Bioactive Isomalabaricane Triterpenes from the Marine Sponge *Rhabdastrella globostellata*

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Two new isomalabaricane triterpenes, stellettin H (**1**) and stellettin I (**2**), have been isolated from the marine sponge *Rhabdastrella globostellata*, collected from the Philippines. Stellettins A−D (**3**−**6**), (−)-stellettin E (**7**), and rhabdastrellic acid-A (**8**) were also isolated and characterized. Stellettin B (**4**) and (−)-stellettin E (**7**) showed selective cytotoxicity toward $p21^{WAF1/Cip1}$-deficient human colon tumor (HCT-116) cells with IC_{50} values of 0.043 and 0.039 μM, respectively.

The malabaricanes, first isolated from the wood of the *Alianthus malabarica* tree, are yellow pigments characterized by a tricyclic terpenoid core and a conjugated, polyene side chain.[1,2] Isomalabaricane triterpenes, which have been reported from several genera of marine sponges,[3−16] differ from the malabaricanes by having a *trans*−*syn*−*trans* ring junction about the tricyclic nucleus instead of *trans*−*anti*−*trans*. The sponge sources of the isomalibaricanes are confusing, as a recent taxonomic reevaluation of the "*Jaspis stellifera*" complex has determined that many isomalabaricane-producing specimens previously reported in the literature are misidentified specimens of *Rhabdastrella globostellata*.[17] Specifically, Kennedy has reassigned *Stelletta globostellata* from Japan[5,7] and *Jaspis* sp. from Vanuatu[10] as *R. globostellata*. Additionally, he has suggested, on the basis of chemotaxonomic evidence, that *Jaspis stellifera* from Japan,[8,9] Fiji,[13] and the Great Barrier Reef[14] are misidentified specimens of *S. globostellata* and thus also should be reassigned as *R. globostellata*. The specimen from Northern Australia reported as *Jaspis* sp.[6] was reexamined by one of us (J.N.A.H.) and is also *R. globostellata*. A study of a Fijian sponge reported the isolation of isomalabaricanes from a specimen of *Geodia globostellifera*.[15] Reexamination of that specimen by one of us (M.K.H.) confirms its identity as *R. globostellata*. The *Jaspis* sp. from Tonga[11] was incorrectly cited and is actually *R. globostellata* (Michelle Kelly, personal communication 2001). In addition, there are three reports of isomalabaricanes from *R. globostellata* from China,[12] New Caledonia,[16] and the Philippines (present study). The affinity of isomalabaricane producing *Stelletta* sp. from Somalia[3] and *Stelletta tenuis* from China[4] remains unclear; however the latter specimen was collected from the identical locale (Hainan Island) as *R. globostellata* cited in ref 12. It is now clear that the isomalabaricanes are chemotaxonomic markers for *R. globostellata*.

It is well known that isomalabaricane terpenes readily isomerize upon exposure to light.[6,7,9−11] During the isolation and characterization process, and also during storage, they rapidly undergo equilibration to a 1:1 mixture of 13*E* and 13*Z* isomers, rendering their workup and characterization difficult. However, because of their significant cytotoxic activity,[11,18] these compounds continue to receive a great deal of attention.

Cell cycle progression is controlled by the induction of cyclins and activation of cyclin-dependent kinases. Cyclin-dependent kinase (cdk) inhibitors may serve as potential chemotherapeutic targets because of their control in cellular proliferation.[19] The overexpression of the cdk inhibitory protein, p21 ($p21^{WAF1/Cip1}$), arrests the cell cycle by inhibiting the following cdks: cdk2, cdk3, cdk4, and cdk6. The expression of p21 is predominantly and directly induced by p53, the most commonly mutated gene in human cancers. Additionally, p21 expression may be regulated independently of p53. This p21-dependent cell cycle arrest allows for cellular repair before continuation of the cell cycle. In the absence of p21 arrest, mutated cells may continue to proliferate unchecked, leading to potential tumor growth.

