzawaic acid H. In a previous report [25], the absolute configuration of tanzawaic acid H was deduced based on the proposed biogenetic pathway. To make a solid evidence, we determined the absolute configuration of 1 by X-ray diffraction following chemical derivatization. Firstly, Compound 1 was esterified to generate the methyl ester (1a) and then the single crystal (CCDC 1537543) of 1a was successfully obtained (Figure 4) with Flack parameter = 0.02 (11). Consequently, the absolute configuration of 1 was elucidated to be 6R, 7R, 8R, 10S, 12S, 13S (Figure 1).

Tanzawaic acid S (2) was isolated as a pale yellow oil. The molecular formula of 2 was established as C_{19}H_{28}O_{4} according to the HRESIMS ions detected at m/z 319.1906 [M – H]^−. The \(^1^H\) NMR data of 2 was similar to those of 1 (Table 1), and the only difference was the existence of signal for an additional methoxy (δ_H 3.33). Further analysis of the 2D NMR spectra indicated the additional methoxy was attached at C-13 (δ_H 7.63) in 2 (Figure 2). The relative configuration of 2 cannot be completely assigned by NOESY data as the signals for H-7, H-8 and H-12 were overlapped. We further resorted to chemical derivatization. Both Compounds 1 and 2 were successfully transformed into Compound 1b when methylated by NaH and CH\textsubscript{3}I in DMF solution, which suggested they share the same relative configuration. Moreover, 1 and 2 showed almost the same CD curve (Figure 5), further confirming their same absolute configuration.

### Table 1. \(^1^H\) (500 MHz) and \(^1^3^C\) (125 MHz) NMR Data of Compounds 1 and 2 (Methanol-\textsubscript{d}_4, δ ppm).

| No.   | \(δ_C\) | \(δ_H\) (J in Hz) | \(δ_C\) | \(δ_H\) (J in Hz) |
|-------|---------|-------------------|---------|-------------------|
| 1     | 170.1   | -                 | 169.3   | -                 |
| 2     | 120.3   | 5.82 overlap      | 119.4   | 5.80 d (15.3)     |
| 3     | 144.6   | 7.22 dd (11.1, 15.3) | 145.1   | 7.23 dd (11.0, 15.3) |
| 4     | 129.8   | 6.32 dd (10.6, 15.3) | 129.4   | 6.29 dd (11.0, 15.3) |
| 5     | 149.1   | 6.00 dd (9.4, 15.3) | 149.3   | 5.96 overlap      |
| 6     | 50.3    | 2.61 t (8.0)      | 50.1    | 2.61 dd (4.7, 9.0) |
| 7     | 43.7    | 1.33 m             | 44.5    | 1.32 overlap      |
| 8     | 39.6    | 1.37 m             | 39.7    | 1.32 overlap      |
| 9     | 39.6    | 1.69 overlap       | 39.5    | 1.69 overlap      |
| 10    | 39.9    | 0.74 q (12.2)      | 39.9    | 0.72 q (11.8)     |
| 11    | 32.6    | 1.66 overlap       | 32.7    | 1.68 overlap      |
| 12    | 42.6    | 1.29 m             | 42.4    | 1.32 overlap      |
| 13    | 67.0    | 3.83 dd (2.3, 6.4) | 76.3    | 3.46 d (6.1)      |
| 14    | 125.5   | 5.83 overlap       | 123.7   | 5.96 overlap      |
| 15    | 138.2   | -                 | 139.7   | -                 |
| 16    | 21.1    | 1.61 s             | 21.1    | 1.63 s            |
| 17    | 67.3    | 3.37 d (6.2)       | 67.3    | 3.35 d (6.6)      |
| 18    | 21.5    | 0.97 d (6.1)       | 21.1    | 0.95 d (5.8)      |
| 13-OCH\textsubscript{3} | -       | -                 | 55.5    | 3.33 s            |
Figure 3. Key NOESY correlations of Compounds 1, 3–6, and 11.

Figure 4. X-ray crystallographic structures of 1a, 7, and 8 (black, blue and red on behalf of the elements of C, H, O, respectively).

