Regular Article

Iriomoteolides-10a and 12a, Cytotoxic Macrolides from Marine Dinoflagellate *Amphidinium* Species

Mai Akakabe,^{a,†} Keiko Kumagai,^a Masayuki Tsuda,^a Yuko Konishi,^a Akira Tominaga,^b Daisuke Kaneno,^c Eri Fukushi,^d Jun Kawabata,^d Atsunori Masuda,^{e,‡} and Masashi Tsuda^{a,*}

^aScience Research Center, Kochi University; B200 Monobe, Nankoku, Kochi 783–8505, Japan; ^bGraduate School of Kuroshio Science and Kochi Medical School, Kochi University; B200 Monobe, Nankoku, Kochi 783–8505, Japan;

^cResearch Faculty of Agriculture, Hokkaido University; Sapporo 060–8589, Japan; ^dFaculty of Science, Kochi University; Kochi 780–8520, Japan; ^eYanmar Environmental Sustainability Support Association; Osaka 530–0014, Japan; and ^fCenter for Advanced Marine Core Research, Kochi University; B200 Monobe, Nankoku, Kochi 783–8505, Japan.

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Two new macrolides, iriomoteolides-10a (**1**) and -12a (**2**), have been isolated from a marine dinoflagellate *Amphidinium* sp. (KCA09053 strain), and their structures were elucidated on the basis of a detailed two dimensional (2D)-NMR analysis. Compound **1** is a novel 21-membered *Amphidinium* macrolide, which contains one tetrahydrofuran ring, two ketone carbonyls, two hydroxyl groups, and six one-carbon branches. Compound **2** is a new 12-membered macrolide related to amphidinolide Q. Compound **1** exhibited cytotoxic activity against human cervix adenocarcinoma HeLa and murine hepatocellular carcinoma MH134 cells.

Key words macrolide; dinoflagellate; *Amphidinium*; cytotoxic activity

Marine dinoflagellates of the genus *Amphidinium* have been proven to produce unique polyketide-like metabolites with interesting biological activities.¹⁾ A series of macrolides, represented by amphidinolides, are well-known secondary metabolites of *Amphidinium* dinoflagellates^{2,3)} with various carbon skeletons forming 12–29-membered (including odd-numbered) macrolactone rings and biosynthetically unique partial structures such as vicinally located C₁ branches. During our investigation of bioactive *Amphidinium* metabolites,^{4,5)} we have recently reported the isolation of 22- and 23-membered *Amphidinium* macrolides, i.e., iriomoteolides-13a⁶⁾ and -2a,⁷⁾ respectively, from the marine benthic dinoflagellate *Amphidinium* species (KCA09053 and HYA024 strains, respectively). Further investigation of the cytotoxic fractions of KCA09053 strain led to the discovery of the first 21-membered *Amphidinium* macrolide, iriomoteolide-10a (**1**), together with a new 12-membered macrolide, iriomoteolide-12a (**2**) (Fig. 1). Herein, we describe the isolation and structural elucidation of **1** and **2**.

Results and Discussion

The dinoflagellate *Amphidinium* sp. (strain KCA09053) was cultivated at 25°C for 2 weeks in seawater medium under illumination. The algal cells were extracted with methanol (MeOH)–toluene (3:1). The toluene-soluble materials of the extract were subjected to cytotoxic assay-guided fractionation using a silica gel column with chloroform (CHCl₃)–MeOH. One of the cytotoxic fractions was separated with an octadecylsilyl (ODS) column followed by reversed-phase HPLC to afford iriomoteolide-10a (**1**, 0.069% from dry weight). On the other hand, iriomoteolide-12a (**2**) was obtained from the less

polar fraction of the first silica gel column chromatography (CC) in 0.016% yield. Known macrolides, iriomoteolides-1a,⁸⁾ -3a,⁹⁾ and -13a,⁶⁾ have been separated by the first SiO₂ gel column from a more polar fraction than those containing **1** and **2**.

