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Penipyridones A–F, Pyridone Alkaloids from *Penicillium funiculosum*

Haibo Zhou,[†,⊥] Liyuan Li,[†,⊥] Chongming Wu,[‡] Tibor Kurtán,[§] Attila Mándi,[§] Yankai Liu,[†] Qianqun Gu,[†] Tianjiao Zhu,[*,†] Peng Guo,[*,‡] and Dehai Li[*,†]

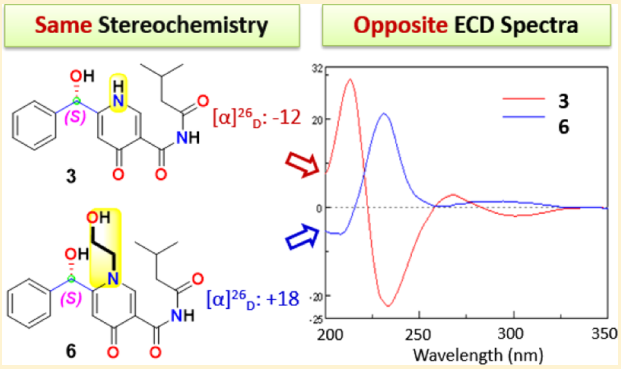
[†]Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China

[‡]Pharmacology and Toxicology Research Center, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, People's Republic of China

[§]Department of Organic Chemistry, University of Debrecen, POB 20, 4010 Debrecen, Hungary

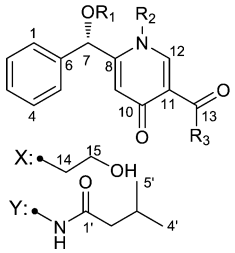
Supporting Information

ABSTRACT: Six new pyridone alkaloids, named penipyridones A–F (**1**–**6**), were isolated from the fermentation broth of an Antarctic moss-derived fungus, *Penicillium funiculosum* GWT2-24. Their structures were elucidated from extensive NMR and MS data. Although they possess the same major chromophore and some of them presented almost mirror ECD spectra, their absolute configurations were found to be uniformly *S*, as evidenced by X-ray single-crystal diffraction analysis, stereocontrolled total synthesis, and chemical conversions. TDDFT-ECD calculations of compounds **3** and **6** revealed that subtle conformational changes are responsible for the significantly different ECD curves. None of the compounds were cytotoxic (IC_{50} > 50 μM), while compounds **1**, **2**, **5**, and **7** elicited lipid-lowering activity in HepG2 hepatocytes.



Structure elucidation, especially the determination of stereochemistry, is essential to natural products research. All current methods, X-ray diffraction, chemical synthesis, electronic circular dichroism (ECD), and NMR, have unique advantages and limitations. Among them, X-ray diffraction is the most reliable. In recent years, ECD especially assisted by quantum mechanical computations has found broad applicability and has been widely used to solve the absolute configurations of complex natural products.[1] In fact, the ECD data depend on both the conformation and the absolute configuration.[2]

During our exploration of bioactive molecules from Antarctic-derived fungi,[3] a series of alkaloids with the unusual phenylpyridone skeleton were isolated, including six new compounds, which we named penipyridones A–F (**1**–**6**), and the known berkeleyamide C (**7**).[4] Although **1**–**7** have the same skeleton and they all possess only one stereogenic center, their ECD spectra are significantly different. In particular, **3** and **6** have the same chromophore, yet they present almost mirror image ECD curves. All the compounds proved to have the same absolute configurations by X-ray diffraction, total synthesis, and chemical conversions. On the grounds of time-dependent density functional theory (TDDFT)-ECD analysis, the differences in the ECD curves of the closely related analogues (**3** and **6**) were explained by the different relative arrangement of the two isolated chromophores, influenced by the different substitution pattern of attached achiral groups. In addition, compounds **1**, **2**, **5**, and **7** showed lipid-lowering activity. Herein, we report the isolation, structural determination, and bioactivity of these compounds.



1 R_1 = H R_2 = H R_3 = NH_2
2 R_1 = H R_2 = H R_3 = OCH_3
3 R_1 = H R_2 = H R_3 = Y
4 R_1 = H R_2 = X R_3 = NH_2
5 R_1 = $COCH_3$ R_2 = X R_3 = NH_2
6 R_1 = H R_2 = X R_3 = Y
7 R_1 = $COCH_3$ R_2 = X R_3 = Y

RESULTS AND DISCUSSION

The fungal *Penicillium funiculosum* strain GWT2-24 was fermented (30 L) under static conditions.[3e] The EtOAc extract of the fermentation was fractionated by Sephadex LH-20 chromatography, ODS MPLC, and finally HPLC to yield compounds **1** (49 mg), **2** (3 mg), **3** (28 mg), **4** (24 mg), **5** (31 mg), **6** (36 mg), and **7** (25 mg).

Penipyridone A (**1**) was isolated as a white solid and has the molecular formula $C_{13}H_{12}O_3N_2$ as evidenced by the HRESIMS protonated ion at *m*/*z* 245.0926. The 1D NMR data (Table 1) revealed the presence of 13 carbons, including two carbonyls (δ_C 178.0, 165.9), one oxymethine (δ_C 71.3), and 10 olefinic carbons with two of them (153.9, 142.0) attached to nitrogen. These data were similar to those for aspernigrin A,[5] with the replacement of a methylene in asperningrin A by a hydroxylated

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of Compounds 1−3 in DMSO-d_6

