Antitumor Agents. 228. Five New Agarofurans, Reissantins A−E, and Cytotoxic Principles from *Reissantia buchananii*

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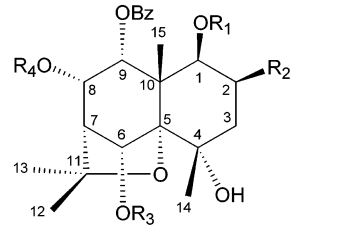
Twenty-one compounds, including five new agarofuran sesquiterpenes, reissantins A−E (**1**−**5**), were isolated from *Reissantia buchananii* by means of bioassay-directed fractionation and their structures identified from spectral data. Reissantins A−C are the first reported simple agarofuran sesquiterpenes to contain a 5-carboxy-*N*-methyl-2-pyridone (CNMP) substituent, which has previously been found only in macroring agarofuran pyridine alkaloids. The major terpenoid components, celastrol (**6**) and its methyl ester derivative, pristimerin (**7**), were significantly active against nine cancer cell lines, including A549, MCF-7, HCT-8, KB, KB-VIN, U-87-MG, PC-3, 1A9, and PTX10 cell lines, with ED_{50} values ranging from 0.076 to 0.34 μg/mL.

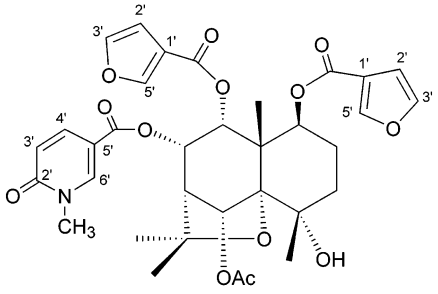
The genus *Reissantia* has been split off from the larger genus *Hippocratea* and belongs to the family Celastraceae. Extensive phytochemical investigation of *Reissantia buchananii* (Loes.) N. Hellé (Celastraceae) has not been performed, and only rare triterpenes have been isolated from this genus.[1−3] In the course of our continuing search for bioactive plant components, we found that a methanol extract of this plant showed effective growth inhibition against several tumor cell lines. Twenty-one compounds were isolated by means of bioassay-directed fractionation and characterized by physical and spectral data. These compounds included five agarofuran sesquiterpenes, reissantins A−E (**1**−**5**), and nine triterpenoids, celastrol (**6**),[4,5] 3-hydroxy-2-oxo-24-nor-friedela-1(10),3,5,7-tetraen-29-oic acid methyl ester (**7**),[6] 6-oxo-pristimerol (**8**),[7] a mixture of 5α-daturadione (**9**) and 5β-daturadione (**10**),[8] a mixture of 5α-3β-hydroxyolean-12-enone (**11**) and 5β-3β-hydroxyolean-12-enone (**12**),[8,9] friedelan (**13**),[10] 29-hydroxyfriedelan-3-one (**14**),[11] 3-oxofriedoolean-29-al (**15**),[12] 3,7,11,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaen-1-ol (**16**),[13] two steroids, β-sitosterol (**17**)[14,15] and β-sitosterol-β-D-glycoside (**18**),[14,15] and two miscellaneous compounds, a mixture of *trans*-docosanylferulate (**19**) and *trans*-lignocerylferulate (**20**),[16] and methyl *N*-methyl-2-oxohydropyridine-5-carboxylate (**21**).[17] Structures of the known compounds (**8**−**21**) are given in the Supporting Information.

Reissantins A−E (**1**−**5**) are new compounds, and their structures were identified on the basis of their spectral data. In addition, compounds **1**−**8**, **11**, **12**−**14**, and **17**−**21** were evaluated in vitro against nine human cancer cell lines, including A549 (lung cancer), MCF-7 (breast), HCT-8 (ileocecal), KB (epidermoid nasopharyngeal), KB-VIN (vincristine-resistant KB), U-87-MG (glioblastoma), PC-3 (prostate), and 1A9 (ovarian) and its subline PTX10 with β-tubulin mutation.