Iriomoteolide-10a (**1**) was obtained as optically active and colorless amorphous solid, and the molecular formula of C₃₅H₆₀O₇ was revealed by electrospray ionization (ESI)-MS data (*m/z* 615.4315 [M+Na]⁺, Δ+0.1 mmu). The ¹³C-NMR spectrum in benzene-*d*₆ (C₆D₆) (Table 1) showed the 35 carbon resonances, and their chemical shifts and multiplicities assigned by heteronuclear multiple quantum coherence (HMQC) and CH₂-selected heteronuclear single quantum coherence

Fig. 1. Structures of Iriomoteolides-10a (**1**) and -12a (**2**)

C-3, C-6, C-8, C-9 and C-11 and C-14, C-16, C-17, C-18, and C-20 of **1** were independently relative.

[†]Present address: Synthetic Organic Chemistry Laboratory, RIKEN; 2–1 Hirosawa, Wako, Saitama 351–0198, Japan.

[‡]Present address: College of Agriculture, Tamagawa University; Machida, Tokyo 194–861, Japan.

*To whom correspondence should be addressed. e-mail: mtsuda@kochi-u.ac.jp

Table 1. ^1H - and ^{13}C -NMR Data for Iriomoteolide-10a (**1**) in C_6D_6

Position	^{13}C	^1H (mult, J =in Hz)	Position	^{13}C	^1H (mult, J =in Hz)
1	171.3 C		17	90.6 CH	3.77 dd, 7.0, 2.8
2	42.9 CH_2	2.36 dd, 14.0, 4.7 2.21 dd, 14.0, 9.8	18	34.5 CH	1.94 m
3	34.3 CH	2.78 m	19	33.1 CH_2	2.03 ddd, 15.0, 9.7, 3.3 1.58 ddd, 15.0, 10.0, 3.1
4	133.7 CH	5.50 dd, 15.0, 7.2	20	75.3 CH	5.37 dd, 9.7, 3.1
5	135.4 CH	5.30 dd, 15.0, 8.6	21	210.0 C	
6	34.1 CH	2.54 m	22	42.8 CH	2.61 m
7	38.7 CH_2	1.43 m 1.27 m	23	32.5 CH_2	1.95 m 1.17 m
8	71.8 CH	3.74 ddd, 9.8, 3.5, 1.8	24	37.2 CH_2	1.11 ^a m
9	37.1 CH	1.81 m	25	23.8 CH_2	1.51 m 1.34 m
10	41.5 CH_2	1.41 m 0.96 m	26	41.7 CH_2	1.92 ^a t, 7.0
11	31.3 CH	1.49 m	27	210.1 C	
12	33.6 CH_2	1.52 m 1.20 m	28	35.7 CH_2	1.91 ^a q, 7.0
13	34.5 CH_2	1.57 m 1.54 m	29	7.9 CH_3	0.94 ^b t, 7.0
14	78.1 CH	4.17 m	30	20.5 CH_3	0.98 ^b d, 7.0
15	41.8 CH_2	1.84 m 1.64 ddd, 14.5, 8.0, 6.6	31	22.6 CH_3	1.10 ^b d, 7.0
16	74.0 CH	4.12 ddd, 7.0, 6.6, 3.5	32	15.2 CH_3	0.99 ^b d, 7.0
			33	20.6 CH_3	0.92 ^b d, 7.0
			34	16.5 CH_3	1.15 ^b d, 7.0
			35	17.9 CH_3	1.22 ^b d, 7.0

a) 2H. b) 3H.

(HSQC) spectra indicated the presence of 2 ketones, 1 ester carboxyl, 13 methines including two sp^2 ones, 12 methylenes, and 7 methyl carbons. One olefin and three carbonyl groups accounted for four out of the six degrees of unsaturation; thus, the remaining double bond equivalents were attributed to the presence of two rings in the molecule of **1**.