	1		2		3	
no.	δ_C	δ_H (Jin Hz)	δ_C	δ_H (Jin Hz)	δ_C	δ_H (Jin Hz)
1/5	127.0, CH	7.45, d (7.7)	126.8, CH	7.40, d (7.1)	126.2, CH	7.44, d (7.1)
2/4	129.0, CH	7.38, dd (7.1, 7.7)	129.1, CH	7.33, dd (7.1, 7.7)	128.2, CH	7.36, dd (7.1, 7.8)
3	128.5, CH	7.30, dd (7.7, 7.1)	128.5, CH	7.26, dd (7.7, 7.1)	127.6, CH	7.30, dd (7.1, 7.8)
6	142.4, C		142.1, C		141.6, C	
7	71.3, CH	5.66, d (4.0)	72.4, CH	5.54, s	70.8, CH	5.67, s
8	153.9, C		157.0, C		155.2, C	
9	116.5, CH	6.35, s	116.1, CH	6.38, s	115.4, CH	6.47, s
10	178.0, C		176.2, C		177.1, C	
11	118.4, C		116.3, C		115.8, C	
12	142.0, CH	8.29, s	145.8, CH	8.25, s	143.8, CH	8.40, s
13	165.9, C		166.6, C		162.8, C	
1′			52.2, CH_3	3.69, s	173.3, C	
2′					46.3, CH_2	2.59, d (7.2)
3′					24.2, CH	2.07, m
4′/5′					22.0, CH_3	0.92, d (6.6)
7-OH		6.62, d (4.0)				6.58, brs
8-NH		12.06, s				
13-NH		9.50, d (4.4); 7.41, d (4.4)				13.11, s

methine (δ_H/δ_C 5.66/71.3) in **1**, which was further confirmed by COSY (7-OH/H-7) and HMBC correlations from H-7 to C-1/5, C-6, C-8, and C-9 (Figure 1). The absolute configuration of C-7 in **1** was determined as *S* by X-ray diffraction analysis using Cu Kα radiation with a Flack parameter of −0.03(13) (Figure 2).

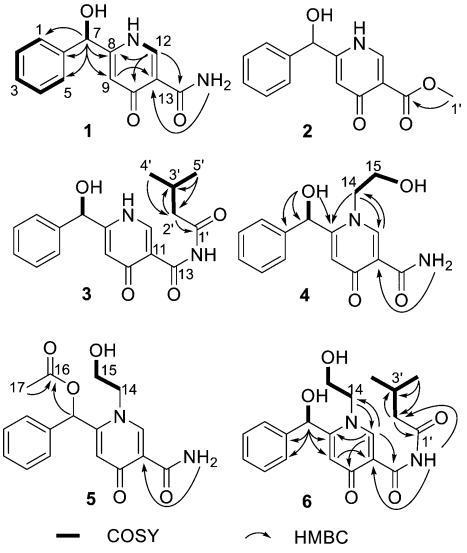

Figure 1. Key COSY and HMBC correlations of **1**−**6**.

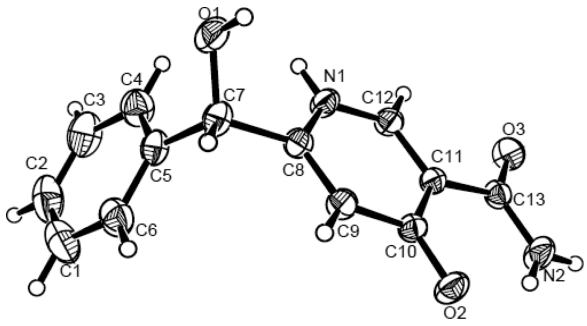

Figure 2. X-ray ORTEP diagram of compound **1**.

Penipyridones B (**2**) and C (**3**) were also isolated as white solids. The 1D NMR data of **2** and **3** (Table 1) showed that they share the same 2-benzylpyridin-4(1*H*)-one skeleton as **1**. The main differences between them and **1** were the replacement of the amine group in **1** by a methoxy group (δ_H/δ_C 3.69/52.2) in **2** and by an isovaleryl amide in **3**, which were in agreement with the 2D NMR correlations (Figure 1), as well as the chemical shifts.[4]

Penipyridones D−F (**4**−**6**) were also found to have similar NMR data (Table 2) to **1**. Compared to **1**, compound **4** showed additional signals of a hydroxyethyl group, which was located on the nitrogen of the pyridinone ring. The main difference between **4** and **5** was the presence of an additional acetate group attached to oxygen at C-7 in **5**, consistent with the significant downfield shift of H-7 (from δ_H 5.91 to δ_H 6.95). The NMR data also demonstrated that compound **6** was a hydroxyethylation derivative of **3**. The planar structures of **4**−**6** were further confirmed from the 2D NMR correlations (Figure 1). Compound **7** was determined to be the known compound berkeleyamide C, whose absolute configuration has not been reported yet.[4]

In order to determine the absolute configurations of **2**−**7**, comparisons of their ECD spectra and specific rotation values were initially performed. However, their ECD curves were not consistent, especially those of **1**−**3**, which presented almost opposite spectra of **6** and **7** (Figure 3). Similarly, the specific rotations of compounds **1**−**3** were negative, while those of **4**−**7** were positive. Indeed, the occurrence of natural product analogues with opposite absolute configurations has been observed previously.[3a]

To solve the absolute configurations of **2**−**7** thoroughly, we employed total synthesis and chemical interconversion. Compound **2** was synthesized starting from (*S*)-(+)-mandelic acid. The secondary alcohol of mandelic acid was acetylated, after which treatment with oxalyl chloride yielded **8**,[6] which was reacted with **9** to afford **10**.[7,8] Finally, compound (*S*)-(−)-**2** (**2a**) was obtained by deacetylation of **10** (Scheme 1). Using the same method, enantiomer (*R*)-(+)-**2** (**2b**) was also synthesized from (*R*)-(+)-mandelic acid. The absolute configuration of the natural **2** was determined as 7*S* from its identical ECD spectra to synthetic **2a** (Figure S3, Supporting Information, SI). Conversion

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Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of 4−6 in DMSO-d_6.

	4		5		6	
no.	δ_C	δ_H (Jin Hz)	δ_C	δ_H (Jin Hz)	δ_C	δ_H (Jin Hz)
1/5	127.5, CH	7.35, d (7.1)	128.3, CH	7.43[a]	127.5, CH	7.38[a]
2/4	129.2, CH	7.40, dd (7.1, 7.8)	129.8, CH	7.43[a]	129.2, CH	7.38[a]
3	128.6, CH	7.32, dd (7.1, 7.8)	129.6, CH	7.43[a]	128.7, CH	7.38[a]
6	141.2, C		136.2, C		140.9, C	
7	70.6, CH	5.91, s	71.6, CH	6.95, s	70.5, CH	5.95, d (5.4)
8	154.4, C		150.2, C		155.5, C	
9	120.0, CH	6.38, s	119.7, CH	6.45, s	120.3, CH	6.53, s
10	177.2, C		177.0, C		177.2, C	
11	118.3, C		118.7, C		116.1, C	
12	148.4, CH	8.45, s	148.6, CH	8.48, s	150.0, CH	8.58, s
13	165.8, C		165.5, C		163.1, C	
14	55.1, CH_2	4.08, m	55.5, CH_2	4.05, m	55.5, CH_2	4.15, m
15	60.9, CH_2	3.48, m	60.5, CH_2	3.61/3.52, m	60.6, CH_2	3.48, m
16			169.9, C			
17			21.2, CH_3	2.18, s		
1′					174.1, C	
2′					47.1, CH_2	2.61, d (7.2)
3′					25.1, CH	2.08, m
4′/5′					22.9, CH_3	0.92, d (6.6)
7-OH		6.51, d (4.0)				6.68, brs
13-NH		9.50, d (4.9); 7.46, d (4.9)		9.41, d (4.4); 7.51, d (4.4)		12.95, s
15-OH		5.12, s		5.17, brs		5.20, brs

