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# SECALONIC ACIDS H AND I, TWO NEW SECONDARY METABOLITES FROM THE MARINE-DERIVED FUNGUS *PENICILLIUM OXALICUM*

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**Abstract** – Two new secalonic acid derivatives, secalonic acids H and I (**1** and **2**), and one known compound, secalonic acid D (**3**), were isolated from the marine-derived fungus *Penicillum oxalicum*. The planar structures and relative configurations of the new compounds were elucidated by the analyses of their 1D and 2D NMR and high-resolution mass spectrometric data. The absolute configurations were established by comparison of their experimental and calculated electronic circular dichroism spectra. The in *vitro* cytotoxic activities of compounds **1** and **2** were evaluated against selected human cell lines.

Cancer is one of the leading causes of human death in the world nowadays. Since the early 20th century, chemotherapy has been making great progress in cancer treatment.[1] However, the search of new chemotherapeutic drugs with improved efficacy is still a challenging task for researchers. Natural products have been and continue to be a rich source of anticancer drugs.[2] The nature-derived anticancer drugs, including taxane,[3] bleomycin,[4] and vinca alkaloids,[5] played significant role in the history of cancer therapy. Marine-derived fungi have been known for producing structurally unique secondary metabolites with diverse biological effects, presumably due to their highly adaptive metabolic systems that evolved

during the natural selection process.[6] Therefore, they represent a huge source for discovery of novel bioactive secondary metabolites.[7]

In 1952, Stoll et al.[8] firstly reported secalonic acid A from a fungus. Total seven homologues, secalonic acids A–G, have been reported to date.[9] In this study, we isolated a marine-derived fungus, *Penicillium oxalicum*, from a sediment sample of the southeast coast of China. The same species from terrestrial sources was reported to be an important source of secalonic acids.[10] Chemical study of this *P. oxalicum* led to the identification of two new secalonic acid derivatives, secalonic acids H and I (**1** and **2**). In this paper, the isolation, structural elucidation and bioactivities of the new compounds **1** and **2** are reported. To the best of our knowledge, this study is the first report on secalonic acid derivatives with a B'-ring-opened skeleton.