Bioactivity-guided isolation using the wild type (WT) and the corresponding p21-deficient human colon tumor (HCT-116) cell lines was carried out on the $CHCl_3$ extract of *R. globostellata*. This study yielded two new isomalabaricane triterpenes, stellettin H (**1**) and stellettin I (**2**), and six known compounds, stellettins A (**3**), B (**4**), C (**5**), and D (**6**), the optical antipode of stellettin E (**7**), and rhabdastrellic acid-A (**8**). The cytotoxicity of compounds **1**−**8** toward a panel of isogenic HCT-116 cell lines is also discussed.

The specimen of *R. globostellata* collected from Mindanao, the Philippines, was extracted with MeOH. The crude extract was subjected to a solvent partition scheme to yield hexane, $CHCl_3$, and aqueous MeOH extracts (see Experimental Section). The $CHCl_3$ phase exhibited significant differential cytotoxicity toward the p21-deficient HCT-116 cell line. Bioactivity-guided fractionation of this material by C_{18} flash column chromatography followed by silica HPLC afforded compounds **1**−**8**. Due to the tendency of isomalabaricane triterpenes to photoisomerize, we avoided light exposure by wrapping all glassware with aluminum foil and also storing the fractions and pure compounds at −80 °C.

Stellettin H (**1**) was obtained as an optically active ($[\alpha]_D$ −83°) orange-yellow glass. Both ESIMS and FABMS

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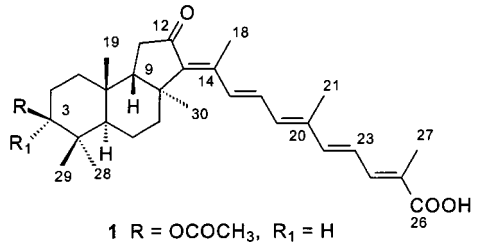

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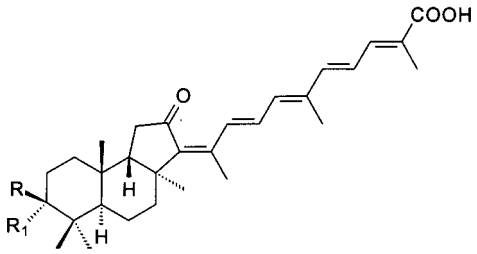

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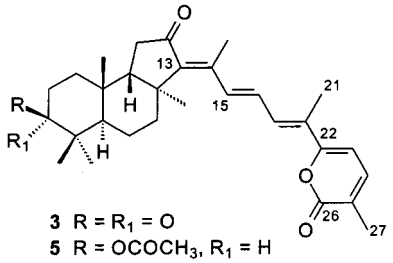

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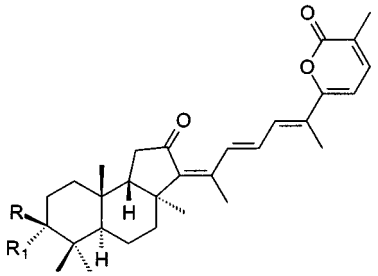

