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# Antineoplastic Agents. 520. Isolation and Structure of Irciniastatins A and B from the Indo-Pacific Marine Sponge *Ircinia ramosa*<sup>1</sup>

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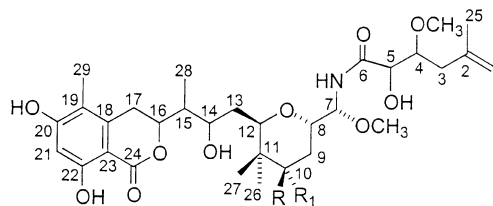
The Indo-Pacific marine sponge *Ircinia ramosa* has been found to contain two powerful (GI<sub>50</sub> from 0.001 to <0.0001  $\mu$ g/mL) murine and human cancer cell growth inhibitors. Both were isolated (10<sup>-3</sup>–10<sup>-4</sup>% yields) by cancer cell line bioassay-guided techniques and named irciniastatins A (**1**) and B (**2**). Structural elucidation by a combination of spectral analyses, primarily high resolution mass and 2D-NMR (principally APT, HMQC, HMBC, and ROESY) spectroscopy, revealed the unusual structures **1** and **2**.

An encouraging number of marine-organism-derived anticancer drugs are either already in human cancer clinical trials<sup>2a–g</sup> or advancing in preclinical development toward that vitally important objective.<sup>2h–j</sup> Others<sup>2k–m</sup> are in earlier stages of development. As another productive advance in our research efforts<sup>3</sup> to discover increasingly useful anticancer drug candidates based on marine organism constituents, we have investigated a promising lead offered by the marine sponge *Ircinia ramosa*. In the sponge phylum Porifera, the order Dictyoceratida contains very productive families in terms of biologically active constituents, and *I. ramosa* is a member of one, the Ircinidae family. The *Ircinia* genus is known in the Indo-Pacific area<sup>4</sup> including Malaysia, where we collected it (~1 kg wet weight) near Samporna, Borneo in 1991. The reddish-brown exterior was more typical of an *Ircinia* sp. from Papua New Guinea.<sup>4b</sup>

The dichloromethane–methanol extract gave strong (GI<sub>50</sub> 10<sup>-2</sup>  $\mu$ g/mL) activity against the P388 lymphocytic leukemia and a minipanel of human cancer cell lines. Unfortunately, our investigation of this lead was seriously delayed and made more challenging by political problems that prevented recollection on a larger scale. However, rapid advances in our overall techniques this past decade have allowed discovery and structural elucidation of two constituents, designated irciniastatins A (**1**) and B (**2**), that are quite remarkable in terms of both cancer cell growth inhibition and novel structure. Since 1972, the *Ircinia* genus has been part of over 135 published studies where some 11<sup>5a–k</sup> have led to cancer cell growth inhibitors such as the marine alkaloid ircinamine.<sup>5a</sup>

The sponge was preserved in methanol and extracted with dichloromethane–methanol (1:1). The dichloromethane fraction was subjected to solvent partition separation (*n*-hexane and 9:1 CH<sub>3</sub>OH–water followed by CH<sub>2</sub>Cl<sub>2</sub> and 3:2 CH<sub>3</sub>OH–water). The resulting dichloromethane-soluble fraction was separated, guided by

P388 leukemia cell line bioassay, employing gel permeation and partition column chromatographic procedures on Sephadex LH-20 with CH<sub>3</sub>OH, CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (3:2), and *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (5:1:1) as eluents. Final separation and purification procedures were performed by utilizing reversed phase (C18) HPLC (30% and 38% CH<sub>3</sub>CN in H<sub>2</sub>O) to yield 34.7 and 2.2 mg of the most potent constituents, irciniastatins A (**1**) and B (**2**), respectively.