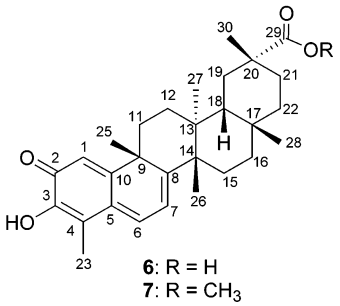


1: $R_1 = R_4 = Ac$, $R_2 = H$, $R_3 = CNMP$[a]
2: $R_1 = R_4 = Ac$, $R_2 = OBz$, $R_3 = CNMP$
4: $R_1 = Ac$, $R_2 = R_3 = R_4 = H$
5: $R_1 = R_4 = Ac$, $R_2 = R_3 = H$

[a]CNMP = 5-carboxy-N-methyl-2-pyridone



3



6: R = H
7: R = CH_3

Results and Discussion

Compound **1** was assigned the formula $C_{33}H_{39}O_{11}N$ on the basis of a HRFABMS molecular ion at *m*/*z* 626.2603

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Table 1. ^{1}H NMR Chemical Shifts for Compounds **1**−**5** (in $CDCl_3$, 500 MHz)[a]

proton	1	2	3	4	5
1	5.34 (dd, 12.5, 5.0)	5.61 (d, 3.6)	5.46 (dd, 12.0, 4.5)	5.37 (dd, 12.0, 4.0)	5.34 (dd, 11.9, 4.0)
2	1.96 (m)	5.81 (d, 3.6)	1.57 (m)	2.01 (m)	1.98 (m)
	1.94 (m)		1.85 (m)	1.49 (m)	1.47 (m)
3	1.74 (m)	2.28 (m)	1.93 (m)	1.92 (m)	1.96 (m)
	1.71 (m)	2.23 (m)	1.67 (m)	1.69 (m)	1.72 (m)
6	5.52 (s)	5.69 (s)	5.71 (s)	4.37 (s)	4.47 (d, 5.0)
7	2.59 (d, 3.5)	2.65 (d, 3.7)	2.64 (d, 3.5)	2.51 (d, 3.0)	2.46 (d, 3.5)
8	5.69 (dd, 6.5, 3.5)	5.76 (dd, 7.7, 3.7)	5.28 (dd, 10.0, 3.5)	4.39 (dd, 6.5, 3.0)	5.44 (dd, 6.4, 3.5)
9	5.37 (d, 6.5)	5.37 (d, 7.7)	6.05 (d, 10.0)	5.25 (d, 6.5)	5.29 (d, 6.4)
12	1.65 (s)	1.69 (s)	1.70 (s)	1.66 (s)	1.65 (s)
13	1.51 (s)	1.56 (s)	1.54 (s)	1.58 (s)	1.58 (s)
14	1.33 (s)	1.61 (s)	1.36 (s)	1.59 (s)	1.60 (s)
15	1.44 (s)	1.71 (s)	1.48 (s)	1.36 (s)	1.39 (s)
Bz-2					
2′ and 6′		7.95 (br d, 7.2)			
3′ and 5′		7.45 (br t, 7.2)			
4′		7.58 (br t, 7.2)			
Bz-9					
2′ and 6′	8.08 (br d, 7.5)	8.08 (br d, 7.2)		8.09 (br t, 7.5)	8.06 (br t, 7.2)
3′ and 5′	7.48 (br t, 7.5)	7.49 (br t, 7.2)		7.47 (br t, 7.5)	7.47 (br t, 7.2)
4′	7.60 (br t, 7.5)	7.61 (br t, 7.2)		7.60 (br t, 7.5)	7.59 (br t, 7.2)
CNMP					
3′	6.56 (d, 9.5)	6.57 (d, 9.5)	6.45 (d, 9.5)		
4′	7.97 (dd, 9.5, 2.5)	7.98 (dd, 9.5, 2.5)	7.68 (dd, 9.5, 2.5)		
6′	8.45 (d, 2.5)	8.44 (d, 2.5)	8.00 (d, 2.5)		
N-Me	3.60 (s)	3.61 (s)	3.50 (s)		
Ac-1					
CH_3	1.66 (s)	1.66 (s)		1.61 (s)	1.63 (s)
Ac-6					
CH_3			2.16 (s)		
Ac-8					
CH_3	1.91 (s)	1.91 (s)			1.91 (s)
Fu-1					
2′			6.45 (t, 1.0)		
3′			7.16 (t, 1.5)		
5′			7.57 (d, 1.0)		
Fu-9					
2′			6.33 (t, 1.0)		
3′			7.26 (s)		
5′			7.68 (t, 1.0)		