4 R = R_1 = O
6 R = $OCOCH_3$, R_1 = H

spectra of **1** showed a protonated molecular ion peak at *m/z* 509 (509.3267 by HRFABMS). These data were in accordance with a molecular formula of $C_{32}H_{44}O_5$ and the presence of 11 degrees of unsaturation. The UV absorptions (λ_{max} 234, 294, 392, 410 nm) and IR data (ν_{max} 1731, 1709, 1691, 1675 cm^{-1}) suggested the presence of a highly conjugated structure. The ^1H NMR spectrum revealed resonances for six vinylic protons [δ 6.39−7.35 (Table 1)], four methyls on sp^3 quaternary carbons (δ 0.87, 0.91, 1.01, and 1.40), three vinylic methyls (δ 2.00, 2.02, and 2.31), an acetyl (δ 2.05), and an oxymethine proton at δ 4.55. The ^{13}C NMR spectrum correlated well with the ^1H NMR data and indicated the presence of five double bonds (10 sp^2 resonances between δ 120−150, see Table 1), an ester carbonyl (δ 171.0), a carboxylic acid (δ 172.5), a ketone (δ 207.5), 11 sp^3 resonances (five CH_2, three CH, and three quaternary carbons), and eight methyl groups, thereby indicating **1** was tricyclic. These observations also accounted for an isomalabaricane triterpene possessing a pentaene moiety in association with extra acetyl and carboxylic acid functionalities. The acetoxy group was assigned to C-3 on the basis of scalar coupling between H-3 and H_2-2 as well as HMBC cross-peaks observed from H-3 to CH_3-28 (δ 29.0), CH_3-29 (δ 16.9), and an acetoxy carbonyl (δ 171.0). The chemical shift of H-3 (δ 4.55, m) indicated the equatorial (β) position of the acetoxy function.[5−7,11] This stereochemistry was confirmed by NOE correlations between H-3/H-5 and H-3/H_3-28. Additional COSY couplings between H_2-1/H_2-2, H-5/H_2-6, H_2-6/H_2-7, and H-9/H_2-11 together with the corresponding HMBC correlations (Table 1) allowed the assemblage of the tricyclic nucleus. The carboxylic acid function reported in previous isomalabaricanes was attached either at C-4 or at the end of the side chain. The observation of a *gem*-dimethyl group showing HMBC correlations to C-3, C-4, C-5 and also to each other eliminated the first possibility. Instead, the HMBC correlations traced from a methyl signal at δ_H 2.00 (δ_C 12.6, CH_3-27) to C-24 (δ_C 140.4), C-25 (δ_C 126.2), and COOH (δ_C 172.5, C-26) indicated that the polyene side chain contained the COOH terminus. Complete assignments of the side chain and the geometry of the pentaene system were accomplished by analysis of the 1D and 2D NMR data. The chemical shift of H_3-18 (δ 2.31) implied that this methyl group was deshielded by the C-12 carbonyl function; therefore, Δ^{13} had an *E*-orientation. On the basis of homonuclear couplings in the COSY spectrum, the proton resonances at δ 6.68 (d, J = 14.7 Hz), δ 6.99 (dd, J = 11.0, 14.7 Hz), and δ 6.39 (d, J = 11.0 Hz) were attributed to H-15, H-16, and H-17, respectively. A *trans* (*E*) geometry was defined for Δ^{15} based on the magnitude of the coupling constant between H-15 and H-16 (J = 14.7 Hz) in addition to the key NOE correlation between H_3-30 and H-15. The appearance of CH_3-21 at δ 13.0 in the ^{13}C NMR spectrum indicated that the C-17(20) trisubstituted double bond also adopted an *E* geometry. The DQF-COSY experiment established the connectivity of the remaining olefinic protons. The proton signal at δ 6.61 (d, J = 14.7 Hz, H-22) was coupled to δ 6.59 (dd, J = 11.0, 14.7 Hz, H-23), which in turn coupled to the proton resonance at δ 7.35 that was assigned as H-24. The chemical shift of H-24 (δ 7.35 d, J = 11.0 Hz) indicated that this proton was in the deshielding zone of the carboxylic acid (C-26), consistent with a 24(*E*) geometry. The observation of CH_3-27 at δ 12.6 in the ^{13}C NMR spectrum presented further evidence for a 24(*E*) configuration. All NMR assignments were verified by HMBC correlations, as shown in Table 1.

The relative stereochemistry of the tricyclic nucleus was fixed through NOE correlations obtained in the NOESY spectrum of **1**. NOE cross-peaks between the pairs H-5/H_3-28, H-5/H_3-30, as well as H-9/H_3-19 and H_3-19/H_3-29, clearly established a *trans*−*syn*−*trans* stereochemistry, corresponding to an isomalabaricane skeleton. Thus, compound **1** was identified as (13*E*,15*E*,17*E*,22*E*,24*E*)-3β-acetoxy-12-oxo-isomalabarica-13,15,17,22,24-pentaen-26-oic acid. The trivial name of stellettin H is proposed for this compound.