Figure 5. Experimental ECD spectra of Compounds 1–9 and 11–14.
Tanzawaic acids T (3) and U (4) were obtained as pale yellow oil, and had the same molecular formula (C_{19}H_{28}O_{3}), established based on the same HRESIMS ions both detected at m/z 303.1962 [M – H]^{-}. Their 1D NMR data (Table 2) suggested that they possessed similar tanzawaic acid scaffold to Compound 2. Further analysis of the 2D NMR spectra indicated that Compounds 3 and 4 shared the same planar structure (Figure 2), and the structural difference between 3 (or 4) and 2 was that the hydroxymethyl in 2 was replaced by a methyl in 3 and 4 (δ_H 0.89 in 3, δ_H 0.88 in 4). The NOESY spectroscopic data of 3 and 4 indicated that they are C-13 epimers (Figure 3). The NOESY correlations between H-7 and H-13 suggested the β orientation of H-13 in Compound 3. The NOESY correlations between H-13 and H-11a (δ_H 1.57), H-11a and H-10, and between H-11b (δ_H 1.16) and H-17/13-OCH3 in Compound 4 indicated an α oriented H-13 in 4 (Figure 3). Although the relative configuration of C-13 in Compound 3 was different from 1, the CD curves of them are almost identical (Figure 5), indicating that the cotton effects, especially those at 270 nm, were predominated by the (2E, 4E)-penta-2,4-dienoic acid moiety. Consequently, the absolute configurations of 3 and 4 were deduced as 6R, 7R, 8R, 10S, 12S, 13R and 6R, 7R, 8R, 10S, 12S, 13S, respectively.

### Table 2. 1H (500 MHz) and 13C (125 MHz) NMR Data of Compounds 3 and 4 (CDCl3, δ ppm).

| No. | δC | δH (J in Hz) | δC | δH (J in Hz) |
|-----|----|-------------|----|-------------|
| 1   | 172.0 | -            | 172.3 | -            |
| 2   | 118.5 | 5.80 d (15.4) | 118.5 | 5.79 d (15.3) |
| 3   | 147.0 | 7.33 dd (10.9, 15.1) | 147.2 | 7.33 dd (11.0, 15.3) |
| 4   | 129.0 | 6.19 dd (11.0, 15.2) | 129.0 | 6.21 dd (11.2, 15.3) |
| 5   | 150.7 | 5.92 dd (9.4, 15.2) | 151.0 | 6.04 dd (9.4, 15.3) |
| 6   | 49.9 | 2.57 t (8.4) | 50.3 | 2.53 d (5.9, 8.1) |
| 7   | 48.7 | 0.94 m | 44.1 | 1.30 overlap |
| 8   | 39.7 | 1.38 m | 40.1 | 1.30 overlap |
| 9   | 45.7 | 1.62 overlap | 45.2 | 1.55 overlap |
| 10  | 31.5 | 1.44 m | 32.3 | 1.46 m |
| 11  | 38.2 | 2.19 d (3.2, 12.6) | 38.5 | 1.57 overlap |
| 12  | 38.2 | 0.61 q (12.2) | 38.5 | 1.16 q (12.0) |
| 13  | 43.9 | 1.23 m | 42.8 | 1.30 overlap |
| 14  | 80.6 | 3.32 d (10.2) | 76.4 | 3.41 d (6.2) |
| 15  | 126.0 | 5.66 s | 124.3 | 5.92 d (6.9) |
| 16  | 134.7 | - | 139.5 | - |
| 17  | 21.8 | 1.58 s | 22.5 | 1.61 s |
| 18  | 22.5 | 0.89 overlap | 22.4 | 0.88 overlap |
| 19  | 22.4 | 0.89 overlap | 21.8 | 0.88 overlap |
| 13-OCH3 | 56.0 | 3.37 s | 56.7 | 3.35 s |

Tanzawaic acid V (5) was isolated as a pale yellow oil with the molecular formula of C_{18}H_{26}O_{4} based on the HRESIMS ions detected at m/z 305.1751 [M – H]^{-}. The 1D NMR data (Table 3) indicated the presence of three methyls, two methylene, ten methines (including six sp^2 methines), and three non-protonated carbons (including one carbonyl and two oxygenated carbons). These data are similar to those of the isolated known compound, tanzawaic acid C (7) [19], except for the appearance of an additional oxygenated non-protonated carbon and the absence of a methine in 5, indicating that Compound 5 is a hydroxylated analogue of 7. Analysis of the 2D NMR spectra located the additional hydroxyl group on C-10 (Figure 2), and the methyl group on C-10 in 5 oriented to the same side as in Compound 7 according to the NOESY correlation between H3-17 and H3-18 (Figure 3). The absolute configuration of 5 was determined by comparing CD with that of 7 whose absolute configuration...
was determined using X-ray diffraction with Flack parameter = 0.06 (9) in this work (Figure 4). Their identical CD curve indicated the same absolute configuration (Figure 5).

Tanzawaic acid W (6) was isolated as a pale yellow oil, whose molecular formula was established as identical to that of compound 5 based on the HRESIMS ions detected at \( m/z \) 305.1752 \([M - H]^-\). The 1D and 2D NMR spectra indicated that the difference to 5 was the lack of the hydroxyl group at C-10 and the methyl on C-15 faced to the same orientation to H-7 according to the NOESY correlations between H-7 and H-16. Compound 6 also had similar CD curve with 7, which indicated the same absolute configuration (Figure 5).

