Cytotoxic Activity of Some Natural and Synthetic Guaianolides

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Several natural guaianolides and synthetic derivatives of repin (**1**) were tested and found to be active against tumor cell replication. Repin (**1**) and both mono- and di-halohydrin analogues (**2**, **7**−**9**, **11**, **12**) showed significant antitumor potency. A more effective compound (**17**) was obtained by esterificating repin with the paclitaxel side chain.

Sesquiterpene lactones have drawn significant interest due to their interesting biological properties, particularly antitumor and cytotoxic activities. Prior studies of natural sesquiterpenes[1−4] and synthetic analogues[5] showed that the main determining factor responsible for cytotoxicity of the compounds studied is the presence of a O=C−CH=CH_2 system.[1−4] This α,β-unsaturated system likely serves as an alkylating center and can be part of an ester, ketone, or lactone moiety. In helenalin analogues, an endocyclic α,β-unsaturated ketone moiety (cyclopentenone) was found to be more important than an exocyclic α-methylene-γ-lactone system,[1−4] and addition of lipophilic character, particularly by incorporation of conjugated ester groups, enhanced cytotoxicity.[3]

Results and Discussion

Recently, we described the isolation of four known [repin (**1**), chlorohyssopifolin C (**2**), janerin (**3**), and cebellin J (**4**)] and two unreported [babylin A (**5**) and babylin B (**6**)] sesquiterpene 8,12-lactones with a guaiane skeleton[6] from *Centaurea babylonica* (Asteraceae), a species used in Lebanese folk medicine. These compounds were first tested against replication of A549 and MCF-7 tumor cell lines (Table 1). While compounds **4**−**6**, which contain a diol rather than a chlorohydrin or epoxide on the skeleton or the ester side chain, were not active against either cell line, compounds **2** and **1** showed good activity against MCF-7 and both cell lines, respectively. In light of these results, we carried out the following chemical modifications on repin (**1**), which was readily available, to study the structure−activity relationships (SAR) of this compound type.

The presence of two oxirane rings in **1** suggested the preparation of various halohydrins containing chlorine, bromine, and iodine. Accordingly, treatment of **1** with 6 equiv of the appropriate lithium halide gave excellent yields of the dichlorohydrin chlorohyssopifolin A (**7**), a natural product previously isolated from *Centaurea hyssopifolia*,[7] and its two analogues dibromohydrin **8** and diiodohydrin **9**. The structures of these compounds were clearly indicated by the absence of ^{1}H NMR signals for the two epoxide methylenes of repin and by the presence of two pairs of AB systems [δ 4.33 and 3.95 (J = 11.8 Hz, H-15a and H-15b) and δ 3.87 and 3.63 (J = 11.1 Hz, H-3′a and H-3′b) for compound **7**, δ 4.26 and 3.88 (J = 11.1 Hz, H-15a and H-15b) and δ 3.76 and 3.56 (J = 10.4 Hz, H-3′a and H-3′b) for compound **8**, and δ 4.06 and 3.71 (J = 10.8 Hz, H-15a and H-15b) and δ 3.59 and 3.45 (J = 10.3 Hz, H-3′a and H-3′b) for compound **9**]. The ^{13}C NMR spectra of compounds **7**−**9** also confirmed the presence of the halogens on C-15 and C-3′. When only 1 equiv of lithium halide was used, different products were obtained, depending on the reagent used (see Experimental Section). With LiCl, the two monochlorohydrins chlorohyssopifolin C (**2**), found for the first time in *Centaurea hyssopifolia*,[7] and solstitiolide (**10**), a natural product isolated from *Centaurea solstitialis*,[8] were obtained. However, reaction with LiI produced the diiodohydrin **9** and one monoiodohydrin, **11**, while LiBr gave three derivatives: the dibromohydrin **8** and both possible monobromohydrins **12** and **13**.

Because compound **14**, with the iodohydrin on the guaiane skeleton, was not obtained with the former method, diiodohydrin **9** was treated with *t*-BuOK to effect a differential ring closure with formation of the oxirane ring only on the ester side chain, most likely due to steric hindrance of the other hydroxyl group.

Furthermore, with the aim of enhancing the cytotoxic activity, we esterified the free C-3 hydroxy group of repin with the side chain of paclitaxel. According to a procedure previously reported,[9] the commercially available (2*R*,3*S*)-3-phenylisoserine hydrochloride was converted to compound **15**, which was then esterified with repin (**1**). Acidic hydrolysis of the resulting ester **16** gave compound **17**, with the same side chain as paclitaxel (Scheme 1).

Compounds **10** and **13** were not stable and decomposed completely within 1 week, even with storage at 5 °C. The cytotoxic activities of the remaining synthetic derivatives and natural guaianolides were evaluated, and the data are reported in Table 1.

Repin (**1**) was tested and found to be significantly potent against seven cell lines [A549 (lung cancer), MCF-7 (breast cancer), 1A9 (ovarian cancer), KB (nasopharyngeal cancer), KB-VIN (KB drug-resistant variant), HCT-8 (ileocecal cancer), and SK-MEL-2 (melanoma)]. This compound and the subsequent derivatives were generally least potent against A549 cell replication.