The planar structure of **1** was elucidated on the basis of detailed NMR studies, namely ^1H - ^1H correlated spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple bond correlation (HMBC), and CH_2 -selected HSQC-TOCSY spectra measured in benzene- d_6 . ^1H - ^1H COSY and TOCSY spectra revealed three ^1H - ^1H networks from H_2 -2 to H_2 -20, H_3 -30, H_3 -31, H_3 -32, H_3 -33, and H_3 -34, from H_2 -22 to H_2 -26 and H_3 -35, and from H_2 -28 to H_3 -29 (Fig. 2). The *E*-geometry was assigned to the disubstituted C-4–C-5 double bond, as suggested by the $J(\text{H-4/H-5})$ value (15.0 Hz). The long-range ^1H - ^{13}C correlations for H_2 -26 (δ_{H} 1.92)/C-24 (δ_{C} 37.2) and H_3 -35 (δ_{H} 1.22)/C-24 observed in CH_2 -selected HSQC-TOCSY spectrum supported an aliphatic substructure for the C-22–C-26 portion. HMBC correlations from H_2 -20 (δ_{H} 5.37) and H_3 -35 to a ketone carbonyl (C-21: δ_{C} 210.0) and from H_2 -26 (δ_{H} 1.92) and H_3 -29 (δ_{H} 0.94) to another ketone carbonyl (C-27: δ_{C} 210.1) suggested that the ketone groups connect C-20 to C-22 and C-26 to C-28. The HMBC correlation for H_2 -17 (δ_{H} 3.77)/C-14 (δ_{C} 78.1) revealed the presence of a tetrahydrofuran ring at C-14–C-17. The ester linkage between C-1 and C-20 was established by the HMBC correlations for H_2 -2 (δ_{H} 2.36 and 2.21)/C-1 (δ_{C} 171.3) and H_2 -20/C-1. Thus, the gross structure of **1** was concluded to contain a 21-membered macrolactone ring, as shown in Fig. 1.

The relative stereochemistry of **1** was investigated on the basis of nuclear Overhauser effect spectroscopy (NOESY) correlations and coupling constants. Magnitude of the ^1H - ^1H couplings was estimated from the intensity of the ^1H - ^1H

Fig. 2. Selected 2D-NMR Correlations for Iriomoteolide-10a (**1**)

COSY correlations, when signals overlapped with other signals or showed multiple couplings. The *J*-based configuration analysis¹⁰⁾ was applied to the investigation of C-8–C-9 and C-17–C-18 bonds. Long-range ^{13}C - ^1H coupling constants were obtained from analyses of the hetero half-filtered TOCSY (HETLOC) correlations or from the cross-peak intensities in the phase-sensitive HMBC spectrum.

The antiperiplanar relationship for H_3 – H_4 and H_5 – H_6 was deduced from NOESY correlations for H_3 /H-5 and H_4 /H-6 and from the relatively large $J(\text{H}_3/\text{H}_4)$ (7.2 Hz) and $J(\text{H}_5/\text{H}_6)$ values (8.6 Hz), thus suggesting an equatorial orientation for both C-30 and C-31 methyl groups (Fig. 3a). The 1,3-*syn* relation for C-31 and 8-OH was indicated by the coupling constants for H_6 /H-7a ($>8\text{ Hz}$), H_6 /H-7b ($<4\text{ Hz}$), $\text{H}_7\text{a}/\text{H}_8$ (9.8 Hz), and $\text{H}_7\text{b}/\text{H}_8$ (3.5 Hz), and by NOESY correlations for H_5 /H-7b and H_5 /H-8. Considering the coupling constants for H_8 /H-9 (1.8 Hz), C-8/H-9 (-1 Hz), C-10/H-8 ($<3\text{ Hz}$), and C-32/H-8 ($<6\text{ Hz}$), the C-8–C-9 bond was suggested to have the *threo* configuration (Fig. 3b). The NOESY correlation observed for H_8 /H-11 indicated that the methine

Fig. 3. Stereostructures for (a) C-2-C-8, (b) C-8-C-11, and (c) C-14-C-20 Portions in Iriomoteolide-10a (1)

