Cytotoxic Activity of Some Natural and Synthetic *ent*-Kauranes[⊥]

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Atractyligenin (**1**) and several synthetic derivatives were tested and found to be active against tumor cell replication. Compound **1** was readily converted to the 2,15-diketo (**3**) or 15-keto (**4**) derivatives, which contain an α,β-unsaturated ketone. Compounds **3** and **4** showed significant cytotoxic activity against all six tested cancer cell lines and were most potent against 1A9 ovarian cancer cells with EC_{50} values of 0.2 and 0.3 μM, respectively. These two **1**-analogues are promising lead compounds for further investigation.

ent-Kauranes are naturally occurring diterpenoids isolated from several plant families, such as the Asteraceae and Lamiaceae. These compounds have attracted interest because of their structures and biological activities, including antitumor, anti-HIV, and antibacterial effects.[1] Extensive chemical work[2,3] has been carried out on the structure of atractyligenin, the nor-diterpene aglycon of the glucoside atractyloside, which occurs, together with its homologous diterpene, carboxyatractyloside, in the roots of *Atractylis gummifera* L. (Asteraceae). Interest in these compounds was stimulated by the high toxicity[4] of both glucosides, which have caused many deadly poisonings, and by the antimicrobial and cytotoxic activities of some derivatives.[5]

In this paper, we report the conversion of the 15-hydroxy group of atractyligenin (**1**, Figure 1) to a ketone in order to incorporate an α,β-unsaturated ketone into the *ent*-kaurane skeleton. It is well known that a main structural determinant for cytotoxicity is the presence of an α,β-unsaturated system, which likely serves as an alkylating center and can be part of an ester, ketone, or lactone moiety.[6] We also report the derivatization of the 2-hydroxy group.

Results and Discussion

Atractyligenin methyl ester (**2**, Figure 1), prepared by treating **1** with CH_2N_2, was reacted with MnO_2 for 15 min until the starting material disappeared completely on TLC. A single product (**3**) was obtained. Its ^1H and ^{13}C NMR spectra showed a downfield shift of the exocyclic protons (δ_H 5.96, H-17a; δ_H 5.28, H-17b), the presence of two ketones at δ_C 207.7 (C-2) and δ_C 209.6 (C-15), and the absence of hydroxy groups. Consequently, compound **3** is consistent with the structure of 2,15-diketoatractyligenin methyl ester. However, when the same reaction was stopped after 3 min, TLC showed the presence of **3** as a minor product and a more polar compound (**4**) as the main product (Scheme 1). The ^1H and ^{13}C NMR spectra of **4** also indicated a downfield shift of the exocyclic protons (δ_H 5.95, H-17a; δ_H 5.27, H-17b), but showed only one ketone at δ_C 210.3 (C-15) with a hydroxy group (δ_H 4.25, H-2), rather than a ketone, at C-2. Compound **4** was assigned the structure of 15-ketoatractyligenin methyl ester.

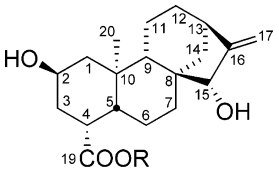
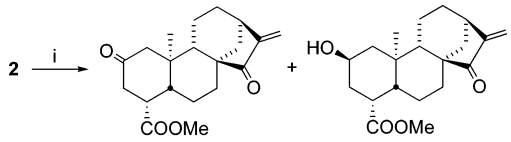


Figure 1.

Scheme 1[a]



[a] (i) MnO_2, dry THF, rt.

The presence of a free hydroxy group allowed us to prepare ester derivatives of **4** in order to evaluate the influence of an ester side chain on the biological properties of atractyligenin (Scheme 2). First, we synthesized the more lipophilic acetyl derivative **5** by treating **4** with Ac_2O−pyridine. Because it was observed previously that the introduction of piperonyl esters enhanced the biological properties of some *ent*-kaurane derivatives,[7] we prepared the piperonyl ester **6** by treating **4** with piperonylic acid, DCC, and DMPA. Finally, as previously reported by us for some guaiane derivatives,[8] in order to enhance the cytotoxic activity, the free C-2 hydroxyl group of compound **4** was esterified with the side chain of paclitaxel. Treatment of alcohol **4** with compound **7**, prepared according to a previously reported procedure[9] from commercially available (2*R*,3*S*)-3-phenylisoserine hydrochloride, gave ester **8**. Acidic hydrolysis yielded **9**, with the same side chain as paclitaxel (Scheme 2).