[a] Multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. *J* values are given in parentheses.

($[M + H]^+$, calcd 626.2601). The IR spectrum indicated the presence of hydroxyl (3537 cm^{-1}) and carboxyl (1722 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 1) showed signals for two acetyl methyls (δ 1.66 and 1.91), one benzoate group, and one 5-carboxy-*N*-methyl-2-pyridone (CNMP) moiety.[17,18] The ^{13}C NMR and DEPT spectra (Table 2) indicated that **1** has an agarofuran skeleton[18] with 15 carbons, including four methyl carbons at δ 26.4 (C-12), 30.4 (C-13), 23.8 (C-14), and 19.5 (C-15), two methylene carbons at δ 23.2 (C-2) and 38.7 (C-3), five methine carbons at δ 72.2 (C-1), 78.3 (C-6), 53.6 (C-7), 68.6 (C-8), and 72.1 (C-9), and four quaternary carbons at δ 70.8 (C-4), 91.1 (C-5), 49.6 (C-10), and 84.7 (C-11). The ^{13}C NMR quaternary signals at δ 91.1 (C-5) and 84.7 (C-11) are characteristic of agarofurans. The structure of **1** and the positions of its functional groups were fully determined on the basis of the HMBC spectrum (Figure 1, Supporting Information). Key correlations were found between δ_C 163.3/δ_H 5.52 (H-6), δ_C 165.6/δ_H 8.08 (BzO-9, 2′, and 6′) and 5.37 (H-9), δ_C 169.8/δ_H 5.34 (H-1) and 1.66 (acetyl methyl), and δ_C 169.2/δ_H 5.69 (H-8) and 1.91 (acetyl methyl). The relative stereochemistry was determined by a NOESY experiment (Figure 2, Supporting Information). In this class of compounds, the stereochemistry of the ring junctions is generally trans and H-1 and H-6 are axial,[19,20] as confirmed by the NOESY spectrum of **1**. Because H-6 correlated with H-14 and H-15, the corresponding methyls have axial orientations. H-8 and H-9 were determined to be axial and equatorial, respectively, on the basis of the cross-peaks between H-8/H-15 and H-9/H-15. The singlet at δ 5.52 (1H, s) was assigned to H_{ax}-6 because of weak coupling between H_{ax}-6 and H_{eq}-7, which occurs in all compounds of this class.[20] Methyl *N*-methyl-2-oxohydropyridine-5-carboxylate (**21**) was also isolated from this plant, which provides biogenetic evidence of the CNMP substitution of **1**. Thus, the structure of **1** was determined as illustrated and named reissantin A.

Compound **2** was analyzed as $C_{40}H_{43}O_{13}N$ by HRFABMS. The IR spectrum indicated the presence of a hydroxyl group at 3460 cm^{-1} and carboxyl groups at 1721 and 1750 cm^{-1}. The NMR signals closely resembled those of **1**, except for an additional benzoic moiety, as also seen from the mass fragmentation. The ^{1}H NMR data of **2** are shown in Table 1 and the ^{13}C NMR data in Table 2. The ^{13}C NMR and DEPT spectra indicated that **2** also possesses an agarofuran skeleton, especially the characteristic ^{13}C NMR quaternary signals at δ 91.1 (C-5) and 85.1 (C-11). The R_3−R_5 substitutions of **2** are identical with those of **1** on the basis of similar spectral features. However, different R_2 (H in **1**, Bn in **2**) substitutions were confirmed from the HMBC and NOESY spectra (Figures 3 and 4, Supporting Information). The HMBC spectrum of **2** exhibited cross-peaks due to long-range correlations between δ_C 163.3/δ_H 5.69 (H-6), δ_C 165.6/δ_H 8.08 (BzO-9, 2′, and 6′) and δ_H 5.37 (H-9), δ_C 165.7/δ_H 7.95 (BzO-2, 2′, and 6′), δ_C 169.5/δ_H 1.66 (acetyl methyl), and δ_C 169.2/δ_H 1.91 (acetyl methyl). The relative stereochemistry was assigned from NOESY experiments. In conclusion, the structure of **2** was determined as shown and named reissantin B.

