Antineoplastic Agents. 536. New Sources of Naturally Occurring Cancer Cell Growth Inhibitors from Marine Organisms, Terrestrial Plants, and Microorganisms[1a,⊥]

George R. Pettit,*[,†] Fiona Hogan,[†] Jun-Ping Xu,[†] Rui Tan,[†] Toshihiko Nogawa,[†] Zbigniew Cichacz,[†] Robin K. Pettit,[†] Jiang Du,[†] Qing-Hua Ye,[†] Gordon M. Cragg,[‡] Cherry L. Herald,[†] Michael S. Hoard,[†] Animesh Goswami,[†] Justin Searcy,[†] Larry Tackett,[†] Dennis L. Doubek,[†] Lee Williams,[†] John N. A. Hooper,[§] Jean M. Schmidt,[†] Jean-Charles Chapuis,[†] Denise N. Tackett,[†] and Felicia Craciunescu[†]

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 872404, Tempe, Arizona 85287-2404, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, P.O. Box B, Frederick, Maryland 21702-1201, and Queensland Museum, P.O. Box 3300, S. Brisbane, Queensland, 4101 Australia

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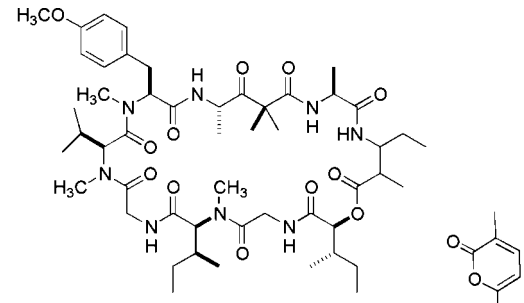
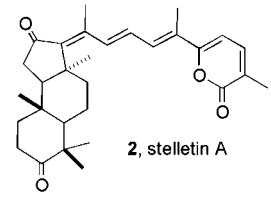
Bioassay-guided fractionation of extracts of various plants, marine organisms, and microorganisms has led to the discovery of new natural sources of a number of known compounds that have significant biological activity. The isolation of interesting and valuable cancer cell growth inhibitors including majusculamide C (**1**), axinastatin 5 (**5**), bengazoles A (**6**), B (**7**), and E (**8**), manzamine A (**10**), jaspamide (**11**), and neoechinulin A (**19**) has been summarized.

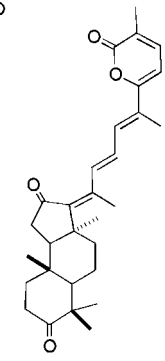
As an inevitable result of our long-term and broad research program directed at discovery of new anticancer drugs from plants, marine organisms, and microorganisms, we continue to isolate constituents that show significant activity in our murine leukemia and human cancer cell lines and that prove to be known substances. The following is a report of such previously discovered compounds that have been isolated in our laboratories and would not otherwise be described in our literature record. New sources of many of these compounds are reported here. The information may lead to greater availability of some promising anticancer drug candidates and may also help other research groups to avoid pursuing natural product leads that have already been investigated for their anticancer constituents. Table 1 lists these compounds and the species from which they were newly extracted as well as the organisms from which they were previously isolated. Many of the known compounds already had biological activity ascribed to them, but fewer had been reported to have cancer cell growth inhibitory activity or had been tested against the cell lines that we use (Table 2).



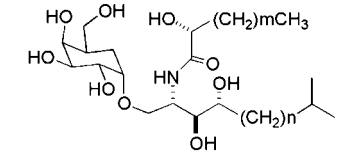
1, majusculamide C



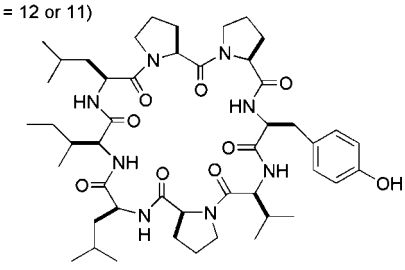
2, stelletin A



3, stelletin B



4, agelasphin-9b
(m = 20 or 21, n = 12 or 11)



5, axinastatin 5

Results and Discussion

Plant and animal samples were preserved for shipment from the collection site in CH_3OH, CH_2Cl_2–CH_3OH, or C_2H_5OH. Upon arrival in our laboratories, the shipping solution was decanted, concentrated, and extracted with CH_2Cl_2. Unless otherwise described in the individual entries, the residual sample material was extracted with a 1:1 mixture of CH_2Cl_2−CH_3OH, to which was added water in order to effect separation of the phases, and the CH_2Cl_2 layer was removed and added to the initial CH_2Cl_2 extract. The combined CH_2Cl_2 solution was dried and the residue dissolved in a 9:1 mixture of CH_3OH–H_2O. Following extraction with hexane, the aqueous layer was adjusted to 3:2 CH_3OH–H_2O and extracted with CH_2Cl_2. The CH_3OH–H_2O, hexane, and CH_2Cl_2 layers were each dried, and the residues were evaluated for anticancer activity. Fungal and bacterial broths were initially extracted with either ethyl acetate or CH_2Cl_2, and the extract was dried to give a residue that was taken up in 9:1 CH_3OH–H_2O and partitioned according to the above method[1b] to yield residues that were similarly tested for activity. Extracts that showed activity were subjected to a series of chromatographic separations on columns of Sephadex LH-20 in suitable solvent systems such as CH_3OH, hexane–toluene–CH_3OH (3:1:1), and toluene–CH_3OH–2-propanol (8:1:1); final purification of the active compounds was performed by HPLC. Now follows a summary of the most promising cell growth inhibitors that we

⊥ Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. Tel: (480) 965-3351. Fax: (480) 965-2747. E-mail: bpettit@asu.edu.

† Arizona State University.

‡ National Cancer Institute, Frederick.

§ Queensland Museum.

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Table 1. New Sources of Previously Isolated Biologically Active Compounds

