Research article

Cytotoxic diaporindene and tenellone derivatives from the fungus Phomopsis lithocarpus

LIU Hui-Bo1,2Δ, LIU Zhao-Ming1Δ, CHEN Yu-Chan1, Tan Hai-Bo3, LI Sai-Ni1, LI Dong-Li2, LIU Hong-Xin1*, ZHANG Wei-Min1*

1 State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou 510070, China; 2 School of Chemical and Environmental Engineering, Wuyi University, Jiangmen 529020, China; 3 Program for Natural Products Chemical Biology, Key Laboratory of Plant Resources Conservation and Sustainable Utilization, Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

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[ABSTRACT] Nine new compounds, including five natural rarely-occurring 2, 3-dihydro-1H-indene derivatives named diaporindenones E−I (1−5), and four new benzophenone analogues named tenellones J−M (6−9) were isolated from the deep-sea sediment-derived fungus Phomopsis lithocarpus FS508. All the structures for these new compounds were fully characterized on the basis of spectroscopic data, NMR spectra, and ECD calculation and single-crystal X-ray diffraction analysis. The potential anti-tumor activities of compounds 1−9 against four tumor cell lines SF-268, MCF-7, HepG-2, and A549 were evaluated using the SRB method. Compound 7 exhibited cytotoxic activity against the SF-268 cell line with an IC50 value of 11.36 μmol·L−1.

[KEY WORDS] Deep-see-derived fungus; Phomopsis lithocarpus; 2, 3-Dihydro-1H-indene isomers; Benzophenone derivatives

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Introduction

Nowadays, marine natural products have emerged as one of the most important strategic sources for drug discovery and pharmaceutical usage. Their diverse structures and pharmacological effects in medical industry have been significantly reflected by the fruitful achievements for bioactive natural products [1−3]. Moreover, the therapeutic potential of marine natural products have also been further immensely adv

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[Corresponding author] E-mails: liuhx@gdim.cn (LIU Hong-Xin); wnzhang@gdim.cn (ZHANG Wei-Min)

ΔThese authors contributed equally to this work.
These authors have no conflict of interest to declare.

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Herein, the details of isolation, structural elucidation by NMR spectral interpretation, quantum molecular calculation, X-ray diffraction, and biological evaluation were described.

**Results and Discussion**

Diaporindene E (1) was obtained as white needles. Its molecular formula was determined as C_{25}H_{28}O_{5} on the basis of positive HR-ESI-MS data with a molecular ion at m/z 409.2011 [M + H]^+ (Calcd. for C_{25}H_{29}O_{5}, 409.2010), indicating the existence of 12 indices of hydrogen deficiency. The IR spectrum of 1 showed two unambiguous absorption bands at 3354 and 1651 cm\(^{-1}\), which were the characteristics of hydroxyl and carbonyl functionalities, respectively. With careful interpretation of its \(^{13}\)C NMR (Table S6) and HSQC spectra, 25 carbon signals were successfully distinguished and ascribed to a carbonyl moiety, four methyls, three methylenes, seven methines, and ten quaternary carbons.

The proton-proton connectivity of 1 clearly clarified the existence of three spin-coupling systems: a (H-4/H-5), b (H-7/H-8/H-12), and c (H-8'/H-9') as depicted in Fig. 2. These spectroscopic features were similar to those of the known compound diaporindene A \(^{24}\), excepting for the presence of two olefinic carbons (\(\delta_{c} 111.8, 145.0\)) as well as the absence of a methyl functional group and a quaternary carbon in 1, which strongly suggested that the 1-propoxy unit should be replaced by an isopropenyl unit. The aforementioned deduction was further established by the HMBC correlations from H-10 and H-11 to C-8 and C-9, H-4 to C-2 and C-6, as well as H-5 to C-3, C-1, and C-7 along with the spin-coupling system b from the COSY spectrum. Moreover, the HMBC and COSY correlations as shown in Fig. 2 indicated that the propan-2-ol fragment was linked to 2, 3-dihydrobenzo[\(b\)]\([1,4]\)dioxine moiety.