Monoiodohydrin **14**, with the halohydrin on the cyclic skeleton and the epoxide in the side chain, displayed weak activity (IC_{50} 4.1 μM) against the KB cell line and was inactive in the other tested cell lines. In contrast, significant activity (IC_{50} 0.8−2.0 μM) was seen against 1A9, KB, and HCT-8 cell lines when the halohydrin was on the side chain and the epoxide on the skeleton (**2**, **11**, and **12**). However, these three monohalohydrins were generally slightly less active than the parent diepoxide repin (**1**) (IC_{50}

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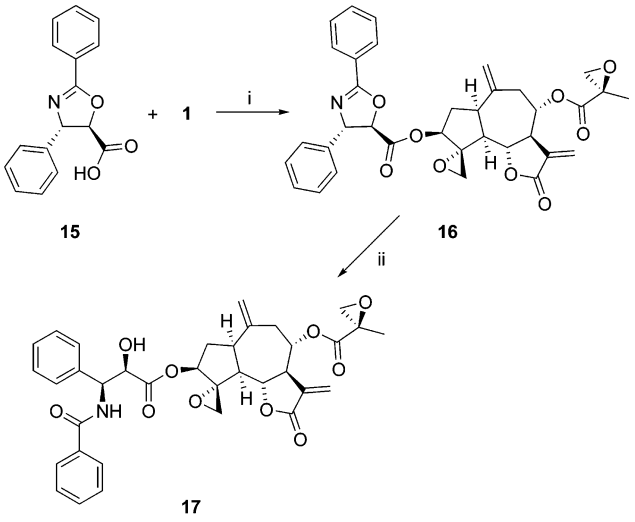
Table 1. Effect of Compounds **1**−**9**, **11**, **12**, **14**, **16**, and **17** against Tumor Cell Lines

	IC_{50} (μM)/Cell Line[a]						
compound	A549	MCF-7	1A9	KB	KB-V	HCT-8	SK-MEL-2
1	2.5	1.1	0.3	1.4	0.8	0.8	2.5
2	9.3	1.5	0.8	2.0	1.5	1.6	
3	19.9		5.0	3.0	25.9	5.5	8.0
4	>24.0	>24.0					
5	>26.3	>26.3					
6	>26.3	>26.3					
7	10.3		0.7	4.1	2.8		
8	4.2		0.4	1.1	1.3		
9	3.6		1.3	1.0	5.5	1.3	2.6
11	7.3		0.8	2.0	1.8		
12	4.3		1.1	1.1	6.3	1.3	3.6
14	13.7		5.6	4.1	18.6	4.5	9.4
16	3.4		0.8	2.6	2.6	0.5	1.5
17	0.8		0.2	0.3	7.5	0.5	1.0
cynaropicrin	16.5[b]	3.2[b]		15.9[c]			5.8[c]
chlorojanerin				15.1[c]			5.8[c]
aguerin B	26.4[b]	3.3[b]					
paclitaxel			0.002	0.001		0.013	
mitomycin C	0.3	1.5		0.6		0.6	

[a] Cell line: A549 = lung; MCF-7 = breast; 1A9 = ovarian; KB = nasopharynx; KB-V = nasopharynx MDR; HCT-8 = ileocecal; SK-MEL-2 = melanoma. [b] Taken from ref 10. [c] Taken from ref 11.

Scheme 1

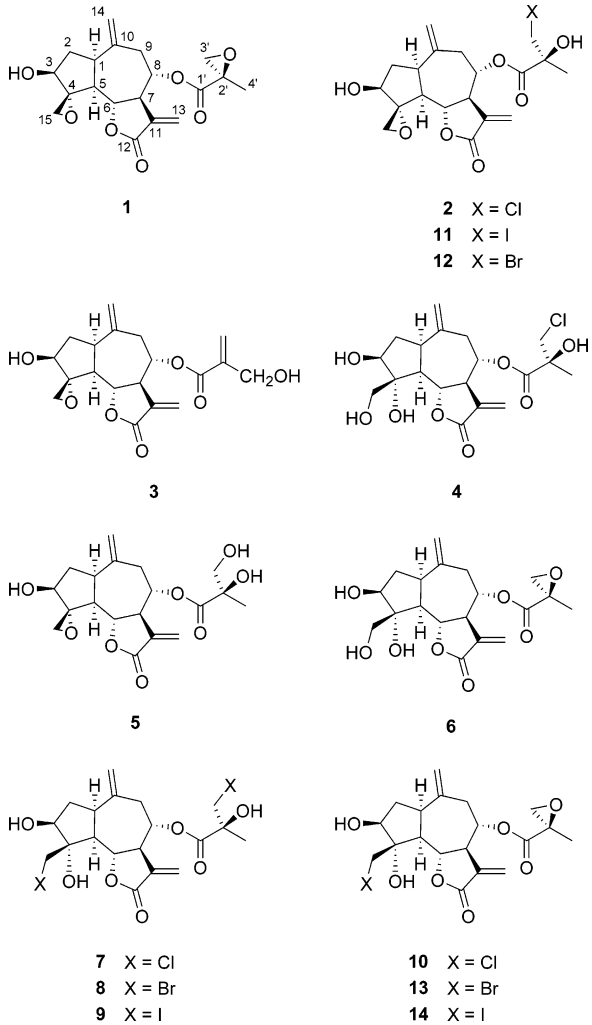


0.3−1.4 μM). The three dihalohydrins (**7**, **8**, and **9**) were also quite active (IC_{50} 0.4−4.1 μM) against most tested cell lines; however, the potency range was broader. The effect of the halogen (chlorine, bromine, iodine) was not consistent among the cell lines.