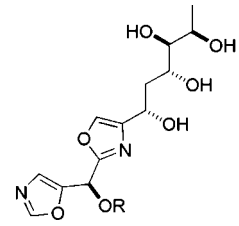
compound	species	previously reported sources
1, majusculamide C	*Dolabella auricularia* (Mollusca)	*Lyngbya majuscula*[2a] (Cyanophyta), *Ptilocaulis trachys*[2c] (Porifera)
2, stellettin A	*Stelletta globostellata*[4a] (Porifera)	*Stelletta tenuis*,[4b] *Rhabdastrella globostellata*,[4a,d] *Geodia japonica*[4f] (Porifera)
3, stellettin B	*Stelletta globostellata*[4a] (Porifera)	*Jaspis stellifera*,[4c] *Rhabdastrella globostellata*[4a,d] (Porifera)
4, agelasphin-9b	*Agelas* sp. (Porifera)	*Agelas mauritianus*[5a] (Porifera)
5, axinastatin 5	*Stylissa flabelliformis* (Porifera)	*Axinella* cf. *carteri*[6] (Porifera)
6, bengazole A	*Dorypleres splendens* (Porifera)	Jaspidae sp.,[7a] *Jaspis* sp.[7c] (Porifera)
7, bengazole B	*Dorypleres splendens* (Porifera)	Jaspidae sp.,[7a] *Jaspis* sp.[7c] (Porifera)
8, bengazole E	*Dorypleres splendens* (Porifera)	*Jaspis* sp.[7c] (Porifera)
9, bengamide A	*Dorypleres splendens* (Porifera)	*Jaspis* cf. *coriacea*,[8a,b] *Jaspis carteri*,[8c] *Pachastrissa* sp.[9] (Porifera)
10, manzamine A	*Petrosia* sp. (Porifera)	*Haliclona* sp.,[10a] *Pellina* sp.,[10b] *Acanthostrongylophora* sp.[10d] (Porifera)
11, jaspamide (jasplakinolide)	*Jaspis* spp. (Porifera)	*Jaspis* spp.[11a,b] *Jaspis johnstoni*,[11c] *Auletta* cf. *constricta*, *Hemiastrella minor*, *Cymbastela* sp.[11d] (Porifera)
12, toyocamycin	*Jaspis* spp. (Porifera), *Streptomyces* sp. (bacteria)	*Jaspis johnstoni*[12a] (Porifera), *Tolypothrix tenuis*[12b] (Cyanophyta), *Streptomyces toyocaensis*[12c] (bacteria)
13, hymenialdisine	*Pseudaxinella* sp., *Phakellia dendyi* (Porifera)	*Axinella* sp., *Axinella carteri, Hymeniacidon* spp.[13a] *Hymeniacidon aldis*,[13b] *Axinella verrucosa*, *Acanthella aurantiaca*,[13c] *Stylissa flabelliformis*[13d] (Porifera)
14, debromohymenialdisine	*Pseudaxinella* sp. (Porifera)	*Axinella carteri, Hymeniacidon* spp.,[13a] *Hymeniacidon aldis,*[13b] *Stylissa flabelliformis*[13d] (Porifera)
15, β-peltatin	*Bridelia ferruginea* (Euphorbiaceae),	*Bridelia ferruginea*[14a] (Euphorbiaceae), *Podophyllum peltatum*,[14b,h] *Podophyllum hexandrum (P. emodi)*[14h] (Podophyllaceae), *Linum* spp.[14c] (Linaceae)
16, deoxypodophyllotoxin (silicicolin, anthricin, hernandion)	*Bridelia ferruginea* (Euphorbiaceae)	*Bridelia ferruginea*[14a] (Euphorbiaceae), *Thuja occidentalis*[14d] (Cupressaceae), various genera[14e,h]
17, β-peltatin 5-*O*-β-D-glucopyranoside	*Bridelia ferruginea (Euphorbiaceae)*	*Bridelia ferruginea*[14a] (Euphorbiaceae), *Podophyllum peltatum*[14c,f] (Podophyllaceae), *Linum* spp.[14c] (Linaceae)
18, isopicrodeoxypodophyllotoxin	*Bridelia ferruginea* (Euphorbiaceae)	*Thuja occidentalis*[14d] (Cupressaceae)
19, neoechinulin A	*Bridelia ferruginea* (Euphorbiaceae)	*Aspergillus ruber*,[15a] *Aspergillus amstelodami*[15b] (fungi)
20, cephaeline	*Alangium villosum* (Alangiaceae)	*Alangium lamarckii*[16] (Alangiaceae)
21, ursolic acid	*Carissa edulis* (Apocynaceae)	*Pyrus malus*[17a] (Rosaceae), *Vitex negundo*[17b] (Verbenaceae)
22, brefeldin A	two unidentified soil fungi	*Penicillium* spp.[18a–c] (fungi)
23, 4-hydroxy-6-methoxy-γ,7-dimethyl-3-oxo-phthalansorbic acid	*Penicillium rugulosum* (fungi)	synthetic derivative of fungal metabolite[19a]
24, genistein	*Phoma glomerata* (fungi)	Leguminosae spp.,[20a] Actinomycetes[20b]
25, daidzein	*Phoma glomerata* (fungi)	Leguminosae spp.,[20a] Actinomycetes[20b]
26, echinomycin (quinomycin A)	*Streptomyces* sp. (bacteria)	*Streptomyces* spp.[21a] (bacteria)
27, FD-991	*Streptomyces* sp. (bacteria)	*Streptomyces echinatus*[21d] (bacteria)
28	*Streptomyces* sp. (bacteria)	synthetic oxide of bacterial metabolite[21e]
29, actinomycin D	*Streptomyces annulatus*	*Streptomyces* spp.[22]

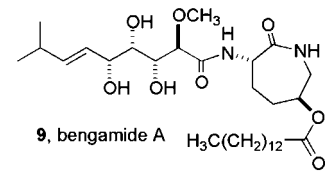
isolated from previously unknown sources. Full details about each lead investigated may be obtained from the corresponding author.

Majusculamide C (**1**) was isolated from the sea-hare *Dolabella auricularia*. Compound **1** was previously found in a cyanobacterium from the Marshall Islands (Table 1) and was reported to have antifungal activity;[2a,b] more recently it was isolated from a sponge collected in the same area.[2c] It is now generally accepted that cyanobacteria are the sources of many biologically active compounds isolated from marine invertebrates.[3] Compound **1** has been described as cytotoxic,[2b] and it showed strong activity against several of our cancer cell lines (Table 2).

Of the compounds isolated from the sponge *Stelletta globostellata* (possibly a misidentified *Rhabdastrella globostellata*[4a]), stellettin A (**2**) showed specific activity against two of our human cell lines, and stelletin B (**3**) was active in the P388 line. Compound **2** was first isolated from *Stelletta tenuis* (or *R. globostellata*)[4a,b] and has also been isolated from other marine sponge genera, and **3** was reported as an isolate from *Jaspis stellifera*[4c] and from a *Stelletta* species[4d] (or misidentified collections of *R. globostellata*[4a]). A mixture of compounds **2** and **3** showed activity in an NCI cancer screen, with particular sensitivity toward leukemia cells,[4e] and **2** itself was recently reported to induce oxidative stress and apoptosis in human leukemia and prostate cancer cell lines.[4f]

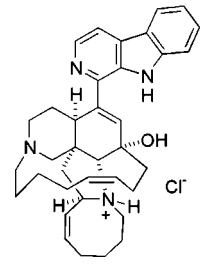
Agelasphin-9b (**4**), isolated from an *Agelas* species, was earlier reported[5a] to have potent antitumor activity in vivo, but because



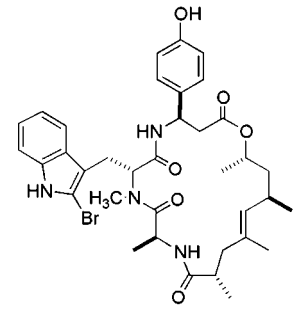


9, bengamide A

6, bengazole A, R = $CO(CH_2)_{12}CH_3$
7, bengazole B, R = $CO(CH_2)_{11}CH(CH_3)_2$
8, bengazole E, R = $CO(CH_2)_{13}CH_3$



10, manzamine A (keramamine-A) hydrochloride



11, jaspamide (jasplakinolide)

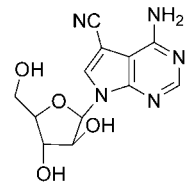
of the lack of material isolated from its original source and the difficulty of its synthesis, an analogue (KRN7000) was chosen as a potential clinical agent[5b,c] and is in phase 1 clinical trials.[5d] Axinastatin 5 (**5**) had been previously isolated in our laboratories from a different sponge genus and shown to have cancer cell growth inhibitory activity.[6]

Fractionation of an extract of the sponge *Dorypleres splendens* led to isolation of active compounds that were identified as bengazoles A (**6**), B (**7**), and E (**8**) and bengamide A (**9**). Compounds **6** and **7** had been reported earlier to have antihelminthic activity and potent antifungal activity,[7a–d] and **8** was shown to be antifungal.[7c] Compound **9** also had antifungal and antimicrobial activity[8a] and was later found to have cytotoxic activity against the NCI 60-cell-line screen,[8b] which is confirmed by the potent activity data shown in Table 2. It had been thought that these compounds were confined to *Jaspis* and *Pachastrissa* species;[8b,c,9] to our knowledge, this is the first time **6**–**9** were isolated from another genus.