**1**, R = OH, R<sub>1</sub> = H, Irciniastatin A

**2**, R, R<sub>1</sub> = O, Irciniastatin B

Irciniastatins A (**1**) and B (**2**) were obtained as colorless amorphous powders. The high-resolution FAB mass spectrum of **1** showed a pseudomolecular ion peak at *m/z* 610.3228 [M + H]<sup>+</sup>, which revealed the molecular formula as C<sub>31</sub>H<sub>48</sub>NO<sub>11</sub> (calcd 610.3228), implying nine degrees of unsaturation. Both **1** and **2** exhibited similar resonance patterns in their <sup>1</sup>H and <sup>13</sup>C NMR spectra. Interpretation of 2D-COSY, TOCSY, and HMQC spectra of irciniastatin A (**1**) revealed three spin–spin systems (I, CH<sub>2</sub>=CH(CH<sub>3</sub>)–CH<sub>2</sub>–CH(OCH<sub>3</sub>)–CH(OH)–CO–; II, NH–CH(OCH<sub>3</sub>)–CH(O–)–CH<sub>2</sub>–CH(OH)–; and III, CH(O–)–CH<sub>2</sub>–CH(OH)–CH(CH<sub>3</sub>)–CH(O–)–CH<sub>2</sub>–). A methyl, a carbonyl, and six aromatic carbons remained and were deduced to be a 1'-carbonyl-2',4'-dihydroxyl-5'-methylbenzene unit based upon HMBC correlations (Table 1). One proton on the benzene ring had a quite low downfield chemical shift ( $\delta$  11.13 s), which indicated that the proton was hydrogen bonded with an adjacent carbonyl oxygen atom. An HMBC correlation from the NH ( $\delta$  7.09 d) to a carbonyl carbon ( $\delta$  173.49 s) defined an amide linkage between spin–spin systems I and II. A series of HMBC correlations around spin–spin system

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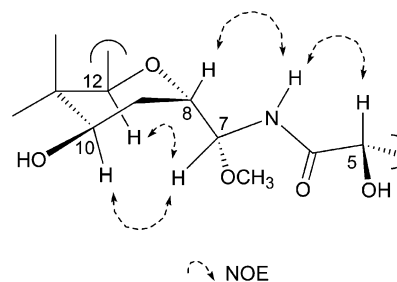
**Table 1.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR Assignments (recorded in  $\text{CDCl}_3$ ) for Irciniastatins A (**1**) and B (**2**)

