Dolabellane Diterpenoids from the Xisha Soft Coral *Clavularia viridis*

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**ABSTRACT:** Twelve new members (1–12) of the dolabellane family, co-occurring with three related known diterpenoids (13–15), were isolated from the Xisha soft coral *Clavularia viridis*. Their structures were determined by extensive spectroscopic analysis, modified Mosher’s method, and X-ray diffraction analysis. Clavuperoxylides A (3) and B (4) represent the first examples of dolabellanes containing peroxyl groups, especially the novel peroxide bridge in 4, whereas clavufuranolides A–C (9–11) are the first example of dolabellane diterpenoids comprising a tetrahydrofuran ring. The possible biogenetic relationship of all the isolates was proposed. In bioassay, several compounds exhibited considerable cytotoxicity against A549 and P388 cell lines. Compound 7 exhibited inhibitory activity against protein tyrosine phosphatases 1B (PTP1B), an anti-diabetic target, representing the first report of PTP1B inhibitory activity for dolabellane diterpenoids.

**INTRODUCTION**

Soft corals of the genus *Clavularia* (class Octocorallia, order Alcyonacea, and family Clavulariidae), widely distributed in waters of the Pacific, were recognized as prolific sources for structurally diverse and biologically active secondary metabolites,1–5 especially dolabellane diterpenoids.6–8 Dolabellanes comprise a 5/11-fused bicyclic carbon skeleton, with a variety of oxidation patterns. They were first reported from the marine mollusk *Dolabella californica* in the 1970s9 and were later discovered from marine algae, soft corals, sponges, terrestrial plants, and even fungi.10–14 The complex and unique structures of dolabellane diterpenoids and their broad biological activities such as cytotoxic, antimicrobial, and phototoxic properties, have attracted great attention from chemists and pharmacologists.15,16

In the course of our ongoing search for bioactive metabolites from the South China Sea Cnidarians,17–19 the soft coral *Clavularia viridis* was collected from the Xisha Islands, Hainan Province, China. A systematic chemical investigation of the title animals led to the isolation of 12 new dolabellanes (1–12) and three known related analogues (13–15).20,21 Structurally, clavuperoxylides A (3) and B (4) represent the first examples of dolabellanes containing peroxyl groups, especially the novel peroxide bridge in 4, and clavufuranolides A–C (9–11) are the first example of dolabellane diterpenoids comprising a tetrahydrofuran ring. This paper describes the isolation, structure elucidation, determination of the absolute configuration, possible biosynthetic pathways, and the bioactivity evaluation of all isolated metabolites.

**RESULTS AND DISCUSSION**

The usual work-up of the title animal yielded 15 pure metabolites 1–15.17–21 Preliminary 1H NMR analysis of the isolated molecules revealed close structural similarities. Fortunately, the structural determination of the most abundant compound 13 was straightforward because it could be crystallized for X-ray diffraction analysis, which allowed to unambiguously recognize its structure as depicted in Figure 1, the same as clavudiol A, a dolabellane-type diterpenoid previously isolated from the same species.22,23 Therefore, the structures of the other compounds could be identified as possessing the same dolabellane skeleton as compound 13. On the basis of detailed spectroscopic analysis and by comparison with the reported data, the other two known compounds 14 and 15 were readily identified as dolabellane diterpenoids clavirolide C and clavirolide G, respectively.21,23