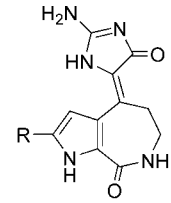
Manzamine A,[10a,b] here isolated as the hydrochloride salt (**10**) from a *Petrosia* sponge, was the first of its type of complex alkaloid to be identified and was shown to have anti-P388[10a] and antimalarial[10c,d] activity. The promising activity of this compound has led to numerous synthetic investigations; its total synthesis was recently accomplished (via 25 steps),[10e] and higher-yielding methods are being investigated.[10f] Jaspamide (jasplakinolide, **11**) was isolated in our laboratories from two *Jaspis* species collected in Malaysia; it had been previously isolated from *Jaspis* species found in Fiji, Palau, and New Guinea[11a–c] and later from other sponge genera,[11d] and it was shown to have a range of interesting biological activities, including induction of apoptosis in HL-60 cells,[11e] because of its ability to interact with actin filaments.[11f] A total synthesis of (+)-jaspamide was recently published,[11g] and less toxic analogues are being pursued.[11h] Toyocamycin (**12**), a known antitumor and antibiotic nucleoside,[12a–d] was also isolated by us from these two *Jaspis* sponges as well as from an unidentified *Streptomyces* bacterium; analogues of this compound are under investigation for cancer chemotherapy.[12d]

Hymenialdisine (**13**) and debromohymenialdisine (**14**) were isolated from a *Pseudaxinella* species, and compound **13** was found in an extract of the sponge *Phakellia dendyi*; these compounds had been isolated previously by us[13a] and others[13b–d] from various sponges. Compound **13** was shown to be a potent inhibitor of cyclin-dependent and other kinases,[13e,f] and the development of analogues with greater or more selective activity is under way;[13f] **14** was reported to inhibit a narrow range of protein kinases.[13d]

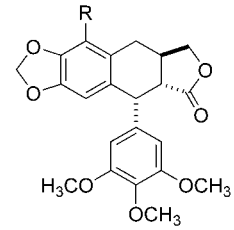
Of the higher plant extracts investigated, a sample from *Bridelia ferruginea* (central Africa) yielded the known lignans β-peltatin (**15**),[14a–c] deoxypodophyllotoxin (**16**),[14a,d,e,h] and the glucoside (**17**) of β-peltatin,[14a,c,f] together with isopicrodeoxypodophyllotoxin (**18**).[14d] Compounds **15** and **16** showed exceptional activity against our cancer cell lines (Table 2). These related lignans have been of interest since podophyllotoxin itself was shown to have antitumor and mitotoxic activities.[14f,g] Natural supplies of podophyllotoxin from the endangered Himalayan *Podophyllum emodi* (*P. hexandrum*[14h]) are precursors for the widely used anticancer analogues etoposide, etopophos, and teniposide. The American mayapple (*P. peltatum*) is being investigated as an alternative, renewable source.[14i] Studies into less toxic semisynthetic analogues of podophyllotoxin and of etoposide are ongoing.[14g,j,k,l] Also isolated from the sample of *B. ferruginea* was the less active indole alkaloid neoechinulin A (**19**), previously found in fungi[15a,b] and recently synthesized.[15c] An extract from the stem bark of *Alangium villosum* yielded the alkaloid cephaeline (**20**), which had already been isolated from another species of that genus.[16] From the twigs of *Carissa edulis*, also collected in Africa, was isolated ursolic acid (**21**), a higher-plant metabolite that is found in the wax-like coating on apples[17a] and other fruits and was recently shown to have antifeedant activity.[17b]



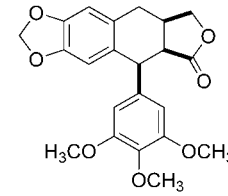
12, toyocamycin



13, R = Br, hymenialdisine
14, R = H, debromohymenialdisine



15, β-peltatin, R = OH
16, deoxypodophyllotoxin, R = H
17, β-peltatin 5-*O*-β-D-glucopyranoside, R = O-glc

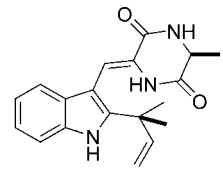


18, isopicrodeoxypodophyllotoxin

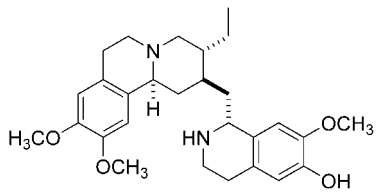
Brefeldin A (**22**) was isolated by us from extracts of two soil fungi and showed good activity in our cell lines (Table 2). Previously it had been isolated from several *Penicillium* species and was found to have a wide range of antibiotic activity[18a–c] and to induce cell differentiation and apoptosis;[18d] because of its poor solubility and other limitations, structure–activity studies of its derivatives are being examined.[18d,e] An extract of *P. rugulosum* yielded compound **23**, which had not been reported as a natural product before but was known[19a] as an unsaturated derivative of mycophenolic acid, an immunosuppressive and antibiotic fungal metabolite.[19b] The known[20a–c] antioxidant phytoestrogens genistein (**24**) and daidzein (**25**) were isolated from an extract of *Phoma glomerata* that had been grown from a soil sample collected in Canada. Compound **24** is thought to be the breast cancer preventive ingredient in soy,[20d] and it also appears to have potential in prevention of colon[20e] and prostate cancers.[20f] From the broth of a *Streptomyces* species, echinomycin (**26**) and two related compounds, the isomeric oxides **27** and **28**, were isolated. Echinomycin had been previously isolated from *S. echinatus*[21a,b] and other *Streptomyces* species and shown to have potent antitumor activity by way of apoptosis and inhibition of cellular signaling.[21c] Compound **27** was described in a patent as a product of fermentation of *S. echinatus* and named FD-991; against the human cancer cell lines HL-60 (leukemia), HeLa S-3 (uterine cancer), and A549 (lung cancer), it had IC_{50}'s of 0.2–2.0 μg/mL.[21d] Compound **28** was reported, with some in vitro and in vivo data that showed it also to be less active than the parent, as a semisynthetic oxide of **26**.[21e] Another bacterium, identified as *S. annulatus*, yielded the well-known antibiotic antitumor agent actinomycin D (dactinomycin, **29**), which has been isolated from many *Streptomyces* species.[22]

Experimental Section

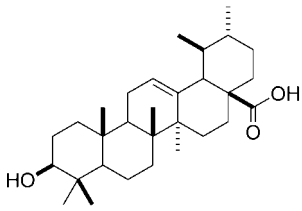
General Experimental Procedures. Solvents used for the chromatographic procedure were redistilled. Sephadex LH-20 employed for gel permeation and partition chromatography was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. The silica gel GHLF Uniplates for thin-layer chromatography were supplied by Analtech, Inc., Newark, DE. The TLC plates were viewed under UV light and developed with ceric sulfate–sulfuric acid (heating for 3 min). Analytical HPLC was conducted with a Hewlett-Packard model 1100 HPLC coupled with a diode-array detector and an evaporative light scattering detector. Reversed-phase HPLC was performed on a Zorbax SB C_{18} column attached to a Gilson instrument with a Gilson UV detector or to a Waters 600E instrument with a 2487 dual λ absorbance detector.