Tanzawaic acid X (11) was isolated as pale yellow oil with the molecular formula of \( \text{C}_{18}\text{H}_{22}\text{O}_3 \) based on the HRESIMS ions detected at \( m/z \) 285.1493 \([M - H]^-\). The 1D NMR data (Table 3) indicated the presence of two methyls, three methylenes (with one oxygenated), eight methines (including six sp\(^2\) ones), and five non-protonated carbons (including one carbonyl). Further analysis of the 2D NMR spectra indicated that the planar structure 11 was similar to tanzawaic acid A (13) [19] and the only difference was the methyl at C-10 in 13 was replaced by a hydroxymethyl. The relative configuration of 11 was determined by NOESY correlations between H-8 (\( \delta_H 3.26 \)) and H-10 (\( \delta_H 1.85 \)), which suggested the cofacial of H-8 and H-10. Moreover, the coincident CD curves of 11 and 13 indicated the 8\( R \), 9\( S \) absolute configuration of 11 (Figure 5).

| No. | 5 \(^a\) | 6 \(^b\) | 11 \(^b\) |
|-----|---------|---------|---------|
| 1   | 169.8   | -       | 171.5   | -       |
| 2   | 120.0   | 5.84 d (15.3) | 120.0 | 5.90 d (15.6) | 119.8 | 5.94 d (15.3) |
| 3   | 144.9   | 7.34 dd (10.0, 15.4) | 145.9 | 7.42 dd (11.6, 14.4) | 147.1 | 7.58 dd (11.0, 15.2) |
| 4   | 122.3   | 6.47 dd (9.9, 15.4) | 125.4 | 6.51 dd (11.5, 14.4) | 131.2 | 6.43 dd (11.0, 15.8) |
| 5   | 153.5   | 6.52 d (15.3) | 149.0 | 6.40 d (15.2) | 141.4 | 7.12 d (15.9) |
| 6   | 75.8    | -       | 77.9    | -       | 135.0  | -       |
| 7   | 49.1    | 1.18 overlap | 51.9 | 1.35 t (9.8) | 140.8 | -       |
| 8   | 29.0    | 2.00 m  | 33.7    | 1.69 m  | 30.4   | 3.26 m  |
| 9   | 50.1    | 1.62 dt (3.2, 14.1) | 47.1 | 1.66 overlap | 35.0 | 2.18 m  |
| 10  | 69.1    | -       | 32.6    | 1.56 m  | 37.3   | 1.85 m  |
| 11  | 45.3    | 1.72 dt (3.0, 13.4) | 41.4 | 1.78 d (12.2) | 33.6 | 2.77 dt (3.05,14,5) |
| 12  | 33.4    | 2.58 m  | 38.3    | 2.21 t (10.7) | 135.2 | -       |
| 13  | 130.1   | 5.36 d (9.8) | 132.5 | 5.49 d (9.9) | 128.7 | 6.98 overlap |
| 14  | 129.1   | 5.50 m  | 130.1   | 5.40 d (9.9) | 127.8 | 6.98 overlap |
| 15  | 45.7    | -       | 75.0    | -       | 134.1  | -       |
| 16  | 18.3    | 0.97 d (7.1) | 27.4 | 1.27 s  | 21.1   | 2.28 s  |
| 17  | 30.1    | 1.18 s  | 22.2    | 0.88 d (6.5) | 68.0 | 3.66 overlap |
| 18  | 22.0    | 0.91 d (6.6) | 23.5 | 0.95 d (6.1) | 24.0 | 1.16 d (7.0) |

\(^{a}\) The NMR data were recorded in Methanol-d\(_4\); \(^{b}\) the NMR data were recorded in CDCl\(_3\).
configurations of Compounds 7 and 8 were determined under X-ray (CCDC numbers 1537544 and 1537542) with the Flack parameters = 0.06 (9) and 0.07 (10), respectively (Figure 4). The absolute configurations of Compounds 9 and 14 were determined by comparing CD spectra with Compounds 8 and 13, respectively (Figure 5). The absolute configuration of Compound 12 was assigned by chemical transformation. We found the Pd/C reduction products (established as 12a) of Compounds 12 and 13 showed the same 1D NMR data and similar optical rotations ([α]20 D ± 7.78 (c 0.042, MeOH) and [α]20 D ± 6.09 (c 0.042, MeOH), respectively), which suggested Compounds 12 and 13 had the same relative and absolute configurations, which is also in accord with the same CD curves of 12 and 13 (Figure 5).