It is known that the C-13 side chain of paclitaxel is crucial for its strong antitumor activity.[10,11] Esterification of repin (**1**) with the side chain of paclitaxel to give compound **17** led to increased potency (IC_{50} 0.2−1.0 μM) in all tested cell lines, with the exception of KB-VIN. The dihydro-oxazole-protected intermediate (**16**) was generally less potent, except against KB-VIN and HCT-8 cells.

In summary, the following structure/activity trends were found with this series of repin analogues. The presence of a diol (**4**, **6**) rather than an epoxide on the cyclic skeleton of repin (**1**) abolished activity. Likewise, compounds with an allylhydroxy (**3**) or diol (**5**) in the side chain also lost activity. However, the side chain epoxide or both epoxide moieties of repin (**1**) could be modified to halohydrins (**2**, **11**, **12** and **7**, **8**, **9**, respectively) and still result in active compounds.

To compare the cytotoxic effects of the compounds presented in this report, we included the IC_{50} (μM) values



of three structurally related guaianolides, whose activity has been previously reported,[12,13] and two positive controls as paclitaxel and mitomycin C in Table 1.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1010 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250 E

MHz NMR spectrometer, using the residual solvent signal (δ = 7.27 in ^{1}H and δ = 77.00 in ^{13}C for $CDCl_3$ as reference. ^{13}C NMR assignments were determined by DEPT spectra. ESIMS was obtained with an Applied Biosystem API-2000 mass spectrometer. Elemental analysis was carried out with a Perkin-Elmer 240 apparatus. Merck Si gel (70−230 mesh), deactivated with 15% H_2O, was used for column chromatography. The optically pure (2*R*,3*S*)-3-phenylisoserine hydrochloride was purchased from Industrial Chemistry Research (Poland). CH_2Cl_2 was dried by distillation over calcium hydride. Tetrahydrofuran was dried by distillation from sodium metal under a nitrogen atmosphere using benzophenone ketyl as indicator.

General Halohydrin Synthesis Procedure. Repin (**1**, 50 mg, 0.138 mmol) was solubilized in 4 mL of dry THF and added to either 1 or 6 equiv of the appropriate lithium halide and 1 (0.01 mL) or 2 (0.02 mL) equiv of acetic acid at room temperature under an argon atmosphere. After stirring overnight, the reaction was subjected to the usual workup by adding brine and extracting with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. Generally, the residue was purified by column chromatography. This procedure gave the following derivatives.

Chlorohyssopifolin A (7). Treatment of 50 mg of repin (**1**) with 6 equiv of lithium chloride (36 mg) gave, after column chromatography (Si gel, 47:3 CH_2Cl_2−MeOH), 48 mg of chlorohyssopifolin A (**7**), whose spectroscopic data were in perfect agreement with literature values:[7] ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.21 (1H, d, *J* = 3.5 Hz, H-13a), 5.57 (1H, d, *J* = 3.1 Hz, H-13b), 5.20 (1H, ddd, *J* = 9.6, 5.2, 1.6 Hz, H-8), 5.16 (1H, br s, H-14a), 5.00 (1H, br s, H-14b), 4.73 (1H, dd, *J* = 11.3, 9.0 Hz, H-6), 4.33 (1H, d, *J* = 11.8 Hz, H-15a), 4.16 (1H, dd, *J* = 6.6, 1.5 Hz, H-3), 3.95 (1H, d, *J* = 11.8 Hz, H-15b), 3.87 (1H, d, *J* = 11.1 Hz, H-3′a), 3.63 (1H, d, *J* = 11.1 Hz, H-3′b), 3.62 (1H, ddd, *J* = 11.1, 8.5, 8.0 Hz, H-1), 3.13 (1H, dddd, *J* = 9.6, 9.0, 3.5, 3.1 Hz, H-7), 2.65 (1H, dd, *J* = 15.3, 5.2 Hz, H-9a), 2.54 (1H, ddd, *J* = 15.0, 11.1, 6.6 Hz, H-2a), 2.49 (1H, dd, *J* = 15.3, 1.6 Hz, H-9b), 2.31 (1H, dd, *J* = 11.3, 8.5 Hz, H-5), 1.56 (1H, ddd, *J* = 15.0, 8.0, 1.5 Hz, H-2b), 1.55 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 173.05 (C, C-1′), 168.32 (C, C-12), 141.80 (C, C-10), 136.87 (C, C-11), 122.39 (CH_2, C-13), 118.51 (CH_2, C-14), 84.62 (C, C-4), 77.15 (CH, C-6), 76.03 (CH, C-3), 75.85 (CH, C-8), 74.69 (C, C-2′), 57.54 (CH, C-5), 51.19 (CH_2, C-3′), 49.99 (CH_2, C-15), 47.26 (CH, C-7), 46.39 (CH, C-1), 37.89 (CH_2, C-9), 34.56 (CH_2, C-2), 23.40 (CH_3, C-4′).