HRFABMS measurement of **3** gave a molecular formula of $C_{34}H_{37}O_{13}N$. Its structure was deduced from ^{13}C and ^{1}H NMR spectral data, together with IR absorption bands at

Table 2. ^{13}C NMR Chemical Shifts for Compounds **1**−**5** (in $CDCl_3$, 500 MHz)

carbon	1	2	3	4	5
1	72.2	69.9	77.4	72.5	72.1
2	23.2	69.2	24.5	23.0	23.1
3	38.7	42.5	38.1	37.1	37.1
4	70.8	70.2	70.4	73.0	73.0
5	91.1	91.1	92.6	91.0	91.1
6	78.3	78.0	76.2	78.5	78.3
7	53.6	53.7	51.9	56.2	55.0
8	68.6	68.3	74.7	70.9	69.2
9	72.1	71.8	75.8	75.4	72.4
10	49.6	49.7	47.9	48.7	48.8
11	84.7	85.1	84.0	84.8	84.7
12	26.4	26.4	25.5	26.9	27.1
13	30.4	30.4	29.6	30.8	30.7
14	23.8	25.4	23.6	23.7	23.7
15	19.5	21.7	13.2	20.1	19.7
Bz-2					
C═O		165.7			
1′		129.8			
2′ and 6′		129.5			
3′ and 5′		128.7			
4′		133.3			
Bz-9					
C═O	165.6	165.6		168.0	165.7
1′	129.1	128.9		128.9	129.2
2′ and 6′	130.1	130.2		130.3	130.1
3′ and 5′	128.4	128.4		128.4	128.4
4′	133.5	133.6		133.7	133.4
CNMP					
C═O	163.3	163.3	163.2		
N-methyl	38.5	38.6	38.3		
2′	162.9	162.9	162.8		
3′	119.7	119.8	119.6		
4′	138.7	138.6	138.3		
5′	109.1	109.1	108.7		
6′	145.0	145.0	143.8		
Ac-1					
C═O	169.8	169.5		169.9	169.5
CH_3	20.7	20.3		20.7	20.8
Ac-6					
C═O			169.9		
CH_3			21.5		
Ac-8					
C═O	169.2	169.2			169.9
CH_3	20.7	20.67			20.7
Fu-1					
C═O			161.9		
1′			119.1		
2′			109.3		
3′			147.3		
5′			143.1		
Fu-9					
C═O			161.9		
1′			119.0		
2′			109.0		
3′			147.3		
5′			144.0		