Compounds 1–4 were isolated as amorphous powder. Their molecular formulas were deduced by high-resolution mass spectrometry (HRMS) to be C_{20}H_{32}O (EI: m/z 288.2463, [M]⁺, calcd 288.2458), C_{22}H_{34}O_{3} (EI: m/z 346.2506, [M]⁺, calcd 346.2508), C_{23}H_{36}O_{4} (ESI: m/z 385.2355, [M + Na]⁺, calcd 385.2349), and C_{23}H_{34}O_{2} (EI: m/z 302.2248, [M]⁺, calcd 302.2246), respectively. Their NMR data (Tables 1 and S1) were reminiscent of those of co-occurring compound **S1** were reminiscent of those of co-occurring compound...
clavudiol A (13). In fact, compounds 1–4 and 13 display the same structural unit extending from C-1 to C-14, including one affiliated methyl at C-1, one exomethylene at C-4, and one isopropyl group at C-12. The only differences in the NMR data of these compounds are attributed to the different oxidative patterns at C-10 and C-18 (Figure 1). Particularly, compound 1 is the dehydroxy product of 13 at C-18 position as indicated by an aliphatic methine at δC 28.0 in 1 instead of an oxygenated quaternary carbon at δC 72.9 in 13. Compound 2 is the acylation derivative of 13 at C-10 because the H-10 at δH 4.15 of 13 was downfield shifted to δH 5.43 of 2 and additional signals supporting an acyl group are present in the spectra of 2 (Tables 1 and S1). Compound 3 is the peroxy derivative of 2 at C-18 position as supported by an obvious upfield shift of C-2 in compound 2 relative to the chemical shift of the same carbon in 3 (δC 84.0 for 3 and δC 72.7 for 2) and additional 16 mass units in its molecular weight. Compound 4 was initially believed to be the C-18 peroxy product of 13 because its 1H and 13C NMR data of CH-10 are extremely similar as those of 1, while its 13C NMR data of C-18 are nearly the same of 3. However, the 18 mass units less of its real molecular weight than that of our proposed structure suggested the formation of a peroxide bridge between C-10 and C-18. The structures of

Figure 1. Structures of compounds 1–15.

Table 1. 13C NMR (150 MHz) Spectroscopic Data for 1–13 in CDCl3 (δ in ppm)

| no. | 1         | 2         | 3         | 4         | 5         | 6         | 7         | 8         | 9         | 10        | 11        | 12        |
|-----|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1   | 49.9      | 51.6      | 52.8      | 52.4      | 51.3      | 45.3      | 45.5      | 45.1      | 43.5      | 45.1      | 38.2      | 48.2      |
| 2   | 39.8      | 39.8      | 39.4      | 39.6      | 40.0      | 36.9      | 36.5      | 37.1      | 39.7      | 37.2      | 40.6      | 38.4      |
| 3   | 31.3      | 31.3      | 31.3      | 31.3      | 30.2      | 30.0      | 31.3      | 31.3      | 30.5      | 30.4      | 29.6      | 30.8      |
| 4   | 153.0     | 152.6     | 152.4     | 152.6     | 152.8     | 152.1     | 152.0     | 151.7     | 152.0     | 152.1     | 152.1     | 152.0     |
| 5   | 36.8      | 36.9      | 37.0      | 37.0      | 36.9      | 37.6      | 37.5      | 35.8      | 37.8      | 35.9      | 37.4      | 37.3      |
| 6   | 29.6      | 29.5      | 29.8      | 29.7      | 29.5      | 29.4      | 29.5      | 28.3      | 29.8      | 28.8      | 28.6      | 29.0      |
| 7   | 128.3     | 129.3     | 128.5     | 128.1     | 128.0     | 128.8     | 129.2     | 128.7     | 128.2     | 129.5     | 129.6     | 129.5     |
| 8   | 130.7     | 129.9     | 130.6     | 130.7     | 130.8     | 131.9     | 130.9     | 131.7     | 131.4     | 131.5     | 131.4     | 131.1     |
| 9   | 47.8      | 47.2      | 44.5      | 48.1      | 49.9      | 49.4      | 48.4      | 46.5      | 49.4      | 47.5      | 49.0      | 49.2      |
| 10  | 66.5      | 68.6      | 68.4      | 66.7      | 66.0      | 67.1      | 67.7      | 69.6      | 69.4      | 70.6      | 74.8      | 74.1      |
| 11  | 141.5     | 136.4     | 136.4     | 140.0     | 141.7     | 50.6      | 49.4      | 51.7      | 47.4      | 57.8      | 58.5      | 60.5      |
| 12  | 148.7     | 150.4     | 146.5     | 143.1     | 145.7     | 135.8     | 140.1     | 150.2     | 90.3      | 90.2      | 59.5      | 157.6     |
| 13  | 28.6      | 35.1      | 35.2      | 34.9      | 34.4      | 31.1      | 31.4      | 122.6     | 36.4      | 37.1      | 21.1      | 114.6     |
| 14  | 35.2      | 35.0      | 33.5      | 34.0      | 35.3      | 36.2      | 35.3      | 43.6      | 33.0      | 32.7      | 41.8      | 48.6      |
| 15  | 29.6      | 29.7      | 29.1      | 29.5      | 29.8      | 24.6      | 24.4      | 24.5      | 28.6      | 23.9      | 26.5      | 23.6      |
| 16  | 110.6     | 110.9     | 111.0     | 110.8     | 110.7     | 111.2     | 111.3     | 111.0     | 111.5     | 111.2     | 111.5     | 111.4     |
| 17  | 16.8      | 16.7      | 16.6      | 17.1      | 16.9      | 17.7      | 17.9      | 18.5      | 17.4      | 18.9      | 16.7      | 16.1      |
| 18  | 28.0      | 27.7      | 84.0      | 84.3      | 72.9      | 128.6     | 131.2     | 28.7      | 76.8      | 75.1      | 76.5      | 76.3      |
| 19  | 21.2      | 28.6      | 27.2      | 26.5      | 30.0      | 23.1      | 19.7      | 21.6      | 26.9      | 23.8      | 27.2      | 13.0      |
| 20  | 21.9      | 32.0      | 27.4      | 26.7      | 31.9      | 24.1      | 65.8      | 24.3      | 27.1      | 27.1      | 29.0      | 29.1      |
| 10-OAc| 168.7     | 168.6     |           |           |           |           |           |           |           |           |           |           |