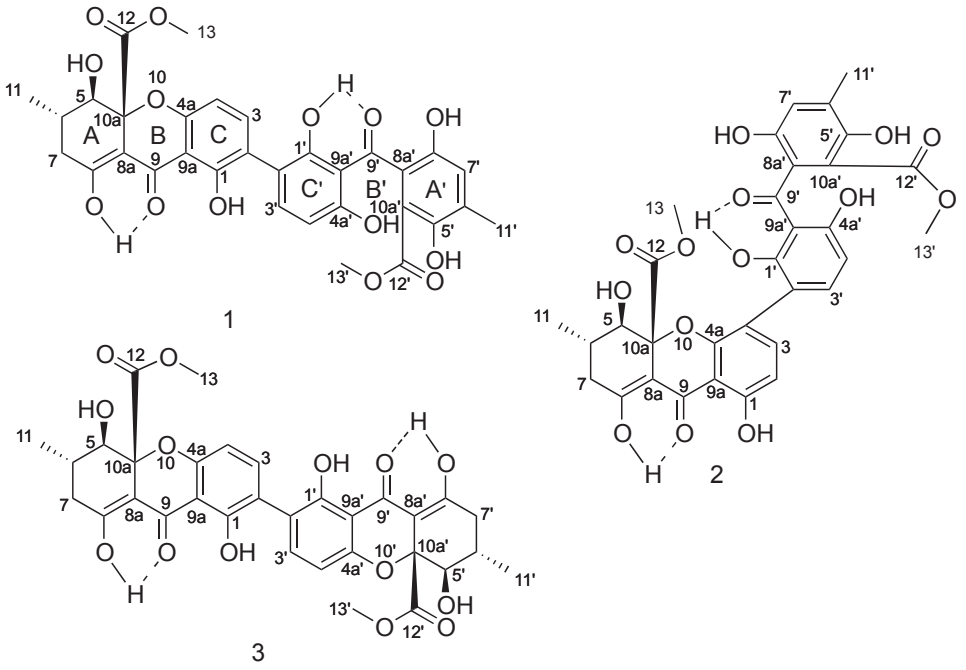


**Figure 1.** Structure of compounds **1**–**3** isolated from *P. oxalicum*

Secalonic acid H (**1**) was obtained as yellow gum. The molecular formula was determined to be $C_{32}H_{28}O_{14}$ on the basis of HRESI-MS (*m/z*: 659.1370 $[M + Na]^+$, calcd for $C_{32}H_{28}NaO_{14}$, 659.1377). Its NMR data (Table 1), combined with DEPT and HMQC spectrum analyses, revealed the presence of thirty-two carbons, including twenty quaternary carbons, seven methines, one methylene, and four methyls. The careful analysis and comparison of the 1D NMR of **1** with those of **3** suggested that **1** is an asymmetric secalonic acid analogue with structural similarity to **3**.[11] Further structure elucidation through COSY and HMBC correlations confirmed the rings A–C of **1** was the same as those of **3**.

**Table 1.** $^{1}$H and $^{13}$C NMR Date for **1** and **2** in DMSO-$d_6$[a]

| No. | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 158.7 s | | 160.1 s | |
| 2 | 118.6 s | | 109.1 d | 6.55 (1H, d, 8.6) |
| 3 | 140.6 d | 7.46 (1H, d, 8.1) | 141.4 d | 7.52 (1H, d, 8.6) |
| 4 | 107.4 d | 6.63 (1H, d, 8.1) | 116.1 s | |
| 4a | 158.6 s | | 156.4 s | |
| 5 | 75.3 d | 3.82 (1H, d, 11.0) | 75.1 d | 3.69 (1H, dd, 10.6, 6.1) |
| 6 | 29.9 d | 2.31 (1H, m) | 30.6 d | 2.29 (1H, m) |
| 7 | 35.8 t | e 2.66 (1H, dd, 19.1, 4.8) | 36.0 t | e 2.62 (1H, dd, 19.1, 5.4) |
| | | a 2.49 (1H, overlap) | | a 2.42 (1H, dd, 19.1, 11.0) |
| 8 | 178.1 s | | 178.4 s | |
| 8a | 101.8 s | | 102.0 s | |
| 9 | 186.7 s | | 186.2 s | |
| 9a | 106.3 s | | 106.9 s | |
| 10a | 85.1 s | | 85.0 s | |
| 11 | 17.8 q | 1.04 (3H, d, 5.5) | 17.6 q | 0.96 (3H, d, 6.4) |
| 12 | 170.1 s | | 169.2 s | |
| 13 | 52.8 q | 3.62 (3H, s) | 52.3 q | 3.62 (3H, s) |
| OH-1 | | 11.57 (1H, s) | | 11.27 (1H, brs) |
| OH-5 | | 6.06 (1H, brs) | | 5.59 (1H, brd, 6.1) |
| OH-8 | | 13.64 (1H, brs) | | 13.83 (1H, brs) |
| 1' | 160.4 s | | 160.3 s | |
| 2' | 114.6 s | | 114.2 s | |
| 3' | 138.3 d | 7.22 (1H, d, 8.3) | 139.3 d | 7.86 (1H, d, 8.6) |
| 4' | 106.1 d | 6.27 (1H, d, 8.3) | 106.3 d | 6.27 (1H, d, 8.6) |
| 4a' | 159.8 s | | 159.6 s | |
| 5' | 150.7 s | | 150.7 s | |
| 6' | 126.7 s | | 126.8 s | |
| 7' | 124.2 d | 6.94 (1H, s) | 124.2 d | 6.94 (1H, s) |
| 8' | 145.1 s | | 145.0 s | |
| 8a' | 128.8 s | | 128.9 s | |
| 9' | 200.4 s | | 200.4 s | |
| 9a' | 110.7 s | | 110.8 s | |
| 10a' | 110.0 s | | 110.1 s | |
| 11' | 15.8 q | 2.17 (3H, s) | 15.8 q | 2.17 (3H, s) |
| 12' | 169.1 s | | 170.1 s | |
| 13' | 52.2 q | 3.60 (3H, s) | 52.9 q | 3.62 (3H, s) |
| OH-1' | | 12.94 (1H, s) | | 13.03 (1H, s) |
| OH-4a' | | 10.38 (1H, s) | | 10.31 (1H, s) |
| OH-5' | | 10.05 (1H, brs) | | 10.05 (1H, s) |
| OH-8' | | 9.23 (1H, brs) | | 9.23 (1H, s) |

[a] $^{1}$H NMR (500 MHz, $\delta$, $J$ in Hz in parentheses), $^{13}$C NMR (126 MHz, $\delta$).