Stellettin I (**2**) was found by HRFABMS to have the same molecular formula ($C_{32}H_{44}O_5$) as **1**. A careful inspection of the NMR data showed that **2** had essentially the same ^{13}C NMR data as those of **1**. The only striking difference between these two compounds was the emergence of a new proton signal at δ 8.11 (d, J = 14.7 Hz) in place of δ 6.68 (H-15 in **1**) and the high-field shift of H_3-18 from δ 2.31 to 2.02 in the ^1H NMR spectrum of **2**. These data were in good agreement with a *Z*-oriented $\Delta^{13,14}$ in **2**. Further support for the 13*Z* geometry came from the NOESY spectrum of

Table 1. NMR Data of Stellettin H (**1**) and Stellettin I (**2**) (500 MHz, $CDCl_3$)

	1			**2**	
position	δ_H mult (*J* in Hz)	δ_C mult	HMBC (H to C)	δ_H mult (*J* in Hz)	δ_C mult
1	1.38 m	33.0 t		1.44 m	33.1 t
	1.60 m				
2	1.64 m	25.1 t	10	1.63 m	25.1 t
	1.82 m			1.85 m	
3	4.55 m	80.8 d	28, 29, $OCOCH_3$	4.55 m	80.8 d
4		38.2 s			39.3 s
5	1.78 m	46.7 d	4, 7, 10, 29	1.78 m	46.6 d
6	1.45 m	18.4 t		1.50 m	18.3 t
	1.70 m			1.68 m	
7	2.15 m	39.6 t	6, 8, 9, 30	2.10 m	38.2 t
8		44.8 s			44.7 s
9	1.83 m	50.1 d	8, 11, 30	1.83 m	50.2 d
10		35.5 s			35.5 s
11	2.20 m	36.6 t	9, 12	2.20 m	36.8 t
12		207.5 s			208.4 s
13		147.6 s			147.2 s
14		141.0 s			140.8 s
15	6.68 (d, 14.7)	134.8 d	17, 18	8.11 (d, 14.7)	135.1 d
16	6.99 (dd, 11.0, 14.7)	131.6 d		6.95 (dd, 11.0, 14.7)	130.2 d
17	6.39 (d, 11.0)	135.6 d		6.45 (d, 11.0)	136.4 d
18	2.31 s	14.5 q	13, 14, 15	2.02 s	15.9 q
19	1.01 s	23.3 q	1, 5, 9, 10	1.01 s	23.3 q
20		138.2 s			137.7 s
21	2.02 s	13.0 q	17, 20, 22	1.98 s	12.9 q
22	6.61 (d, 14.7)	144.3 d	23	6.67 (d, 14.7)	144.8 d
23	6.59 (dd, 11.0, 14.7)	124.6 d	20, 24	6.57 (dd, 11.0, 14.7)	123.9 d
24	7.35 (d, 11.0)	140.4 d		7.37 (d, 11.0)	141.9 d
25		126.2 s			125.8 s
26		172.5 s			172.3 s
27	2.00 s	12.6 q	24, 25, 26	1.99 s	12.6 q
28	0.91 s	29.0 q	3, 4, 5, 29	0.93 s	29.0 q
29	0.87 s	16.9 q	3, 4, 5, 28	0.88 s	17.0 q
30	1.40 s	25.9 q	7, 8, 9, 13	1.36 s	24.7 q
$OCOCH_3$	2.05	21.2 q	$OCOCH_3$	2.04 s	21.2 q
$OCOCH_3$		171.0 s			171.0 s

2, which contained correlations between the H_3-30 and H_3-18 methyl groups. The 1D and 2D NMR data and coupling constant analyses demonstrated an all-*E* configuration for the 15,17,22,24-tetraene system, as in the case of **1**. The relative stereochemistry of the tricyclic core was also found to be the same as **1** by a NOESY experiment. Hence, the structure of the compound **2** was determined as (13*Z*,15*E*,-17*E*,22*E*,24*E*)-3β-acetoxy-12-oxoisomalabarica-13,15,17,-22,24-pentaen-26-oic acid.