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compounds 1−4 were further confirmed by the careful analysis of their 1H−1H COSY, HSQC, and HMBC spectra (Figure 2).

The relative stereochemistry of 1−4 was assigned the same as those of 13 through analysis of their NMR data (Tables 1 and S1) and interpretation of their NOESY spectra (Figure 3). The appearance of a cross-peak between CH3-17 and H2-6 in the NOESY spectrum of 1−4 supported the E configuration for Δ7,8 whereas the correlations of CH3-15/H-10 indicated the same orientation of them. On the basis of biogenetic considerations, the absolute configurations at C-1 and C-10 of 1−4 were tentatively suggested as R and R, respectively, the same as those of already determined clavudiol A (13). In order to confirm the absolute configuration, modified Mosher’s method was applied for compound 1. It was treated with (R)- or (S)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(R)- or (S)-MTPA-Cl] in the presence of pyridine to yield the (S)- and (R)-MTPA esters, respectively. The chemical shift differences (Δδ = δ(S)-MTPA-ester − δ(R)-MTPA-ester) of the protons surrounding the C-10 chiral center are disclosed in Figure 4. A consistent negative δ value was recorded for proton CH3-15, whereas positive δ values were observed for the protons H2-9 and H-7. According to Mosher’s rule,24,25 the absolute configuration at C-10 in 1 was unambiguously established as R. Therefore, compounds 1−4 were determined as shown in Figure 1, namely, clavurols A and B and clavuperoxylides A and B, respectively. It is worth mentioning that 3 and 4 are the first

Figure 2. Key 1H−1H COSY (red lines) and HMBC (arrows, from 1H to 13C) correlations of compounds 1−12.

Figure 3. Key NOESY correlations of compounds 1−7 and 9−11.
example of dolabellanes containing peroxy groups, especially the novel peroxide bridge in 4.

Clavurols C–E (5–7) showed extremely similar NMR data as those of 1, especially 5 and 7 possess the same molecular formula as 1. Detailed NMR data comparison suggested that 5 and 7 were the double bond migration product from $\Delta^{11,12}$ in 1 to $\Delta^{12,18}$ in 5 and $\Delta^{12,13}$ in 7, respectively, whereas 6 is the C-19 oxidation product of 5 because one of the gem-dimethyl in 5 was oxidized to be hydroxymethyl ($\delta_H$ 3.76 and $\delta_C$ 65.8) in 6. The same relative configuration of 5–7 was deduced by the obvious NOE correlations of their CH$_3$-15 and H-10. The absolute configuration in C-10 of 5 was further determined by using the same Mosher’s method as 1 to be R (Figure 4).