To evaluate whether an oxygenated function at C-2 was needed for biological activity, we planned to remove the C-2 keto group of **3**. Our synthetic design included the selective formation of a C-2 thioketal followed by a Mozingo reduction. However, because the α,β-unsaturated ketone in **3** could undergo an unwanted Michael reaction, we decided to remove this system temporarily. As shown in Scheme 3, compound **3** was treated with ethylene glycol and PTSA in benzene to give **10**. The ^1H and ^{13}C NMR spectra showed signals for the protecting group in the range δ_H 4.05−3.76 (m, 4H), the presence of a ketal carbon (δ_C 108.1 s), and the absence of the carbonyl group at δ_C 207.7 (C-2). Reduction of compound **10** with

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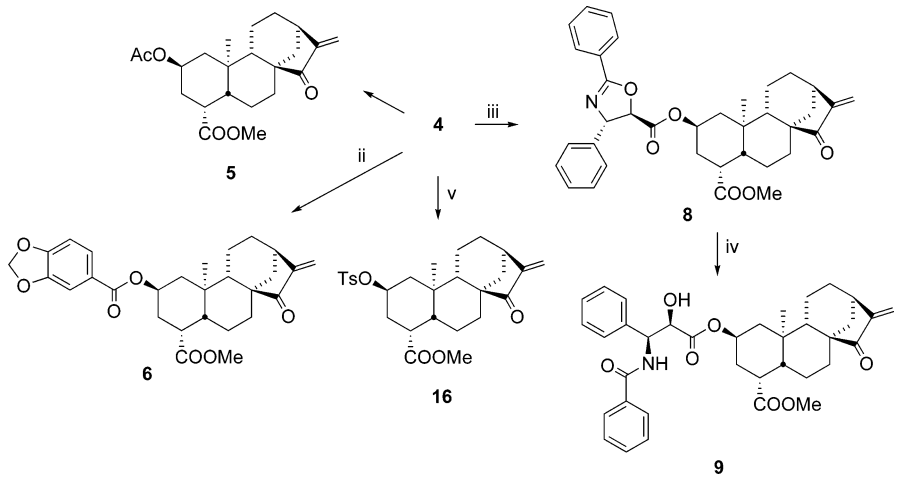
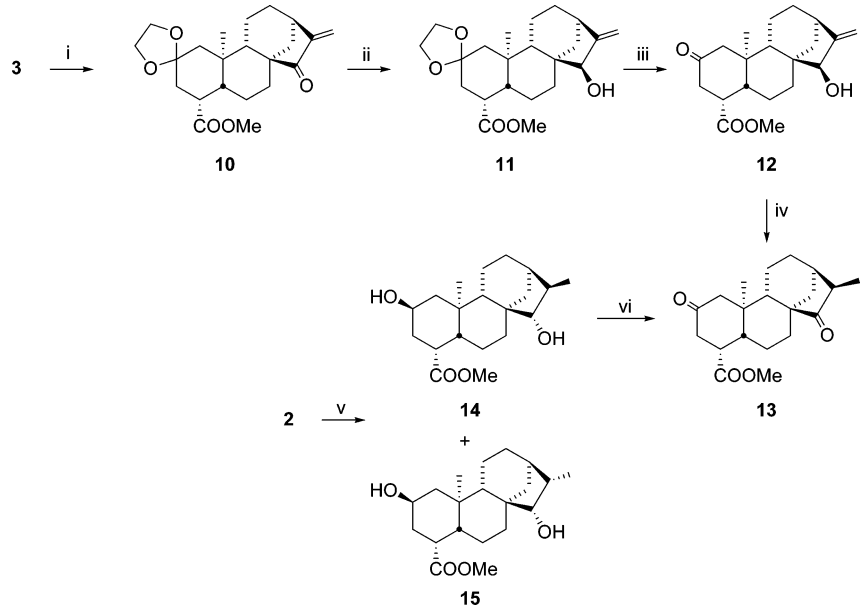
Scheme 2[a]



[a] (i) Ac_2O, Py, rt; (ii) piperonylic acid, DCC, DMAP, CH_2Cl_2, rt; (iii) (4*S*,5*R*)-2,4-diphenyl-4,5-dihydro-oxazol-5-carboxylic acid (**7**), DCC, DMAP, CH_2Cl_2, rt; (iv) *p*-TsOH, CH_2Cl_2, rt; (v) TsCl, Py, rt;