Likewise, the 2, 3-dihydrobenzo[\(b\)]\([1,4]\)dioxine moiety could be unambiguously confirmed by the key HMBC correlations from the cross-peaks of H-4'/C-2', C-3', and C-7' in conjunction with H-7' to C-2' as referring to the substructure c. Additionally, the aldehyde functionality could be readily located at C-2 position by the critical HMBC correlations from H-13 to C-2 and C-3. Furthermore, the HMBC correlations from H-2' to C-4', C-5', and C-6' chemically evidenced that the C-7' methyl functional group was connected to the aromatic phenyl ring at C-5'. Based on the above informative deduction, the planar structure was finally elucidated as shown in Fig. 1. The obvious NOESY correlation of H-12/H-8 and H-7'/H-11 suggested that H-12 and H-8 were co-facially oriented. Furthermore, the HMBC correlations from H-2' to C-4', C-5', and C-6' chemo-logically evidenced that the C-7' methyl functional group was connected to the aromatic phenyl ring at C-5'. Based on the above informative deduction, the planar structure was finally elucidated as shown in Fig. 1. The obvious NOESY correlation of H-12/H-8 and H-7'/H-11 suggested that H-12 and H-8 were co-facially oriented. In order to substantiate the above deduction and establish the stereochemistry of 1, the X-ray single-crystallographic analysis was conducted. Fortunately, the single crystals were obtained from a MeOH-H\(_2\)O solvent system, and an X-ray diffraction experiment was performed with CuK\(\alpha\) radiation (Fig. 3). The crystal data unambiguously assigned its absolute configuration as \(8R,12R,9'S\). Finally, the structure of 1 was determined and given the trivial name as diaporindene E.

Diaporindene F (2) was obtained as yellow oil with the molecular formula of C_{25}H_{28}O_{5} deduced from the HR-ESI-MS. The \(^{13}\)C NMR (Table S6) and HSQC spectra of 2 showed 25 carbon signals, which displayed very close similarity in most profiles of chemical shifts for those of 1. The slight differences of the NMR chemical shifts (upshifted or downshifted) between 2 and 1 were clearly distinguished in Table S6. These data logically indicated that compounds 2 and 1 shared the same carbon core skeleton, and the vari-
The presence of different chiral genetic centers. Therefore, the planar structure of 2 was tentatively assigned to be the same as 1 shown in Fig. 1.

In the NOESY spectrum, the key NOE correlations of H-12/H-7α strongly suggested that these protons should be cofacial and arbitrarily assigned to be in α-orientation. Meanwhile, the NOE correlation of H-7β/H-11 indicated that the isopentyl group was β-orientation. Compound 2 displayed a reversal and symmetric ECD absorption curve comparing to 1 (Fig. S1), which strongly suggested that the absolute configurations of the C-8 and C-12 stereogenic centers, close to critical chromophores in 2, was opposite to those of compound 1, thus tentatively deducing to be 8S and 12S. With respect to the same biosynthetic pathway and the weak ECD absorption of C-9' stereogenic center at 200–300 nm for this kind of compounds, the absolute configuration of C-9' stereogenic center for 2 was assumed to be identical to that of compound 1. Moreover, the absolute configuration of 2 was further confirmed by the ECD calculations. As showed in Fig. S1, the calculated ECD curve of 8S, 12S, 9'S-2 was perfectly consistent with the experimental curve, which successfully evidenced the aforementioned deduction. Thus, the absolute configuration of 2 was determined as 8S, 12S, 9'S and given the trivial name diaporindene F.

Diaporindene G (3) was obtained as yellow needles. The molecular formula was assigned as C_{25}H_{33}O_{3} based on the positive mode with the HR-ESI-MS [M + H]^+ ion discovered at m/z 409.2008 (Calcd. for 409.2010), indicating the presence of 12 degrees of unsaturation in the molecule. The $^1$H and $^{13}$C NMR spectra of 3 closely resembled to those of 1 except for the presence of an additional methyl functionality (δ_C 1.56/20.2) and the absence of a methine carbon in 3, which collectively indicated that the double bond of C-9/C-10 might be rearranged to be the olefin at the carbons C-8/C-9; these findings were confirmed by the HMBC correlations from H_{12}-10 (δ_C 20.2) and H_{12}-11 (δ_C 21.8) to C-8 (δ_C 135.8) and C-9 (δ_C 126.8). Thus, the planar structure of 3 was unambiguously elucidated as depicted in Fig. 1.