Clavurol F (8) was isolated as a colorless crystal. Its HREIMS showed an ion peak at m/z [M]$^+$ 322.2510 (calcd 322.2508), indicating the molecular formula to be C$_{20}$H$_{32}$O$_2$. Its NMR data were closely similar as those of clavudiol A (13), with only difference on the cyclopentane ring. Detailed NMR analysis revealed that the $\Delta^{11,12}$ in 13 was substituted with a 12-OH ($\delta_C$ 90.3, C-12 and $\delta_C$ 47.4, C-11) in 8 (Tables 1 and S2). The determination of the stereochemistry at C-12 of compound 8 was extremely difficult because it is a quaternary carbon with no available proton for the NOESY experiment. Fortunately, a suitable crystal of 8 was obtained by careful crystallization from petroleum ether (PE)/dichloromethane and submitted for the crystallographic study. The X-ray diffraction analysis of 8 employing graphite-monochromated Cu Kα radiation ($\lambda$ = 1.54178 Å) with a small Flack parameter 0.04(5) allowed the deduction of its absolute configuration and confirmed its structure as depicted in Figure 5.

Compound 9 encompassed a suit of highly similar NMR data as those of 8, even those of double bonds and oxidative carbons. However, its molecular formula C$_{20}$H$_{32}$O$_2$ (HREIMS: m/z 304.2407, [M]$^+$ calcd 304.2401) revealed an additional degree of unsaturation than that of 8. Bearing in mind the 18 mass unit difference in the molecular weight, an elimination of H$_2$O from two hydroxyls in 8 toward an epoxide in 9 was envisaged. Detailed comparison of their carbon NMR data of C-10, C-12, and C-18 revealed that it should be the condensation of 10-OH and 18-OH in 8 toward a stable tetrahydrofurann ring of 9.

Careful analysis of the NMR data of compounds 10 and 11 indicated that they were close structural analogues of 9 (Tables 1 and S2). Particularly, the presence of the methine signal at C-12 ($\delta_H$ 2.49 and $\delta_C$ 59.5) revealed that 10 is the precursor of 9, whereas 11 is the $\Delta^{12,13}$ product of 9 as revealed by the trisubstituted double bond signals at C-13 ($\delta_H$ 5.18 and $\delta_C$ 114.6) and C-12 ($\delta_C$ 157.6). The relative configurations of 9–11 were elucidated by the analysis of NOE correlations as shown in Figure 3. On the basis of the biogenetic consideration and by analogy to 8, the absolute configurations of 9–11 were tentatively assigned as shown in Figure 1. Compounds 9–11 are the first example of dolabellane diterpenoids comprising a tetrahydrofuran ring and was named herein as clavufuranolides A–C.

Compound 12 was obtained as an optically active, colorless crystal. The molecular formula, C$_{20}$H$_{32}$O$_2$ consistent with seven degrees of unsaturation, was determined by HREIMS (m/z 316.2038 [M]$^+$, calcd 316.2043) suggesting the diterpenoid nature of the metabolite. Furthermore, $^1$H and $^{13}$C NMR data (Tables 1 and S2) showed similarities with those of the co-occurring 14 and 15, indicating that 12 is also a dolabellane-type diterpenelactone. Detailed 1D and 2D NMR data analysis revealed that compound 12 was a 7,8-epoxy derivative of 15 from the characteristic epoxide signals at C-7 ($\delta_H$ 2.91 and $\delta_C$ 71.0) and C-8 ($\delta_C$ 59.6). The proposed structure, especially absolute configuration, of 12 was confirmed by X-ray diffraction analysis employing graphite-monochromated Cu Kα radiation ($\lambda$ = 1.54178 Å) with a small Flack parameter 0.07(4) (Figure 5). Compound 12 was thus named, after the family of clavurolides, clavirolide I.