Compounds **3**−**6** and **8** were identified as stellettins A (**3**),[4] B (**4**),[13] C (**5**),[6] and D (**6**)[6] and rhabdastrellic acid-A (**8**)[12] on the basis of 1D and 2D NMR, MS data, and $[\alpha]_D$ values. The structure of compound **7** was found to be the same as stellettin E;[6] however, the sign of the optical rotation value ($[\alpha]_D$ −68.5° in **7**, $[\alpha]_D$ +36°) for the C-26 methyl ester of stellettin E,[6] both in $CHCl_3$, indicated that **7** was the optical enantiomer of stellettin E. Due to the minor amount of stellettin E isolated, only partial ^1H and ^{13}C NMR data have been reported.[6] We include the complete NMR data of **7** in the Experimental Section.

Immediately after isolation, all compounds were tested against a set of isogenic colorectal cancer cells, wild-type HCT-116 and the corresponding p21-deficient mutant cell line in which the p21 gene was disrupted through homologous recombination. Stellettin B (**4**) and (−)-stellettin E (**7**) displayed selective toxicity toward the p21-deficient HCT cell line with IC_{50} values of 0.043 and 0.039 μM, respectively. The other isomalabaricanes were either less active or inactive (Table 2). In addition, stellettin B (**4**) and (−)-stellettin E (**7**) were tested in the corresponding WT and p53-deficient HCT-116 cell lines in which the p53 gene was disrupted through homologous recombination. Neither compound showed differential cytotoxicity against these cell lines. These data suggest several interpretations. First, the mechanism of action of stellettin B and (−)-stellettin E may be p53 independent and p21 dependent. Second, this result may mean that the cells lacking the p21 genes are more sensitive to these compounds than wild type cells, causing them to preferentially undergo cell death. In the absence of growth arrest genes the death-inducing effects of p53 may be more evident or efficacious.[20] Further experiments to understand the mechanism of action of the stellettins are currently in progress. Of note, it is interesting that both stellettin B (**4**) and (−)-stellettin E (**7**) contain a keto function at C-3 and adopt *Z* geometry at Δ.[13] It appears that the terminus of the conjugated side chain does not play a major role for the activity. Because of their instability, the data presented in the past have reflected testing of isomeric mixtures of the isomalabaricanes.[14,18] In fact, the isolation of nearly equal mixtures of *E* and *Z* isomers in this study as well as previous ones raises the possibility (probability) that one of the isomers is an

Table 2. IC_{50} Values (μM) of **1**−**8** Toward Wild Type (p21+ and p53+) and Mutated (p21− and p53−) HCT-116 Cell Lines

compound	p21+	p21−	p53+	p53−
stellettin H (**1**, 13*E*)	>4.92	3.98	n.t.[a]	n.t.
stellettin I (**2**, 13*Z*)	>4.92	3.86	n.t.	n.t.
stellettin A (**3**, 13*E*)	4.46	0.26	n.t.	n.t.
stellettin B (**4**, 13*Z*)	0.65	0.043	7.25	8.23
stellettin C (**5**, 13*E*)	2.45	0.30	n.t.	n.t.
stellettin D (**6**, 13*Z*)	0.43	0.31	n.t.	n.t.
(−)-stellettin E (**7**, 13*Z*)	4.57	0.039	>5.39	>5.39
rhabdastrellic acid-A (**8**, 13*E*)	>5.39	3.49	n.t.	n.t.