irciniastatin A ( <b>1</b> )				HMBC (from H to C)	irciniastatin B ( <b>2</b> )				HMBC (from H to C)
C no.	$^1\text{H}$ $\delta$	$J$ (Hz)	$^{13}\text{C}$ $\delta$		C no.	$^1\text{H}$ $\delta$	$J$ (Hz)	$^{13}\text{C}$ $\delta$	
1	4.80 s		113.06 t	2, 3	1	4.80 s		113.07 t	2, 3
2			141.96 s		2			141.89 s	
3a	2.18 m		37.58 t	1, 2, 4, 5, 25	3a	2.16 m		37.21 t	1, 2, 4, 25
3b	2.38 dd	4.0, 15		1, 2, 4, 5, 25	3b	2.36 m			1, 2, 4, 25
4	3.74 m		80.53 d	3, 6, $\text{OCH}_3$	4	3.74 m		80.36 d	$\text{OCH}_3$
$\text{OCH}_3$	3.38 s		57.92 q	4	$\text{OCH}_3$	3.36 s		56.41 q	
5	4.11 d	2.5	73.11 d	3, 4, 6	5	4.44 d	2.5	72.23 d	3, 4, 6
OH	5.76 s <sup>a</sup>								
6			173.49 s		6			172.77 s	
NH	7.09 d	10.5		6	NH	7.36 d	10		6
7	5.45 t	4.5	78.31 d	6, 8, $\text{OCH}_3$	7	5.20 t	4.5	80.24 d	$\text{OCH}_3$
$\text{OCH}_3$	3.38 s		56.25 q	7	$\text{OCH}_3$	3.38 s		56.67 q	
8	3.89 m		73.11 d	7, 12	8	3.89 m		73.58 d	7, 10, 12
9a	1.81 m		29.70 t	7, 8, 10, 11	9	2.62 m		38.58 t	7, 8, 10
9b	2.06 m			7, 8, 10, 11					
10	3.67 dd	4.0, 10.5	71.41 d	11, 12, 27	10			210.00 s	
11			38.75 s		11			49.40 s	
12	3.53 d	10.5	81.90 d	8, 10, 11, 13, 14, 26	12	4.00 d	11	82.96 d	8, 10, 11, 13, 26
13	1.62 m		32.11 t	12	13	1.58 m		32.17 t	
14	3.96 m		73.75 d	12, 16, 26	14	4.08 m		72.40 d	16
OH	4.36 s			13, 14, 15					
15	1.84 m		42.62 d	14, 16	15	1.90 m		42.64 d	
16	4.53 m		79.41 d	14, 15, 18, 26	16	4.57 m		80.09 d	
17a	2.82 m		28.39 t	15, 16, 18, 19, 23	17a	2.88 m		28.18 t	
17b	2.89 m			16, 18, 19, 23					
18			139.68 s		18			139.69 s	
19			113.20 s		19			113.07 s	
20			160.08 s		20			162.36 s	
OH	4.60 s <sup>a</sup>			21					
21	6.31 s		101.28 d	19, 20, 22, 23, 24	21	6.32 s		101.37 d	
22			162.29 s		22			162.36 s	
OH	11.13 s			21, 22, 23					
23			101.59 s		23			101.37 s	
24			170.44 s		24			170.48 s	
25	1.76 s		22.68 q	1, 2, 3	25	1.75 s		22.66 q	1, 2, 3
26	0.92 s		13.67 q	11, 27	26	1.10 s		19.21 q	10, 11, 27
27	0.97 s		23.06 q	11, 26	27	1.16 s		22.16 q	10, 11, 26
28	1.10 d	6.0	9.36 q	14, 16	28	1.11 d		9.02 q	14, 15, 16
29	2.02 s		10.44 q	17, 18, 19	29	2.08 s		10.53 q	17, 18, 19

<sup>a</sup> The data were obtained using  $\text{CD}_3\text{OH}$  as solvent.

II and III as well as one quaternary carbon ( $\delta$  38.75 s) and two geminal methyl groups ( $\delta$  0.92 s/13.67 q and  $\delta$  0.97/23.06 q), with cross-peaks of H-8/C-12, H-12/C-8, H-12/C-10, H-10/C-12 and H-9/C-11 (Table 1), established a *gem*-dimethyltetrahydropyran unit between spin-spin systems II and III. Between spin-spin system III and the benzene ring, many HMBC correlations were observed, namely from H-17 ( $\delta$  2.82 and 2.89) to C-18 ( $\delta$  139.68 s), C-19 ( $\delta$  113.20 s), and C-23 ( $\delta$  101.59 s), from H-16 ( $\delta$  4.53) to C-18, and from methyl protons at the benzene ring (C-29,  $\delta$  2.02 s) to C-17 ( $\delta$  28.39 t). Thus, connection of one side of spin-spin system III with the 1'-carbonyl-2',4'-dihydroxyl-5'-methylbenzene portion was confirmed. Also, according to HMBC cross-peaks, two methoxyl groups ( $\delta$  3.38 s) were identified and linked at C-4 ( $\delta$  80.53 d) and C-7 ( $\delta$  78.31 d), respectively.