All the compounds were evaluated for their cytotoxicity (on HL-60, HCT-116, K562, Hela and A549 cell lines), but none of them presented a cytotoxic effect at 30 μM. The antiviral (influenza A H1N1 virus) and NF-κB inhibitory activities were also evaluated, with no activity detected under the concentration of 30 μM. In light of the structural similarity with lovastatin, with the exception of Compound 9 (limited quantity), all the compounds were evaluated for their lowering effects against oleic acid (OA)-elicited lipid accumulation in HepG2 liver cells. Five compounds (2, 3, 6, 10 and 12) significantly decreased the lipid accumulation elicited by OA, determined by oil-red O staining, at the concentration of 10 μM. Compounds 6 and 12 showed comparable efficiency with simvastatin (Figure 6). Additionally, four compounds (3, 6, 10 and 12) could significantly decreased intracellular total cholesterol (TC) levels and three compounds (3, 6, and 10) significantly decreased intracellular triglyceride (TG) levels (Figure 7). It’s worth mentioning that the TG-lowering efficiency of Compounds 6 and 10 were comparable with simvastatin and the TG levels were nearly equal to blank control (p > 0.05) (Figure 7B).

**Figure 6.** Effects of compounds on OA-elicited intracellular lipid accumulation. Cells were treated with 10 μM of indicated compounds or simvastatin (as positive control) in DMEM + 100 μM OA or with DMEM alone (as blank) or DMEM + 100 μM OA (as negative control) for 24 h. Neutral lipids were determined by spectrophotometry at 358 nm after oil-red O staining. Bars depict the means ± SEM of at least three experiments. # # p < 0.01, OA versus Blank; * p < 0.05, ** p < 0.01, test group versus OA group. OA: oleic acid.
Figure 7. Inhibitory effects of compounds on intracellular (A) total cholesterol (TC) and (B) triglycerides (TG). Cells were treated with 10 µM of indicated compounds or simvastatin (as positive control) in DMEM + 100 µM OA or with DMEM alone (as blank) or DMEM + 100 µM OA (as negative control) for 24 h. Intracellular TC and TG concentration were measured by kits according to the manufacturer’s instructions. Bars depict the means ± SEM of at least three experiments. * p < 0.05, ** p < 0.01, test group versus OA group. OA: oleic acid.

3. Materials and Methods

3.1. General Experimental Procedures

UV spectra were recorded on Beckman DU 640 spectrophotometer (Beckman Coulter Inc., Brea, CA, USA). IR spectra were taken on Bruker tensor-27 spectrophotometer in KBr discs (Bruker Corporation, Billerica, MA, USA). Specific rotations were measured on JASCO P-1020 digital polarimeter (JASCO Corporation, Tokyo, Japan). ESIMS were obtained on Thermo Scientific LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). CD spectra were measured on JASCO J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were recorded on Agilent 500 MHz DD2 spectrometer using TMS as internal standard and chemical shifts were recorded as δ-values (Agilent Technologies Inc., Santa Clara, CA, USA). Semi-preparative HPLC was performed on an ODS column (HPLC (YMC-Pack ODS-A, 10 × 250 mm, 5 µm, 3 mL/min)) (YMC Co., Ltd., Kyoto, Japan). Medium-pressure preparation liquid chromatography (MPLC) was performed on a Bona-Agela CHEETAHTM HP100 (Beijing Agela Technologies Co., Ltd., Beijing, China). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Amersham Biosciences, San Francisco, CA, USA), respectively [27].

3.2. Fungal Material

The fungal strain *P. steckii* HDN13-279 was isolated from the leaf of *Sonneratia caseolaris* collected from mangrove conservation area of Hainan, China. It was identified by ITS sequence and the sequence data have been submitted to GenBank (accession number: KY399997). The voucher specimen was deposited in our laboratory at −20 °C.

3.3. Fermentation and Extraction

The fungus *P. steckii* HDN13-279 was cultured at 28 °C on a rotary platform shakers at 180 rpm for 9 days in 500 mL Erlenmeyer flasks containing 150 mL of liquid culture medium, composed of maltose (20.0 g/L), mannitol (20.0 g/L), glucose (10.0 g/L), monosodium glutamate (10.0 g/L), MgSO₄·7H₂O (0.3 g/L), KH₂PO₄ (0.5 g/L), yeast extract (3.0 g/L), corn steep liquor (1.0 g/L), and seawater (Huiquan Bay, Yellow Sea, Qingdao, China). After 9 days of cultivation, 45 L of whole broth was filtered through cheesecloth to separate the supernatant from the mycelia. The supernatant was extracted three times
with EtOAc. The water-containing mycelia was extracted three times with acetone, and the mixed solution was concentrated under reduced pressure to afford an aqueous solution without acetone, then extracted three times with EtOAc. Both EtOAc solutions were combined and concentrated under reduced pressure to give the organic extract (40 g) [28].