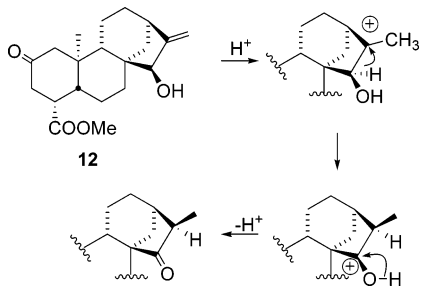
Scheme 3[a]



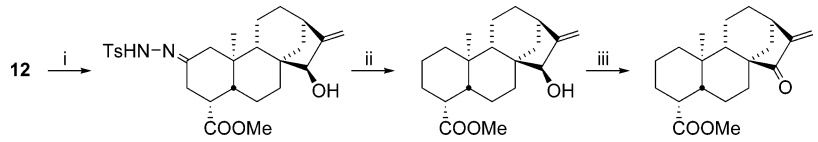
[a] (i) $HOCH_2CH_2OH$, *p*-TsOH, C_6H_6, reflux; (ii) $NaBH_4$, MeOH, rt; (iii) *p*-TsOH, MeOH/H_2O, rt; (iv) $HSCH_2CH_2SH$, *p*-TsOH, C_6H_6 reflux; (v) H_2, Pd/C, MeOH, rt; (vi) PCC, CH_2Cl_2, rt.

$NaBH_4$ in MeOH gave alcohol **11** with a 15β-hydroxy group (δ_H 3.76, H-15α), the opposite stereochemistry of that of atractyligenin (**1**). Removal of the C-2 protecting group gave ketone **12**, as clearly indicated by a carbonyl signal at δ_C 208.8 (C-2). At this point, we attempted the thioketalization of **12** by reflux with ethanedithiol and PTSA. However, the ^1H and ^{13}C NMR spectra of the resulting product showed unexpected signals. In fact, there were no signals for the thioketal group, the exocyclic double bond, or the C-15 hydroxy group, whereas resonances for a methyl group (δ_H 1.09, CH_3-17; δ_C 9.9, C-17) and for two ketones (δ_C 207.8, C-2; δ_C 223.7, C-15) were observed. Consequently, this product was assigned the structure **13**. Hypothetically, compound **13** could be produced through formation of a C-16 carbocation in the strongly acidic medium, followed by proton transposition and formation of a saturated ketone (Scheme 4). This proposed mechanism would require the C-17 methyl group to be β. To verify this stereochemistry, atractyligenin methyl ester (**2**) was catalytically reduced with H_2 and Pd/C to give two products in a 4:1 ratio (Scheme 3). The major product showed ^1H NMR signals for a secondary methyl group (δ_H 1.14, d, 3H, CH_3-17) and a secondary alcohol (δ_H 3.26 d, 1H, $J = 4.2$ Hz, H-15). Similar signals (δ_H 0.96, d, 3H, CH_3-17; δ_H 3.62, d, 1H, $J = 7.6$ Hz, H-15) were observed in the ^1H NMR spectrum of the minor product. The values of the H-15/H-16 coupling constant allowed us to assign the major product as **14** with a β-oriented C-17 methyl group and the anomeric **15** to the minor product with α-orientation. Oxidation of **14** with PDC gave **13**, confirming the proposed stereochemistry. Since our first reduction strategy failed, we next planned to remove the C-2 oxygenated moiety by forming the C-2 tosylate, followed by $LiAlH_4$ reduction and reoxidation of the C-15 hydroxyl group. Treatment

Scheme 4



Scheme 5[a]



[a] (i) *p*-TsNHNH$_2$, EtOH, 70 °C; (ii) NaBH$_3$CN, *p*-TsOH, DMF/sulfolane, 120 °C; (iii) PCC, CH$_2$Cl$_2$, rt.