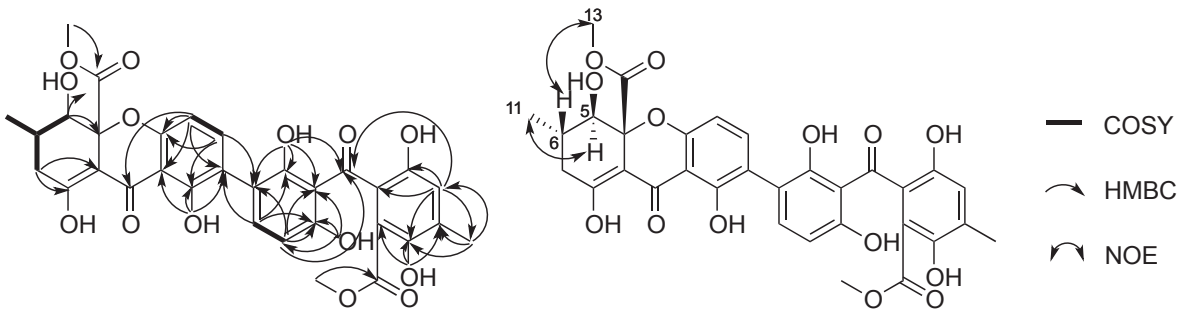


**Figure 2.** COSY, HMBC and NOE correlations for compound **1**

Additional, the HMBC correlations from 5'-OH to C-5', C-6', and C-10a', from H-11' to C-5', C-6', and C-7', and from H-7' to C-5', C-8', and C-8a', suggested ring A' to be a multi-substituted benzene dehydrogenated from ring A, which was further supported by five obviously downfield shifts of C-5', C-6', C-7', 8a', and 10a', and an obviously upfield shift of C-8'. The COSY correlation of H-3' and H-4', and the HMBC correlations from 1'-OH to C-1', C-2', and C-9a', from H-3' to C-1' and C-4a', from H-4' to C-2', C-4a', and C-9a', and from 4a'-OH to C-4', C-4a', and C-9a' indicated that the ring C' was the same as ring C. In view of only one less unsaturation degree of **1** than that of **3**, the appearance of OH-4a' (10.38 s), and the non-oxidized chemical shift value of C-10a' (110.0 s), the ether linkage in ring B' between C-4a' and C-10a' should be disconnected. Finally, the key HMBC correlations from H-3' to C-2 and from H-3 to C-2' suggested the two independent segments were linked through C-2 and C-2'. Until now, the planar structure of **1** was established (Figure 1).

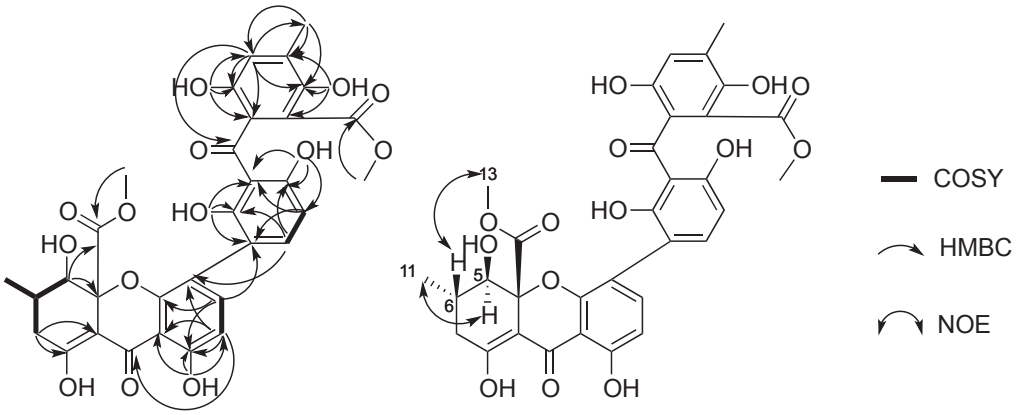


**Figure 3.** COSY, HMBC and NOE correlations for compound **2**

Secalonic acid I (**2**), which was obtained as yellow gum, was analyzed to have the same molecular formula as **1** on the basis of HRESIMS (*m/z*: 659.1377 $[M + Na]^+$, calcd for $C_{32}H_{28}NaO_{14}$, 659.1377). Further careful comparison of the similar NMR spectral data (Table 1) of **2** with that of **1**, an obviously downfield shifts of H-3' (from 7.22 to 7.86) was observed. Considering the HMBC correlations (Figure 3) from OH-1 to C-1, C-2, and C-9a, from H-3 to C-2', from OH-1' to C-1', C-2', and C-9a', and from H-3' to C-4, it was confirmed that **2** owned different linkage sites from **1** shown in Figure **3**. Therefore, the planar structure of **2** was determined.