Diaporindene H (4) was obtained as white needles with the molecular formula of C_{25}H_{33}O_{3} as deduced from the HR-ESI-MS spectrum. Similar to compound 3, the $^{13}$C NMR data as shown in Table S7 and HSQC spectrum of 4 also showed 25 carbon signals. Moreover, the chemical shifts for all the carbons displayed close similarity with those of compound 3. The slight differences of the NMR chemical shifts between 4 and 3 were clearly distinguished in the NMR data (Table S7), which strongly suggested that these two compounds existed as a pair of related diastereoisomers. The absolute structures of compounds 3 and 4 were established as 12S, 9'S and 12R,9'S respectively by ECD calculations as shown in Figs. S2 and S3. Therefore, the final structure of 4 was determined to be the diastereoisomer of compound 3 and given the trivial name as diaporindene H.

Diaporindene I (5) was purified as white powder. Its molecular formula was assigned as C_{25}H_{33}O_{3} based on the positive mode with a HR-ESI-MS [M + H]^+ ion peak at m/z 485.2160 (Calcd. for 485.2170). The 1D NMR data (Table S8) of 5 was similar to those of 1, indicating that 5 should also share a very similar diaporindene skeleton. After careful inspection of their 1D NMR data, the major differences between 1 and 5 were disclosed to be the presence of an acetyl moiety (δ_C 22.0, 170.3) and the absence of two olefinic carbons in 5. The HMBC correlations of H_{12}-10 (δ_H 1.51) and H_{12}-11 (δ_H 1.52) to C-8 (δ_C 53.7) and C-9 (δ_C 84.8) strongly suggested that the 2-propenyl functional group should be replaced by a propan-2-ol unit, which was further confirmed by the up-shifted carbon resonances for C-9 (δ_C 84.8) and C-10 (δ_C 27.1). Similarly, the down-shifted quaternary carbon resonance for C-12 (δ_C 84.2) confirmed that the hydroxyl moiety should link at C-12 position. As referring to the NOESY cross peaks of H-14'/H-11' and H-14'/H-12', the HMBC correlation from H-14' to C-13' strongly suggested the acetoxy functionality to be located at C-10' position.

The relative configuration of compound 5 was investig-
The connectivity of the units A and B was initially speculated to conjunct through the carbonyl carbon atom C-12 (δC 203.2) with the formation of a benzenophenone architecture, which mainly referred to the HMBC correlation between H-6′ and C-12 as well as correlation from H-5 to C-12. In light of the aforementioned findings, the structure of 6 was concluded as shown in Fig. 2 and given the trivial name as tenellone J.

Tenellone K (7) was isolated as yellow oil. Its molecular formula was assigned as C20H20O3 based on the positive HR-ESI-MS mode with a molecular ion at m/z 485.2173 [M + H]⁺ (Calcd. for C20H20O3, 485.2170). A detailed inspection and comparison of the 1D NMR spectra between 8 and 6 clearly revealed that both of them possessed the same benzophenone architecture, which was further confirmed by the 2D NMR correlations. The notable differences between the two compounds were ascribed to the presence of seven additional carbons (δC 20.8, 25.7, 26.0, 69.7, 71.3, 77.9, 170.6) in 8, which were the characteristics for an isopentyl moiety and an acetoxy functionality. The HMBC correlations of H₂-14’ to C-13’, H-9’ to C-13’ suggested that the acetoxy functionality might be located at C-9’ position, whereas the informative HMBC correlation from H₂-8’ to C-3’ verified the attachment of the highly oxygenated isopentyl moiety at C-3’ in the benzophenone architecture. Thus, compound 8 was finally determined and given the trivial name as tenellone K.