Table 1. Effect of **1**−**6**, **8**, **9**, **16**, **17**, and **19** against Tumor Cell Line Replication

	EC$_{50}$ (μM)					
compound	A549	PC-3	1A9	MCF-7	KB	KB-VIN
1	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA
3	3.4	1.1	0.2	1.0	1.6	1.6
4	1.0	4.0	0.3	2.0	1.5	2.6
5	3.3	1.4	0.5	0.9	3.6	4.0
6	19.0	9.2	3.0	21.4	1.1	3.0
8	2.2	2.2	1.8	11.8	3.0	4.8
9	1.7	0.6	0.4	1.5	2.6	3.5
16	2.3	2.6	0.5	4.3	1.4	3.5
17	24.5	15.8	7.5	18.0	18.1	20.4
19	4.4	3.1	0.7	5.0	1.3	1.3
doxorubicin	0.9	2.0	0.1	0.2	0.4	1.7

[a] Cell line: A549 = lung; PC-3 = prostate; 1A9 = ovarian; MCF-7 = breast; KB = nasopharynx; KB-VIN = nasopharynx MDR.

of **4** with *p*-toluenesulfonyl chloride cleanly gave tosylate **16** (Scheme 2), but reduction with LiAlH$_4$ gave a complex mixture of unidentifiable products. Since this synthetic strategy also failed, we decided to prepare the tosylhydrazone of **12**. Reduction of intermediate **17** with NaBH$_3$CN afforded alcohol **18**, which was oxidized with PCC to give the desired ketone **19** (Scheme 5).

Compounds **1**−**6**, **8**, **9**, **16**, **17**, and **19** were screened against a panel of human tumor cell lines including A549 (lung), PC-3 (prostate), 1A9 (ovarian), MCF-7 (breast), KB (nasopharyngeal), and KB-VIN (multidrug-resistant KB subline) in order to explore their anticancer spectra and critical drug-resistance profile.[10] The results are shown in Table 1. Compounds **1**, **2**, and **17**, which do not contain an α,β-unsaturated ketone, were inactive, while the remaining compounds, which do contain this moiety, were active against all or some cell lines. Thus, as proposed in the literature,[6] the α,β-unsaturated ketone is likely the active center, possibly acting as an alkylation site. Compounds **3**−**5** and **9** showed significant activity against all six tested cell lines, while **16** and **19** were slightly less active against the MCF-7 or A549 cell lines. Accordingly, a wide range of substituents (ketone, hydroxyl, acetate, paclitaxel side chain, tosylate, and hydrogen, respectively) could be present at the C-2 position, without losing significant potency. However, compounds **6** (2-piperonyl ester) and **8** (2-oxazole ester) lost activity against certain cell lines. The 1A9 cell line was highly susceptible to all active tested compounds, particularly to compounds **3** and **4** with EC$_{50}$ values of 0.2 and 0.3 μM.

In conclusion, the ready conversion of **1** to the 2,15-diketo (**3**) or 15-keto (**4**) derivatives with an α,β-unsaturated ketone provided new lead compounds for further investigation, including in vivo evaluation as new anticancer drug candidates.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1010 digital polarimeter. IR spectra were obtained on a Shimadzu FTIR-8300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-250 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$_2$O, was used for column chromatography. Preparative TLC was performed using Merck glass plates (product code 1.13895.0001). The optically pure (2*R*,3*S*)-3-phenylisoserine hydrochloride was purchased from Industrial Chemistry Research (Warsaw, Poland). CH$_2$Cl$_2$ was dried by distillation over calcium hydride.

Synthesis of Compound 2. Compound **2** was prepared from atractyligenin (**1**) and CH$_2$N$_2$ as previously reported,[11] and its physical and spectroscopic data agreed with those reported in the literature.[12]

Oxidation of Atractyligenin Methyl Ester (2). A solution of **2** (3 g, 9 mmol) in 300 mL of dry THF was added to 3.6 g (42 mmol) of MnO$_2$ and left to stir for 3 min at room temperature. After filtration through a Millipore filter (45 μm) and column chromatography (Si gel, 2:1 petroleum ether−EtOAc as eluent), compounds **3** (600 mg, 20% yield) and **4** (2.1 g, 70% yield) were obtained.