[a] n.t.= not tested

artifact. Precautions were taken during isolation and assay to avoid isomerization. Specifically, the compounds and assays were maintained in a dark room. The differences observed for bioactivity of the 13*Z* and 13*E* isomers (Table 2) indicate that this was at least partially successful, although the possibility that some isomerization occurred cannot be ruled out. The results do nonetheless illustrate that the geometry of Δ^{13} does impact biological activity.

Experimental Section

General Experimental Procedures. UV spectra were recorded in MeOH on a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded using a JASCO FTIR-420 spectrophotometer. A Varian instrument (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) with a Nalorac MDBG 3 mm probe and spinner was used to obtain NMR data. NMR spectra were recorded in $CDCl_3$, using the residual signal of nondeuterated solvents as internal standards. Mass spectra were taken on Finnigan MAT 95 (FABMS) and Finnigan LCQ DECA ion trap (ESIMS) spectrometers. C_{18} material (J. T. Baker, 40 mm, 275 Å) was used for flash chromatography. HPLC separations were performed on a Varian Si gel 60 semipreparative column (25 × 10 cm i.d, 4 mL/min) using a Beckman 168 photodiode array system (395 nm). Optical rotations were recorded at room temperature on a JASCO DIP-370 polarimeter.

Animal Material. The specimen of *Rhabdastrella globostellata* (Carter, 1883) was collected by scuba in Mindanao (Guimputlan, Dakak, Dipolog City; N 08° 43.420′, E 123° 23.202′), Philippines, in 1998. The sponge was identified by Mary Kay Harper, and a voucher specimen ($PDZ_1$98-1-10) is held at the University of Utah.

Extraction and Isolation. Thawed sponge material (250 g) was extracted with MeOH (3 × 250 mL). The MeOH extracts were combined, filtered, and evaporated to dryness in vacuo to give a dark yellow residue (8.1 g). This residue was dissolved in 10% water in MeOH (200 mL) and partitioned against hexane (3 × 200 mL). The water content of the MeOH phase was then adjusted to 30% by adding 80 mL of water before partitioning against $CHCl_3$. The $CHCl_3$ extract (2.9 g) was fractionated by C_{18} flash column chromatography (CC) using MeOH gradients in H_2O. The fractions eluted with 80, 90, and 100% MeOH (fractions 8−10) showed selective cytotoxicity against p21-deficient HCT-116 cells and were pooled together based on their similarity in TLC and ^{1}H NMR analyses. An aliquot (100.1 mg) of the fractions 8−10 was further rechromatographed by C_{18} flash CC. Employment of step gradient H_2O−MeOH mixtures (60−100% MeOH) yielded two bioactive fractions, 9a and 9b, both eluted with 90% MeOH. Fraction 9a (40.4 mg) was further purified by Si HPLC using CH_2Cl_2−EtOAc mixtures (95:5 for 15 min, then 30 min gradient to CH_2Cl_2−EtOAc, 85:5) to afford stellettin A (**3**, 5.7 mg), stellettin B (**4**, 4.8 mg), rhabdastrellic acid-A (**8**, 4.1 mg), and (−)-stellettin E (**7**, 5.2 mg). Purification of fraction 9b (32.8 mg) was also performed by Si HPLC using the same conditions as above to provide stellettin C (**5**, 4.9 mg), stellettin D (**6**, 5.2 mg), stellettin H (**1**, 2.6 mg), and stellettin I (**2**, 2.9 mg), respectively.

Stellettin H (1): orange-yellow glass; $[\alpha]_D$ −83° (*c* 0.19, $CHCl_3$), UV (MeOH) λ_{max} (log ϵ) 234 (3.46), 294 (3.62), 392 (4.03), 410 (3.99) nm; IR (NaCl) ν_{max} 3466 (broad), 2925, 1731, 1709, 1691, 1675, 1578, 1375, 1246 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; ESIMS *m*/*z* 509 $[M + H]^+$; FABMS *m*/*z* 509 $[M + H]^+$ (2), 307 (12), 289 (9), 154 (100), 136 (83); HRFABMS 509.3267 *m*/*z* (calcd for $C_{32}H_{45}O_5$, 509.3267).