"a" and "b" for geminal proton pairs denoted low- and high-field resonances, respectively. ^1H - ^1H coupling constants (Hz) (H/H): 4.7 (2a/3), 9.8 (2b/3), 7.2 (3/4), 7.0 (3/30), 15.0 (4/5), 8.6 (5/6), >8 (6/7a), <4 (6/7b), 7.0 (6/31), 9.8 (7a/8), 3.5 (7b/8), 1.8 (8/9), >8 (9/10a), <4 (9/10b), 7.0 (9/32), >8 (10a/11), <4 (10b/11), 7.0 (11/33), <4 (14/15a), 8.0 (14/15b), 3.5 (15a/16), 6.6 (15b/16), 7.0 (16/17), 2.8 (17/18), 3.3 (18/19a), 10.0 (18/19b), 7.0 (18/34), 9.7 (19a/20), 3.1 (19b/20). ^{13}C - ^1H coupling constants (Hz) (C/H): -1 (8/9), <3 (10/8), ca. 0 (17/18), 6 (19/17), <6 (32/8), 2 (34/17).

proton of C-8 was oriented toward the inside of the macrocyclic ring. The relatively large $J(\text{H-9}/\text{H-10a})$ and $J(\text{H-10a}/\text{H-11})$ values (both >8 Hz) were deduced to be an antiperiplanar relationship between these protons and an equatorial orientation for both C-32 and C-33 methyl groups, which was corroborated by the NOESY correlation for H-9/H-11.

For the tetrahydrofuran portion at C-14-C-17 (Fig. 3c), NOESY correlations for H-14/H-17 and H-16/H-18 revealed *syn* and *anti* configurations for H-14-H-17 and H-16-H-17, respectively. The *erythro* configuration of the C-17-C-18 bond was elucidated from the coupling constants of H-17/H-18 (2.8 Hz), C-17/H-18 (ca. 0 Hz), C-19/H-17 (6 Hz), and C-34/H-17 (2 Hz). The *anti* relations for H-18-H-19b and H-19a-H-20 were implied by their relatively large coupling constants (10.0, 9.7 Hz, respectively). Moreover, a 1,3-*syn* configuration for the C-18-C-20 portion was suggested by NOESY correlations for H-16/H-18, H-16/H-19a, H-17/H-19b, H-18/H-20, and H-20/H₃-34. On the other hand, the stereochemical relationship between C-20 and C-22 through the C-21 ketone could not be determined, although NOESY correlations were found for H-20/H-22, H-20/H-23b, and H-20/H₃-35. In addition, because of the overlap between H-12a and H₂-13, the relative stereochemistry between C-11 and C-14 could not be unambiguously assigned. In order to determine the absolute configuration at C-8 and C-16, the modified Mosher's method was applied using a small amount of **1**; however, **1** decomposed in the reaction with α -methoxy- α -trifluoromethylphenylacetyl chloride (MTPACl).

The molecular formula, C₂₅H₄₀O₅, of iriomotrolide-12a (**2**) was established by ESI-MS (m/z 443.2768 [$\text{M}+\text{Na}$]⁺, Δ +0.0 mmu). The ^{13}C -NMR spectrum in chloroform-*d*₁ (CDCl₃)

Fig. 4. Selected 2D-NMR Correlations for Iriomoteolide-12a (**2**)