Furthermore, the possible biosynthetic pathways of dolabellane-type diterpenoids is proposed as shown in Scheme 1. Geranylgeranyl pyrophosphate is a well-known starting material for diterpenoids. The pathway involved acetylation, reduction, oxidation, elimination, epoxidation, hydrolysis, cyclization, rearrangement, and so on. In addition, the proposed biosynthetic pathways supported the assignment of
the absolute configurations to the other new compounds because those of 8, 12, and 13 were proved by single crystal X-ray diffraction analysis.

All of the compounds were evaluated for their cytotoxic and protein tyrosine phosphatases 1B (PTP1B) inhibitory activities. Compounds 4, 5, and 10 were found to display moderate cytotoxic activity against the P388 cell line with IC\textsubscript{50} values of 37.6, 18.1, and 32.1 μM, respectively, with adriamycin as the positive control (IC\textsubscript{50} = 0.10 μM). Compound 4 also exhibited moderate cytotoxicity against the A549 cell line with an IC\textsubscript{50} value of 17.3 μM, with adriamycin as the positive control (IC\textsubscript{50} = 0.13 μM). Previously, compounds with an endoperoxide moiety were found to exhibit cytotoxicities. It is suggested that the endoperoxide moiety is probably responsible for the cytotoxicity of the novel compound 4. Compound 7 was found to display considerable PTP1B inhibitory activity with an IC\textsubscript{50} value of 14.5 μg/mL, with oleanolic acid as the positive control (IC\textsubscript{50} = 1.3 μg/mL).

**CONCLUSIONS**

In summary, 12 new biogenetically related dolabellane-type diterpenoids (1–12), along with three known analogues (13–15), were isolated from the soft coral C. viridis collected from the Xisha Island, South China Sea. Among them, compounds 9–11 represent the first examples of tetrahydrofuran-containing dolabellane skeletons, whereas 3 and 4 were the first dolabellanes comprising peroxyls. The absolute configurations of 8, 12, and 13 were established by X-ray diffraction analysis employing graphite-monochromated Cu Kα radiation. The absolute configurations of compounds 1 and 5 were determined by modified Mosher’s method, and the configurational assignments of other analogues were determined by biogenetic consideration due to their close similarities to the above unambiguously elucidated compounds. In bioassay, the cytotoxicity of clavuperoxylide B (4) on two cancer cell lines revealed the importance of the peroxyl group, whereas clavurol E (7) is first dolabellane diterpenoid discovered to have the PTP1B inhibitory activity. As a new type of skeleton for the aforementioned biological activities, further studies would be worth conducting for their structure modification toward novel and more bioactive drug leads.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Commercial silica gel (200–300 and 400–500 mesh) was used for column chromatography (CC), and precoated silica gel plates (GF254) were used for analytical thin-layer chromatography. Sephadex LH-20 gel was used for CC. NMR spectra were measured on either a Bruker DRX-500 or a Bruker DRX-400 spectrometer with the residual CHCl\textsubscript{3} (δ\textsubscript{H} 7.26 ppm and δ\textsubscript{C} 77.16 ppm) as an internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) are expressed in Hz. The \textsuperscript{1}H NMR spectra were recorded at an operating frequency of 500 or 400 MHz. The \textsuperscript{13}C NMR spectra were recorded at an operating frequency of 125 MHz. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. EIMS and HREIMS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Q-TOF Micro LC–MS–MS mass spectrometer.

**Biological Materials.** The specimens of C. viridis, identified by Prof. H. Hang of the South China Sea Institute of Oceanology, Chinese Academy of Sciences (CAS), were collected from the Xisha Islands, Hainan Province, P.R. China, on March 21, 2013 at a depth of −20 m and were frozen immediately after collection. A voucher specimen is available.
for inspection at the Shanghai Institute of Materia Medica, CAS, under registration no. 13XS-21.