Stellettin I (2): yellow oil; $[\alpha]_D$ −31° (*c* 0.22, $CHCl_3$), UV (MeOH) λ_{max} (log ϵ) 234 (3.28), 294 (3.46), 394 (3.91), 410 (3.88) nm; IR (NaCl) ν_{max} 3474 (broad), 2925, 1729, 1707, 1691, 1676, 1579, 1375, 1246 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; ESIMS *m*/*z* 507 $[M - H]^-$; FABMS *m*/*z* 508 $[M]^+$ (1), 509 $[M + H]^+$ (2), 391 (8), 307 (18), 289 (10), 154 (100), 136 (72); HRFABMS 508.3185 *m*/*z* (calcd for $C_{32}H_{44}O_5$, 508.3189), 509.3234 (calcd for $C_{32}H_{45}O_5$, 509.3267).

(−)-Stellettin E (7): yellow oil; $[\alpha]_D$ −68.5° (*c* 0.2, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 14.7, H-15), 7.36 (d, J = 11.0, H-24), 6.99 (dd, J = 11.0, 14.7, H-16), 6.64 (d, J = 14.7, H-22), 6.56 (dd, J = 11.0, 14.7, H-23), 6.44 (d, J = 11.0, H-17), 2.72 (m, H-2b), 2.36 (m, H-5), 2.34 (m, H-2a), 2.20 (m, H_2-11), 2.14 (m, H-1b), 2.05 (s, H_3-18), 2.04 (m, H_2-7), 1.98 (s, H_3-21), 1.97 (s, H_3-27), 1.85 (m, H-9), 1.59 (m, H-6b), 1.50 (m, H-6a), 1.48 (H-1a), 1.38 (s, H_3-30), 1.09 (s, H_3-28), 1.03 (s, H_3-29), 0.83 (s, H_3-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 219.1 (s, C-3), 206.1 (s, C-12), 172.9 (s, COOH), 146.1 (s, C-13), 144.7 (d, C-22), 142.6 (s, C-14), 140.6 (d, C-24), 137.9 (s, C-20), 136.3 (d, C-17), 134.9 (d, C-15), 130.5 (d, C-16), 126.0 (s, C-25), 124.0 (d, C-23), 47.9 (d, C-9), 46.8 (s, C-4), 45.4 (d, C-5), 44.9 (s, C-8), 37.2 (t, C-7), 36.8 (t, C-11), 34.8 (s, C-10), 33.5 (t, C-2), 31.3 (t, C-1), 29.2 (q, C-28), 24.7 (q, C-30), 23.5 (q, C-19), 19.7 (t, C-6), 19.4 (q, C-29), 15.9 (q, C-18), 12.9 (q, C-21), 12.6 (q, C-27).

Cells and Culture Conditions. Human colon tumor (HCT-116) cell lines, both wild type and p53- and p21-deficient (provided by Dr. Bert Vogelstein at Johns Hopkins University, Baltimore, MD), were propagated in McCoy's 5A media (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 0.05 units/mL penicillin, 0.05 μg/mL streptomycin (Life Technologies), 200 mM L-glutamine (Life Technologies), and 100 mM MEM sodium pyruvate (Life Technologies). The cells were incubated at 37 °C in a humidified 5% carbon dioxide atmosphere in T-75 cm^2 tissue culture flasks.

Cell Proliferation Assay. Cells were seeded in flat bottom 96-well plates at the concentration of 3000 cells per well for p53+ and p53− cell lines and 4000 and 6000 cells per well for p21+ and p21−, respectively, in 0.2 mL of media. After a 24 h sit down period, the media was changed and cells were incubated with varying concentrations of drug for 48 h. Cell viability was measured by a colorimetric assay based on the bioreduction of MTS tetrazolium salt (cell proliferation reagent, CellTiter 96 AQueous One Solution; Promega) at 490 nm.

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