disclosed the presence of a total of 25 carbon signals due to 2 ketones, 1 ester carbonyl, 2 sp^2 quaternary carbons, 2 sp^2 methines, 6 sp^3 methines including 2 oxygenated ones, 5 sp^3 methylenes, and 7 methyl groups. The ^1H -NMR spectrum showed the presence of two singlet sp^2 methines [δ 5.99 (H-17), 5.89 (H-2)] and three singlet methyl groups [δ 2.12 (H₃-19), 2.07 (H₃-20), and 2.06 (H₃-25)], all presumably adjacent to quaternary carbons. ^1H - ^1H COSY and TOCSY spectra revealed two ^1H - ^1H networks from H-4 to H₂-5 and from H-7 to H₂-15, H₃-21, H₃-22, H₃-23, and H₃-24 (Fig. 4). HMBC correlations for H-2/C-3 (δ_{C} 154.9), H₃-20/C-3, and H₃-20/C-4 (δ_{C} 73.1) indicated that C-2 and C-20 were connected to C-4 through the sp^2 quaternary carbon C-3. The presence of a ketone carbonyl at C-6 was implied by HMBC correlations for H₂-5 (δ_{H} 3.02, 2.48)/C-6 (δ_{C} 217.0) and H₃-21 (δ_{H} 1.03)/C-6. Attachment of a pent-2-en-3-one terminus at C-15 was deduced from HMBC correlations for H₂-15 (δ_{H} 2.07, 1.84)/C-16 (δ_{C} 157.9), H-17/C-18 (δ_{C} 198.7), H₃-19/C-18, H₃-25/C-16, and H₃-25/C-17. The *E*-geometries for the two trisubstituted double bonds at C-2-C-3 and C-16-C-17 were inferred from the ^{13}C chemical shifts of the C-20 and C-25 methyl groups (δ_{C} 16.8, 14.7, respectively). A four-bond HMBC correlation was observed from H₃-20 to C-1 (δ_{C} 167.7), thus indicating that an ester carbonyl was attached to C-2. The relatively low-field resonance for the oxymethine H-11 (δ_{H} 5.06) suggested that C-11 was involved in an ester linkage with C-1. Thus, the planar structure of **2** was found to consist of a 12-membered macrolide associated with two α,β -unsaturated carbonyls, a saturated ketone, six C₁ branches, and a hydroxyl group. Moreover, the gross structure of the macrocyclic portion of **2** corresponded to that of amphidinolide Q¹¹⁻¹³ (**3**), a known 12-membered macrolide.

In order to elucidate the stereochemistry of **2**, the ^{13}C chemical shifts of **2** in CDCl₃ were compared with the data reported¹²⁾ for **3**. Figure 5 shows the absolute values of chemical shift differences [$\Delta\delta$ (in ppm) = δ (**2** in CDCl₃) - δ (**3** in CDCl₃)] for C-1-C-13 and four branched methyl groups at C-3, C-7, C-9, and C-13. The absolute $\Delta\delta$ values for C-1-C-11 and methyls at C-3, C-7, and C-9 were extremely small (≤ 0.2 ppm), strongly suggesting that the relative stereochemistries for C-4, C-7, C-9, and C-11 in the macrolactone ring of **2** were the same as those of **3**. On the other hand, because of side-chain structural differences between **2** and **3**, the $\Delta\delta$ values for C-12, C-13, and 13-Me were high. C-11 and C-13 of **2** were assumed to take a *syn* configuration, as that in **3**, because the strongly coupled signal for H₂-12 [δ_{H} 1.42 (2H)] of **2** was reminiscent of the signal pattern of **3**.¹²⁾ Moreover, the optical rotation ($[\alpha]_{\text{D}}$) of **2** showed the same sign and similar magnitude as that reported for **3**, presumably indicating that the absolute stereochemistries for C-4, C-7, C-9, C-11, and

Fig. 5. Chemical Shift Differences ($\Delta\delta$) between Iriomoteolide-12a (**2**) and Amphidinolide Q (**3**)

C-13 of **2** corresponded to those of **3**. The relatively small coupling constant (≤ 4 Hz) for H-13–H-14 suggested a *gauche* relation between H-13 and H-14. Nevertheless, the relative stereochemistry of the C-13–C-14 bond could not be assigned, because no information on long-range ^{13}C – ^1H coupling around the C-13–C-14 bond was obtained from the HETLOC spectrum.