3.4. Isolation

The organic extract was subjected to vacuum liquid chromatography over a silica gel column using a gradient elution with petroleum ether-CHCl₃-MeOH to give six fractions (fractions 1–6). Fraction 3 (5.1 g) eluted with 98:2 CH₂Cl₂-MeOH was applied on a C-18 ODS column using a stepped gradient elution of MeOH-H₂O yielding five subfractions (fractions 3.1–3.5). Fraction 3.3 that eluted with 50:50 MeOH-H₂O was separated by semi-preparative HPLC using a stepped gradient elution of CH₂CN-H₂O (25:75 to 65:35, 3 mL/min) to furnish Compounds 1 (10.0 mg, tR 14.5 min), 11 (7.0 mg, tR 16.7 min), 5 (4.0 mg, tR 19.2 min), 10 (5.0 mg, tR 23.8 min), 2 (4.0 mg, tR 25.5 min), and 9 (4.0 mg, tR 28.2 min). Fraction 3.4 that eluted with 75:25 MeOH-H₂O was chromatographed on Sephadex LH-20 CH₂Cl₂-MeOH (1:1) and further separated by MPLC (C-18 ODS) using MeOH-H₂O (70:30) to furnish four subfractions (fractions 3.4.1–3.4.4). Fraction 3.4.1 was purified by semi-preparative HPLC (63:37 MeCN:H₂O, 3 mL/min) to afford Compounds 3 (14.0 mg, tR 20.5 min) and 4 (15.0 mg, tR 24.2 min). Fraction 3.4.2 was purified by semi-preparative HPLC (73:27 MeOH:H₂O, 3 mL/min) to afford Compounds 14 (8.0 mg, tR 15.4 min), 6 (5.0 mg, tR 21.4 min), 12 (10.0 mg, tR 24.4 min), and 7 (18.0 mg, tR 27.5 min). Fraction 3.4.4 was purified by semi-preparative HPLC (80:20 MeOH:H₂O, 3 mL/min) to afford Compounds 13 (20.0 mg, tR 16.4 min) and 8 (25.0 mg, tR 20.4 min).

**Tanzawaic acid R (1):** Pale yellow oil; [α]D²⁰ +49.4 (c 0.57, MeOH); CD (1.63 × 10⁻³ M, MeOH) λ [nm] (Δc): 263 (76.2), 219 (−2.9); IR (KBr) νmax 3415, 2921, 1685, 1413, 1207, 1005, 724 cm⁻¹; UV (MeOH) λmax (log ε): 221 (1.10), 268 (4.06) nm; ¹H and ¹³C NMR data, see Table 1; HRESIMS [M − H]⁻ m/z 305.1750 (calcd. for C₁₉H₂₅O₄, 305.1747).

**Tanzawaic acid S (2):** Pale yellow oil; [α]D²⁰ +126.4 (c 0.25, MeOH); CD (1.56 × 10⁻³ M, MeOH) λ [nm] (Δc): 263 (85.8), 220 (−10.1); IR (KBr) νmax 3414, 2927, 1684, 1412, 1006, 622 cm⁻¹; UV (MeOH) λmax (log ε): 221 (1.04), 268 (4.01) nm; ¹H and ¹³C NMR data, see Table 1; HRESIMS [M − H]⁻ m/z 319.1906 (calcd. C₁₉H₂₇O₄, 319.1915).

**Tanzawaic acid T (3):** Pale yellow oil; [α]D²⁰ +98.2 (c 0.27, MeOH); CD (1.64 × 10⁻³ M, MeOH) λ [nm] (Δc): 266 (102.4), 216 (−5.6); IR (KBr) νmax 3416, 2910, 1688, 1453, 1272, 1092, 503, 562 cm⁻¹; UV (MeOH) λmax (log ε): 221 (1.05), 261 (3.99) nm; ¹H and ¹³C NMR data, see Table 2; HRESIMS [M − H]⁻ m/z 303.1962 (calcd. C₁₉H₂₇O₃, 303.1955).

**Tanzawaic acid U (4):** Pale yellow oil; [α]D²⁰ +66.9 (c 0.20, MeOH); CD (1.64 × 10⁻³ M, MeOH) λ [nm] (Δc): 266 (96.0), 220 (−10.1); IR (KBr) νmax 2949, 2910, 1688, 1417, 1272, 1092, 1003 cm⁻¹; UV (MeOH) λmax (log ε): 221 (1.02), 261 (3.96) nm; ¹H and ¹³C NMR data, see Table 2; HRESIMS [M − H]⁻ m/z 303.1962 (calcd. C₁₉H₂₇O₃, 303.1955).