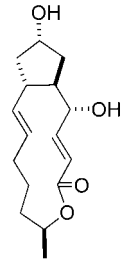
19, neoechinulin A



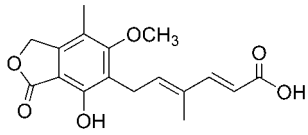
20, cephaeline



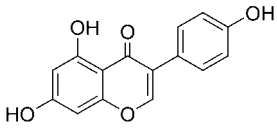
21, ursolic acid



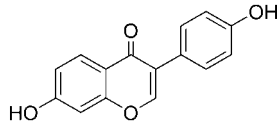
22, brefeldin A



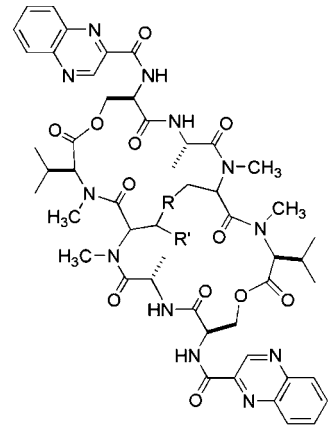
23, 4-hydroxy-6-methoxy-γ,7-dimethyl-3-oxo-5-phthalansorbic acid



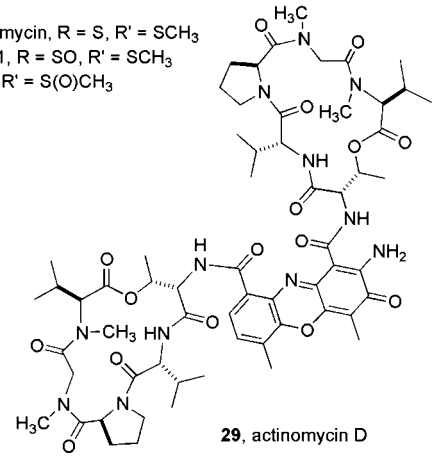
24, genistein



25, daidzein



26, echinomycin, R = S, R' = SCH_3
27, FD-991, R = SO, R' = SCH_3
28, R = S, R' = $S(O)CH_3$



29, actinomycin D

The melting points were recorded with a Kofler melting point instrument. The optical rotation data were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Perkin-Elmer Lambda 3β UV/vis spectrophotometer equipped with a Hewlett-Packard laser jet 2000 plotter. IR spectra were obtained using an Avatar 360 FT-IR instrument with the sample prepared in $CHCl_3$ film. The NMR experiments were conducted using a Varian Unity INOVA-500 spectrometer operating at 500 or 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR. High-resolution mass spectra were obtained on a JEOL LCmate magnetic sector instrument by APCI+ with a poly(ethylene glycol) reference. X-ray spectra were acquired on a Bruker SMART 6000 diffractometer. For fermentation procedures please refer to our recent publications.[23a,b]

Majusculamide C (1). From *Dolabella auricularia* (1000 kg) that was collected in Papua New Guinea (1983), the CH_2Cl_2-soluble extract was fractionated by LH-20 column chromatography followed by HPLC to give a solid (4.8 mg), which by spectroscopic analyses was identified as **1**.

Stelletin A (2). From *Stelletta globostellata* (269 g) that was collected in Kubat, Malaysia (1991), the CH_2Cl_2-soluble extract was fractionated by LH-20 column chromatography followed by HPLC to give a solid (21.0 mg), which by spectroscopic analyses was identified as **2**.

Stelletin B (3). Further chromatographic separation by HPLC of the CH_2Cl_2-soluble mixtures from *S. globostellata* yielded a solid (15.5 mg), which by spectroscopic analyses was identified as **3**.

Agelasphin-9b (4). From an *Agelas* species (450 kg) that was collected in Papua, New Guinea (1983), the CH_2Cl_2-soluble extract was fractionated by LH-20 column chromatography followed by HPLC to give a solid (4.6 mg), which by spectroscopic analyses was identified as **4**.

Axinastatin 5 (5). After removal of the shipping solution (methanol–water) from the sample of *Stylissa flabelliformis* (a dark orange-brown sponge) that was collected in the Maldive Islands in 1995, the sponge was partitioned according to the general procedure. The initial CH_2Cl_2–CH_3OH-soluble extract (1030 g) yielded a CH_2Cl_2-soluble extract (72.5 g), which was fractionated by successive LH-20 column chromatography [sequence: CH_2Cl_2–CH_3OH (3:2); toluene−CH_3OH−2-propanol (8:1:1); and hexane–toluene–CH_3OH (3:1:1)]. The CH_3OH-soluble portion of the combined active fractions was eluted through Sephadex LH-20 [hexane–2-propanol–CH_3 (8:1:1)] to give four fractions, the most active of which was purified by semipreparative HPLC [Zorbax SB C_{18} (42% acetonitrile–H_2O at 6 mL/min) followed by Luna C_8 (72% CH_3–H_2O at 1 mL/min)] to yield a colorless, amorphous powder (4.6 mg), which by mass and NMR spectroscopy and by comparison with an authentic specimen was identified as **5**.

Bengazole A (6). A CH_2Cl_2-soluble extract of a sample of *Dorypleres splendens* (4.28 g) (possibly *Stelleta splendens*, per a private communication from Dr. Michelle Kelly, NIWA, Auckland, to Dr. David Newman at the NCI) that had been collected in Fiji in 1996 was received from the National Cancer Institute (NCI) and was partitioned according to the general procedure, followed by an extraction of the aqueous phase with ethyl acetate. The active CH_2Cl_2-soluble extract (1.77 g) was subjected to LH-20 column chromatography (CH_3OH) to give nine fractions (A−I), of which three were P388-active. Further chromatography of fraction B on LH-20 [hexane–toluene–CH_3OH (3:1:1)], followed by HPLC of the most active eluate [LiChrospher 100 RP C_{18} (50–60% acetonitrile–H_2O at 1 mL/min)], afforded a colorless, amorphous powder (16.5 mg), which by HRMS and by 1D and 2D NMR spectroscopy was identified as **6**.

Bengazole B (7). From the initial LH-20 (CH_3OH) separation above (see **6**), active fraction C was purified by HPLC [LiChrospher 100 RP C_{18} (55% acetonitrile–H_2O at 1 mL/min)] to give a solid (7.5 mg), identified as **7** by HRMS and by 1D and 2D NMR spectroscopy.