Compound 3: amorphous solid; $[\alpha]_D^{25}$−154.6 (*c* 0.05, CHCl$_3$); IR (film) ν_{max} 2985, 1724, 1647, 1431, 1377, 1247, 1193, 1173, 1138, 959 cm^{-1}; ^{1}H NMR (CDCl$_3$, 250 MHz) δ 5.96 (1H, brs, H-17a), 5.28 (1H, brs, H-17b), 3.65 (3H, s, OCH$_3$), 3.07 (1H, m, H-13), 3.05 (1H, m, H-4), 2.87 (1H, ddd, J = 14.8, 2.3, 2.3 Hz, H-3α), 2.58 (1H, dd, J = 14.1, 2.3 Hz, H-1α), 2.42 (1H, dd, J = 14.8, 7.5 Hz, H-3β), 2.28 (1H, d, J = 12.1 Hz, H-14a), 1.84 (1H, d, J = 14.1 Hz, H-1β), 0.94 (3H, s, Me-20); ^{13}C NMR (CDCl$_3$, 62.7 MHz) δ 209.6 (C, C-15), 207.7 (C, C-2), 173.7 (C, C-19), 148.7 (C, C-16), 115.0 (CH$_2$, C-17), 55.2 (CH$_2$, C-1), 51.6 (CH$_3$, OMe), 51.6 (C, C-8), 50.0 (CH, C-9), 47.4 (CH, C-5), 45.1 (CH, C-4), 42.9 (C, C-10), 42.8 (CH$_2$, C-3), 37.7 (CH, C-13), 35.8 (CH$_2$, C-14), 32.7 (CH$_2$, C-7), 31.6 (CH$_2$, C-12), 24.0 (CH$_2$, C-6), 18.0 (CH$_2$, C-11), 16.6 (CH$_3$, C-20); EIMS *m*/*z* 330 [M]$^+$ (4), 296 (100), 268 (21), 189 (16), 143 (11), 107 (12), 91 (62); *anal.* C 72.72%, H 7.90%, calcd for C$_{20}$H$_{26}$O$_4$, C 72.70%, H 7.93%.

Compound 4: amorphous solid; $[\alpha]_D^{25}$ −170.4 (*c* 0.11, CHCl$_3$); IR (film) ν_{max} 3433, 3055, 2933, 1718, 1672, 1643, 1448, 1265, 1198, 1047, 931 cm^{-1}; ^{1}H NMR (CDCl$_3$, 250 MHz) δ 5.95 (1H, brs, H-17a), 5.27 (1H, brs, H-17b), 4.25 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 3.67 (3H, s, OCH$_3$), 3.06 (1H, m, H-13), 2.70 (1H, m, H-4), 2.42 (1H, m, H-3α), 2.35 (1H, d, J = 12.0 Hz, H-14a), 2.20 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 0.96 (3H, s, Me-20), 0.74 (1H, dd, J = 11.8, 11.8 Hz, H-1β); ^{13}C NMR (CDCl$_3$, 62.7 MHz) δ 210.3 (C, C-15), 175.3 (C, C-19), 149.1 (C, C-16), 114.9 (CH$_2$, C-17), 64.0 (CH, C-2), 52.2 (C, C-8), 51.4 (CH$_3$, OMe), 50.8 (CH, C-9), 48.3 (CH$_2$, C-1), 48.3 (CH, C-5), 43.7 (CH, C-4), 40.8 (C, C-10), 38.0 (CH, C-13), 37.4 (CH$_2$, C-3), 36.5 (CH$_2$, C-14), 33.2 (CH$_2$, C-7), 32.0 (CH$_2$, C-12), 24.4 (CH$_2$, C-6), 18.2 (CH$_2$, C-11), 16.2 (CH$_3$, C-20); EIMS *m*/*z* 332 [M]$^+$ (12), 314 (71), 282 (100), 267 (37), 255 (63), 198 (23), 131 (23), 119 (26), 105 (63), 91 (67); *anal.* C 72.23%, H 8.52%, calcd for C$_{20}$H$_{28}$O$_4$, C 72.26%, H 8.49%.

Synthesis of Acetyl 15-Ketoatractyligenin Methyl Ester (5). Compound **4** (50 mg, 0.15 mmol) was dissolved in a mixture of Ac$_2$O−pyridine 1:1 (5 mL) and allowed to stir for 24 h at room temperature. The solution was diluted with 30 mL of CH$_2$Cl$_2$ and 20 mL of aqueous HCl. The organic layer was separated, washed with H$_2$O, and dried over Na$_2$SO$_4$. After evaporation of the solvent, 45 mg (80% yield) of acetyl 15-ketoatractyligenin methyl ester (**5**) was obtained.