**Tanzawaic acid V (5):** Pale yellow oil; [α]D²⁰ +18.9 (c 0.30, MeOH); CD (1.63 × 10⁻³ M, MeOH) λ [nm] (Δc): 275 (4.9), 253 (−2.2), 224 (1.4); IR (KBr) νmax 3396, 2929, 1689, 1377, 1152, 1011, 732 cm⁻¹; UV (MeOH) λmax (log ε): 221 (2.30), 239 (3.56) nm, 284 (4.06) nm; ¹H and ¹³C NMR data, see Table 3; HRESIMS [M − H]⁻ m/z 305.1751 (calcd. for C₁₉H₂₅O₅, 305.1751).

**Tanzawaic acid W (6):** Pale yellow oil; [α]D²⁰ −5.9 (c 0.17, MeOH); CD (1.63 × 10⁻³ M, MeOH) λ [nm] (Δc): 298 (2.6), 258 (−9.6), 227 (1.5); IR (KBr) νmax 3416, 2924, 1702, 1459, 1377, 1261, 1013, 748 cm⁻¹; UV (MeOH) λmax (log ε): 221 (1.20), 264 (4.09) nm; ¹H and ¹³C NMR data, see Table 3; HRESIMS [M − H]⁻ m/z 305.1752 (calcd. for C₁₉H₂₅O₅, 305.1747).

**Tanzawaic acid C (7):** CD (1.72 × 10⁻³ M, MeOH) λ [nm] (Δc): 251 (−1.7), 227 (0.5), 220 (−3.2).

**Tanzawaic acid B (8):** CD (1.82 × 10⁻³ M, MeOH) λ [nm] (Δc): 270 (12.7), 220 (−3.2).
**Tanzawaic acid M (9):** CD (1.72 × 10⁻³ M, MeOH) λ [nm] (Δε): 269 (8.0), 220 (–3.7).

**Tanzawaic acid X (11):** pale yellow oil; [α]D²⁰ +131.1 (c 0.20, MeOH); CD (1.75 × 10⁻³ M, MeOH) λ [nm] (Δε): 294 (28.5), 238 (–4.0), 218 (3.3); IR (KBr) νmax 3392, 2925, 1695, 1390, 1273, 996, 871, 808 cm⁻¹; UV (MeOH) λmax (log ε): 221 (3.22), 239 (3.50) nm, 293 (4.09) nm; ¹H and ¹³C NMR data, see Table 3; HRESIMS [M − H]⁻ m/z 285.1493 (calcd. for C₁₈H₂₁O₃, 285.1485).

**Arohnapepe B (12):** CD (1.75 × 10⁻³ M, MeOH) λ [nm] (Δε): 292 (26.5), 235 (–3.6), 217 (1.6).

**Tanzawaic acid A (13):** CD (1.85 × 10⁻³ M, MeOH) λ [nm] (Δε): 295 (31.5), 236 (–4.6), 217 (0.1).

**Tanzawaic acid D (14):** CD (1.75 × 10⁻³ M, MeOH) λ [nm] (Δε): 291 (33.7), 237 (–3.7), 218 (7.1).

3.5. **Crystal Data for 1a**

Orthorhombic, C₄₆H₇₇O₄, space group P2₁2₁2₁, a = 7.69910 (10) Å, b = 12.8933 (2) Å, c = 18.6839 (3) Å, V = 1854.69 (5) Å³, Z = 4, T = 290 (2) K, μ (CuKα) = 0.635 mm⁻¹, Dcalcd. = 1.147 g/cm³, 13,363 reflections measured (8.332° ≤ 2θ ≤ 139.834°), 3474 unique (Rint = 0.0298, Rsigma = 0.0268) which were used in all calculations. The final R₁ was 0.0383 and wR₂ was 0.0955 (I > 2σ(I)). Flack parameter = 0.02 (11).

3.6. **Crystal Data for 7**

Orthorhombic, C₁₈H₂₅O₃, space group P2₁2₁2₁, a = 7.1226 (2) Å, b = 12.8012 (3) Å, c = 37.9453 (12) Å, V = 3459.77 (17) Å³, Z = 8, T = 291 (2) K, μ (CuKα) = 0.589 mm⁻¹, Dcalcd. = 1.115 g/cm³, 30,947 reflections measured (7.288° ≤ 2θ ≤ 142.662°), 6596 unique (Rint = 0.0332, Rsigma = 0.0254) which were used in all calculations. The final R₁ was 0.0408 and wR₂ was 0.0982 (I > 2σ(I)). Flack parameter = 0.06 (9).