Compound 8. Treatment of 50 mg of repin (**1**) with 6 equiv of lithium bromide (73 mg) gave, after column chromatography (Si gel, 97:3 CH_2Cl_2−MeOH), 66 mg of dibromo derivative **8**: amorphous solid; $[\alpha]^{25}_D$ +60.5° (*c* 0.4, CH_3COCH_3); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.24 (1H, d, *J* = 3.6 Hz, H-13a), 5.59 (1H, d, *J* = 3.2 Hz, H-13b), 5.25 (1H, br dd, *J* = 9.6, 5.2 Hz, H-8), 5.19 (1H, br s, H-14a), 5.08 (1H, br s, H-14b), 4.74 (1H, dd, *J* = 11.2, 9.0 Hz, H-6), 4.26 (1H, d, *J* = 11.1 Hz, H-15a), 4.18 (1H, br d, *J* = 6.6 Hz, H-3), 3.88 (1H, d, *J* = 11.1 Hz, H-15b), 3.76 (1H, d, *J* = 10.4 Hz, H-3′a), 3.68 (1H, ddd, *J* = 11.1, 8.5, 7.6 Hz, H-1), 3.56 (1H, d, *J* = 10.4 Hz, H-3′b), 3.17 (1H, dddd, *J* = 9.6, 9.0, 3.6, 3.2 Hz, H-7), 2.68 (1H, dd, *J* = 15.3, 5.2 Hz, H-9a), 2.54 (1H, ddd, *J* = 15.0, 11.1, 6.6 Hz, H-2a), 2.54 (1H, br d, *J* = 15.3 Hz, H-9b), 2.35 (1H, dd, *J* = 11.2, 8.5 Hz, H-5), 1.62 (3H, s, Me-4′), 1.56 (1H, br dd, *J* = 15.0, 7.6 Hz, H-2b); ^{13}C NMR ($CDCl_3$−CD_3OD, 9:1, 62.7 MHz) δ 173.37 (C, C-1′), 170.25 (C, C-12), 143.75 (C, C-10), 138.17 (C, C-11), 122.75 (CH_2, C-13), 118.08 (CH_2, C-14), 84.61 (C, C-4), 77.95 (CH, C-6), 77.16 (CH, C-3), 76.06 (CH, C-8), 74.76 (C, C-2′), 58.81 (CH, C-5), 48.65 (CH, C-7), 46.96 (CH, C-1), 40.38 (CH_2, C-3′), 40.00 (CH_2, C-9), 39.38 (CH_2, C-15), 35.08 (CH_2, C-2), 24.67 (CH_3, C-4′); ESIMS *m/z* (positive mode) 549 $[(M + 4) + Na]^+$ (23), 547 $[(M + 2) + Na]^+$ (48), 545 $[M + Na]^+$ (25), 365 $[(M + 2) + Na - C_4H_7O_3Br]^+$ (59), 363 $[M + Na - C_4H_7O_3Br]^+$ (61); *anal.* C 43.58%, H 4.64%, Br 30.52%, calcd for $C_{19}H_{24}Br_2O_7$, C 43.53%, H 4.61%, Br 30.49%.

Compound 9. Treatment of 50 mg of repin (**1**) with 6 equiv of lithium iodide (112 mg) gave, after column chromatography (Si gel, 97:3 CH_2Cl_2−MeOH), 82 mg of diiodo derivative **9**: amorphous solid; $[\alpha]^{25}_D$ +15.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.24 (1H, d, *J* = 3.4 Hz, H-13a), 5.60 (1H, d, *J* = 3.0 Hz, H-13b), 5.24 (1H, br dd, *J* = 9.4, 5.1 Hz, H-8), 5.20 (1H, br s, H-14a), 5.12 (1H, br s, H-14b), 4.75 (1H, dd, *J* = 11.2, 9.1 Hz, H-6), 4.11 (1H, br d, *J* = 6.5 Hz, H-3), 4.06 (1H, d, *J* = 10.8 Hz, H-15a), 3.71 (1H, d, *J* = 10.8 Hz, H-15b), 3.68 (1H, ddd, *J* = 11.1, 8.5, 7.0 Hz, H-1), 3.59 (1H, d, *J* = 10.3 Hz, H-3′a), 3.45 (1H, d, *J* = 10.3 Hz, H-3′b), 3.16 (1H, dddd, *J* = 9.4, 9.1, 3.4, 3.0 Hz, H-7), 2.69 (1H, dd, *J* = 15.0, 5.1 Hz, H-9a), 2.58 (1H, br d, *J* = 15.0 Hz, H-9b), 2.54 (1H, ddd, *J* = 14.8, 11.1, 6.5 Hz, H-2a), 2.37 (1H, dd, *J* = 11.1, 8.5 Hz, H-5), 1.69 (3H, s, Me-4′), 1.60 (1H, br dd, *J* = 14.8, 7.0 Hz, H-2b); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 174.58 (C, C-1′), 168.39 (C, C-12), 142.37 (C, C-10), 136.87 (C, C-11), 122.41 (CH_2, C-13), 118.51 (CH_2, C-14), 83.47 (C, C-4), 79.81 (CH, C-6), 76.32 (CH, C-3), 75.86 (CH, C-8), 73.26 (C, C-2′), 56.52 (CH, C-5), 48.55 (CH, C-7), 46.45 (CH, C-1), 37.82 (CH_2, C-9), 34.59 (CH_2, C-2), 22.68 (CH_3, C-4′), 19.11 (CH_2, C-15), 15.13 (CH_2, C-3′); ESIMS *m/z* (positive mode) 641 $[M + Na]^+$ (48), 411 $[M + Na - C_4H_7O_3I]^+$ (62); *anal.* C 36.94%, H 3.90%, I 41.03%, calcd for $C_{19}H_{24}I_2O_7$, C 36.91%, H 3.91%, I 41.06%.

Compounds 2 and 10. Treatment of 50 mg of repin (**1**) with 1 equiv of lithium chloride (6 mg) gave a mixture of two compounds, which were separated by column chromatography (Si gel, 24:1 CH_2Cl_2−MeOH as eluent), giving 10 mg of chlorohyssopifolin C[6] (**2**) and 12 mg of solstitiolide (**10**), recovering 30 mg of repin (**1**).