[a]Signals were overlapped.

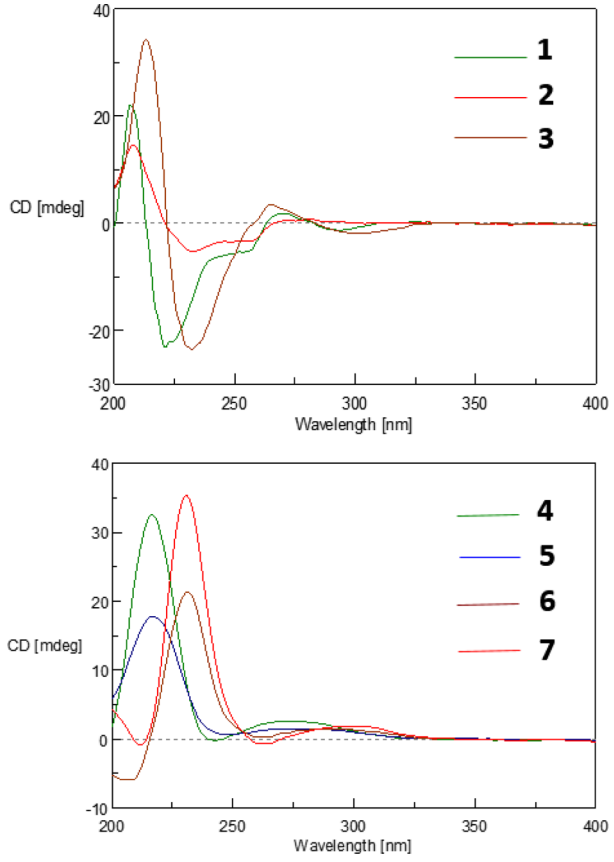

Figure 3. ECD spectra of compounds 1−7.

of 1 and 3 to 11 via acidic hydrolysis (Scheme 1) established the absolute configuration of 3 as 7*S*, the same as 1. Similarly, compounds 4−7 were also converted into the synthetic 13, which can be derived from the intermediate (*S*)-10 (Scheme 1), indicating that they shared the same 7*S* absolute configuration.

In order to reveal the origin of the significant differences in the ECD spectra of the homochiral compounds 1−7 and to check the applicability of ECD calculations for the configurational assignment, TDDFT-ECD calculations were carried out using both gas-phase and solution conformers of the two representative compounds 3 and 6. Compounds 3 and 6 have the same chromophore with considerable conformational freedom around the C-6 to C-8 single bonds. The relative arrangement of the two isolated chromophores and hence the conformations adopted by rotation around the above bonds were expected to govern the ECD properties. The initial MMFF conformational search of (*S*)-3 resulted in 42 conformers within a 21 kJ/mol energy window, the reoptimization of which using both the B3LYP/6-31G(d) *in vacuo* and B97D/TZVP[9] with PCM MeOH solvent basis sets afforded three (Figure 4) and seven (Figure S4, SI) low-energy conformers over 2% population, respectively.

In the lowest energy B3LYP/6-31G(d) *in vacuo* conformer, A (population 53.6%), the characteristic $\omega_{7\text{-H,C-7,C-6,C-1}}$ and $\omega_{7\text{-H,C-7,C-8,N}}$ torsional angles were −3.8° and −8.9°, respectively, which implies near coplanarity between the H-7−C-7 bond and the phenyl ring, as well as between the O-7−C-7 bond and the pyridinone. The experimental ECD spectrum of 3 showed negative Cotton effects at 301 and 233 nm and positive Cotton effects at 268 and 213 nm. The ECD spectrum of the lowest energy conformer, A, computed with different methods (BH&HLYP/TZVP, B3LYP/TZVP, and PBE0/TZVP) did not reproduce well the experimental ECD data, and additionally the BH&HLYP/TZVP ECD spectrum was quite different from those from the other two methods (Figure 5a). In conformer B (35.8%), the relative orientation of the two chromophores was slightly different from that of conformer A, with $\omega_{7\text{-H,C-7,C-6,C-1}}$ and $\omega_{7\text{-H,C-7,C-8,N}}$ torsional angles of 17.4° and 17.0°, respectively. However, these small changes in the torsional angles of conformer B made the computed ECDs

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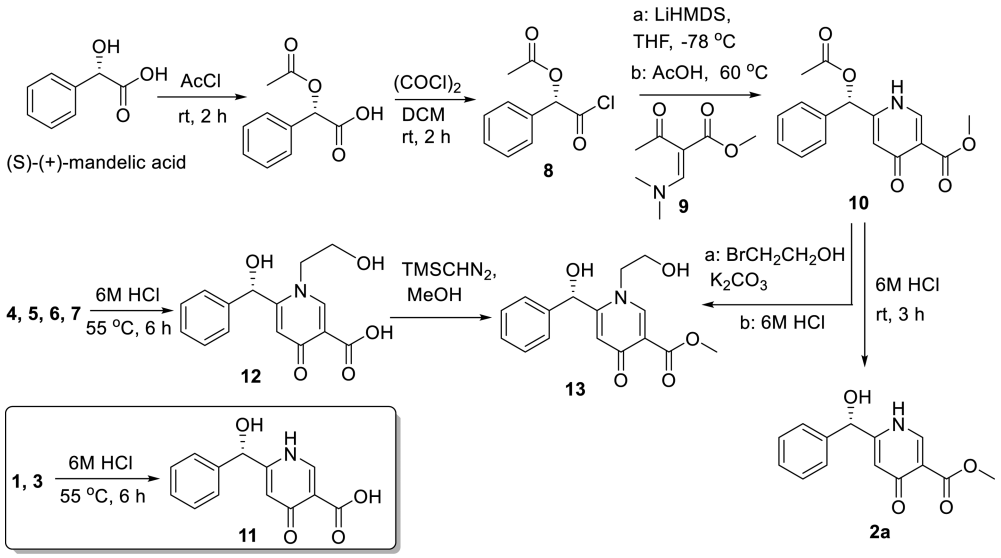
Scheme 1. Synthesis of Compounds 2a and 13 and Acid Hydrolysis of Compounds 1 and 3−7