3.7. **Crystal Data for 8**

Orthorhombic, C₁₈H₂₆O₂, space group P2₁2₁2₁, a = 7.2748 (2) Å, b = 12.7225 (3) Å, c = 36.8100 (12) Å, V = 3406.90 (17) Å³, Z = 8, T = 289 (2) K, μ (CuKα) = 0.527 mm⁻¹, Dcalcd. = 1.070 g/cm³, 22,920 reflections measured (7.352° ≤ 2θ ≤ 140.302°), 6368 unique (Rint = 0.0271, Rsigma = 0.0284) which were used in all calculations. The final R₁ was 0.0448 and wR₂ was 0.1130 (I > 2σ(I)). Flack parameter = 0.07 (10).

3.8. **X-ray Crystallographic Analysis of Compound 1a, 7 and 8**

Crystals of 1a, 7 and 8 were obtained in the mixed solvent of CHCl₃-MeOH, and crystallographic data for 1a, 7 and 8 (Cu Kα radiation) have been deposited in the Cambridge Crystallographic Data Center with the deposition numbers CCDC 1537543, 1537544 and 1537542, respectively. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via the link of reference [29].

3.9. **Esterification of 1**

To a solution of 1 (3.8 mg) in MeOH (0.5 mL) was added excess TMS-CH₂₂ in n-hexane (300 μL), and the mixture was stirred at r. t. for 30 min. Then the reaction mixture was concentrated in vacuo and the residue was purified by preparative HPLC (MeOH: H₂O = 50–100%) to yield methyl ester 1a (2.0 mg). 1a: ¹H NMR (500 MHz, Methanol-d₄) δ 7.26 (1H, dd, J = 11.1, 15.4 Hz, H-3), 6.33 (1H, dd, J = 11.0, 15.3 Hz, H-4), 6.05 (1H, dd, J = 9.5, 15.3 Hz, H-5), 5.85 (1H, d, J = 15.4 Hz, H-2), 5.82 (1H, d, J = 6.5 Hz, H-14), 3.82 (1H, dd, J = 1.7, 6.5 Hz, H-13), 3.71 (3H, s, -CH₃), 3.36 (2H, d, J = 6.3 Hz, H-17), 2.61 (1H, t, J = 7.9 Hz, H-6), 1.68 (1H, overlap, H-11a), 1.66 (1H, overlap, H-9a), 1.60 (3H, s, H₂-16), 1.60 (1H, overlap, H-10), 1.36 (1H, overlap, H-8), 1.33 (1H, overlap, H-7), 1.28 (1H, overlap, H-12), 1.12 (1H, q, J = 12.1 Hz, H-11b), 0.96 (3H, d, J = 6.3 Hz, H₃-18), 0.73 (1H, q, J = 12.0 Hz, H-9b).

3.10. **Methylation of 1 and 2**

To a solution of 1 (1.6 mg) or 2 (1.6 mg) and excess NaH in DMF (0.2 mL) were added excess CH₃I, and the mixtures were stirred at 80 °C for 6 h. Then the reaction mixtures were filtered and purified by
preparative HPLC (MeOH:H$_2$O = 50–100%) to yield $1b$ (0.8 mg from $1$ and 0.7 from $2$), respectively.

$1b$: $^1$H NMR (500 MHz, Methanol-d$_4$) δ 7.24 (1H, dd, $J = 11.6, 15.3$ Hz, H-3), 6.30 (1H, dd, $J = 11.0, 15.3$ Hz, H-4), 5.96 (1H, overlap, H-14), 5.96 (1H, overlap, H-5), 5.81 (1H, d, $J = 15.4$ Hz, H-2), 3.45 (1H, d, $J = 5.9$ Hz, H-13), 3.33 (3H, s, OCH$_3$-13), 3.31 (3H, s, OCH$_3$-17), 3.21 (2H, d, $J = 6.3$ Hz, H-2), 2.61 (1H, dd, $J = 4.6, 8.8$ Hz, H-6), 1.70 (1H, overlap, H-11a), 1.68 (1H, overlap, H-9a), 1.64 (3H, s, H$_3$-16), 1.61 (1H, overlap, H-10), 1.31 (1H, overlap, H-8), 1.31 (1H, overlap, H-7), 1.31 (1H, overlap, H-12), 1.19 (1H, q, $J = 11.6$ Hz, H-11b), 0.95 (3H, d, $J = 5.7$ Hz, H$_3$-18), 0.75 (1H, q, $J = 10.8$ Hz, H-9b). $^{13}$C NMR (125 MHz, Methanol-d$_4$) δ 174.7, 144.9, 140.4, 139.6, 130.4, 127.0, 123.1, 78.3, 76.3, 57.6, 55.3, 50.0, 44.5, 42.5, 39.7, 39.7, 37.5, 33.0, 21.2, 21.1. ESI-MS [M − H]$^{-}$/m/z 333.45.