Iriomoteolide-10a (**1**) is the first 21-membered *Amphidinium* macrolide, although several macrolides with odd-numbered, e.g. 13-, 15-, 17-, 19-, 23-, 25-, 27-, and 29-membered, lactone rings were isolated from the marine dinoflagellates *Amphidinium* species.³⁾ Isoapoptolidin^{14,15)} has been reported as the first example of 21-membered macrolide from natural sources, and corresponds to a ring-expanded isomer of the 20-membered macrolide, apoptolidin.^{16,17)} The interconversion between apoptolidin and isoapoptolidin was described to occur in dilute aqueous solution. However, such isomerization was not observed for **1**, in alcohol or water. Furthermore, linear fatty acid precursors for **1** and **2** have not been observed in the extract. Iriomoteolide-12a (**2**) is a new congener of amphidinolide Q (**3**), containing a 12-membered lactone ring. Compound **1** exhibited cytotoxic activity against human cervix adenocarcinoma HeLa, human B lymphocyte DG-75, and murine hepatocellular carcinoma MH134 cells (IC_{50} : 1.5, 1.2, 3.3 μM , respectively). Compound **2** showed moderate cytotoxicity against DG-75 cells (IC_{50} : 50 μM).

Experimental

General Experimental Procedures Optical rotation and IR data were measured on a JASCO DIP-370 polarimeter and a JASCO FT/IR-5300 spectrophotometer, respectively. NMR data were recorded using 2.5 mm microcells (Shigemi Co., Ltd., Japan). NMR spectra were measured on a Bruker AMX-500 spectrometer or a Varian-NMR500 spectrometer equipped with a triple resonance PFG Cold Probe or CH “Xsens” PFG Cold Probe. Chemical shifts were reported in ppm with reference to the residual proton and carbon signals of C_6D_6 (δ_{H} 7.20 and δ_{C} 128.0, respectively) and CDCl_3 (δ_{H} 7.26 and δ_{C} 77.0, respectively). ESI-MS spectra were recorded on a JEOL JMS-T100LC spectrometer.

Materials A dinoflagellate *Amphidinium* species (strain

KCA09053) was monoclonally separated from benthic sea sands collected off Iriomote Island, Japan in May 2009.⁶⁾ The voucher specimen was deposited at the Center for Advanced Marine Core Research, Kochi University.

Extraction and Isolation For the cultivation of the dinoflagellate, deep seawater pumped up from –344 m, offshore of Muroto, Japan, was sterilized by filtration performed using an ultrafiltration membrane, and was then utilized. Cultivation was performed using a 50 L fermenter (Earth Co., Japan) composed of polycarbonate resin. The culture was maintained in seawater supplemented with 1% Provasoli’s enriched seawater at 25–30°C with mechanical stirring at 180 rpm stirring using a MAZELA Z-1300 mechanical stirrer (Tokyo Rikakikai Co., Ltd., Japan) under illumination of approximately 30 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by fluorescent lights with a 16 h:8 h light/dark cycle. The cultured algal cells were filtered and concentrated by using a MOLSEP® Fiber FS03-FC-FUS1582 ultrafiltration membrane (Daicel Membrane-Systems Ltd., Japan). The concentrated cells (ca. 2 L from 50 L culture) were centrifuged at 3000 rpm at 4°C for 10 min by using a Hitachi CR 22GIII high speed refrigerated centrifuge (Hitachi Koki Co., Ltd., Japan) and then lyophilized using an EYELA FD-511 freeze dryer (Tokyo Kasei Co., Ltd., Japan). The dried algal cells (30.3 g) obtained from 350 L of medium were extracted with MeOH–toluene (3:1, 300 mL $\times$ 3), and partitioned between toluene (500 mL $\times$ 3) and water (H_2O , 500 mL). The toluene-soluble materials (7.0 g) of the extract were subjected to silica gel CC using a gradient elution of 0–2% MeOH in CHCl_3 . The fraction eluted with CHCl_3 –MeOH (98:2) was then chromatographed using an ODS column [Cosmosil 140C₁₈-PREP; eluent: acetonitrile (CH_3CN)– H_2O , 7:3] to give a cytotoxic fraction with >80% inhibition of HeLa cells proliferation. The cytotoxic fraction was separated by reversed-phase HPLC [YMC-Pack Pro C₁₈, 5 μm , YMC Co., Ltd. (Japan) 10 mm $\times$ 250 mm; eluent, CH_3CN – H_2O (70:30); flow rate, 2 mL/min; UV detection at 210 nm] to afford **1** [1.4 mg, 0.069%, retention time (t_{R}) 24 min]. The less polar fraction of the first silica column, eluted with CHCl_3 and CHCl_3 –MeOH (98:2), was separated by ODS CC using a gradient of CH_3CN in H_2O (70 to 100%). The fraction eluted with 70% aqueous CH_3CN was purified by reversed-phase HPLC, using the