Tenellone M (9) was obtained as yellow needles. Its molecular formula was assigned as C24H24O3 based on the positive HR-ESI-MS mode with a molecular ion at m/z 409.2012 [M + H]⁺ (Calcd. for C24H24O3, 409.2010). The 1D NMR data (Table S10) of 9 were similar to those of the known compound tenellone D [23], indicating that they should also share a similar tenellone skeleton, except for the absence of the aldehyde moiety in conjunction with the presence of an oxygenated methylene (δC 65.2) in 9. This deduction could be further rationalized by the critical HMBC correlations from H₂-13 (δH 5.22) to C-1 (δC 140.7), C-2 (δC 122.8), C-1’ (δC 150.8), and C-6’ (δC 153.1), which strongly suggested that the aldehyde moiety was replaced by an oxygenated methylene group, which ought to be linked at C-6’ position in the benzene ring. Therefore, compound 9 was fi-
nally determined and given the trivial name as tenellone M.
Compounds 1–9 were evaluated for their cytotoxic activities against the SF-268, MCF-7, HepG-2, and A-549 tumor cell lines, with cisplatin as the positive control. Compound 7 exhibited moderate inhibitory activity against SF-268 cell line with an IC₅₀ value of 11.36 μmol·L⁻¹, as well as weak inhibitory activity against HepG-2 and A-549 cell lines with IC₅₀ values of 34.85 and 47.60 μmol·L⁻¹, respectively. Meanwhile, compounds 4, 6, and 9 showed weak inhibitory effects against SF-268 cell line with the IC₅₀ values ranging from 29.49 to 44.48 μmol·L⁻¹. The other five compounds did not exhibit cytotoxic activity even at the concentration of 50 μmol L⁻¹ (Table 1).

**Conclusion**

In summary, five 2,3-dihydro-1H-indene isomers and four new benzophenone derivatives, were isolated from the marine-derived fungus *P. lithocarpus* FS508. The chemical structures of nine compounds were elucidated by NMR analysis. The anti-tumor activities of compounds 1–9 were evaluated, wherein compound 7 exhibited moderate growth inhibitory effects against SF-268 cell line with an IC₅₀ value of 11.36 μmol·L⁻¹, as well as weak inhibitory activity against HepG-2 and A-549 cell lines with IC₅₀ values of 34.85 and 47.60 μmol·L⁻¹, respectively. Meanwhile, compounds 4, 6, and 9 displayed weak inhibitory effects against SF-268 cell line with the IC₅₀ values ranging from 29.49 to 44.48 μmol·L⁻¹. However, the other five compounds did not exhibit cytotoxic activity against these tumor cell lines even at 50 μmol L⁻¹.

**Experimental**

**General procedures**

UV spectra were taken on a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). IR data were recorded on a Shimadzu IR Affinity-1 spectrometer (Shimadzu, Kyoto, Japan). Optical rotation values were measured on an Anton Paar MCP-500 spectropolarimeter (Anton Paar, Graz, Austria) at 25 °C. Circular dichroism (CD) spectra were obtained under N₂ gas on a Jasco 820 spectropolarimeter (Jasco Corporation, Kyoto, Japan). The NMR spectra were acquired using a Bruker Avance 600 MHz NMR spectrometer with TMS as an internal standard (Bruker, Fallanden, Switzerland). ESI-MS data were collected on an Agilent Technologies 1290-6430A Triple Quad LC/MS (Agilent Technologies, Palo Alto, CA, USA). HR-ESI-MS were done with a Thermo MAT95XP high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Preparative HPLC separation were carried out using a YMC-pack ODS-A column (250 mm × 20 mm, 5 μm, 12 nm, YMC Co., Ltd., Kyoto, Japan). Semi-preparative HPLC separation was performed utilizing a YMC-pack ODS-A/AQ column (250 mm × 10 mm, 5 μm, 12 nm, YMC Co., Ltd., Kyoto, Japan), a S-Chiral A column (250 mm × 10 mm, 5 μm, 10 nm, 3.5 mm, Chiral Technologies Co., Ltd., Beijing, China) and a YMC-pack Cellulose-SC column (250 mm × 10 mm, 5 μm, 12 nm, 3.5 mm, YMC Co., Ltd., Kyoto, Japan). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), respectively. Thin-layer chromatography (TLC) was conducted with precoated glass plates GF-254 (Merck KGaA, Darmstadt, Germany).