1733 cm^{-1} (carbonyl) and 3419 cm^{-1} (hydroxyl). The ^1H NMR spectrum of **3** (Table 1) showed the presence of one acetyl singlet at δ 2.16, two furancarboxylate signals,[20] and one 5-carboxy-*N*-methyl-2-pyridone (CNMP). The ^{13}C NMR and DEPT spectra indicated that **3** contained a 15-carbon agarofuran skeleton, including four methyl carbons at δ 25.5 (C-12), 29.6 (C-13), 23.6 (C-14), and 13.2 (C-15), two methylene carbons at δ 24.5 (C-2) and 38.1 (C-3), five methine carbons at δ 77.4 (C-1), 76.2 (C-6), 51.9 (C-7), 74.7 (C-8), and 75.8 (C-9), and four quaternary carbons at δ 70.4 (C-4), 92.6 (C-5), 47.8 (C-10), and 84.0 (C-11). Key HMBC correlations (Figure 5, Supporting Information) between δ_C 161.90/δ_H 5.46 (H-1), δ_C 161.88/δ_H 6.05 (H-9), δ_C 169.89/δ_H 5.71 (H-6) and 2.16 (acetyl methyl), and δ_C 163.21/δ_H 5.28 (H-8) combined with the HMQC data indicated that the acetyl, CNMP, and two furancarboxylate moieties could be assigned to C-6, C-8, C-1, and C-9, respectively. The relative stereochemistry was determined from NOESY experiments (Figure 6, Supporting Information) and coupling constants. As usual in agarofurans, H-1 and H-6 are axial. The C-14 and C-15 methyls are also axial, on the basis of the HMBC and NOE correlations of H-14 and H-15 with H-6. An unusual coupling constant (10.0 Hz) between H-8 and H-9 indicated a trans-orientation, and both protons are axial in compound **3**. NOESY peaks between H-8/H-15 and H-9/H-1 also confirmed these orientations. Thus, the structure of **3** was determined as illustrated and named reissantin C.

The structure of compound **4** was also established from mass and ^1H and ^{13}C NMR spectral data. The molecular formula $C_{24}H_{32}O_8$ was assigned by HRFABMS. The IR spectrum suggested the presence of hydroxyl (3419 cm^{-1}) and carboxyl (1719 cm^{-1}) groups. The NMR signals characteristic for CNMP and furancarboxylate moieties were absent, and the mass spectral data indicated an agarofuran with one benzoic and one acetyl group. The ^1H NMR spectrum (Table 1) of **4** contained one acetyl methyl signal at δ 1.61 and one benzoyl group at δ 8.09 (2H, br d, J = 7.5), 7.47 (2H, br t, J = 7.5), and 7.60 (1H, br t, J = 7.5). The ^{13}C NMR and DEPT spectra were consistent with these assignments. In the HMBC spectrum of **4**, key correlations were found between δ_C 169.87/δ_H 5.37 (H-1) and 1.61 (acetyl methyl) and between δ_C 168.01/δ_H 8.09 (Bz-9, 2′, and 6′) and 5.25 (H-9). The coupling patterns were similar to those of **1**. Compared with those of **1**, the H-6 and H-8 signals of **4** were shifted upfield from δ 5.52 and 5.69 to δ 4.37 and 4.39, respectively, which is consistent with the absence of ester substitution. NOESY data were used for stereochemistry determination. Thus, the structure of **4** was elucidated as illustrated and named reissantin D.

Compound **5** was analyzed for $C_{26}H_{34}O_9$ by HRFABMS and contained one more acetyl group (C_2H_2O) in comparison with **4**. The IR spectrum indicated characteristic ester absorption at 1741 cm^{-1} and free hydroxyl absorption at 3418 cm^{-1}. The NMR spectral data of **5** were quite similar to those of **4**, except for the proton signals assignable to H-8. The ^1H NMR spectrum of **5** contained one benzoate and two acetate esters. The coupling patterns were similar to those of **1** and **4**. The $^1H-^1H$ COSY and HMBC spectra confirmed the basic dihydroagarofuran skeleton and ester linage sites. The data clearly indicated that the one benzoyl and two acetyl esters are present at C-9, C-1, and C-8, respectively. Consequently, the structure of **5** was determined as illustrated and named reissantin E.

Known compounds **6**−**21** were identified by comparison of their physical and spectral data with those reported in the literature.

In previous literature, some macroring agarofuran pyridine alkaloids were reported to contain 5-carboxy-*N*-methyl-2-pyridone (CNMP) groups.[17,18,21] These macroring alkaloids and simple agarofuran derivatives have the same sesquiterpene nuclei, but the two compound classes often have different bioactivities.[17] To our best knowledge, new compounds **1**−**3** are the first simple agarofuran sesquiterpenes to contain the unique substitution 5-carboxy-*N*-methyl-2-pyridone (CNMP).