conditions described above, to afford **2** (0.32 mg, 0.016%, t_R 17 min).

Cytotoxic Assay A cytotoxicity assay involving HeLa, DG-75, and MH134 cells was performed at 37°C in 5% carbon dioxide, at a density of 5000 cells per well in 96-well plates, using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. After 72 h, the number of viable cells was counted using Cell Counting Kit 8 (Dojindo Co., Kumamoto, Japan) according to the manufacturer's instructions. The assay reagent was a tetrazolium compound (WST-8) that is reduced by live cells into a colored formazan product; the absorbance was measured at 450 nm using a microplate reader (Bio-Rad, U.S.A.). The experiments were repeated in triplicate wells. The viability of the treated groups was estimated as a percentage of that of the control groups. The cytotoxicity is expressed as IC_{50} . 5-Fluorouracil was used as an authentic sample, and IC_{50} values against HeLa, DG-75, and MH134 cells were 30, 9, and 0.6 μM , respectively.

Iriomoteolide-10a (**1**)

Colorless amorphous solid; $[\alpha]_D^{23}$ -20 ($c=0.25$, $CHCl_3$); IR (KBr) cm^{-1} : 3443 (broad), 2920, 1714; 1H - and ^{13}C -NMR (500 MHz, C_6D_6): Table 1; ESI-MS m/z : 615.4315 $[M+Na]^+$ (Calcd for $C_{33}H_{60}O_{10}Na$, 615.4316).

Iriomoteolide-12a (**2**)

Colorless amorphous solid; $[\alpha]_D^{23}$ $+37$ ($c=0.16$, MeOH); UV λ_{max} (MeOH) nm (ϵ): 223 (21300); IR (KBr) cm^{-1} : 3438 (broad), 2920, 1700; 1H -NMR (500 MHz, $CDCl_3$) δ : 0.68 (3H, d, $J=7.0$ Hz, H_3-24), 0.76 (3H, d, $J=7.0$ Hz, H_3-23), 0.90 (3H, d, $J=7.0$ Hz, H_3-22), 0.90 (1H, m, H-8b), 0.91 (1H, m, H-9), 1.03 (3H, d, $J=7.0$ Hz, H_3-21), 1.27 (1H, brd, $J=14.0$ Hz, H-10b), 1.41 (1H, m, H-10a), 1.42 (2H, t $J=7.0$ Hz, H_2-12), 1.51 (1H, m, H-13), 1.84 (1H, dd, $J=15.0, 8.4$ Hz, H-15b), 1.91 (1H, m, H-14), 2.05 (1H, m, H-8a), 2.06 (3H, s, H_3-25), 2.07 (1H, m, H-15a), 2.07 (3H, s, H_3-20), 2.12 (1H, m, H-7), 2.12 (3H, s, H_3-19), 2.48 (1H, dd, $J=12.6, 5.4$ Hz, H-5b), 3.02 (1H, dd, $J=12.6, 2.9$ Hz, H-5a), 4.42 (1H, m, H-4), 5.06 (1H, m, H-11), 5.89 (1H, s, H-2), 5.99 (1H, s, H-17); ^{13}C -NMR (500 MHz, $CDCl_3$) δ : 13.4 (CH_3 , C-24), 14.7 (CH_3 , C-25), 16.8 (CH_3 , C-20), 17.9 (CH_3 , C-21), 19.2 (CH_3 , C-25), 23.2 (CH_3 , C-22), 31.8 (CH_3 , C-19), 33.18 (CH, C-9), 33.23 (CH, C-13), 33.4 (CH, C-14), 39.8 (CH_2 , C-8), 40.4 (CH_2 , C-12), 44.6 (CH_2 , C-5), 44.9 (CH_2 , C-10), 46.9 (CH_2 , C-15), 50.7 (CH, C-7), 73.1 (CH, C-4), 74.2 (CH, C-11), 116.9 (CH, C-2), 124.9 (CH, C-17), 154.9 (C, C-3), 157.9 (C, C-16), 167.7 (C, C-1), 198.7 (C, C-18), 217.0 (C, C-6); ESI-MS m/z : 443.2768 $[M+Na]^+$ (Calcd for $C_{25}H_{40}O_5Na$, 443.2768).