The  $^{13}\text{C}$  chemical shifts of C-5 ( $\delta$  73.11 d), C-10 ( $\delta$  71.41 d), and C-14 ( $\delta$  73.75 d) indicated the presence of a hydroxyl group linked to each of the three carbons. A special deuterated solvent ( $\text{CD}_3\text{OH}$ ) was used in 1D- and 2D-NMR experiments for defining each hydroxyl group location. Four active hydroxyl protons were determined to be located at C-5, C-14, C-20, and C-22. Since a few signals were found to overlap with the large  $\text{D}_2\text{O}$  resonance, the hydroxyl proton at C-10 was not observed. Comparison of the  $^{13}\text{C}$  chemical shift of C-16 with those of C-5, C-10, and C-14 showed that C-16 was shifted downfield by 5.66 to 8.0 ppm. Those data clearly showed C-16 was the site of another ring involving an

**Figure 1.** NOE correlations around C-5–C-12 in **1**.

oxygen atom, which satisfied the last one degree of unsaturation. In NMR experiments employing  $\text{CD}_3\text{OH}$  solvent, the lactone carbonyl group (C-14,  $\delta$  170.44 s) was found linked to the benzene ring, which suggested the formation of a six-member lactone with C-16. On the basis of the above extensive analysis, the structure of irciniastatin A (**1**) was established.

A review of 2D-NOESY and ROESY experiments afforded valuable stereochemical information for assignment of four chiral centers. Because both protons at C-5 and C-8 exhibited NOE correlations with the amide proton (NH), these three protons were clearly oriented from the same side in space (Figure 1). Two other NOE correlations were observed from H-7 to the two axial protons on the tetrahydropyran, H-10 and H-12, and implied that these three protons were in close proximity on another side. Therefore, the relative configuration of the four chiral carbons was deduced as  $7R^*$ ,  $8S^*$ ,  $10R^*$ , and  $12R^*$ .

**Table 2.** Inhibition of Cancer Cell Line Growth (GI<sub>50</sub>,  $\mu\text{g/mL}$ ) by Irciniastatins A (**1**) and B (**2**)

human cancer cell line		irciniastatin A	irciniastatin B
pancreas	BXPC-3	0.0038	0.00073
breast	MCF-7	0.0032	0.00050
CNS	SF268	0.0034	0.00066
lung	NCI-H460	<0.0001	0.0012
colon	KM20L2	0.0027	0.0021
prostate	DU-145	0.0024	0.0016
leukemia <sup>a</sup>	P388	0.00413	0.006
<b>normal endothelial</b>	HUVEC <sup>b</sup>	<0.0005	ND

<sup>a</sup> Murine. <sup>b</sup> BD-Biosciences Clontech.

Irciniastatin B (**2**) was found to correspond to molecular formula  $\text{C}_{31}\text{H}_{45}\text{NO}_{11}$ , as determined by high-resolution FAB mass spectroscopy, indicating two hydrogens less and one degree of unsaturation more than irciniastatin A (**1**). In the  $^{13}\text{C}$  NMR spectrum one hydroxyl disappeared and one ketone carbon signal ( $\delta$  210.00 s) appeared compared to that of **1**. HMBC correlations from H-8, H-9, H-12, and two geminal methyls (C-12 and C-27) to the ketone carbon were observed, suggesting that the ketone was at C-10. Also, the chemical shifts of two neighboring carbons (C-9 and C-11) were shifted downfield by 8.88 and 10.65 ppm, respectively. The resonance shifts supported the assignment from the HMBC experiment. Therefore, irciniastatin B was assigned structure **2**. Both irciniastatins A and B resisted a variety of attempts at crystallization, and that has so far precluded completing the stereochemical assignments by X-ray crystal structure determination. Synthetic approaches to these important new anticancer drug candidates will now be undertaken to define the remaining chiral centers and increase the availability of both irciniastatins and derivatives for further development.