Seventeen compounds, including the five compounds **1**−**5**, as well as compounds **6**−**8**, **11**−**14**, and **17**−**21**, were evaluated. Compounds **6** and **7** were the most potent compounds toward the nine cancer cell lines, with ED_{50} values ranging from 0.076 to 0.34 μg/mL (Table 3). In addition, they showed similar activities toward 1A9 and

Table 3. In Vitro Cytotoxicity of Isolates from *Reissantia buchananii*

	cell lines[a]/ED_{50} (μg/mL)										
compound	A549	MCF-7	HCT-8	KB	KB-VIN	U-87-MG	PC-3	1A9 (3 day)	PTX10 (3 day)	1A9 (6 day)	PTX10 (6 day)
1	>20	>20	>20	>20	NA[b]	NA[b]	>20	>20	>20	>20	>20
2	15.3	>20	>20	11.7	17.1	>20	>20	17.1	>20	13.7	15.3
4	>20	16.2	NA[b]	>20	NA[b]	NA[b]	>20	>20	>20	>20	>20
5	>20	>20	>20	14.4	16.5	NA[b]	>20	>20	>20	NA[b]	>20
6	0.25	0.21	0.23	0.20	0.34	0.22	0.27	0.091	0.10	0.11	0.11
7	0.31	0.14	0.15	0.10	0.29	0.17	0.23	0.10	0.11	0.11	0.076
8	16.4	12.8	14.1	14.5	16.6	>20	>20	11.7	12.4	10.7	7.1
11, **12**	13.3	14.6	13.7	13.0	14.7	6.7	15.1	11.4	11.0	11.3	11.2
17	>20	>20	>20	>20	>20	>20	>20	16.8	20.0	10.6	9.5
paclitaxel	0.005	ND	0.011	0,001	ND	ND	ND	0.002	ND	0.041	ND

[a] A549, lung cancer; MCF-7, breast cancer; HCT-8, ileocecal cancer; KB, epidermoid nasopharyngeal carcinoma; KB-VIN, vincristine-resistant KB; U-87-MG, glioblastoma; PC-3, prostate cancer; 1A9, ovarian cancer; PTX10, ovarian cancer cell line with β-tubulin mutation. [b] NA = not active at 20 μg/mL.

its subline PTX10 with ED_{50} values of 0.076−0.11 μg/mL in both 3- and 6-day bioassays. These two cytotoxic principles of this plant are pristimerin-type triterpenoids and have a planar structure between the A and B rings and a carbonyl group at the C-2 position.[22] Except for the mild activity of compound **2** toward A549, KB, KB-VIN, 1A9 (3-day and 6-day), and PTX10 (6-day), the new compounds were not active in the cytotoxicity bioassay.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 200−20 spectrophotometer, and IR spectra were measured on a Mattson Genesis II spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 500, Varian Unity Plus 400 MHz, or Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in hertz. LREIMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC−MS spectrometer having a direct inlet system. HREIMS were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography, while TLC analysis was carried out on Si gel GF_{254} precoated plates with detection using 50% H_2SO_4 followed by heating on a hot plate. HPLC was performed on a Shimadzu LC-10AT apparatus equipped with a Shimadzu SPD-10A UV−vis detector. Hypersil ODS-5 (250 × 4.6 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant Material. The plant extract (8 g, NIH-N031145-T-31) was prepared in MeOH from the root bark of *Reissantia buchananii* (Loes.) N. Hellé collected in January 1989, in the Iringa District of Tanzania by Drs. Roy Gereau and John Lovett of Missouri Botanical Garden. The plant was identified by Dr. Gereau. A voucher specimen is deposited in the Botany Department, Museum of Natural History, Smithsonian Institution.