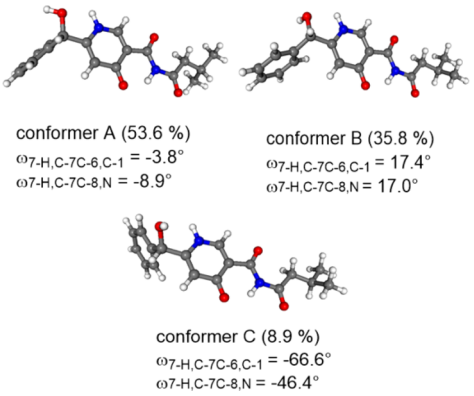


Figure 4. Structure, population, and characteristic torsional angles of the low-energy B3LYP/6-31G(d) *in vacuo* conformers of (*S*)-**3**.

nearly congruent with the experimental curves, with all three methods giving consistent results (Figure 5b). The geometry of conformer C (8.9%) was markedly different from those of the lower energy conformers, as demonstrated by the −66.6° and −46.4° values for the $\omega_{7\text{-H,C-7,C-6,C-1}}$ and $\omega_{7\text{-H,C-7,C-8,N}}$ torsional angles. In this conformer, the H-7−C-7 bond was near coplanar with the pyridinone and the O-7−C-7 bond with the phenyl ring, which again gave rise to computed ECD spectra completely different from the experimental one (Figure 5c).

The comparison of the Boltzmann-weighted computed ECD spectra of (*S*)-**3** with the experimental ECD (Figure 5d) suggested the *S* absolute configuration for **3**, but the positive Cotton effect at 268 nm was completely missing from the computed spectra, and agreement was far from perfect. This could be clearly attributed to the lower estimated population of conformer B *in vacuo*, which presumably is the dominant conformer in solution based on the closer resemblance of its calculated ECD spectrum to that obtained experimentally. Interestingly, the seven B97D/TZVP-reoptimized conformers with the PCM solvent model for MeOH (Figure S5, SI) were all different from the *in vacuo* conformer B, and the Boltzmann-weighted computed ECD spectra of the B97D/TZVP (PCM) conformers showed even less agreement with the experimental ECD than the *in vacuo* spectra.

The ECD spectrum of **6** in methanol showed positive Cotton effects at 295 (broad) and 231 (sharp) nm and negative Cotton effect at 208 nm. The initial MMFF conformational search of (*S*)-**6** resulted in 219 conformers, the reoptimization of which yielded seven low-energy conformers above 2% at the B3LYP/6-31G(d) level *in vacuo* (Figure S5, SI). In the lowest energy conformer A of (*S*)-**6**, the H-7−C-7 bond is *syn*-coplanar with the H-9−C-9 bond, while the O-7−C-7 bond is almost coplanar with the plane of the benzene ring with 67.4° and 54.7° values for the $\omega_{7\text{-H, C-7, C-6, C-1}}$ and $\omega_{7\text{-H, C-7, C-8, N}}$ torsional angles, respectively (Figure 6a). Similar conformations are adopted by conformers B, E, and F, and thus in the more populated conformers of (*S*)-**6**, the relative arrangements of the two isolated chromophores are different from that of (*S*)-**3**, which is responsible for their markedly different ECD spectra. The different torsional angles of conformers A and B of (*S*)-**6**, the main contributors to the overall ECD spectrum, are stabilized by an intramolecular hydrogen-bonding interaction between 7-OH and 15-OH, which is not possible for (*S*)-**3**. The Boltzmann-weighted TDDFT-ECD spectra calculated for the B3LYP/6-31G(d) *in vacuo* conformers of (*S*)-**6** (Figure 6b) reproduced the main features of the experimental ECD spectrum, confirming the *S* absolute configuration of **6**. The results indicate that achiral substituents significantly modify the electronic properties of the chromophores and the conformational ensemble in compounds **3** and **6**, resulting in different ECD sepctra.

Compounds **1**−**7** are proposed to be biosynthesized from phenylacetic acid (Scheme 2). With elongation of two acetate units and methylation, the intermediate **i** is formed and then transformed to **ii** by generation of a pyridine ring.[4,10] Compound **2** is proposed to be generated by methylation of **ii**. By forming the amide group at C-13 in **ii**, compounds **1**, **4**, and **5** are generated, and compounds **3**, **6**, and **7** are formed by further modification of isovaleryl-CoA originating from leucine.[11]

None of **1**−**7** were cytotoxic (IC_{50} > 50 μM) to K562, HL-60, HeLa, or A-549 cells.[12,13] Compounds **1**−**7** were also screened for lowering of oleic acid (OA)-elicited lipid accumulation in HepG2 hepatocytes. At 10 μM, compounds **1**, **2**,[14] **5**, and **7** significantly decreased intracellular lipid accumulation and both the total cholesterol and triglyceride quantification (Figure 7).