Irciniastatins A (**1**) and B (**2**) displayed powerful cancer cell growth inhibition against the murine P388 leukemia cell line and six human cancer cell lines with GI<sub>50</sub> values of  $10^{-3}$ – $10^{-4}$   $\mu\text{g/mL}$  (Table 2). Although there was only one minor difference at C-10 between irciniastatins A and B, the cell growth inhibition of **2** proved to be 10 times stronger than that of **1** against the human pancreas (BXPC-3), breast (MCF-7), and central nervous system (SF268) cancer cell lines. However, against lung cancer cells (NCI-H460), irciniastatin A was at least 10 times more active than irciniastatin B. Human umbilical vein endothelial cells (HUVEC) were strongly inhibited by irciniastatin A (Table 2), with no evidence of tube formation. That powerful antivasculature activity may in part be assisted by cytotoxicity. Irciniastatin A (**1**) was available in sufficient quantity to evaluate possible antimicrobial activity. Irciniastatin A (**1**) had marginal antifungal and antibacterial activities, with minimum inhibitory concentrations of 16  $\mu\text{g/mL}$  for *Cryptococcus neoformans* and 64  $\mu\text{g/mL}$  for *Neisseria gonorrhoeae*.

In summary, irciniastatins A (**1**) and B (**2**) are very promising anticancer agents and their development, including synthetic approaches and investigation of possible microorganism sources, is in progress.

## Experimental Section

**General Procedures.** Organic solvents used for column chromatography were freshly distilled. Sephadex LH-20, particle size 25–100  $\mu\text{m}$ , used in gel permeation and partition column chromatographic separations was obtained from Phar-

macia Fine Chemicals AB, Uppsala, Sweden. The TLC plates were viewed under shortwave UV light and then developed by 20%  $\text{H}_2\text{SO}_4$  or 3% ceric sulfate–3 N sulfuric acid spray reagent following by heating at approximately 150  $^\circ\text{C}$ . For HPLC separations, a Phenomenex Zorbax SB C18 (particle size 10  $\mu\text{m}$ ,  $\phi$  9.4 mm  $\times$  25 cm) C18 column and a Phenomenex IB-SIL (particle size 5  $\mu\text{m}$ ,  $\phi$  4.6 mm  $\times$  25 cm) C-18 column were used in reversed-phase mode with Waters Delta (model 600) solvent metering pumps in conjunction with a Waters 2487 Dual  $\lambda$  Absorbance Detector (at  $\lambda$  254 nm), with Gilson (model 306) solvent metering pumps and Gilson 118 UV/VIS detection at  $\lambda$  254 nm. HPLC grade organic solvents were purchased from EM Science, and pure water was produced by Barnstead Easypure PF compact ultrapure water system model D7031. The solvent partitioning sequence was a modification of the original procedure of Bligh and Dyer.<sup>6</sup>

Optical rotation was determined by employing a Perkin-Elmer Model 241 polarimeter. The UV spectrum was recorded with a Hewlett-Packard 8450 UV-vis spectrometer. The HRFABMS was measured with a JEOL JMS-LC mate LCMS system. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR, APT,  $^1\text{H}$ – $^1\text{H}$ -COSY, TOCSY (mixing time of 45 ms and 60 ms), HMQC (optimized for  $^1J_{\text{H-C}}$  = 8.2 Hz), ROESY (mixing time of 100 ms and 150 ms), and 2D J-solution data were recorded using a Varian VXR-500 instrument in  $\text{CDCl}_3$ .

**Collection of *I. ramosa*.** On July 6, 1991, an approximate 1 kg sample of wet *I. cf. ramosa* Bergquist, 1965 (Demospongiae: Dictyoceratida: Ircinidae) was collected using SCUBA at –20 to –36 M on a barrier reef near Semporna, Sabah (Borneo), Malaysia. The reddish brown (darkens on exposure to air) encrusting (with vertical branching) sponge with a light brown exterior was preserved in methyl alcohol. Reference specimens are maintained at ASU-CRI and Queensland Museum.