Bengazole E (8). From the initial LH-20 (CH_3OH) separation above (see **6**), active fraction D was purified by HPLC [LiChrospher 100 RP C_{18} (55% acetonitrile–H_2O at 1 mL/min)] to give a solid (7.1 mg), identified as **8** by HRMS and by 1D and 2D NMR spectroscopy.

Bengamide A (9). The hexane extract from the general partitioning above (see **6**) was subjected to LH-20 chromatography (CH_3OH) followed by semipreparative HPLC [Prepex C_8 (63% acetonitrile–H_2O at 6 mL/min)] to give a solid (7.9 mg), which by HRMS and by 1D and 2D NMR spectroscopy was identified as **9**.

Manzamine A hydrochloride (10). From a sample of a *Petrosia* species (260 kg) that was collected in Indonesia (1996), the CH_3OH-soluble extract (1.6 kg) was fractionated by LH-20 column chroma-

Table 2. Murine and Human Cancer Cell Line Results (ED_{50} and GI_{50} values in μg/mL)

	cell line[a]									
compound	P388	OVCAR-3	A498	NCI-H460	KM20L2	SK-MEL-5	DU-145	BXPC-3	MCF-7	SF-268	SF-295
1		0.51	0.058	0.0032	0.0013	0.0068					0.13
2	0.012							0.078	0.752		
3	0.037										
4		4.2		0.57							
5	1.9			0.82	0.28		0.87	0.68	1.4	1.8	
6	0.14	0.28		0.21	0.0031		0.15	0.14			0.19
7	0.053	0.37		0.20	0.33		0.15	0.18			0.19
8	0.074	0.16		0.14	0.18		0.11	0.081			0.13
9	0.12	0.01		0.00054	0.0049		0.0056	0.027			0.001
10	0.0067			0.36	0.37		0.60	0.35	0.41	0.42	
11	0.0080										
12	0.0023										
13	2.4										
14	5.0										
15	0.0031	0.00055		0.00077	0.0076		0.0021	0.0012			0.0004
16	0.029	0.0027		0.0018	0.0014		0.0017	0.00044			0.00056
17	0.21	0.85		0.28	>1		>1	>1			0.27
18	<0.01										
19	0.21	0.24		0.21	0.19		0.27	0.25			0.21
20	0.0027	0.032		0.022	0.022		0.0098	0.024			0.018
21	>1										
22	0.23	0.041		0.28	0.022		0.13	0.07	0.047	0.21	0.38
23	0.11										
24				1.5	3.7		1.9	2.5	2.5	1.7	
25				>10	8.8		7.3	6.2	5.1	4.4	
26	0.00147										
29	<0.001										

[a] Cancer cell lines in order: murine lymphocytic leukemia (P388); ovarian (OVCAR-3); renal (A498); lung (NCI-H460); colon (KM20L2); melanoma (SK-MEL-5); prostate (DU-145); pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); CNS (SF-295).

tography to yield a colorless solid (200 mg; 7.7×10^{-5}%) that crystallized from CH_2Cl_2–CH_3OH (3:2) and was shown by X-ray spectroscopy to be the hydrochloride salt of manzamine A (**10**). As well as the cell-line activity given in Table 2, **10** showed 99% inhibition of *Mycobacterium tuberculosis* with an MIC of 6.25 μg/mL and an IC_{50} of 1.7 μg/mL in Vero cells.

Jaspamide (11). After removal of the shipping solution from a sample of a *Jaspis* species collected on the east coast of Malaysia (1994), the sponge was extracted according to the general procedure. The resulting CH_2Cl_2-soluble residue (2.17 g) was fractionated by Sephadex LH-20 column chromatography to yield **11** (28.8 mg), as identified by mass and by 1D and 2D NMR spectroscopy. A similar procedure was used to isolate **11** from another *Jaspis* species that was collected at Kudat, Malaysia, in 1991.

Toyocamycin (12). A. Methanol was removed from the aqueous phase of each *Jaspis* species collection (see **11**) to leave a 1:9 CH_3OH–H_2O solution, which was extracted with 1-butanol. The residues from each 1-butanol layer were fractionated by Sephadex LH-20 column chromatography to yield **12** (8.8 and 3.6 mg, respectively), as identified by mass and by ^1H and ^{13}C NMR spectroscopy.

B. From a soil sample collected by the Chilkat River in Alaska (2000), a *Streptomyces* species was isolated and grown. After the partitioning of the ethyl acetate extract (9.70 g), the CH_2Cl_2-soluble portion (6.25 g) was fractionated by LH-20 chromatography followed by HPLC to give a solid (102 mg) that was shown by spectroscopic analyses to be **12**.

Hymenialdisine (13). A sample of a *Pseudaxinella* species that was collected in the Republic of Palau in 1979 was extracted successively with 2-propanol and with 2-propanol–CH_2Cl_2 (1:1). Each of the residues was partitioned according to the general procedure, after which CH_3OH was removed from each aqueous phase to leave a 1:9 CH_3OH–H_2O solution, which was extracted with 1-butanol. The two 1-butanol-soluble residues (8.69 and 19.2 g, respectively) were combined and taken up in CH_3OH. The insoluble material (16.0 g) was removed, and upon reduction of the filtrate, a yellow powder (910 mg) precipitated and was also removed. The solvent was removed from the remaining solution, and the residue was passed through a Sephadex LH-20 column in CH_3OH. Yellow needles (72 mg) that precipitated from one of the fractions were added to needles (380 mg) that crystallized from a CH_3OH solution of the powdery precipitate above, and the combined solid was subjected to chromatography on a Lobar silica column (CH_2Cl_2–CH_3OH, 10:1 followed by 5:1) to give **13** (61 mg) as a yellow crystalline solid, which was identified by melting point and HRMS data and by TLC comparison with an authentic specimen. A similar procedure was used to isolate **13** (149 mg) from a sample of *Phakellia dendyi* species that was collected in the Byron Strait, Papua New Guinea, in 2003.

Debromohymenialdisine (14). Further elution of the *Pseudoaxinella* fraction on the Lobar column above (see **13**) yielded yellow needles, which were identified as **14** (12 mg) by melting point and HRMS data and by TLC comparison with an authentic specimen.

***β*-Peltatin (15).** A CH_2Cl_2-soluble extract of dried roots of *Bridelia ferruginea* (collected by an NCI contractor in Gabon, 1987) was received from the NCI, and a 3.24 g portion was partitioned according to the general procedure. The resultant active CH_2Cl_2-soluble extract (1.19 g) was subjected to successive LH-20 column chromatography [CH_3OH; hexane–2-propanol–CH_3OH (8:1:1)] followed by HPLC [LiChrospher 100 RP C_{18} (48% acetonitrile–H_2O at 1 mL/min)] to afford a colorless crystalline material (10 mg), which by X-ray spectroscopy was identified as **15** [mp 226–229 °C; lit.[14b] mp 231–238; *Merck Index* mp 238–241 °C (dec)]. A larger sample (52 g) of the same extract of *B. ferruginea* yielded 430 mg (0.8%) of **15**.