Naturally occurring phenylpyridone alkaloids characterized by the linkage of benzene and 4-pyridone moieties are rare, with

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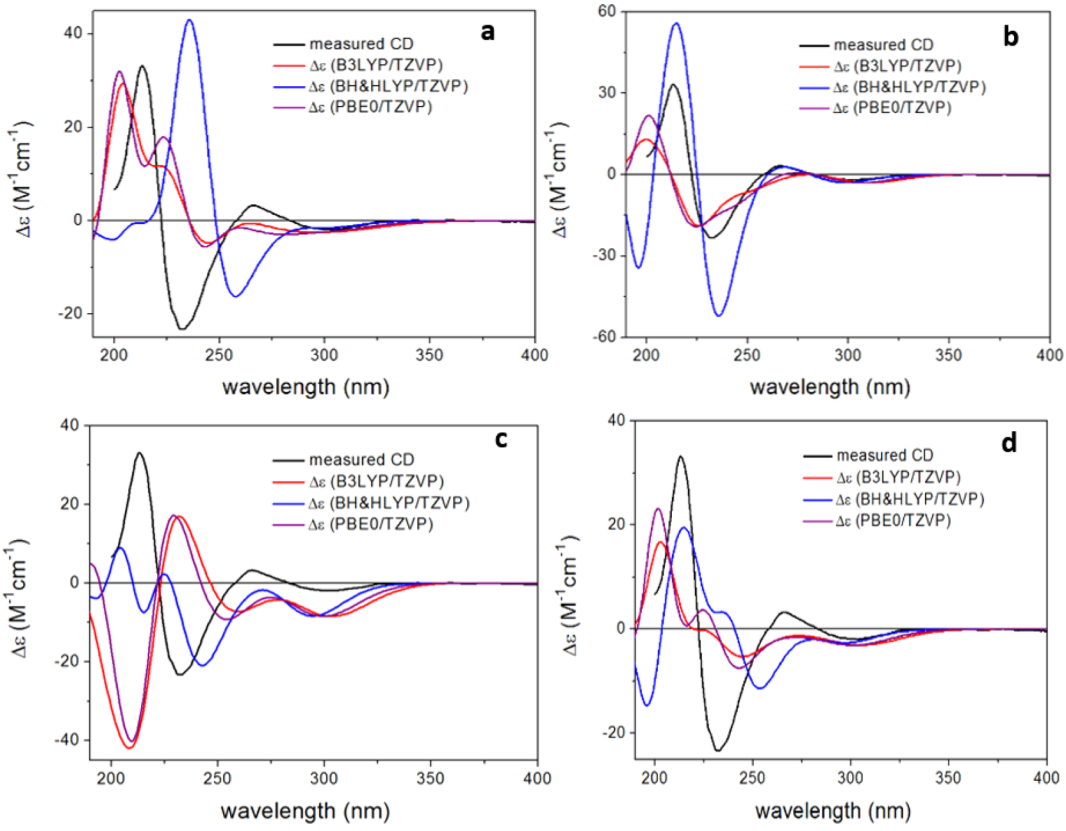


Figure 5. Experimental ECD spectrum of **3** compared with computed ECD spectra of conformer A (53.6%) (a), conformer B (35.8%) (b), conformer C (8.9%) (c), and the Boltzmann-weighted TDDFT-ECD spectra calculated for (*S*)-3 (d).

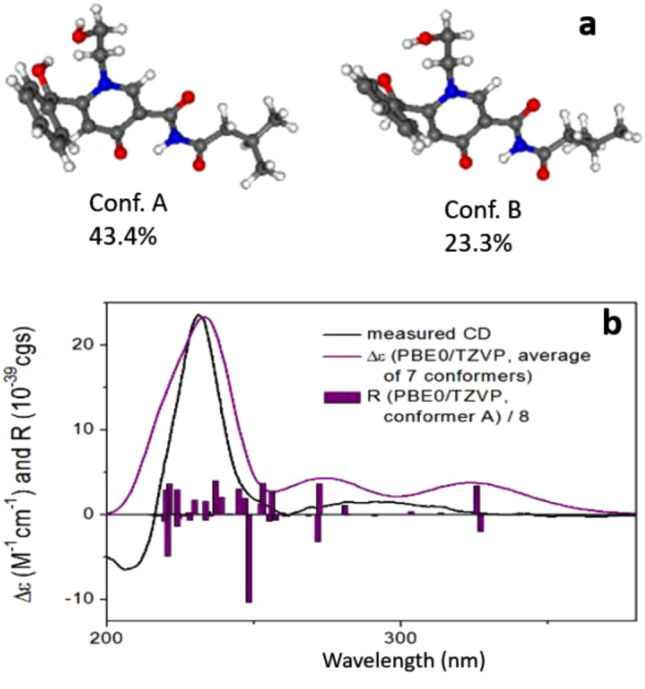
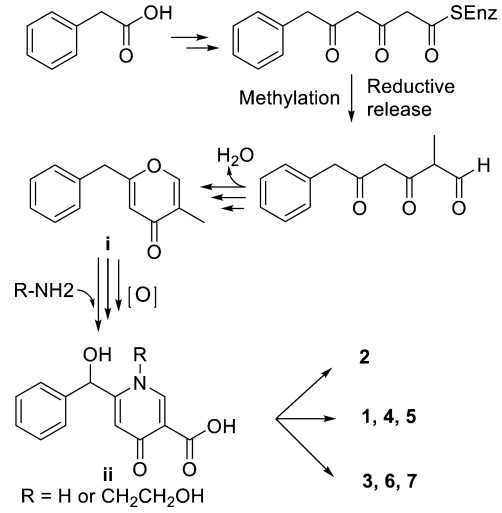


Figure 6. Structure and population of conformers A and B of (*S*)-**6** (a) and experimental ECD spectrum of **6** compared with the calculated one for (*S*)-**6** (b).

Scheme 2. Proposed Biosynthetic Pathway of 1−7



only 11 compounds reported previously from the fungal genera *Penicillium*,[4,15,16] *Aspergillus*,[17−19] *Cladosporium*,[5] and *Pestalotiopsis*.[20] Limited bioactivity data are reported for structural relatives of the penipyridones including berkeleyamide C inhibiting MMP-3 and caspase-14 and aspernigrin B being neuroprotective.[17a] This is the first report of a lipid-lowering effect of this family of alkaloids. Although compounds **1**−**7** possess the same major chromophore and some of them presented almost mirror ECD spectra, their absolute configurations were found to be uniform. Given the popular utilization of comparing experimental and calculated ECD spectra for the assignment of absolute configurations, it may be worth emphasizing repeatedly that simple comparison of ECD spectra for even closely related analogues may lead to the wrong configurational assignment if conformational differences are overlooked.