**Extraction and Solvent Partitioning.** The *I. ramosa* sponge sample (~1 kg wet wt) was extracted with methylene chloride–methanol (1:1) and was dried to a 98.8 g residue. This material was successively partitioned using the system  $\text{MeOH}$ – $\text{H}_2\text{O}$  (9:1 and 3:2) against *n*-hexane and methylene chloride, respectively, to yield the most bioactive methylene chloride fraction (1.78 g, yield 1.8%) with an ED<sub>50</sub> of 0.0077  $\mu\text{g/mL}$  for P388 and GI<sub>50</sub> values of 0.0018, <0.001, 0.00089, <0.001, 0.0014, and 0.0012  $\mu\text{g/mL}$  for BXPC-3, MCF-7, SF268, NCI-H460, KM20L2, and DU-145 cells, respectively.

**Isolation of Irciniastatins A (**1**) and B (**2**).** Two bioactive fractions were obtained from the methylene chloride fraction (1.78 g) by a series of Sephadex LH-20 gel permeation and partition column chromatographic steps using methanol, methylene chloride–methanol (3:2), and *n*-hexane–methylene chloride–methanol (5:1:1) as eluents. Further separation of the two fractions (A, 144 mg and B, 69.5 mg) was performed employing C18 (10  $\mu\text{m}$ ) reversed-phase column (9.4 mm  $\times$  25 cm) HPLC with mobile phases 3:7 and 19:31  $\text{CH}_3\text{CN}$ – $\text{H}_2\text{O}$  to isolate and collect two major peaks. Final purification was achieved by using an ODS (5  $\mu\text{m}$ ) column (4.6 mm  $\times$  25 cm) HPLC techniques with 3:7 and 19:31  $\text{CH}_3\text{CN}$ – $\text{H}_2\text{O}$  as eluents. By this means, pure irciniastatins A (34.7 mg, yield 3.51  $\times 10^{-3}$  %) and B (2.2 mg, yield 2.23  $\times 10^{-4}$  %) were isolated. Irciniastatin A (**1**) was obtained as a colorless amorphous powder:  $[\alpha]_{\text{D}} + 24.40^\circ$  (c, 0.55,  $\text{CH}_3\text{OH}$ ); UV ( $\text{CH}_3\text{OH}$ – $\text{H}_2\text{O}$ )  $\lambda$  310, 270, 230(sh), 215 nm; FAB-MS  $m/z$  610 [M + H]<sup>+</sup>; HRFABMS  $m/z$  610.3228,  $\text{C}_{31}\text{H}_{48}\text{NO}_{11}$  (calcd 610.3228). The  $^1\text{H}$ , APT, HMQC, HMBC, and ROESY (in  $\text{CDCl}_3$ ) assignments have been summarized in Table 1.

Irciniastatin B (**2**) was isolated as a colorless amorphous powder:  $[\alpha]_{\text{D}} - 4.67^\circ$  (c, 0.15,  $\text{CH}_3\text{OH}$ ); UV ( $\text{CH}_3\text{OH}$ – $\text{H}_2\text{O}$ )  $\lambda$  310, 270, 230(sh), 215 nm; FAB-MS  $m/z$  608 [M + H]<sup>+</sup>; HRFABMS  $m/z$  608.3101,  $\text{C}_{31}\text{H}_{46}\text{NO}_{11}$  (calcd 608.3071). Refer to Table 1 for the  $^1\text{H}$ , APT, HMQC, HMBC, and ROESY (in  $\text{CDCl}_3$ ) data.

**Cancer Cell Line Procedures.** Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.<sup>7</sup> Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium



solution were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI<sub>50</sub> of the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software. Mouse leukemia P388 cells were incubated in a 10% horse serum/Fisher medium solution for 24 h followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED<sub>50</sub>) was then calculated using a Z1 Beckman/Coulter particle counter.

**HUVEC and Tube Formation.** Unpolymerized Matrigel (Becton Dickinson) was used to coat the wells (250  $\mu$ L/well) of a 24-well tissue culture plate and allowed to polymerize for 1 h at 37 °C. HUVEC (human umbilical vascular endothelial cells) (BD Biosciences Clontech) were plated ( $6 \times 10^4$  cells/well) in 0.5 mL of EGM-2 complete medium (Clonetics-BioWhittaker Cambrex) to which ircinastatin A doses were added in experimental wells. After ~24 h of incubation, digital photographs were taken. Tube formation in control wells was compared with that in ircinastatin A-exposed wells.