Acetyl 15-Ketoatractyligenin Methyl Ester (5): amorphous solid; $[\alpha]_D^{25}$ −61.6 (*c* 0.25, CHCl$_3$); IR (film) ν_{max} 2950, 1726, 1645, 1446, 1365, 1247, 1173, 1028, 930 cm^{-1}; ^{1}H NMR (CDCl$_3$, 250 MHz) δ 5.96 (1H, brs, H-17a), 5.34 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 5.28 (1H, brs, H-17b), 3.70 (3H, s, OCH$_3$), 3.06 (1H, m, H-13), 2.74 (1H, m, H-4), 2.43 (1H, m, H-3α), 2.36 (1H, d, J = 12.1 Hz, H-14a), 2.29 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 2.03 (3H, s, Ac), 1.02 (3H, s, Me-20), 0.78 (1H, dd, J = 11.8, 11.8 Hz, H-1β); ^{13}C NMR (CDCl$_3$, 62.7 MHz) δ 210.0 (C, C-15), 174.5 (C, C-19), 149.2 (C, C-16), 114.8 (CH$_2$, C-17), 67.9 (CH, C-2), 52.1 (C, C-8), 51.5 (CH$_3$, OMe), 50.8 (CH, C-9), 48.3 (CH, C-5), 44.6 (CH$_2$, C-1), 43.5 (CH, C-4), 40.8 (C, C-10), 38.0 (CH, C-13), 36.5 (CH$_2$, C-14), 33.6 (CH$_2$, C-3), 33.2

(CH_2, C-7), 31.9 (CH_2, C-12), 24.4 (CH_2, C-6), 18.2 (CH_2, C-11), 16.1 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 413 $[M + K]^+$ (100), 397 $[M + Na]^+$ (95), 375 $[M + H]^+$ (4), 315 $[M + H - AcOH]^+$ (8); *anal.* C 70.60%, H 8.03%, calcd for $C_{22}H_{30}O_5$, C 70.56%, H 8.07%.

Synthesis of Ester 6. Compound **4** (50 mg, 0.15 mmol) was dissolved in dry CH_2Cl_2 (5 mL), and this was added to 25 mg of piperonylic acid, 1 equiv of DMAP, and 1 equiv of DCC, under argon, followed by 1 equiv of 1-hydroxybenzotriazole hydrate. The reaction mixture was allowed to stir for 10 h at room temperature. The reaction was stopped by evaporation in vacuo of the solvent, and the residue was purified by preparative TLC (4:1 petroleum ether−EtOAc as eluent) to give 27 mg (20% yield) of compound **6**: amorphous solid; $[\alpha]_D^{25}$ −16.6 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 2923, 1762, 1645, 1443, 1279, 1257, 1159, 968, 933 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.63 (1H, dd, J = 8.1, 1.3 Hz, H-7′), 7.45 (1H, d, J = 1.3 Hz, H-3′), 6.81 (1H, d, J = 8.1 Hz, H-6), 6.03 (2H, s, H-8′), 5.96 (1H, brs, H-17a), 5.55 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 5.27 (1H, brs, H-17b), 3.72 (3H, s, OCH_3), 3.07 (1H, m, H-13), 2.79 (1H, m, H-4), 2.55 (1H, m, H-3α), 2.40 (1H, d, J = 12.0 Hz, H-14a), 2.40 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 1.07 (3H, s, Me-20), 0.91 (1H, dd, J = 11.8, 11.8 Hz, H-1*β*); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 210.2 (C, C-15), 174.5 (C, C-19), 165.2 (C, C-1′), 151.4 (C, C-5′), 149.1 (C, C-16), 147.6 (C, C-4′), 125.2 (CH, C-7′), 124.7 (C, C-2′), 114.9 (CH_2, C-17), 109.5 (CH, C-3′), 107.9 (CH, C-6′), 101.7 (CH_2, C-8′), 68.6 (CH, C-2), 52.1 (C, C-8), 51.6 (CH_3, OMe), 50.8 (CH, C-9), 48.3 (CH, C-5), 44.6 (CH_2, C-1), 43.5 (CH, C-4), 40.8 (C, C-10), 37.9 (CH, C-13), 36.5 (CH_2, C-14), 33.6 (CH_2, C-3), 33.2 (CH_2, C-7), 31.9 (CH_2, C-12), 24.3 (CH_2, C-6), 18.2 (CH_2, C-11), 16.1 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 519 $[M + K]^+$ (36), 503 $[M + Na]^+$ (100), 481 $[M + H]^+$ (11); *anal.* C 69.95%, H 6.75%, calcd for $C_{28}H_{32}O_7$, C 69.98%, H 6.71%.