**Fungal material**

The fungus *P. lithocarpus* FS508 was isolated in 2016 from a deep-sea sediment sample collected in Indian Ocean (111°53.335′ E, 16°50.508′ N; depth 3606 m). The sequence of amplified ITS region of the strain FS508 has been submitted to GenBank (Accession No. MG686131). A BLAST search of ITS region revealed that FS508 has 99% homology with *Phomopsis lithocarpus* CZ105B (Accession No. FJ755236). The strain is preserved at Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology.

**Fermentation, extraction, and isolation**

The fermentation was carried out in 3 L Erlenmeyer flasks, which contained 250 g of rice and 300 mL of 0.5% saline water. Each flask was aseptically inoculated with the seed inocula and statically fermented at 28°C for a month. The fermented rice substrate (40 flasks) was extracted three times with EtOAc, and the solvent was evaporated to dryness under the vacuum to obtain a crude extract. The crude extract was subjected to silica gel chromatography (200–300 mesh) by step gradient elution with petroleum ether/EtOAc (10 : 1 → 0 : 1) followed by CH₂Cl₂/MeOH in linear gradient (5 : 1 → 1 : 1) to yield 8 fractions (Frs. 1–8). Fr. 3 was further rechromatographed by column chromatography over C₁₈ reversed phase (RP) silica gel eluting with a MeOH/H₂O gradient (60 : 40–100 : 0) to produce 4 fractions Fr. 3-1–Fr. 3-4. Fr. 3-3 was further purified by column chromatography on silica gel eluting with a n-hexane/EtOAc.

<table>
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<tr>
<th>Compound</th>
<th>IC₅₀ (μmol·L⁻¹)</th>
<th>SF-268</th>
<th>MCF-7</th>
<th>HepG-2</th>
<th>A549</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
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<tr>
<td>3</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
</tr>
<tr>
<td>4</td>
<td>40.25 ± 0.34</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
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</tr>
<tr>
<td>5</td>
<td>≥ 50</td>
<td>≥ 50</td>
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<tr>
<td>6</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
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<tr>
<td>7</td>
<td>11.36 ± 1.52</td>
<td>≥ 50</td>
<td>34.85 ± 2.47</td>
<td>47.60 ± 5.47</td>
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<tr>
<td>8</td>
<td>44.48 ± 0.98</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
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<tr>
<td>9</td>
<td>29.49 ± 3.69</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
<td>≥ 50</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3.25 ± 0.05</td>
<td>3.02 ± 0.11</td>
<td>2.13 ± 0.17</td>
<td>2.65 ± 0.03</td>
<td></td>
</tr>
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</table>

*Values are expressed as the mean ± SD
Diaporindene \( G \) (3): yellow needles; \([\alpha]_D^{25} + 39.5 \) (c 0.02, MeOH). UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 209 (3.12), 260 (2.32), 350 (1.97) nm; IR \( \nu_{\text{max}} \) 3332, 2947, 2853, 2835, 2018, 1646 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S7; positive ESI-MS: \( m/z \) 409 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 409.2016 [M + H]\(^{\text{+}}\) (Caled. for \( \text{C}_{28}\text{H}_{41}\text{O}_{5}\), 409.2010).  

**Diaporindene \( H \) (4):** yellow needles; \([\alpha]_D^{25} + 24.0 \) (c 0.02, MeOH). UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 208 (3.13), 264 (2.31), 349 (1.95) nm; IR \( \nu_{\text{max}} \) 3309, 2949, 2835, 1647, 1016, 667 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S7; positive ESI-MS: \( m/z \) 409 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 409.2008 [M + H]\(^{\text{+}}\) (Caled. for \( \text{C}_{28}\text{H}_{41}\text{O}_{5}\), 409.2010).  