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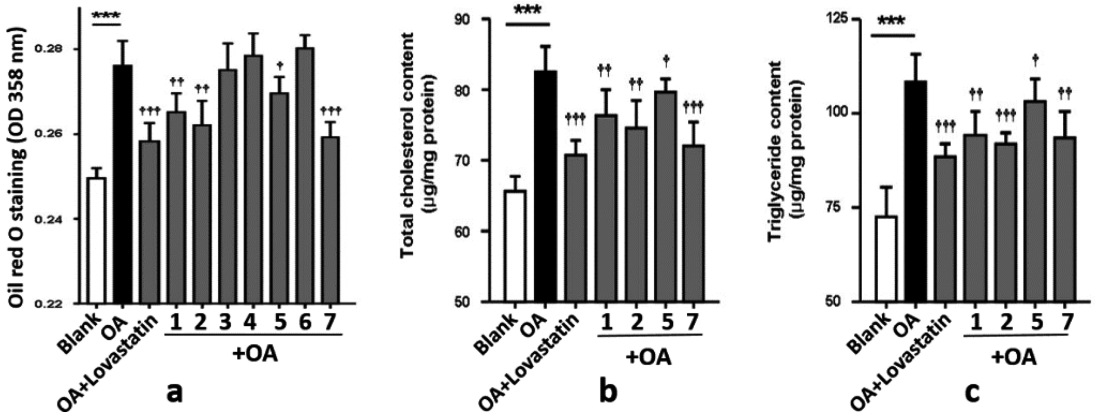

Figure 7. Inhibitory effects of **1**−**7** on oleic acid-elicited intracellular lipid accumulation (a), total cholesterol (b), and triglycerides (c) (lovastatin as positive control). Bars depict the means ± SEM of at least three experiments. ***$p < 0.001$, OA vs blank; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, test group vs OA group.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an RY-1 micromelting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Waters 2487 absorbance detector. ECD spectra were recorded on a JASCO J-815 spectropolarimeter, using MeOH as solvent. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on a JEOL JNMECP 600 spectrometer or an Agilent 500 MHz DD2 spectrometer using tetramethylsilane (TMS) as an internal standard, and chemical shifts were recorded as δ values. ESIMS spectra were measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. HRESIMS spectra were measured on a Micromass EI-4000 (Autospec-Ultima-TOF). X-ray crystal data were measured on an Agilent Gemini Ultra diffractometer (Cu Kα radiation). Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 3 mL/min]. MPLC was performed using an ODS column [25 × 50 cm, 50 μm, 20 mL/min]. TLC and column chromatography were performed on plates precoated with silica gel GF254 (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (GE Healthcare), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory).

Material. The fungal strain *Penicillium funiculosum* GWT2-24 was isolated from an inner part of moss collected around the China Great Wall Station in Antarctica and was identified by the ITS sequence with GenBank accession number JQ670957.[3e] A voucher specimen is deposited in our laboratory at −20 °C. The working strain was prepared on potato dextrose agar slants and stored at 4 °C. Commercial reagents and solvents were purchased from Sigma-Aldrich, Fluka, and Alfa Aesar and used as received, without further purification.

Fermentation and Extraction. The fungus *P. funiculosum* GWT2-24 was cultured and extracted as previously described.[3e]

Purification. The extract (20 g) was subjected to a Sephadex LH-20 column eluting with MeOH, to give four subfractions (Fr.1−Fr.4). Fr.2 was then separated by medium-pressure liquid chromatography (MPLC) with a gradient solvent system MeOH-H_2O (20−100% in 2 h) to afford three subfractions (Fr.2.1−Fr.2.3). Fr.2.3 was chromatographed on a Sephadex LH-20 column eluting with CH_2Cl_2−MeOH (1:1, v/v) and finally purified on semipreparative HPLC (ODS; 5 μm, 250 × 10 mm; MeOH−H_2O, 50:50, v/v; 3 mL/min) to afford compounds **2** (3 mg), **6** (36 mg), and **7** (25 mg). Fr.4 was separated into three subfractions (Fr.4.1−Fr.4.3) by MPLC with a step gradient elution with MeOH−H_2O (20−100% in 2 h). Fr.4.1 was then subjected to a Sephadex LH-20 column eluting with CH_2Cl_2−MeOH (1:1, v/v) and further purified by using semipreparative HPLC with 30% MeOH−H_2O to obtain **1** (49 mg), **4** (24 mg), and **5** (31 mg). Fr.4.3 was purified on semipreparative HPLC with 50% MeOH−H_2O to give **3** (28 mg).

Penipyridone A (1): white solid; mp 262−264 °C; $[\alpha]^{26}_D$ −22 (c 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+2.59), 223 (−4.48), 270 (+0.17), 295 (−0.31) nm; UV (MeOH) λ_{max} (log ε) 212 (1.40), 252 (0.57) nm; IR (KBr) ν_{max} 3355, 1658, 1627, 1543, 1348, 1212, 1187, 1047, 696 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS m/z 245.0926 $[M + H]^+$ (calcd for $C_{13}H_{13}O_3N_2$ 245.0921).

Penipyridone B (2): white solid; $[\alpha]^{26}_D$ −12 (c 0.1, $CHCl_3$); ECD (0.5 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+7.38), 234 (−2.92), 277 (+0.37) nm; UV (MeOH) λ_{max} (log ε) 212 (0.34), 252 (0.12) nm; IR (KBr) ν_{max} 3353, 1715, 1658, 1533, 1350, 1207, 935, 734, 698 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS m/z 260.0923 $[M + H]^+$ (calcd for $C_{14}H_{13}O_4N$ 260.0921).

Penipyridone C (3): white solid; $[\alpha]^{26}_D$ −12 (c 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 213 (+8.81), 233 (−6.77), 268 (+0.88), 301 (−0.56) nm; UV (MeOH) λ_{max} (log ε) 223 (2.26), 297 (0.42) nm; IR (KBr) ν_{max} 3248, 2958, 1742, 1690, 1681, 1642, 1505, 1304, 1190, 1056, 698 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS m/z 329.1494 $[M + H]^+$ (calcd for $C_{20}H_{21}O_4N_2$, 329.1496).

Penipyridone D (4): colorless oil; $[\alpha]^{26}_D$ +16 (c 0.1, $CHCl_3$); ECD (1.2 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+9.87), 280 (+0.75) nm; UV (MeOH) λ_{max} (log ε) 212 (0.58), 262 (0.25) nm; IR (KBr) ν_{max} 3345, 1646, 1538, 1211, 1060, 700 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRESIMS m/z 289.1187 $[M + H]^+$ (calcd for $C_{15}H_{17}O_4N_2$, 289.1183).