Deoxypodophyllotoxin (16). Another active fraction from the LH-20 separation above (see **15**) was further purified by HPLC [Prepex C_8 (40–60% acetonitrile–H_2O at 1 mL/min)] to afford two compounds, one of which was identified by HRMS and by 1D and 2D NMR spectroscopy as **16** (8.0 mg, 0.25%). (For identification of the second compound, see **19** below.) A larger amount (52 g) of the same extract of *B. ferruginea* (see **15**) yielded 400 mg (0.77%) of **16**.

***β*-Peltatin 5-*O*-*β*-D-glucoside (17).** A third active fraction from the LH-20 separation above (see **15**) was further purified by HPLC [LiChrospher 100 RP C_{18} (35% acetonitrile–H_2O at 1 mL/min)] to afford a solid (13.8 mg, 0.425%), which by mass spectroscopy and by ^1H, ^{13}C, and related 2D NMR spectroscopy was identified as **17**.

Isopicrodeoxypodophyllotoxin (18). From the 52 g extract of *B. ferruginea* (see **15** and **16**), a solid was isolated (10 mg, 0.02%), which by spectroscopic analyses was identified as **18**.

Neoechinulin A (19). The second of the purified compounds from the second active *B. ferruginea* fraction above (see **16**) was obtained as a colorless, amorphous compound (3.7 mg, 0.11%), which by UV, IR, NMR, and mass spectroscopic analyses was identified as **19**.

Cephaeline (20). A CH_2Cl_2–CH_3OH-soluble extract (14.7 g) of dried stem bark of *Alangium villosum* (collected in Ceram, Indonesia, by University of Illinois at Chicago personnel for the NCI) was received

from the NCI in 1998 and was partitioned according to the general method. The CH_2Cl_2- and H_2O-soluble extracts were separately fractionated by LH-20 column chromatography (CH_3OH); each column gave one active fraction, and these were combined for further LH-20 chromatography [hexane–toluene–CH_3OH (3:1:1) followed by hexane–ethyl acetate–CH_3OH (5:1:2)]. The resultant active fraction was purified by HPLC [Prepex C_8 (50–60% acetonitrile–H_2O at 1 mL/min)] to afford a yellowish, amorphous powder (304 mg, 2.1%), which by NMR and mass spectroscopy was identified as **20**.

Ursolic acid (21). From dried twigs of *Carisia edulis* (56.7 kg, collected in Kenya in 1972), a 480 g portion of the final CH_2Cl_2-soluble (635 g) residue was fractionated by LH-20 chromatography [eluant: CH_3OH–CH_2Cl_2 (3:2)]. The P388-active fraction (ED_{50} 4.6 mg/mL; 90 g) was taken up in CH_3OH (400 mL). The insoluble portion was filtered and further treated with CH_2Cl_2 to yield an insoluble colorless material (2 g, 0.0047%) whose physical and spectroscopic properties, as well as those of its acetate and methyl ester, matched those of ursolic acid and its corresponding derivatives, respectively. The sample was identical by infrared spectroscopy with an authentic sample of ursolic acid.

Brefeldin A (22). A. From a soil sample collected in Chile (1998), an unidentified fungus was isolated and cultivated. After the partitioning of the ethyl acetate extract (20 g), the CH_2Cl_2-soluble portion (10.89 g) showed activity against the human tumor cell lines and was fractionated by LH-20 chromatography (eluant: CH_3OH). From a methanolic solution of the most active fraction, a crystalline material formed that was removed and recrystallized from CH_3OH. X-ray spectroscopic analysis of the crystal showed the compound to be **22**.

B. A brown, gummy CH_2Cl_2-soluble extract (54.4 mg) of the culture broth of an unknown fungus (no. 0GOS1620) was received from the NCI. Partitioning according to the general method led to an active CH_2Cl_2-soluble extract (42 mg) that was purified by HPLC [Prepex C_8 (60% acetonitrile–H_2O at 1 mL/min) followed by LiChrospher 100 RP C_{18} (50% acetonitrile–H_2O at 1 mL/min)] to afford a solid (6.4 mg, 10.8%), which by X-ray spectroscopic analysis was identified as **22**.

4-Hydroxy-6-methoxy-γ,7-dimethyl-3-oxo-5-phthalansorbic acid (23). From a soil sample collected by the McKenzie River in the Yukon Territory, Canada (1995), a fungus identified as *Penicillium rugulosum* was isolated and grown. A CH_2Cl_2-soluble extract (31.64 g) of the fungus was partitioned according to the general procedure, and the final CH_2Cl_2-soluble portion (3.91 g) was fractionated by LH-20 chromatography (eluant: CH_3OH) followed by HPLC to give a solid (695 mg, 2.19%) that was shown by NMR and X-ray spectroscopy to be compound **23**.

Genistein (24). From a soil sample collected in Manitoba, Canada (1999), a fungus identified as *Phoma glomerata* was isolated and grown. After the partitioning of the ethyl acetate extract, the CH_2Cl_2-soluble portion (10.21 g) was fractionated by LH-20 chromatography (eluant: hexane–CH_2Cl_2–CH_3OH) to give two compounds, the first of which was identified as **24** (10.8 mg) by NMR and mass spectroscopy.

Daidzein (25). The second of the compounds from *Phoma glomerata* (see **23**) was identified as **25** (5.6 mg) by NMR and mass spectroscopy.

Echinomycin (26). From a soil sample collected in the Northwest Territories, Canada (1995), a *Streptomyces* species was isolated and cultivated. The broth was extracted with CH_2Cl_2 to give a residue (15.7 g) that was partitioned according to the general procedure. The CH_2Cl_2-soluble portion (4.3 g) was fractionated by LH-20 chromatography followed by HPLC to give a solid (15.2 mg) that was shown by spectroscopic analyses to be **26**.

FD-991 (27). From the above *Streptomyces* extract (see **26**), LH-20 chromatography followed by HPLC yielded a solid (2.8 mg) that was shown by spectroscopic analyses to be **27**.

Compound 28. From the above *Streptomyces* extract (see **26**), LH-20 chromatography followed by HPLC yielded a solid (1.0 mg) that was shown by spectroscopic analyses to be **28**.

Actinomycin D (29). From a soil sample collected in Alaska (1998), a bacterium identified as *Streptomyces annulatus* was isolated and grown. A CH_2Cl_2-soluble extract (70.9 g) of the broth was partitioned according to the general procedure, and the final CH_2Cl_2-soluble portion (3.9 g) was fractionated by LH-20 chromatography (eluant: CH_3OH) followed by multiple HPLC separations to give a solid (74 mg, 0.1%) that was shown by NMR spectroscopy to be **29**.

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References and Notes

(1) (a) For Antineoplastic Agents Part 535 refer to: Pettit, G. R.; Nogawa, T.; Knight, J. C.; Doubek, D. L.; Hooper, J. N. A. *J. Nat. Prod.* **2004**, *67*, 1611–1613. (b) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.

(2) (a) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236–241. (b) Moore, R. E.; Entzeroth, M. *Phytochemistry* **1988**, *27*, 3101–3103. (c) Williams, D. E.; Burgoyne, D. L.; Rettig, S. J.; Andersen, R. J.; Fathi-Afshar, Z. R.; Allen, T. M. *J. Nat. Prod.* **1993**, *56*, 545–551.