The relative configurations of **1** and **2** were proposed by selected NOE experiments. In the NOE spectrum (Figure 2) of **1**, correlations of H-5 with Me-11 and H-6 with Me-13 help to determine the configuration of cyclohexene ring (ring A), which was further supported by the coupling constant ($J_{5,6}$ = 11.0). The similar phenomenon and coupling constants were observed in **2** and **3**, suggesting the relative configurations of compounds **1**–**3** were the same.

Secalonic acids contain almost the same chromophore system.[12] The Cotton effect around 330 nm corresponds to the n–π* electronic transition and it has been correlated with the configurations of C-10a.[13] For compounds **1** and **2**, positive Cotton effects around 330 nm in the experimental CD spectra agreed well with the spectrum of secalonic acid D,[12] yet were opposite to that of SAA,[13] demonstrating that the same absolute configurations of **1** and **2** to be 5*R*, 6*S*, 10a*R* as **3**, which were further supported by quantum chemical ECD calculation (Figure 4).

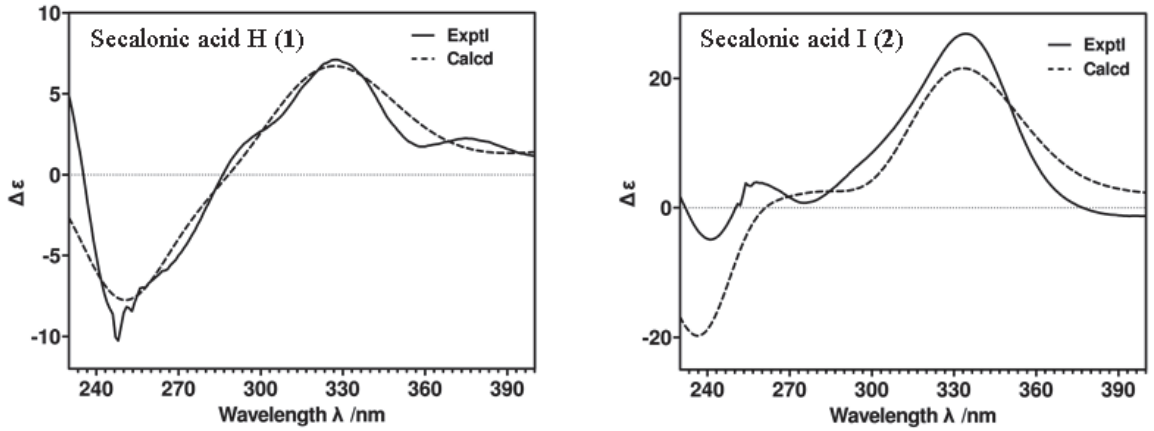


**Figure 4.** Comparison of experimental and calculated ECD spectra of **1** (left) and **2** (right)

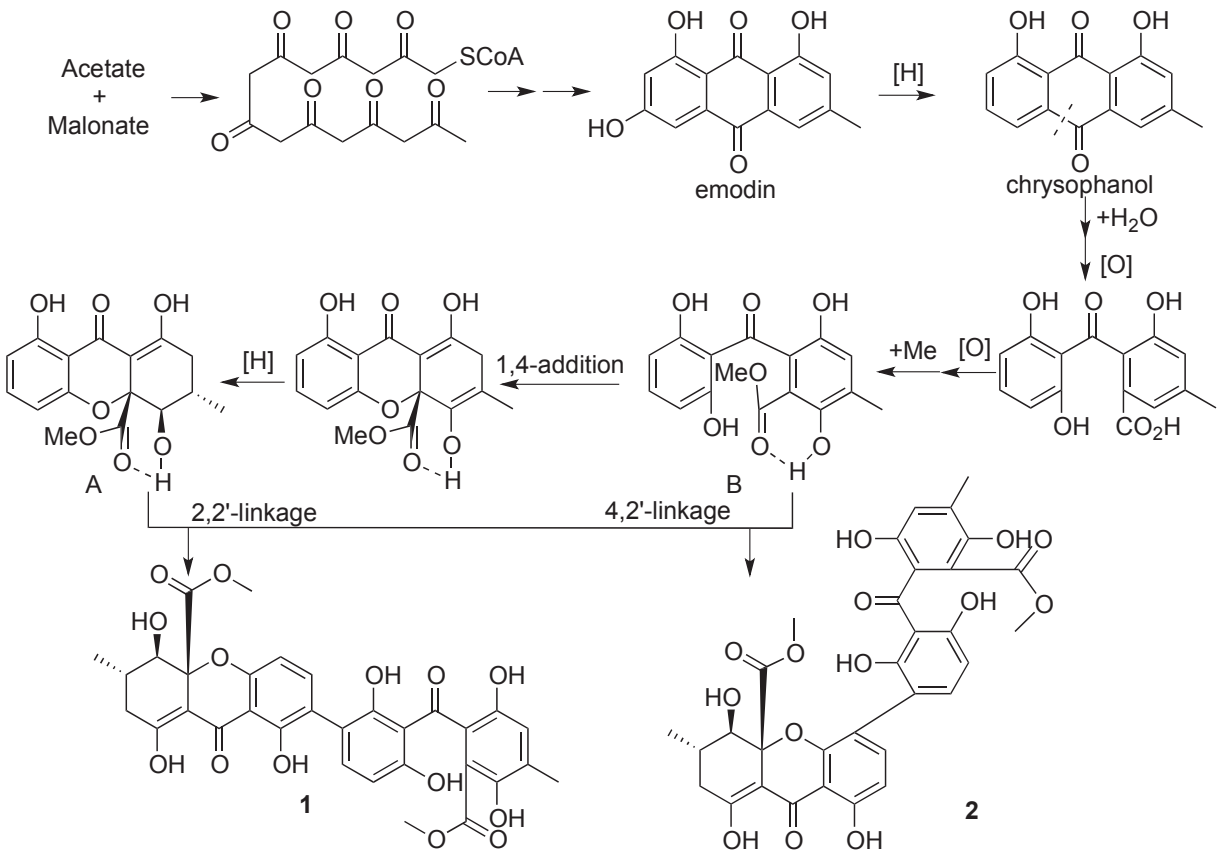


**Figure 5.** Plausible biosynthetic pathway of compounds **1** and **2**

To explain the biogenetic origin of secalonic acids H and I (**1** and **2**), a plausible biosynthetic pathway is proposed in Figure 5. Moreover, compounds **1** and **2** were further tested for cytotoxic effects on HCT116, KB and EC9706 cell lines by MTT method. Compound **1** showed better cytotoxic effect against three cell lines than **2** (Table 2).