Compound 10: amorphous solid; $[\alpha]^{25}_D$ +52.0° (*c* 0.7, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.23 (1H, d, *J* = 3.5 Hz, H-13a), 5.59 (1H, d, *J* = 3.1 Hz, H-13b), 5.16 (1H, br s, H-14a), 5.13 (1H, ddd, *J* = 9.5, 5.0, 1.9 Hz, H-8), 4.86 (1H, br s, H-14b), 4.73 (1H, dd, *J* = 11.2, 9.1 Hz, H-6), 4.33 (1H, d, *J* = 11.8 Hz, H-15a), 4.17 (1H, br d, *J* = 6.6 Hz, H-3), 3.96 (1H, d, *J* = 11.8 Hz, H-15b), 3.62 (1H, ddd, *J* = 11.2, 8.5, 8.0 Hz, H-1), 3.18 (1H, d, *J* = 5.8 Hz, H-3′a), 3.12 (1H, dddd, *J* = 9.5, 9.1, 3.5, 3.1 Hz, H-7), 2.83 (1H, d, *J* = 5.8 Hz, H-3′b), 2.63 (1H, dd, *J* = 15.4, 5.0 Hz, H-9a), 2.52 (1H, ddd, *J* = 15.0, 11.2, 6.6 Hz, H-2a), 2.38 (1H, dd, *J* = 15.4, 1.6 Hz, H-9b), 2.31 (1H, dd, *J* = 11.2, 8.5 Hz, H-5), 1.60 (3H, s, Me-4′), 1.58 (1H, br dd, *J* = 15.0, 8.0 Hz, H-2b); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 169.67 (C, C-1′), 168.51 (C, C-12), 141.64 (C, C-10), 136.85 (C, C-11), 122.48 (CH_2, C-13), 118.28 (CH_2, C-14), 84.51 (C, C-4), 77.21 (CH, C-6), 76.17 (CH, C-3), 74.99 (CH, C-8), 57.50 (CH, C-5), 53.83 (C, C-2′), 52.78 (CH_2, C-3′), 49.86 (CH_2, C-15), 47.07 (CH, C-7), 46.43 (CH, C-1), 37.77 (CH_2, C-9), 34.88 (CH_2, C-2), 17.36 (CH_3, C-4′); ESIMS *m/z* (positive mode) 423 $[(M + 2) + Na]^+$ (12), 421 $[M + Na]^+$ (40), 321 $[(M + 2) + Na - C_4H_6O_3]^+$ (21), 319 $[M + Na - C_4H_6O_3]^+$ (70); *anal.* C 57.26%, H 5.83%, Cl 8.90%, calcd for $C_{19}H_{23}ClO_7$, C 57.22%, H 5.81%, Cl 8.89%.

Compounds 12 and 13. Treatment of 50 mg of repin (**1**) with 1 equiv of lithium bromide (12 mg) gave a mixture of three compounds, which were separated by column chromatography (Si gel, 24:1 CH_2Cl_2−MeOH as eluent), giving 18 mg of **12**, 12 mg of **13**, and 12 mg of **8**, as well as 16 mg of unreacted repin (**1**).

Compound 12: amorphous solid; $[\alpha]^{25}_D$ +40.6° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.23 (1H, d, *J* = 3.5 Hz, H-13a), 5.60 (1H, d, *J* = 3.1 Hz, H-13b), 5.27 (1H, ddd, *J* = 9.0, 5.2, 2.4 Hz, H-8), 5.22 (1H, br s, H-14a), 5.17 (1H, br s, H-14b), 4.68 (1H, dd, *J* = 11.3, 9.1 Hz, H-6), 4.01 (1H, dd, *J* = 7.2, 5.3 Hz, H-3), 3.76 (1H, d, *J* = 10.4 Hz, H-3′a), 3.56 (1H, d, *J* = 10.4 Hz, H-3′b), 3.38 (1H, ddd, *J* = 10.7, 8.6, 8.5 Hz, H-1), 3.36 (1H, d, *J* = 4.8 Hz, H-15a), 3.10 (1H, dddd, *J* = 9.1, 9.0, 3.5, 3.1 Hz, H-7), 3.09 (1H, d, *J* = 4.8 Hz, H-15b), 2.77 (1H, dd, *J* = 14.7, 5.2 Hz, H-9a), 2.52 (1H, dd, *J* = 14.7, 2.4 Hz, H-9b), 2.49 (1H, ddd, *J* = 14.0, 8.6, 7.2 Hz, H-2a), 2.05 (1H, dd, *J* = 11.3, 8.5 Hz, H-5), 1.83 (1H, ddd, *J* = 14.0, 10.7, 5.3 Hz, H-2b), 1.62 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 173.07 (C, C-1′), 169.35 (C, C-12), 141.04 (C, C-10), 137.16 (C, C-11), 122.33 (CH_2, C-13), 119.21 (CH_2, C-14), 76.63 (CH, C-6), 76.30 (CH, C-3), 75.80 (CH, C-8), 74.00 (C, C-2′), 68.15 (C, C-4), 53.62 (CH, C-5), 48.48 (CH_2, C-15), 47.75 (CH, C-7), 46.09 (CH, C-1), 40.07 (CH_2, C-3′), 37.84 (CH_2, C-9), 35.53 (CH_2, C-2), 24.03 (CH_3, C-4′); ESIMS *m/z* (positive mode) 467 $[(M + 2) + Na]^+$ (44), 465 $[M + Na]^+$ (45), 283 $[M + Na - C_4H_7O_3Br]^+$

(58); *anal.* C 51.52%, H 5.18%, Br 18.08%, calcd for $C_{19}H_{23}BrO_7$, C 51.48%, H 5.23%, Br 18.03%.