(3) Dunlap, W. C.; Battershill, C. N.; Liptrot, C. H.; Cobb, R. E.; Bourne, D. G.; Jaspars, M.; Long, P. F.; Newman, D. J. *Methods* **2007**, *42*, 358–376.

(4) (a) Tasdemir, D.; Mangalindan, G. C.; Concepción, G. P.; Verbitski, S. M.; Rabindran, S.; Miranda, M.; Greenstein, M.; Hooper, J. N. A.; Harper, M. K.; Ireland, C. M. *J. Nat. Prod.* **2002**, *65*, 210–214. (b) Su, J. Y.; Meng, Y. H.; Zeng, L. M.; Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1994**, *57*, 1450–1451. (c) Ravi, B. N.; Wells, R. J.; Croft, K. D. *J. Org. Chem.* **1981**, *46*, 1998–2001. (d) McCormick, J. L.; McKee, T. C.; Cardellina, J. H., II; Leid, M.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 1047–1050. (e) McKee, T. C.; Bokesch, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 431–438. (f) Liu, W. K.; Cheung, F. W. K.; Che, C.-T. *J. Nat. Prod.* **2006**, *69*, 934–937.

(5) (a) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592. (b) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayasji, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187. (c) Xia, C.; Yao, Q.; Schümann, J.; Rossy, E.; Chen, W.; Zhu, L.; Zhang, W.; De Libero, G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195–2199. (d) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.

(6) Pettit, G. R.; Gao, F.; Schmidt, J. M.; Chapuis, J.-C.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2935–2940.

(7) (a) Adamczeski, M.; Quiñoà, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110*, 1598–1602. (b) Molinski, T. F. *J. Nat. Prod.* **1993**, *56*, 1–8. (c) Searle, P. A.; Richter, R. K.; Molinski, T. F. *J. Org. Chem.* **1996**, *61*, 4073–4079. (d) Mulder, R. J.; Schafer, C. M.; Molinski, T. F. *J. Org. Chem.* **1999**, *64*, 4995–4998.

(8) (a) Quiñoà, E.; Adamczeski, M.; Crews, P.; Bakus, G. J. *J. Org. Chem.* **1986**, *51*, 4494–4497. (b) Thale, Z.; Kinder, F. R.; Bair, K. W.; Bontempo, J.; Czuchta, A. M.; Versace, R. W.; Phillips, P. E.; Sanders, M. L.; Wattanasin, S.; Crews, P. *J. Org. Chem.* **2001**, *66*, 1733–1741. (c) D'Auria, M. V.; Giannini, C.; Minale, L.; Zampella, A.; Debitus, C.; Frostin, M. *J. Nat. Prod.* **1997**, *60*, 814–816.

(9) Fernández, R.; Dherbomez, M.; Letourneux, Y.; Nabil, M.; Verbist, J. F.; Biard, J. F. *J. Nat. Prod.* **1999**, *62*, 678–680.

(10) (a) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405. (b) Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T.; Hirata, Y. *Tetrahedron Lett.* **1987**, *28*, 621–624. (c) El Sayed, K. A.; Kelly, M.; Kara, U. A. K.; Ang, K. K. H.; Katsuyama, I.; Dunbar, D. C.; Khan, A. A.; Hamann, M. T. *J. Am. Chem. Soc.* **2001**, *123*, 1804–1808. (d) Rao, K. V.; Donia, M. S.; Peng, J.; Garcia-Palomero, E.; Alonso, D.; Martinez, A.; Medina, M.; Franzblau, S. G.; Tekwani, B. L.; Khan, S. I.; Wahyuono, S.; Willett, K. L.; Hamann, M. T. *J. Nat. Prod.* **2006**, *69*, 1034–1040. (e) Humphrey, J. M.; Liao, Y.; Ali, A.; Rein, T.; Wong, Y.-L.; Chen, H.-J.; Courtney, A. K.; Martin, S. F. *J. Am. Chem. Soc.* **2002**, *124*, 8584–8592. (f) Tokumaru, K.; Arai, S.; Nishida, A. *Org. Lett.* **2006**, *8*, 27–30.

(11) (a) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797–2800. (b) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123–3124. (c) Braekman, J. C.; Daloze, D.; Moussiaux, B.; Riccio, R. *J. Nat. Prod.* **1987**, *50*, 994–995. (d) See Murray, L. M.; Johnson, A.; Diaz, M. C.; Crews, P. *J. Org. Chem.* **1997**, *62*, 5638–5641. (e) Cioca, D. P.; Kitano, K. *Cell. Mol. Life Sci.* **2002**, *59*, 1377–1387. (f) Bubb, M. R.; Spector, I.; Beyer, B. B.; Fosen, K. M. *J. Biol. Chem.* **2000**, *275*, 5163–5170. (g) Ghosh, A. K.; Moon,

D. K. *Org. Lett.* **2007**, *9*, 2425–2427. (h) Terracciano, S.; Bruno, I.; Bifulco, G.; Avallone, E.; Smith, C. D.; Gomez-Paloma, L.; Riccio, R. *Bioorg. Med. Chem.* **2005**, *13*, 5225–5239.

(12) (a) Zabriskie, T. M.; Ireland, C. M. *J. Nat. Prod.* **1989**, *52*, 1353–1356. (b) Stewart, J. B.; Bornemann, V.; Chen, J. L.; Moore, R. E.; Caplan, F. R.; Karuso, H.; Larsen, L. K.; Patterson, G. M. L. *J. Antibiot.* **1988**, *41*, 1048–1056. (c) Nishimura, H.; Katagiri, K.; Sato, K.; Mayama, M.; Shimaoka, N. *J. Antibiot.* **1956**, *9A*, 60–62. (d) Girardet, J.-L.; Gunic, E.; Esler, C.; Cieslak, D.; Pietrzkowski, Z.; Wang, G. *J. Med. Chem.* **2000**, *43*, 3704–3713.

(13) (a) Pettit, G. R.; Herald, C. L.; Leet, J. E.; Gupta, R.; Schaufelberger, D. E.; Bates, R. B.; Clewlow, P. J.; Doubek, D. L.; Manfredi, K. P.; Rützler, K.; Schmidt, J. M.; Tackett, L. P.; Ward, F. B.; Bruck, M.; Camou, F. *Can. J. Chem.* **1990**, *68*, 1621–1624. (b) Kitagawa, I.; Kobayashi, M.; Kitanaka, K.; Kido, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1983**, *31*, 2321–2328. (c) Cimino, G.; De Rosa, S.; De Stefano, S.; Mazzarella, L.; Puliti, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 767–768. (d) Curman, D.; Cinel, B.; Williams, D. E.; Rundle, N.; Block, W. D.; Goodarzi, A. A.; Hutchins, J. R.; Clarke, P. R.; Zhou, B.-B.; Lees-Miller, S. P.; Andersen, R. J.; Roberge, M. *J. Biol. Chem.* **2001**, *276*, 17914–17919. (e) Meijer, L.; Thunnissen, A.-M. W. H.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L.-H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y.-Z.; Biernat, J.; Mandelkov, E.-M.; Kim, S.-H.; Pettit, G. R. *Chem. Biol.* **2000**, *7*, 51–63. (f) Wan, Y.; Hur, W.; Cho, C. Y.; Liu, Y.; Adrian, F. J.; Lozach, O.; Bach, S.; Mayer, T.; Fabbro, D.; Meijer, L.; Gray, N. S. *Chem. Biol.* **2004**, *11*, 247–259.