**Extraction and Isolation.** The frozen animals (256 g, dry weight) were cut into pieces and extracted exhaustively with acetone at room temperature (4 × 20 L). The organic extract was evaporated to give a brown residue, which was partitioned between Et<sub>2</sub>O and H<sub>2</sub>O. The Et<sub>2</sub>O solution was concentrated under reduced pressure to give a dark-brown residue (6.6 g), which was fractionated by gradient silica gel CC (Et<sub>2</sub>O/PE, 0–100%), giving eight fractions. Fraction 3 was further purified with Sephadex LH-20 (PE/CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 2:1:1), followed by silica gel CC (Et<sub>2</sub>O/PE, 98:2–95:5) to give 1 (3.0 mg), 5 (16.2 mg), 7 (6.6 mg), and 9 (2.2 mg). Fraction 4 gave compound 10 (9.0 mg) after gel filtration on Sephadex LH-20 (PE/CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 2:1:1) and chromatography on silica gel (Et<sub>2</sub>O/PE, 96:4–93:7). Fraction 5 was eluted with a Sephadex LH-20 column (PE/CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 2:1:1) to give 3 pure (4.8 mg), and 13 pure (2.7 mg), 14 pure (6.6 mg), and 15 pure (0.10, in CH<sub>2</sub>Cl<sub>2</sub>), under reduced pressure to give a dark-brown residue (6.6 g), followed by chromatography on silica gel (Et<sub>2</sub>O/PE, 95:5=90:10) to afford pure 13 (58.6 mg), 14 (16.2 mg), and 15 (11.32, 1077 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>), see Tables 1 and S1; HRMS (EI) m/z: 288.2459 [M]+ (calcld for C<sub>20</sub>H<sub>30</sub>O, 288.2458).

**Compound 9.** Colorless oil; [α]<sub>D</sub> = −102.1 (c 0.17, in CHCl<sub>3</sub>); IR (KBr) <sup>ν</sup>max: 3359, 2966, 2924, 2851, 1660, 1637, 1444, 1195, 1132, 1077 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>), see Tables 1 and S2; HRMS (EI) m/z: 304.2401 [M]+ (calcld for C<sub>20</sub>H<sub>28</sub>O<sub>2</sub>, 304.2401).

**Compound 10.** Colorless oil; [α]<sub>D</sub> = −3.0 (c 0.10, in CHCl<sub>3</sub>); IR (KBr) <sup>ν</sup>max: 3359, 2929, 2850, 1645, 1468, 1195, 1132, 1077 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>), see Tables 1 and S2; HRMS (EI) m/z: 288.2459 [M]+ (calcld for C<sub>20</sub>H<sub>18</sub>O, 288.2458).

**Compound 11.** Colorless oil; [α]<sub>D</sub> = −34.0 (c 0.08, in CHCl<sub>3</sub>); IR (KBr) <sup>ν</sup>max: 3348, 2953, 2927, 2870, 1671, 1453, 1385, 1195, 1132, 1077 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>), see Tables 1 and S2; HRMS (EI) m/z: 316.2043 [M]+ (calcld for C<sub>20</sub>H<sub>20</sub>O<sub>2</sub>, 316.2038).

**X-ray Crystallographic Analysis for Compounds 8, 12, and 13.** The colorless block crystals of 8, 12, and 13 were obtained by recrystallization from PE–CH<sub>2</sub>Cl<sub>2</sub>. A single crystal with dimensions of 0.25 × 0.15 × 0.12 mm was used for X-ray diffraction studies on a Bruker APEX-II CCD diffractometer employing graphite-monochromated Cu Kα radiation (λ=1.54178 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least square difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed at idealized positions and refined as riding atoms with the relative isotropic temperature factors.

**Crystal Data of 8.** C<sub>20</sub>H<sub>28</sub>O<sub>3</sub> (M = 322.47), orthorhombic with the space group P<sub>2</sub>1<sub>2</sub>1<sub>2</sub>1, with = 7.8488 (2) Å, b = 14.8647 (3) Å, c = 15.8378 (3) Å, V = 1847.80 (7) Å<sup>3</sup>, T = 170 K, ZK = 4, D<sub>c</sub> = 1.159 mg/m<sup>3</sup>, and F(000) = 712. Independent reflections 3265 [R<sub>int</sub> = 0.030] and Flack parameter = 0.04 (5) (CCDC no. 2108956).