It is interesting to point out that the KB cell line was first established in 1955 by Eagle, reportedly from an epidermoid carcinoma from the larynx of a male donor. Eagle was also working with HeLa at that time. In 1966, Gartler demonstrated that KB was misidentified and corresponded to HeLa, which has been confirmed multiple times using different methods. Original stocks of KB, deposited at the ATCC by Eagle (www.atcc.org/Products/All/CCL-17.aspx#history), were shown to be the same as HeLa, demonstrating that the KB cell line is in fact a subline of HeLa.[14]

**Table 2.** Cytotoxicity effects of compounds **1** and **2** in three cancer cell lines

| | Cytotoxicity effects ($IC_{50}$, μM) | | |
|---|---|---|---|
| compound | HCT116 | KB | EC9706 |
| **1** | 31.3 | 19.0 | 19.7 |
| **2** | 87.6 | 42.8 | 21.3 |
| **ADM**[a] | 0.2 | 0.5 | 0.4 |

[a]ADM = doxorubicin (positive control)

## EXPERIMENTAL

**General Experimental Procedures.** Optical rotations were obtained from an Anton Paar MCP 200 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer, while CD spectra were measured on a JASCO J-715 spectra polarimeter. $^{1}$H NMR, $^{13}$C NMR, DEPT spectra and 2D NMR were recorded on a BRUKER BIOSPIN AVANCE III spectrometer using TMS as the internal standard. HRESIMS were obtained by an Agilent Q-TOF 6520 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column (ODS-A, 10×250 mm, 5 μm) at 5 mL/min.