Compound 13: amorphous solid; $[\alpha]^{25}_D$ +24.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.25 (1H, d, J = 3.4 Hz, H-13a), 5.60 (1H, d, J = 3.1 Hz, H-13b), 5.17 (1H, br s, H-14a), 5.13 (1H, br dd, J = 9.3, 5.0 Hz, H-8), 4.87 (1H, br s, H-14b), 4.74 (1H, dd, J = 11.0, 9.2 Hz, H-6), 4.25 (1H, d, J = 11.0 Hz, H-15a), 4.16 (1H, br d, J = 6.3 Hz, H-3), 3.88 (1H, d, J = 11.0 Hz, H-15b), 3.65 (1H, ddd, J = 11.2, 8.5, 7.3 Hz, H-1), 3.19 (1H, d, J = 5.7 Hz, H-3′a), 3.15 (1H, dddd, J = 9.3, 9.2, 3.4, 3.1 Hz, H-7), 2.84 (1H, d, J = 5.7 Hz, H-3′b), 2.62 (1H, dd, J = 15.4, 5.0 Hz, H-9a), 2.52 (1H, ddd, J = 15.1, 11.2, 6.3 Hz, H-2a), 2.38 (1H, br d, J = 15.4 Hz, H-9b), 2.30 (1H, dd, J = 11.0, 8.5 Hz, H-5), 1.63 (3H, s, Me-4′), 1.57 (1H, br dd, J = 15.1, 7.3 Hz, H-2b); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 169.87 (C, C-1′), 168.48 (C, C-12), 141.73 (C, C-10), 136.85 (C, C-11), 122.46 (CH_2, C-13), 118.29 (CH_2, C-14), 83.93 (C, C-4), 78.19 (CH, C-6), 76.12 (CH, C-3), 74.96 (CH, C-8), 57.48 (CH, C-5), 53.83 (C, C-2′), 52.78 (CH_2, C-3′), 47.65 (CH, C-7), 46.35 (CH, C-1), 41.40 (CH_2, C-15), 37.72 (CH_2, C-9), 34.66 (CH_2, C-2), 17.37 (CH_3, C-4′); ESIMS *m/z* (positive mode) 467 [(M + 2) + Na]$^+$ (50), 465 [M + Na]$^+$ (52), 365 [(M + 2) + Na − $C_4H_6O_3$]$^+$ (72), 363 [M + Na − $C_4H_6O_3$]$^+$ (74); *anal.* C 51.50%, H 5.20%, Br 18.00%, calcd for $C_{19}H_{23}BrO_7$, C 51.48%, H 5.23%, Br 18.03%.

Compound 11. Treatment of 50 mg of repin (**1**) with 1 equiv of lithium iodide (19 mg) gave a mixture of two compounds, which were separated by column chromatography (Si gel, 24:1 CH_2Cl_2−MeOH as eluent), giving 54 mg of **11**, 8 mg of **9**, and 5 mg of recovered repin (**1**).

Compound 11: amorphous solid; $[\alpha]^{25}_D$ +51.7° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.24 (1H, d, J = 3.5 Hz, H-13a), 5.59 (1H, d, J = 2.9 Hz, H-13b), 5.27 (1H, ddd, J = 9.1, 5.2, 2.9 Hz, H-8), 5.24 (1H, br s, H-14a), 5.21 (1H, br s, H-14b), 4.68 (1H, dd, J = 11.3, 9.2 Hz, H-6), 4.00 (1H, dd, J = 7.2, 5.9 Hz, H-3), 3.58 (1H, d, J = 10.3 Hz, H-3′a), 3.44 (1H, d, J = 10.3 Hz, H-3′b), 3.39 (1H, ddd, J = 10.0, 9.6, 8.3 Hz, H-1), 3.35 (1H, d, J = 4.2 Hz, H-15a), 3.10 (1H, dddd, J = 9.2, 9.1, 3.5, 2.9 Hz, H-7), 3.08 (1H, d, J = 4.2 Hz, H-15b), 2.80 (1H, dd, J = 15.1, 5.2 Hz, H-9a), 2.56 (1H, dd, J = 15.1, 2.9 Hz, H-9b), 2.48 (1H, ddd, J = 14.5, 9.6, 7.2 Hz, H-2a), 2.04 (1H, dd, J = 11.3, 8.3 Hz, H-5), 1.84 (1H, ddd, J = 14.5, 10.0, 5.9 Hz, H-2b), 1.68 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 175.35 (C, C-1′), 168.80 (C, C-12), 141.06 (C, C-10), 137.10 (C, C-11), 122.29 (CH_2, C-13), 119.32 (CH_2, C-14), 76.48 (CH, C-6), 76.48 (CH, C-3), 75.81 (CH, C-8), 73.29 (C, C-2′), 68.16 (C, C-4), 53.54 (CH, C-5), 48.54 (CH_2, C-15), 47.87 (CH, C-7), 46.09 (CH, C-1), 37.78 (CH_2, C-9), 35.71 (CH_2, C-2), 21.16 (CH_3, C-4′), 14.98 (CH_2, C-3′); ESIMS *m/z* (positive mode) 513 [M + Na]$^+$ (45), 283 [M + Na − $C_4H_7O_3I$]$^+$ (58); *anal.* C 46.51%, H 4.77%, I 25.83%, calcd for $C_{19}H_{23}IO_7$, C 46.54%, H 4.73%, I 25.88%.