(14) (a) Rashid, M. A.; Gustafson, K. R.; Cardellina, J. H., II; Boyd, M. R. *Nat. Prod. Lett.* **2000**, *14*, 285–292. (b) Hartwell, J. L.; Detty, W. E. *J. Am. Chem. Soc.* **1948**, *70*, 2833–2833. (c) Broomhead, A. J.; Dewick, P. M. *Phytochemistry* **1990**, *29*, 3839–3844. (d) Chang, L. C.; Song, L. S.; Park, E. J.; Luyengi, L.; Lee, K. J.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2000**, *63*, 1235–1238. (e) Hartwell, J. L.; Schrecker, A. W. *J. Am. Chem. Soc.* **1954**, *76*, 4034–4035. (f) von Wartburg, A.; Angliker, E.; Renz, J. *Helv. Chim. Acta* **1957**, *40*, 1331–1357. (g) Imbert, T. F. *Biochimie* **1998**, *80*, 207–222. (h) Jackson, D. E.; Dewick, P. M. *Phytochemistry* **1984**, *23*, 1147–1152. (i) Bedir, E.; Tellez, M.; Lata, H.; Khan, I.; Cushman, K. E.; Moraes, R. M. *Ind. Crops Prod.* **2006**, *24*, 3–7. (j) Couture, A.; Deniau, E.; Grandclaudon, P.; Lebrun, S.; Léonce, S.; Renard, P.; Pfeiffer, B. *Bioorg. Med. Chem.* **2000**, *8*, 2113–2125. (k) Meresse, P.; Dechaux, E.; Monneret, C.; Bertounesque, E. *Curr. Med. Chem.* **2004**, *11*, 2443–2466. (l) You, Y. J. *Curr. Pharm. Des.* **2005**, *11*, 1695–1717.

(15) (a) Nagasawa, H.; Isogai, A.; Ikeda, K.; Sato, S.; Murakoshi, S.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1975**, *39*, 1901–1902. (b) Dossena, A.; Marchelli, R.; Pochini, A. *J. Chem. Soc., Chem. Commun.* **1974**, 771–772. (c) Aoki, T.; Kamisuki, S.; Kimoto, M.; Ohnishi, K.; Takakusagi, Y.; Kuramochi, K.; Takeda, Y.; Nakazaki, A.; Kuroiwa, K.; Ohuchi, T.; Sugawara, F.; Arai, T.; Kobayashi, S. *Synlett* **2006**, 677–680.

(16) Budzikiewicz, H.; Pakrashi, S. C.; Vorbrüggen, H. *Tetrahedron* **1964**, *20*, 399–408.

(17) (a) Sando, C. E. *J. Biol. Chem.* **1923**, *56*, 457–468. (b) Chandramu, C.; Manohar, R. D.; Krupadanam, D. G. L.; Dashavantha, R. V. *Phytother. Res.* **2003**, *17*, 129–134.

(18) (a) Singleton, V. L.; Bohonos, N.; Ullstrup, A. J. *Nature* **1958**, *181*, 1072–1073. (b) Betina, V.; Nemec, P.; Dobias, J.; Barath, Z. *Folia Microbiol.* **1962**, *7*, 353–357. (c) Härri, E.; Loeffler, W.; Sigg, H. P.; Stähelin, H.; Tamm, Ch. *Helv. Chim. Acta* **1963**, *46*, 1235–1243. (d) Zhu, J.-W.; Nagasawa, H.; Nagura, F.; Mohamad, S. B.; Uto, Y.; Ohkura, K.; Hori, H. *Bioorg. Med. Chem.* **2000**, *8*, 455–463. (e) Anadu, N. O.; Davisson, V. J.; Cushman, M. *J. Med. Chem.* **2006**, *49*, 3897–3905.

(19) (a) Jones, D. F.; Mills, S. D. *J. Med. Chem.* **1971**, *14*, 305–311. (b) Shum, B.; Duffull, S. B.; Taylor, P. J.; Tett, S. E. *Br. J. Clin. Pharmacol.* **2003**, *56*, 188–197.

(20) (a) Harborne, J. B. *Phytochemistry* **1969**, *8*, 1449–1456. (b) Maskey, R. P.; Asolkar, R. N.; Speitling, M.; Hoffmann, V.; Grün-Wollny, I.; Fleck, W. F.; Laatsch, H. *Z. Naturforsch.* **2003**, *58*, 686–691. (c) Miyazawa, M.; Sakano, K.; Nakamura, S.-i.; Kosaka, H. *J. Agric. Food Chem.* **1999**, *47*, 1346–1349. (d) Chen, W.-F.; Huang, M.-H.; Tzang, C.-H.; Yang, M.; Wong, M.-S. *Biochim. Biophys. Acta* **2003**, *1638*, 187–196. (e) Lechner, D.; Cross, H. S. *Recent Results Cancer Res.* **2003**, *164*, 379–391. (f) Miltyk, W.; Craciunescu, C. N.; Fischer, L.; Jeffcoat, R. A.; Koch, M. A.; Lopaczynski, W.; Mahoney, C.; Jeffcoat, R. A.; Crowell, J.; Paglieri, J.; Zeisel, S. H. *Am. J. Clin. Nutr.* **2003**, *77*, 875–882.

(21) (a) Corbaz, R.; Ettlinger, L.; Gäumann, E.; Keller Schierlein, W.; Kradolfer, F.; Neipp, L.; Prelog, V.; Reusser, P.; Zähner, H. *Helv. Chim. Acta* **1957**, *40*, 199–204. (b) Dell, A.; Williams, D. H.; Morris, H. R.; Smith, G. A.; Feeney, J.; Roberts, G. C. K. *J. Am. Chem. Soc.* **1975**, *97*, 2497–2502. (c) Park, J. Y.; Park, S. J.; Shim, K. Y.; Lee, K. J.; Kim, Y.-B.; Kim, Y. H.; Kim, S. K. *Pharmacol. Res.* **2004**, *50*, 201–207. (d) Ko, J.; Chin, S.; Kyo, T.; Mizogami, K.; Hanada, K. Jpn. Kokai Tokkyo Koho JKXXAF 06316595 A 19941115 Heisei, 1994, 6 pp. (e) Park, Y. S.; Kim, Y. H.; Kim, S. K.; Choi, S.-J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 731–734.

(22) DiPaolo, J. A. *Ann. N.Y. Acad. Sci.* **1960**, *89*, 408–420.

(23) (a) Pettit, G. R.; Tan, R.; Pettit, R. K.; Smith, T. H.; Feng, S.; Doubek, D. L.; Richert, L.; Hamblin, J.; Weber, C.; Chapuis, J.-C. *J. Nat. Prod.* **2007**, *70*, 1069–1072. (b) Pettit, G. R.; Du, J.; Pettit, R. K.; Richert, L. A.; Hogan, F.; Mukku, V. J. R. V.; Hoard, M. S. *J. Nat. Prod.* **2006**, *69*, 804–806.

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