**Antimicrobial Susceptibility Testing.** Ircinastatin A (1) was evaluated against the bacteria *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, and *N. gonorrhoeae* ATCC 49226 and the fungi *Candida albicans* ATCC 90028 and *C. neoformans* ATCC 90112, following established broth microdilution susceptibility assays.<sup>8,9</sup> The minimum inhibitory concentration was defined as the lowest concentration of ircinastatin A that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days.

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## References

- (1) For contribution 519, refer to: Nabha et al. Combretastatin A-4 prodrug induces mitotic catastrophe in chronic lymphocytic leukemia cell line independent of caspase activation and poly (ADP-ribose) polymerase cleavage. *Clin. Cancer Res.* **2002**, *8*, 2735–2741.
- (2) (a) Pettit, G. R.; Herald, C. L.; Hogan, F. Biosynthetic products for anticancer drug design and treatment: The bryostatins. In *Anticancer Drug Design*; Baguley, B., Ed.; Academic Press: San Diego, CA, 2002; pp 203–235. (b) Clamp, A.; Jayson, G. C. The clinical development of the bryostatins. *Anti-Cancer Drugs* **2002**, *13*, 673–683. (c) O'Brien, M.; Taylor, N. H.; Thomas, E. J. Synthesis of the C(1)–C(16) fragment of bryostatins. *Tetrahedron Lett.* **2002**, *43*, 5491–5494. (d) Saad, E. D.; Kraut, E. H.; Hoff, P. M.; Moore, D. F.; Jones, D.; Pazdur, R.; Abbruzzese, J. L. Phase II study of dolastatin 10 as first-line treatment for advanced colorectal cancer. *Am. J. Clin. Oncol.* **2002**, *25*, 451–453. (e) van Kesteren, C.; Twelves, C.; Bowman, A.; Hoekman, K.; López-Lázaro, L.; Jimeno, J.; Guzman, C.; Mathôt, R. A. A.; Simpson, A.; Vermorken, J. B.; Smyth, J.; Schellens, J. H. M.; Hillebrand, M. J. X.; Rosing, H.; Beijnen, J. H. Clinical pharmacology of the novel marine-derived anticancer agent ecteinascidin 743 administered as a 1- and 3-h infusion in a phase I study. *Anti-Cancer Drugs* **2002**, *13*, 381–393. (f) Endo, A.; Yanagisawa, A.; Abe, M.; Tohma, S.; Kan, T.; Fukuyama, T. Total synthesis of ecteinascidin 743. *J. Am. Chem. Soc.* **2002**, *124*, 6552–6554. (g) Jimeno, J. M. A clinical armamentarium of marine-derived anticancer compounds. *Anti-Cancer Drugs* **2002**, *13*, S15–S19. (h) Paterson, I.; Coster, M. J. Total synthesis of althohyrin A (spongistatin 1): An alternative synthesis of the CD-spiroacetal subunit. *Tetrahedron Lett.* **2002**, *43*, 3285–3289. (i) Crimmins, M. T.; Katz, J. D.; Washburn, D. G.; Allwein, S. P.; McAtee, L. F. Asymmetric total synthesis of spongistatins 1 and 2. *J. Am. Chem. Soc.* **2002**, *124*, 5661–5663. (j) Cragg, G. M.; Newman, D. J. Medicinals for the millennia. *Ann. New York Acad. Sci.* **2001**, *953*, 3–25. (k) Vedejs, E.; Kongkittitang, C. A total synthesis of (–)-hemisterlin using *N*-Bts methodology. *J. Org. Chem.* **2001**, *66*, 7355–7364. (l) Faulkner, D. J. Marine natural products. *Nat. Prod. Rep.* **2001**, *18*, 1–49. (m) Faulkner, D. J. Marine natural products. *Nat. Prod. Rep.* **2000**, *17*, 7–55.