**Crystal Data of 12.** C<sub>20</sub>H<sub>18</sub>O<sub>3</sub> (M = 316.42), orthorhombic with the space group P<sub>2</sub>1<sub>2</sub>1<sub>2</sub>1, with = 8.3514 (2) Å, b = 8.8762 (2) Å, c = 23.3986 (5) Å, V = 1734.51 (7) Å<sup>3</sup>, T = 170 K, ZK = 4, D<sub>c</sub> = 1.212 mg/m<sup>3</sup>, and F(000) = 688. Independent reflections 3018 [R<sub>int</sub> = 0.032] and Flack parameter = 0.07 (4) (CCDC no. 2108958).

**Crystal Data of 13.** C<sub>20</sub>H<sub>18</sub>O<sub>5</sub> (M = 304.45), orthorhombic with the space group P<sub>2</sub>1<sub>2</sub>1<sub>2</sub>1, with = 8.5414 (3) Å, b = 10.4689 (3) Å, c = 20.7809 (5) Å, V = 1858.21 (10) Å<sup>3</sup>, T = 170 K, ZK = 4, D<sub>c</sub> = 1.088 mg/m<sup>3</sup>, and F(000) = 672. Independent reflections 3018 [R<sub>int</sub> = 0.039] and Flack parameter = 0.03 (7) (CCDC no. 2108773).

**Cytotoxicity Assays.** The antiproliferative activities of the isolated compounds were evaluated against the A549 and P388 cell lines using cell counting kit-8 (CCK-8) assay. Briefly, cells (10 × 10<sup>4</sup> cell/well) were seeded in 96-well plates and grown for 24 h. Cells were then treated with increasing concentrations of compounds and grown for further 72 h. At the end of exposure time, 10 µL of CCK-8 (Dojindo, Kumamoto, Japan) was added to each well, and the plates were kept in an incubator for 4 h and then measured at 450 nm using a multiwell spectrophotometer (SpectraMax, Molecular Devices, U.S.A). The inhibition rate was calculated using the
following relation: \((1 - A_{450\text{ treated}}/A_{450\text{ control}}) \times 100\%\). The cytotoxicity of compounds was expressed as IC\(_{50}\) determined by the Logit method. Adriamycin was used as the positive control with IC\(_{50}\) values of 0.13 and 0.10 \(\mu\)M on A549 and P388 cancer cells, respectively.

**PTP1B Inhibitory Activity Assay.** A recombinant PTP1B catalytic domain was expressed and purified according to a previous report.\(^{28}\) The enzymatic activities of the PTP1B catalytic domain were determined at 30 °C by monitoring the hydrolysis of pNPP. Dephosphorylation of pNPP generated the product pNP, which was monitored at an absorbance of 405 nm with an EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA). In a typical 100 \(\mu\)L assay mixture containing 50 mmol/L 3-morpholinopropanesulfonic acid, pH 6.5, 2 mmol/L pNPP, and 30 nmol/L recombinant PTP1B, activities were continuously monitored and the initial rate of hydrolysis was determined by using the early linear region of the enzymatic reaction kinetic curve. The IC\(_{50}\) was calculated with Prism 4 software (Graphpad, San Diego, CA) from the nonlinear curve fitting of the percentage of inhibition (\% inhibition) versus the inhibitor concentration [I] by using the following equation: \(\%\) inhibition = \(1/(1 + [IC_{50}/[I]]^k)\), where \(k\) is the Hill coefficient; IC\(_{50}\) ≥ 50 \(\mu\)M was considered inactive. Oleanolic acid was used as the positive control with IC\(_{50}\) value of 1.3 \(\mu\)g/mL.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06156.

1D and 2D NMR, HRMS, and IR spectra of 1-12 (PDF)

**Accession Codes**
CCDC 2108956, 2108958, and 2108773 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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**Notes**
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