Synthesis of Compound 14. Compound **9** (20 mg, 0.032 mmol) was solubilized in 2 mL of dry THF and then 1 equiv of potassium *tert*-butoxide (4 mg) was added, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was quenched by adding 2 mL of saturated aqueous NH_4Cl and extracted three times with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (Si gel, 24:1 CH_2Cl_2−MeOH as eluent), giving 7 mg of **14**, 1.5 mg of **13**, and 4 mg of repin (**1**).

Compound 14: amorphous solid; $[\alpha]^{25}_D$ +32.5° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.24 (1H, d, J = 3.5 Hz, H-13a), 5.59 (1H, d, J = 3.0 Hz, H-13b), 5.16 (1H, br s, H-14a), 5.14 (1H, br dd, J = 9.3, 5.0 Hz, H-8), 4.86 (1H, br s, H-14b), 4.73 (1H, dd, J = 11.0, 9.0 Hz, H-6), 4.12 (1H, br d, J = 6.3 Hz, H-3), 4.05 (1H, d, J = 10.8 Hz, H-15a), 3.70 (1H, d, J = 10.8 Hz, H-15b), 3.67 (1H, ddd, J = 11.2, 8.5, 7.4 Hz, H-1), 3.19 (1H, d, J = 5.8 Hz, H-3′a), 3.18 (1H, dddd, J = 9.3, 9.0, 3.5, 3.0 Hz, H-7), 2.83 (1H, d, J = 5.8 Hz, H-3′b), 2.62 (1H, dd, J = 15.4, 5.0 Hz, H-9a), 2.52 (1H, ddd, J = 15.1, 11.2, 6.3 Hz, H-2a), 2.38 (1H, br d, J = 15.4 Hz, H-9b), 2.37 (1H, dd, J = 11.0, 8.5 Hz, H-5), 1.85 (1H, br dd, J = 15.1, 7.4 Hz, H-2b), 1.64 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 169.90 (C, C-1′), 168.55 (C, C-12), 142.03 (C, C-10), 137.00 (C, C-11), 122.44 (CH_2, C-13), 118.14 (CH_2, C-14), 83.77 (C, C-4), 79.84 (CH, C-6), 76.80 (CH, C-3), 74.99 (CH, C-8), 56.53 (CH, C-5), 53.82 (C, C-2′), 52.71 (CH_2, C-3′), 48.38 (CH, C-7), 46.29 (CH, C-1), 37.70 (CH_2, C-9), 34.67 (CH_2, C-2), 19.07 (CH_2, C-15), 17.38 (CH_3, C-4′); ESIMS *m/z* (positive mode) 513 [M + Na]$^+$ (50), 411 [M + Na − $C_4H_6O_3$]$^+$ (78); *anal.* C 46.50%, H 4.70%, I 25.85%, calcd for $C_{19}H_{23}IO_7$, C 46.54%, H 4.73%, I 25.88%.

Compound 16. The (4*S*,5*R*)-2,4-diphenyl-4,5-dihydro-oxazol-5-carboxylic acid **15** (44 mg, 0.165 mmol), synthesized as previously reported,[9] was dissolved in 5 mL of dry CH_2Cl_2 and added to DMAP (5 mg, 0.025 mmol) and DCC (38 mg, 0.184 mmol). After stirring at room temperature for 15 min, repin (**1**) (55 mg, 0.149 mmol) was added, and the mixture was stirred for an additional 3 h. The reaction mixture was filtered, the solution evaporated in vacuo, and the residue purified by chromatography (Si gel, 49:1 CH_2Cl_2−MeOH as eluent) to give 66 mg (yield 72%) of the ester **16**: amorphous solid; $[\alpha]^{25}_D$ +1.0° (*c* 2.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.05 (1H, m, arom), 8.02 (1H, m, arom), 7.55−7.29 (8H, m, arom), 6.12 (1H, d, J = 3.4 Hz, H-13a), 5.41 (1H, d, J = 3.0 Hz, H-13b), 5.40 (1H, d, J = 6.0 Hz, H-3″), 5.16 (1H, br s, H-14a), 5.06 (1H, dd, J = 7.1, 1.6 Hz, H-3), 4.95 (1H, d, J = 6.0 Hz, H-2″), 4.90 (1H, br s, H-14b), 4.71 (1H, ddd, J = 9.6, 4.7, 3.9 Hz, H-8), 4.12 (1H, dd, J = 11.4, 9.4 Hz, H-6), 3.40 (1H, ddd, J = 11.0, 8.8, 8.2 Hz, H-1), 3.27 (1H, d, J = 4.4 Hz, H-15a), 3.18 (1H, d, J = 4.4 Hz, H-15b), 3.14 (1H, d, J = 5.7 Hz, H-3′a), 2.90 (1H, dddd, J = 9.6, 9.4, 3.4, 3.0 Hz, H-7), 2.82 (1H, d, J = 5.7 Hz, H-3′b), 2.67 (1H, ddd, J = 15.5, 11.0, 7.1 Hz, H-2a), 2.39 (1H, dd, J = 14.7, 4.7 Hz, H-9a), 2.23 (1H, dd, J = 14.7, 3.9 Hz, H-9b), 1.97 (1H, dd, J = 11.4, 8.2 Hz, H-5), 1.87 (1H, ddd, J = 15.5, 8.8, 1.6 Hz, H-2b), 1.61 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 169.60 (C, C-1″), 169.07 (C, C-1′), 167.92 (C, C-12), 163.42 (C, C-5″), 140.43 (C, C-10), 139.85 (C, arom), 136.60 (C, C-11), 132.27 (CH, arom), 128.89 (2CH, arom), 128.72 (2CH, arom), 128.40 (2CH, arom), 128.23 (CH, arom), 126.90 (C, arom), 126.41 (2CH, arom), 121.87 (CH_2, C-13), 119.28 (CH_2, C-14), 82.82 (CH, C-2″), 79.64 (CH, C-3), 75.40 (CH, C-6), 74.94 (CH, C-8), 74.55 (CH, C-3″), 66.16 (C, C-4), 53.74 (CH, C-5), 53.62 (C, C-2′), 52.73 (CH_2, C-3′), 49.38 (CH, C-7), 48.06 (CH_2, C-15), 45.71 (CH, C-1), 35.93 (CH_2, C-9), 35.19 (CH_2, C-2), 17.25 (CH_3, C-4′); ESIMS *m/z* (positive mode) 634 [M + Na]$^+$ (20), 266 (52); *anal.* C 68.70%, H 5.47%, N 2.27%, calcd for $C_{35}H_{33}NO_9$, C 68.73%, H 5.44%, N 2.29%.