- (3) (a) Pettit, G. R. Evolutionary Biosynthesis of Anticancer Drugs. In *Anticancer Agents: Frontiers in Cancer Chemotherapy*; Ojima, I.; Vite, G. D.; Altmann, K.-H., Eds.; American Chemical Society, Washington, DC, 2001; pp 16–42. (b) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. Antineoplastic components of marine animals. *Nature* **1970**, *227*, 962–963.
- (4) (a) Gosliner, T. M.; Behrens, D. W.; Williams, G. C. *Coral Reef Animals of the Indo-Pacific*; Sea Challengers: Monterey, CA, 1996; p 25. (b) Colin, P. L.; Arneson, C. *Tropical Pacific Invertebrates*; Coral Reef Research Foundation: Beverly Hills, CA, 1995; p 53.
- (5) (a) Kuramoto, M.; Fujita, T.; Ono, N. Ircinamine, a novel cytotoxic alkaloid from *Ircinia* sp. *Chem. Lett.* **2002**, *4*, 464–465. (b) Salama, A. M.; Estrada, M. G.; Orjuela, M. P. Antimicrobial and antitumor activity of variabiline and its enantiomers isolated from *Ircinia felix*. *Revista Colombiana Ciencias Quim.-Farm.* **2001**, *30*, 74–80. (c) Yan, S.-J.; Zhang, G.-W.; Su, J.-Y.; Zeng, L.-M. Rare long conjugated diterpene ketone from the marine sponge *Ircinia selaginella* (Lamark). *Gaodeng Xuexiao Huaxue Xuebao* **2001**, *22*, 949–951. (d) Takada, N.; Sato, H.; Suenaga, K.; Arimoto, H.; Yamada, K.; Ueda, K.; Uemura, D. Isolation and structures of haterumalides NA, NB, NC, ND, and NE, novel macrolides from an Okinawan sponge *Ircinia* sp. *Tetrahedron Lett.* **1999**, *40*, 6309–6312. (e) Prokof'eva, N. G.; Kalinovskaya, N. I.; Lukyanov, P. A.; Kuznetsova, T. A. Membranotropic effects of cyclic lipopeptides produced by a marine isolate of the bacteria *Bacillus pumilus*. *Biologiya Morya* (Vladivostok) **1996**, *22*, 179–182. (f) Mau, C. M. S.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. Waiakeamide, a cyclic hexapeptide from the sponge *Ircinia dendroides*. *J. Org. Chem.* **1996**, *61*, 6302–6304. (g) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Kobayashi, J.; Sasaki, T. Structures of ircinal A and B, novel alkaloids from the Okinawan marine sponge *Ircinia* sp. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1992**, *34*, 463–469. (h) Kamimura, D.; Yamada, K. Novel antitumor sesterterpenoids. *Jpn. Kokai Tokkyo Koho* **1993**, JP 92–40445 19920131. (i) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. Ircinal A and B from the Okinawan marine sponge *Ircinia* sp.: Plausible biogenetic precursors of manzamine alkaloids. *J. Org. Chem.* **1992**, *57*, 2480–2483. (j) Mihopoulos, N.; Vagias, C.; Chinou, I.; Roussakis, C.; Scoullou, M.; Harvala, C.; Roussis, V. *Ircinia spinosula*. *Z. Naturforsch., C: Biosci.* **1999**, *54*, 417–423. (h) De Rosa, S.; De Giulio, A.; Iodice, C. Biological effects of prenylated hydroquinones: Structure–activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. *J. Nat. Prod.* **1994**, *57*, 1711–1716.
- (6) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 912.
- (7) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paul, K.; Vestica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. Feasibility of high-flux anticancer screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
- (8) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7-A5. NCCLS: Wayne, PA, 2000.
- (9) National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard M27-A. NCCLS: Wayne, PA, 1997.

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