Penipyridone E (5): colorless oil; $[\alpha]^{26}_D$ +29 (c 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+5.37), 280 (+0.43) nm; UV (MeOH) λ_{max} (log ε) 209 (1.98), 263 (1.45) nm; IR (KBr) ν_{max} 3333, 2924, 1739, 1690, 1684, 1626, 1547, 1487, 1347, 1190, 746, 698 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRESIMS m/z 331.1290 $[M + H]^+$ (calcd for $C_{17}H_{19}O_5N_2$, 331.1288).

Penipyridone F (6): white solid; $[\alpha]^{26}_D$ +18 (c 0.1, $CHCl_3$); ECD (0.9 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−0.65), 231 (+7.19), 295 (+0.51) nm; UV (MeOH) λ_{max} (log ε) 223 (2.45), 259 (1.36), 300 (0.72) nm; IR (KBr) ν_{max} 3408, 3268, 2957, 1745, 1690, 1638, 1503, 1453, 1426, 1370, 1168, 1056, 883, 702 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRESIMS m/z 373.1756 $[M + H]^+$ (calcd for $C_{20}H_{25}O_5N_2$, 373.1758).

Berkeleyamide C (7): colorless oil; $[\alpha]^{26}_D$ +39 (c 0.1, $CHCl_3$); [lit. $[\alpha]^{25}_D$ +24.9 (c 0.007, $CHCl_3$)];[4] ECD (1.2 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−0.21), 231 (+8.93), 264 (−0.18), 301 (+0.48) nm.

Synthesis of 2a and 2b.[8] A solution of LiHMDS (1.0 M in THF, 3 mL, 3 mmol) was cooled to −78 °C under Ar, and then THF solutions of freshly prepared (*S*)-*O*-acetylmandelic acid chloride **8**[6] (300.0 mg, 4 mL, 1.4 mmol) and **9**[7] (240.0 mg, 4 mL, 1.4 mmol) were added simultaneously over 10 min at −78 °C. After stirring for an additional 30 min, the mixture was warmed to room temperature, and AcOH (3.0 mL) was added. The mixture was then stirred at 60 °C for 30 min and then concentrated under reduced pressure. The residue was diluted with EtOAc and water. The organic phase was separated, washed with saturated aqueous $NaHCO_3$, and concentrated under reduced pressure. Then the residue was purified by MPLC (20−100% MeOH−H_2O in 1 h) and semipreparative HPLC (40% MeOH−H_2O) to yield compound **10** (45 mg). Compound **10** (10 mg) was hydrolyzed in 6 N HCl (1 mL)

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at room temperature for 3 h and finally purified by semipreparative HPLC (30% MeOH−H_2O) to yield compound **2a** (8 mg). By the same procedure, the enantiomers **10** (*ent*-**10**) and **2b** were obtained from (*R*)-(+)-mandelic acid.

Compound 10: colorless oil; $[\alpha]^{26}_D$ +48 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.46 (1H, s), 7.43 (2H, d, *J* = 7.3 Hz), 7.38 (2H, t, *J* = 7.3 Hz), 7.34 (1H, t, *J* = 7.3 Hz), 6.69 (1H, s), 6.61 (1H, s), 3.77 (3H, s), 2.17 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.4, 169.4, 165.9, 152.6, 143.6, 137.7, 128.6 (2C), 128.5, 127.2 (2C), 114.2, 112.4, 74.9, 51.8, 20.8; ESIMS *m*/*z* 302.2 $[M + H]^+$, 324.1 $[M + Na]^+$, 340.1 $[M + K]^+$.

Compound *ent*-10. colorless oil; $[\alpha]^{26}_D$ −42 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (1H, s), 7.42 (2H, d, *J* = 7.3 Hz), 7.37 (2H, t, *J* = 7.3 Hz), 7.34 (1H, t, *J* = 7.3 Hz), 6.68 (1H, s), 6.60 (1H, s), 3.77 (3H, s), 2.17 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.4, 169.4, 165.8, 152.6, 143.4, 137.6, 128.6 (2C), 128.5, 127.2 (2C), 114.1, 112.4, 74.8, 51.8, 20.8; ESIMS *m*/*z* 302.2 $[M + H]^+$, 324.1 $[M + Na]^+$, 340.1 $[M + K]^+$.

Compound 2a: white solid; $[\alpha]^{26}_D$ −20 (*c* 0.1, $CHCl_3$); ESIMS *m*/*z* 260.1 $[M + H]^+$, 282.1 $[M + Na]^+$; ^{1}H and ^{13}C NMR and ECD were identical to those of compound **2**.

Compound 2b: white solid; $[\alpha]^{26}_D$ +16 (*c* 0.1, $CHCl_3$); ECD (1.0 × 10^{-3} M in MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−7.38), 234 (+2.92), 277 (−0.37) nm; ESIMS *m*/*z* 260.1 $[M + H]^+$, 282.1 $[M + Na]^+$, 541.2 $[2M + Na]^+$; ^{1}H and ^{13}C NMR were identical to those of compound **2**.

Acid Hydrolysis of Compounds 1 and 3−7. Compounds **1** and **3**−**7** were hydrolyzed with 1 mL of 6 M HCl for 6 h at 55 °C. The mixture was directly prepared by semipreparative HPLC to yield compound **11** from **1** and **3**. Under the same procedure, compounds **4**−**7** lead to **12**.

Compound 11: colorless oil; $[\alpha]^{26}_D$ −28 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.95 (1H, brs) 8.40 (1H, s), 7.46 (2H, d, *J* = 7.5 Hz), 7.38 (2H, dd, *J* = 7.5, 7.2 Hz), 7.34 (1H, dd, *J* = 7.2, 7.5 Hz), 6.71 (1H, s), 5.78 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.0, 166.0, 156.6, 142.5, 141.3, 137.7, 128.6 (2C), 128.1, 126.5 (2C), 114.5, 114.2, 70.7; HRESIMS *m*/*z* 246.0768 $[M + H]^+$ (calcd for $C_{13}H_{12}O_4N$, 246.0761).