Compound 17. Compound **16** (36 mg, 0.059 mmol), dissolved in CH_2Cl_2 (6 mL), was stirred at room temperature with *p*-toluenesulfonic acid (3 mg, 0.017 mmol). After completion of the reaction the solution was neutralized with saturated aqueous $NaHCO_3$, diluted with water (10 mL), and extracted three times with $CHCl_3$ (15 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness, leaving a residue, which was purified by chromatography (Si gel, 49:1 CH_2Cl_2−MeOH as eluent) to give 28 mg (yield 75%) of compound **17**: amorphous solid; $[\alpha]^{25}_D$ +81.8° (*c* 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.82 (1H, m, arom), 7.79 (1H, m, arom), 7.58−7.30 (8H, m, arom), 7.03 (1H, d, J = 9.7 Hz, NH), 6.28 (1H, d, J = 3.4 Hz, H-13a), 5.88 (1H, dd, J = 9.7, 1.9 Hz, H-3″), 5.62 (1H, d, J = 3.0 Hz, H-13b), 5.40 (1H, ddd, J = 9.1, 4.9, 2.2 Hz, H-8), 5.11 (1H, br s, H-14a), 4.85 (1H, br s, H-14b), 4.82 (1H, dd, J = 8.3, 4.7 Hz, H-3), 4.77 (1H, dd, J = 11.6, 9.3 Hz, H-6), 4.73 (1H, d, J = 1.9 Hz, H-2″), 3.40 (1H, d, J = 4.2 Hz, H-15a), 3.31 (1H, ddd, J = 12.0, 7.3, 7.3 Hz, H-1), 3.17 (1H, d, J = 5.9 Hz, H-3′a), 3.08 (1H, d, J = 4.2 Hz, H-15b), 3.03 (1H, dddd, J = 9.3, 9.1, 3.4, 3.0 Hz, H-7), 2.82 (1H, d, J = 5.9 Hz, H-3′b), 2.63 (1H, ddd, J = 15.3, 8.3, 7.3 Hz, H-2a), 2.63 (1H, dd, J = 15.5, 4.9 Hz, H-9a), 2.10 (1H, dd, J = 15.5, 2.2 Hz, H-9b), 1.93 (1H, dd, J = 11.6, 7.3 Hz, H-5), 1.73 (1H, ddd, J = 15.3, 12.0, 4.7 Hz, H-2b), 1.63 (3H, s, Me-4′); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 172.17 (C, C-1″), 169.40 (C, C-1′), 168.55 (C, C-12), 167.05 (C, C-5″), 140.27 (C, C-10), 138.21 (C, arom), 137.31 (C, C-11), 133.18 (C, arom), 132.21 (CH, arom), 128.92 (2CH, arom), 128.83 (2CH, arom), 128.06 (CH, arom), 127.17 (2CH, arom), 126.99 (2CH, arom), 122.24 (CH_2, C-13), 119.61 (CH_2, C-14), 81.90 (CH, C-3), 75.60 (CH, C-8), 75.06 (CH, C-6), 73.07 (CH, C-2″), 66.04 (C, C-4), 55.14 (CH, C-3″), 53.92 (CH, C-5), 53.92 (C, C-2′), 52.72 (CH_2, C-3′), 49.00 (CH, C-7), 48.35

(CH_2, C-15), 46.73 (CH, C-1), 36.33 (CH_2, C-9), 34.57 (CH_2, C-2), 17.44 (CH_3, C-4′); ESIMS *m/z* (positive mode) 652 $[M + Na]^+$ (11), 268 (78); *anal.* C 66.72%, H 5.58%, N 2.20%, calcd for $C_{35}H_{35}NO_{10}$, C 66.76%, H 5.60%, N 2.22%.

In Vitro Anticancer Assay.[14] 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 added from DMSO-diluted stock. After 3 days in culture, attached cells 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 mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from three determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: A549 (non small cell lung cancer), MCF-7 (estrogen receptor positive breast cancer), HCT-8 (colon cancer), SK-Mel-2 (melanoma), 1A9 (ovarian cancer), KB (nasopharyngeal carcinoma), and KB-VIN (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) or from ATCC (Rockville, MD) and 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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