**Diaporindene \( I \) (5):** white powder; \([\alpha]_D^{25} + 36.5 \) (c 0.02, MeOH). UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 212 (3.38), 266 (2.85), 351 (2.45) nm; IR \( \nu_{\text{max}} \) 3309, 2947, 2835, 1647, 1541, 1506, 1456, 1018, 665 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S8; positive ESI-MS: \( m/z \) 485 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 485.2160 [M + H]\(^{\text{+}}\) (Caled. for \( \text{C}_{29}\text{H}_{43}\text{O}_{5}\), 485.2170).  

**Tenellone \( J \) (6):** purple powder; UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 217 (3.51), 267 (3.05), 343 (2.68) nm; IR \( \nu_{\text{max}} \) 3325, 2927, 1653, 1456, 1313, 1271, 1165, 1018, 750 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S9; negative ESI-MS: \( m/z \) 339 [M – H]\(^{\text{–}}\); HR-ESI-MS \( m/z \) 339.1225 [M – H]\(^{\text{–}}\) (Caled. for \( \text{C}_{29}\text{H}_{41}\text{NO}_{5}\), 339.1238).  

**Tenellone \( K \) (7):** yellow oil; UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 200 (3.59), 221 (3.35), 297 (2.90), 346 (2.55) nm; IR \( \nu_{\text{max}} \) 3354, 1653, 1506, 1456, 1014, 665 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S9; positive ESI-MS: \( m/z \) 443 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 445.1877 [M + Na]\(^{\text{+}}\) (Caled. for \( \text{C}_{32}\text{H}_{29}\text{NaO}_{5}\), 465.1884).  

**Tenellone \( L \) (8):** yellow oil; UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 222 (3.58), 266 (3.30), 346 (3.05) nm; IR \( \nu_{\text{max}} \) 2974, 2926, 1747, 1651, 1456, 1373, 1265, 1240, 1168, 1037, 775, 752 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S10; positive ESI-MS: \( m/z \) 485 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 485.2173 [M + H]\(^{\text{+}}\) (Caled. for \( \text{C}_{29}\text{H}_{43}\text{O}_{5}\), 485.2170).  

**Tenellone \( M \) (9):** yellow needles; UV (MeOH) \( \lambda_{\text{max}} \) (log \( e \) 203 (4.27), 221 (4.04), 298 (3.64), 349 (3.35) nm; IR \( \nu_{\text{max}} \) 3313, 2920, 2833, 1456, 1022, 669 cm\(^{-1}\)). \( ^{1}H \) (600 MHz) and \( ^{13}C \) (150 MHz) NMR spectral data, see Table S10; positive ESI-MS: \( m/z \) 409 [M + H]\(^{\text{+}}\); HR-ESI-MS \( m/z \) 409.2012 [M + H]\(^{\text{+}}\) (Caled. for \( \text{C}_{26}\text{H}_{39}\text{O}_{5}\), 409.2010).  

*X-ray crystallographic analysis of compound 1*  

A suitable crystal was selected and measured on a XtaLAB AFC12 (RINC): Kapla single diffractometer. The crystal was kept at 100(1) K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimisa-
tion.

Crystal data for C$_{23}$H$_{25}$O$_5$ (M = 408.47 g mol$^{-1}$): monoclinic, space group P2$_1$ (no. 4), a = 7.746 00(10) Å, b = 21.1808(4) Å, c = 13.0247(2) Å, $\beta$ = 90.372(2), V = 2136.87(6) Å$^3$, Z = 4, T = 100(1) K, $\mu$(CuK$\alpha$) = 0.709 mm$^{-1}$, Dcalc = 1.270 g cm$^{-3}$, 21346 reflections measured (7.968 $\leq$ 2$\theta$ $\leq$ 134.124$^\circ$), 7613 unique ($R_{int}$ = 0.0414, Rsigma = 0.0479) which were used in all calculations. The final R1 was 0.0459 (I > 2σ(I)) and wR2 was 0.1190 (all data). Crystallographic data for 1 reported in this paper has been deposited in the Cambridge Crystallographic Data Centre. (Deposition number: CCDC 2021998). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

**Cytotoxicity assay**

The cytotoxic activities of compounds (1–9) were evaluated against four human tumor cell lines SF-268, MCF-7, HepG-2, and A549 with cisplatin as the positive control.

**References**