3.11. Reduction of $12$ and $13$

To a solution of $12$ (2.0 mg) or $13$ (2.0 mg) in MeOH (300 µL) were added Pd/C (0.2 mg) under hydrogen atmosphere. After stirring at r.t. for 2 h, the reaction mixtures were evaporated in vacuo, and $12a$ (0.6 mg ([$\alpha$]$^D_{20}$ − 7.78 (c 0.042, MeOH)) from $12$ and 0.6 mg ([$\alpha$]$^D_{20}$ − 6.09 (c 0.042, MeOH)) from $13$ were obtained by semi-preparative HPLC using a gradient solvent system of 30−100% CH$_3$CN/H$_2$O over 30 min, respectively.

$12a$: $^1$H NMR (500 MHz, CDCl$_3$) δ 6.90 (1H, d, $J = 7.6$ Hz), 6.83 (1H, d, $J = 7.6$ Hz), 3.22 (1H, m), 2.72–2.59 (2H, overlap), 2.58 (1H, m), 2.41 (2H, t, $J = 7.5$ Hz), 2.36 (1H, dd, $J = 12.4, 14.1$ Hz), 2.29 (3H, s), 2.19 (1H, m), 1.77 (2H, m), 1.63 (1H, m), 1.51 (2H, m), 1.18 (3H, d, $J = 7.1$ Hz), 1.14 (1H, m), 1.07 (1H, d, $J = 6.6$ Hz). $^{13}$C NMR (125 MHz, CDCl$_3$) δ 178.7, 140.4, 137.9, 136.9, 134.0, 127.5, 126.1, 40.1, 39.0, 33.7, 30.0, 29.9, 29.8, 28.6, 25.6, 25.3, 22.9, 19.8.

3.12. Cell-Based Lipid Accumulation Assay

HepG2 cells, seeded in a 96 wells plate at the concentration of $1 \times 10^5$ cells/well, were cultured in high glucose DMEM medium containing 10% fetal bovine serum (FBS) at 37 °C and 5% CO$_2$. After reaching 90% confluence, cells were incubated with the indicated concentration of compounds (10 µM) or with simvastatin (10 µM) in high glucose DMEM containing OA (100 µM) for 24 h. The blank group was incubated with serum-free high glucose DMEM alone. Oil red O staining was performed as previous reported [30] and the intracellular contents of total cholesterol and triglyceride were determined by kits according to manufacturer’s instructions.

3.13. Assay of Cytotoxicity, Antiviral Activity and NF-κB Inhibitory Activity

These biological evaluations were carried out as previously reported [27,31,32].

3.14. Statistical Analysis

The data of lipid lowering effect were expressed as mean ± SEM, representing at least three different experiments with $n = 8$ in each test. SPSS 17.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Differences were assessed by an unpaired t-test. A probability level ($p$) of 0.05 was considered significant.

4. Conclusions

In summary, seven new tanzawaic acid derivatives, along with seven known compounds were isolated from Penicillium steckii HDN13-279. The absolute configurations of all the compounds (including the known compounds of which the absolute configurations were not confirmed in the literature) were determined by NMR, X-ray diffraction, CD analyses, as well as chemical derivatization. In addition, for the first time, we evaluated the lowering effects against oleic acid (OA)-elicited lipid accumulation in HepG2 liver cells of this kinds of compounds, and five ($2$, $3$, $6$, $10$ and $12$) showed pharmaceutical potential with lipid-lowering activity.

Supplementary Materials: The following are available online at www.mdpi.com/1660-3397/16/1/25/s1, 1D and 2D NMR spectra, IR spectra of $1$–$6$, $11$; $^1$H NMR spectrum of $1a$; 1D NMR spectra of $1b$ and $12a$. Figures S1–S7:
1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid R (1), Figures S8–S14: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid S (2), Figures S15–S21: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid T (3), Figures S22–S28: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid U (4), Figures S29–S35: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid V (5), Figures S36–S42: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid W (6), Figures S43–S49: 1D, 2D NMR, NOESY and HRESIMS spectra of tanzawaic acid X (11), Figure S50: 1H NMR spectrum of 1a, Figures S51–S52: 1D NMR spectra of 1b, Figures S53–S54: 1D NMR spectra of 12a, Figures S55–S61: IR spectrum of tanzawaic acids R–X (1–6, 11).

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