**Fungal Material.** The fungus *P. oxalicum* was isolated from marine sediments collected from Langqi Island, Fujian, China. It was identified according to its morphological characteristics and ITS by Beijing Sunbiotech Co. Ltd, and preserved in our laboratory at –80 ˚C.

**Fermentation and Extraction.** The fungus was cultured under static conditions at 28 ˚C for 30 days in 150×1000 mL conical flasks containing 400 mL liquid medium, composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), $KH_2PO_4$ (0.5 g/L), $MgSO_4$•$7H_2O$ (0.3 g/L), yeast extract (3 g/L), NaCl (15 g/L) and water. The fermented whole broth (60 L) was filtered through cheese cloth to separate supernatant from mycelia. The mycelia were extracted three times with acetone.

The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc and concentrated in vacuo to give mycelia extract (36.5 g).

**Purification.** The mycelia extract (36.5 g) was separated into five fractions on a silica gel column using a step gradient elution of petroleum ether, $CH_2Cl_2$, and MeOH. Fraction D (5.9 g) eluted with $CH_2Cl_2$/MeOH (100:1) was separated into five subfractions (D1 to D5) on a silica gel column again, using a step gradient elution of $CH_2Cl_2$ and MeOH. Subfraction D2 (1.2 g) was further purified on a Sephadex LH-20 column ($CHCl_3$/MeOH) to obtain four subfractions (D2-1 to D2-4). Subfraction D2-3 (212 mg) was finally purified by semipreparative HPLC (55% MeCN with 0.1% TFA), yielding compounds **1** (2.4 mg) and **2** (2.7 mg). Subfraction D3 (1.1 g) was further purified on a reversed-phase column (MeOH:$H_2O$) to obtain four subfractions (D3-1 to D3-4). Subfraction D3-3 (285 mg) was purified by semipreparative HPLC (55% MeCN with 0.1% TFA), yielding compound **3** (68.7 mg).

**Secalonic acid H (1):** yellow gum ($CHCl_3$); $[\alpha]^{25}_D$ +230.3 (*c* 0.07, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 264 (4.34), 336 (4.58) nm; CD ($CHCl_3$) $\lambda_{max}$ ($\Delta\varepsilon$) 247 (–10.4), 327 (+7.2), 376 (+2.4) nm; $^1$H and $^{13}$C NMR see Table 1; HRESIMS $[M + Na]^+$ *m/z*: 659.1370 (calcd for $C_{32}H_{28}NaO_{14}$, 659.1377).

**Secalonic acid I (2):** yellow gum ($CHCl_3$); $[\alpha]^{25}_D$ +29.2 (*c* 0.11, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 264 (4.28), 337 (4.64) nm; CD ($CHCl_3$) $\lambda_{max}$ ($\Delta\varepsilon$) 240 (–4.9), 259 (+4.5), 276 (+1.0), 334 (+27.6) nm; $^1$H and $^{13}$C NMR see Table 1; HRESIMS $[M + Na]^+$ *m/z*: 659.1377 (calcd for $C_{32}H_{28}NaO_{14}$, 659.1377).

**Quantum Chemical ECD Calculations.** A conformational search was carried out with ComputeVOA 0.1 software for the proposed absolute configuration as shown using GMMX method. Geometry and frequency calculations of the lowest-energy conformers resulted from the conformational search were carried out with Gaussian 09 (Gaussian Inc., Wallingford, CT)[15] at the DFT level (B3LYP functional/DGDZVP basis set) in chloroform using the COSMO solvation model. TD-DFT calculations (B3LYP functional/DGDZVP basis set in chloroform using the COSMO solvation model) provided the single UV and CD spectra of the optimized lowest-energy conformers, which were then added up after a Boltzmann statistical weighting using SpecDis 1.60 (by using a sigma value of 0.3 eV).[16] After applying a UV-shift correction of +10 nm, the computed CD spectra were compared with the CD curves experimentally obtained.

**Biological Assays.** The cytotoxic activity for the HCT116, KB and EC9706 cell lines were evaluated by the MTT method as previous reported.[17]

## ACKNOWLEDGEMENTS

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