Compound 12: colorless oil; $[\alpha]^{26}_D$ +20 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (1H, s), 7.43−7.36 (5H), 6.75 (1H, s), 6.01 (1H, s), 4.20 (2H, dd, *J* = 14.7 Hz), 3.49 (2H, dd, *J* = 14.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 178.2, 165.9, 157.1, 148.8,140.0, 128.7, 128.2, 127.0, 117.4, 114.2, 70.0, 60.0, 55.3; HRESIMS *m*/*z* 290.1022 $[M + H]^+$ (calcd for $C_{15}H_{16}O_5N$, 290.1023).

Methylation of 12 with TMS−CHN_2. To a solution of **12** (3 mg) in MeOH (0.5 mL) was added 400 μL of TMS−CHN_2 (2.0 M in hexanes) until a chartreuse color persisted upon addition. After stirring at room temperature for 10 h, the solvent was removed via a stream of N_2; then the residue was purified by reversed-phase HPLC using a gradient solvent system of MeOH−H_2O (30:70) to yield compound **13** (3 mg).

Syntheses of 13. A mixture of **10** (5 mg), K_2CO_3 (5 mg), and 2-bromoethanol (6 μL) in anhydrous CH_2Cl_2 (1 mL) was stirred for 20 h at room temperature. The reaction mixture was filtered through a small 4.5 μm filter and concentrated under reduced pressure. Then the residue was hydrolyzed in 6 M HCl (1 mL) at room temperature for 3 h and directly separated by HPLC (30:70 MeOH−H_2O) to yield compound **13** (2 mg).

Compound 13: colorless oil; $[\alpha]^{26}_D$ +22 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (1H, s), 7.32−7.40 (5H), 6.47 (1H, s), 6.21 (1H, s), 5.85 (1H, s), 5.10 (1H, s), 4.05 (1H, m), 3.97 (1H, m), 3.70 (3H, s), 3.47 (2H, m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.0, 165.1, 153.1, 148.9, 140.6, 128.6, 128.0, 126.9, 120.4, 116.5, 70.0, 60.3, 54.2, 51.4; HRESIMS *m*/*z* 304.1173 $[M + H]^+$ (calcd for $C_{16}H_{18}O_5N$, 304.1179).

Cell-Based Lipid Accumulation Assay. HepG2 cells, which were originally from the American Type Culture Collection (ATCC) (Manassas, VA, USA), were obtained from the China Union Medical University. Cells were maintained in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and penicillin/streptomycin (100 μg/mL, Gibco). When grown to 70−80% confluence, cells were incubated in DMEM + oleic acid (100 μM, Sigma-Aldrich, Shanghai, China) for 12 h, then treated with 10 μM of indicated compounds or the marketed antihyperlidemic drug lovastatin (Sigma-Aldrich) in DMEM + 100 μM oleic acid or with DMEM + 100 μM oleic acid alone for another 6 h. Subsequently, the cells were subjected to Oil-Red O staining or TC and TG determination as described previously.[21] Each experiment (n = 8 for Oil-Red O staining or n = 3 for TC and TG determination) was repeated at least three times. Data are presented as the means ± SEM. One-way ANOVA was used to determine significant differences among groups, after which the modified Student's *t* test with the Bonferroni correction was used for comparison between individual groups. $P < 0.05$ was considered statistically significant.

Computational Methods. Mixed torsional/low-mode conformational searches were carried out by means of the Macromodel 9.9.223 software[22] using the Merck molecular force field (MMFF) applying a 21 kJ/mol energy window. Geometry reoptimizations of the resultant conformers [B3LYP/6-31G(d) level *in vacuo* and B97D/TZVP[9] with PCM solvent model for MeOH] and TDDFT calculations were performed with Gaussian 09[23] using various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set. DFT-optimized structures were clustered for the heavy (non-hydrogen) atoms and OH hydrogens, but the orientation of the terminal isopropyl group and thus conformers differing only in the orientation of the isopropyl group were neglected.

ECD spectra were generated as the sum of Gaussians[24] with 2400 and 3000 cm^{-1} half-height width (corresponding to ca. 16 and 20 at 260 nm, respectively), using dipole-velocity-computed rotational strengths. Boltzmann distributions were estimated from the ZPVE-corrected B3LYP/6-31G(d) energies in the gas-phase calculations and from the B97D/TZVP energies in the PCM model ones. The MOLEKEL software[25] package was used for visualization of the results.

X-ray Crystallographic Analysis of 1. Single-crystal X-ray diffraction data were collected on an Agilent Gemini Ultra diffractometer with Cu Kα radiation (λ = 1.541 84 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined relatively isotropically with a riding model. A crystal suitable for X-ray diffraction of **1** was obtained by slow evaporation of a solution in MeOH−H_2O. Crystallographic data for **1** have been deposited in the Cambridge Crystallographic Data Centre with the deposition number 968304. Copies of the data can be obtained, free of charge, from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data-request/cif.

Crystal data for 1: orthorhombic, $C_{13}H_{12}N_2O_3$, space group $P2_12_12_1$ with a = 6.5201(1) Å, b = 7.2318(1) Å, c = 24.7168(4) Å, $\alpha = \beta = \gamma = 90°$, V = 1165.45 (3) $Å^3$, Z = 4, T = 290 (2) K, μ(Cu Kα) = 0.836 mm^{-1}, D_c = 1.392 g/mm^3, and F (000) = 512.0. Crystal size: 0.36 × 0.32 × 0.30 mm^3. Independent reflections: 2246 with R_{int} = 0.0198. The final agreement factors are R_1 = 0.0298 and wR_2 = 0.0818 [$I \geq 2\sigma(I)$]. Flack parameter = −0.03(13).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00218.

Structures of compounds **1**−**13**; COSY and HMBC correlations of new compounds **1**−**6**; ECD spectra of **2**, **2a**, and **2b**; HRESIMS and NMR spectra of compounds **1**−**7**, **2a**, **2b**, and **9**−**13** (PDF)

Crystallographic data (CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zhutj@ouc.edu.cn (T. Zhu).

*E-mail: pguo@implad.ac.cn (P. Guo).

*E-mail: dehaili@ouc.edu.cn (D. Li).

Author Contributions

⊥H. Zhou and L. Li contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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