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Conflict of Interest The authors declare no conflict of interest.

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References

- Blunt J. W., Copp B. R., Keyzers R. A., Munro M. H. G., Prinsep M. R., *Nat. Prod. Rep.*, **32**, 116–211 (2015).
- Kobayashi J., Tsuda M., *Nat. Prod. Rep.*, **21**, 77–93 (2004).
- Kobayashi J., Kubota T., *J. Nat. Prod.*, **70**, 451–460 (2007), and references cited therein.
- Minamida M., Kumagai K., Ulanova D., Akakabe M., Konishi Y., Tominaga A., Tanaka H., Tsuda M., Fukushima E., Kawabata J., Masuda A., Tsuda M., *Org. Lett.*, **16**, 4858–4861 (2014).
- Akakabe M., Kumagai K., Tsuda M., Konishi Y., Tominaga A., Tsuda M., Fukushima E., Kawabata J., *Tetrahedron Lett.*, **55**, 3491–3494 (2014).
- Akakabe M., Kumagai K., Tsuda M., Konishi Y., Tominaga A., Tsuda M., Fukushima E., Kawabata J., *Tetrahedron*, **70**, 2962–2965 (2014).
- Kumagai K., Tsuda M., Masuda A., Fukushima E., Kawabata J., *Heterocycles*, **91**, 265–274 (2015).
- Tsuda M., Oguchi K., Iwamoto R., Okamoto Y., Kobayashi J., Fukushima E., Kawabata J., Ozawa T., Masuda A., Kitaya Y., Omasa K., *J. Org. Chem.*, **72**, 4469–4474 (2007).
- Oguchi K., Tsuda M., Iwamoto R., Okamoto Y., Kobayashi J., Fukushima E., Kawabata J., Ozawa T., Masuda A., Kitaya Y., Omasa K., *J. Org. Chem.*, **73**, 1567–1570 (2008).
- Matsumori N., Kaneno D., Murata M., Nakamura H., Tachibana K., *J. Org. Chem.*, **64**, 866–876 (1999).
- Kobayashi J., Takahashi M., Ishibashi M., *Tetrahedron Lett.*, **37**, 1449–1450 (1996).
- Takahashi Y., Kubota T., Fukushima E., Kawabata J., Kobayashi J., *Org. Lett.*, **10**, 3709–3711 (2008).
- Hangyou M., Ishiyama H., Takahashi Y., Kobayashi J., *Org. Lett.*, **11**, 5046–5049 (2009).
- Wender P. A., Gullledge A. V., Jankowski O. D., Seto H., *Org. Lett.*, **4**, 3819–3822 (2002).
- Pennington J. D., Williams H. J., Salomon A. R., Sulikowski G. A., *Org. Lett.*, **4**, 3823–3825 (2002).
- Kim J. W., Adachi H., Shin-ya K., Hayakawa Y., Seto H., *J. Antibiot.*, **50**, 628–630 (1997).
- Hayakawa Y., Kim J. W., Adachi H., Shin-ya K., Fujita K., Seto H., *J. Am. Chem. Soc.*, **120**, 3524–3525 (1998).