Extraction and Isolation. *R. buchananii* extract (8.0 g) was mixed with Celite 545 (35 g) and then was chromatographed on Celite 545 (80 g) eluting first with $CHCl_3$ (1.2 L) then with MeOH (2 L) to provide, after evaporation of solvent, $CHCl_3$ (3.9 g) and MeOH (3.6 g) residues. The $CHCl_3$ extract exhibited significant activity toward A-549 (human lung carcinoma) and HOS (human osteosarcoma) cell lines and was chromatographed on silica gel (10 g), eluted with *n*-hexane/$CHCl_3$ (1:1) to $CHCl_3$/MeOH (4:1) to give 17 fractions. Fraction 4 (452 mg) was chromatographed on silica gel eluting with *n*-hexane/$CHCl_3$/MeOH (2:3:0.01) to give compound **13** (8 mg, $CHCl_3$/MeOH, 11:1, R_f 0.5**)**. Fraction 5 (378 mg) was separated by silica gel chromatography eluting with $CHCl_3$/MeOH (100:1) to give compound **16** (2 mg, $CHCl_3$/MeOH, 100:1, R_f 0.3). Fraction 6 (245 mg) was purified by silica gel eluting with $CHCl_3$/MeOH (90:1) to give a mixture of compounds **19** and **20** (1.5 mg, *n*-hexane/EtOAc, 7:3, R_f 0.3), **17** (5 mg, *n*-hexane/EtOAc, 4:1, R_f 0.6), and a mixture of **9** and **10** (5 mg, *n*-hexane/EtOAc, 4:1, R_f 0.4). Fraction 7 (440 mg) was chromatographed on a Biotage MPLC column eluting with *n*-hexane/EtOAc (10:1 → 1:1) and $CHCl_3$/MeOH (9:1 → 3:2) to provide compounds **7** (100 mg, *n*-hexane/EtOAc, 2:1, R_f 0.7), **15** (5 mg, *n*-hexane/EtOAc, 3:1, R_f 0.5), **14** (3 mg, $CHCl_3$/MeOH, 18:1, R_f 0.6), and a mixture of **11** and **12** (10 mg, $CHCl_3$/MeOH, 18:1, R_f 0.8). Fraction 10 (680 mg) was separated by silica gel chromatography eluting with *n*-hexane/EtOAc (20:1 → 1:1) and $CHCl_3$/MeOH (20:1 → 4:1) to give 12 fractions. Fraction 10-4 was purified using reversed-phase HPLC (63% MeOH) to give compounds **3** (3.7 mg, *n*-hexane/EtOAc/acetone, 10:1:7, R_f 0.4) and **1** (7.0 mg, *n*-hexane/EtOAc/acetone, 10:1:7, R_f 0.4). Fraction 10-4-3 (6 mg) was purified on Sephadex to yield compound **2** (3.2 mg, *n*-hexane/EtOAc, 1:1, R_f 0.3). Fraction 10-7 (23 mg) was purified by silica gel chromatography to give compounds **4** (1 mg, *n*-hexane/EtOAc, 1:1, R_f 0.2), **5** (1.3 mg, $CHCl_3$/MeOH, 25:1, R_f 0.4), and **6** (2.3 mg, $CHCl_3$/MeOH, 25:1, R_f 0.3). Fraction 11 (210 mg) was chromatographed on silica gel eluting with *n*-hexane/EtOAc (11:1 → 1:1) to obtain seven fractions. Fraction 11-4 (102 mg) was purified by silica gel to give compounds **8** (2 mg, $CHCl_3$/MeOH, 11:1, R_f 0.3) and **6** (4 mg, $CHCl_3$/MeOH, 10:1, R_f 0.3). Crystalline fraction 14 (78 mg) was filtered and washed with $CHCl_3$ to give pure compound **18** (18 mg).

Reissantin A (1): white powder; mp 218−220 °C; $[\alpha]^{25.2}_D$ +42.44° (*c* 0.69, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (4.62), 234 (4.20), 263 (4.37), 300 (3.72) nm; IR (neat) ν_{max} 3537, 1722 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRFABMS *m*/*z* 626.2603 [M + H]$^+$ (calcd for $C_{33}H_{40}NO_{11}$, 626.2601).

Reissantin B (2): white powder; mp 168−172 °C; $[\alpha]^{25.1}_D$ +45.7° (*c* 0.32, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.39), 209 (4.32), 231 (4.34), 264 (4.19), 301 (5.89) nm; IR (neat) ν_{max} 3460, 1750, 1721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRFABMS *m*/*z* 746.2825 [M + H]$^+$ (calcd for $C_{40}H_{44}NO_{13}$, 746.2813).

Reissantin C (3): white wax; $[\alpha]^{25.2}_D$ +36.4° (*c* 0.37, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 200 (3.94), 221 (3.91), 267 (4.13) nm; IR (neat) ν_{max} 3419, 1733 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRFABMS *m*/*z* 668.2354 [M + H]$^+$ (calcd for $C_{34}H_{38}NO_{13}$, 668.2343).

Reissantin D (4): white powder; mp 190−192 °C, $[\alpha]^{25.1}_D$ +41.3° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.35), 229 (4.63), 273 (3.78) nm; IR (neat) ν_{max} 3419, 1719 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRFABMS *m*/*z* 471.1997 [M + Na]$^+$ (calcd for $C_{24}H_{32}O_8Na$, 471.1995).

Reissantin E (5): white powder; mp 70−75 °C; $[\alpha]^{24}_D$ +21.54° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.21), 273 (3.07) nm; IR (neat) ν_{max} 3418, 1741 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table

2; FABMS *m/z* 513 $[M + Na]^+$ (41), 327 (28), 246 (43), 197 (46), 165 (44), 147 (51), 105 (100), 91 (100), 73 (83); HRFABMS *m/z* 513.2119 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_9Na$, 513.2101).

Celastrol (6): orange powder; mp 196−199 °C; $[\alpha]^{25}_D$ −61.22° (*c* 0.049, MeOH); IR (neat) ν_{max} 3390, 1707 cm^{-1}; UV (MeOH) λ_{max} 209, 424 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.09 (1H, dd, $J = 7$ and 1; H-6), 6.52 (1H, d, $J = 1$; H-1), 6.28 (1H, d, $J = 7$; H-7), 2.15 (3H, s, 4-CH_3), 1.37 (3H, s, 9-CH_3), 1.21, 1.18, 1.03 (3 × 3H, s, 13-CH_3, 14-CH_3, 17-CH_3), 0.52 (3H, s, 20-CH_3); ESIMS *m/z* 449 $[M - H]^+$ (100).

Pristimerin (7): orange powder; mp 216−218 °C; $[\alpha]^{24.5}_D$ −157.43° (*c* 0.101, MeOH); UV (MeOH) λ_{max} 211, 252, 428 nm; IR (neat) ν_{max} 3730 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.48 (1H, d, $J = 1.5$, H-1), 6.96 (1H, d, $J = 7.05$, 1.5, H-6), 6.29 (1H, d, $J = 6.9$, H-7); ESIMS *m/z* 123 (45), 109 (54), 107 (36), 95 (61), 81 (76), 69 (69), 55 (100).

Bioassays. Compounds were assayed for cytotoxic activity against human tumor cell lines using a reported procedure.[23] All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with compounds from DMSO-diluted stock. After 3 days in culture, cells attached to the plastic substratum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The ED_{50} is the concentration of agent that reduced cell growth by 50% over a 3-day or 6-day assay period.

Human tumor cell lines [A549 (lung), HCT-8 (ileocecal), MCF-7 (breast), KB (nasopharyngeal), PC-3 (prostate)] were obtained from ATCC (Rockville, MA). KB-VIN (vincristine resistant KB subline) was a generous gift of Dr. Y.-C. Cheng (Yale University). 1A9 (ovarian) and PTX10 (a 1A9 subline that has a mutated β-tubulin gene) cell lines were generous gifts of Dr. P. Ginnakakou (NCI MD). All cell lines were cultured in RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin.

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Supporting Information Available: Figures 1−6 and structures and analytical and ^1H NMR data for the known compounds (**8**−**21**). This material is available free of charge via the Internet at http://pubs.acs.org.

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