Synthesis of Ester 8. (4*S*,5*R*)-2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid **7** (71.5 mg, 0.3 mmol), synthesized as previously reported,[9] was dissolved in 10 mL of dry CH_2Cl_2, and this was added to DMAP (5.51 mg, 0.04 mmol) and DCC (62.58 mg, 0.3 mmol). After stirring at room temperature for 15 min, compound **4** (50 mg, 0.15 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, 9:1 petroleum ether−EtOAc as eluent) to give ester **8** (64 mg, 73% yield): amorphous solid; $[\alpha]_D^{25}$ −48.0 (*c* 0.23, $CHCl_3$); IR (film) ν_{max} 2931, 2858, 1751, 1724, 1655, 1450, 1267, 1230, 1064, 1026, 960, 931, 737, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 8.10−8.04 (2H, m, arom), 7.60−7.20 (8H, m, arom), 5.97 (1H, brs, H-17a), 5.56 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 5.40 (1H, d, J = 6.3 Hz, H-3′), 5.28 (1H, brs, H-17b), 4.87 (1H, d, J = 6.3 Hz, H-2′), 3.70 (3H, s, OCH_3), 3.07 (1H, m, H-13), 2.78 (1H, m, H-4), 2.48 (1H, m, H-3α), 2.45 (1H, d, J = 12.0 Hz, H-14a), 2.35 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 1.04 (3H, s, Me-20), 0.90 (1H, dd, J = 11.8, 11.8 Hz, H-1*β*); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 209.8 (C, C-15), 174.3 (C, C-19), 169.4 (C, C-1′), 164.1 (C, C-5′), 149.0 (C, C-16), 141.2 (C, arom), 131.9 (C, arom), 128.8 (2CH, arom), 128.7 (2CH, arom), 128.4 (2CH, arom), 128.0 (CH, arom), 126.8 (CH, arom), 126.4 (2CH, arom), 114.9 (CH_2, C-17), 83.1 (CH, C-2′), 74.6 (CH, C-3′), 69.9 (CH, C-2), 52.0 (C, C-8), 51.5 (CH_3, OMe), 50.7 (CH, C-9), 48.2 (CH, C-5), 44.2 (CH_2, C-1), 43.4 (CH, C-4), 40.6 (C, C-10), 37.9 (CH, C-13), 36.5 (CH_2, C-14), 33.4 (CH_2, C-3), 33.1 (CH_2, C-7), 31.6 (CH_2, C-12), 24.3 (CH_2, C-6), 18.2 (CH_2, C-11), 16.0 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 582 $[M + H]^+$ (28), 474 (100); *anal.* C 74.30%, H 6.73%, N 2.39%, calcd for $C_{36}H_{39}NO_6$, C 74.33%, H 6.76%, N 2.41%.

Synthesis of Ester 9. Compound **8** (50 mg, 0.09 mmol), dissolved in CH_2Cl_2 (5 mL), was stirred at room temperature with *p*-toluenesulfonic acid (3 mg, 0.017 mmol). After completion of the reaction (4 days) 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 preparative TLC (4:1 petroleum ether−EtOAc as eluent) to give 38 mg (75% yield) of compound **9**: amorphous solid; $[\alpha]_D^{25}$ −21.4 (*c* 0.29, $CHCl_3$); IR (film) ν_{max} 3431, 2928, 1724, 1664, 1647, 1514, 1485, 1448, 1265, 1211, 1117, 739, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.77−7.73 (2H, m, arom), 7.48−7.28 (8H, m, arom), 7.03 (1H, d, J = 9.3 Hz, NH), 5.95 (1H, brs, H-17a), 5.76 (1H, dd, J = 9.3, 2.1 Hz, H-3′), 5.47 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 5.25 (1H, brs, H-17b), 4.63 (1H, d, J = 2.1 Hz, H-2′), 3.69 (3H, s, OCH_3), 3.00 (1H, m, H-13), 2.75 (1H, m, H-4), 2.43 (1H, m, H-3), 2.30 (1H, d, J = 12.0 Hz, H-14a), 2.08 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 0.93 (3H, s, Me-20), 0.80 (1H, dd, J = 11.8, 11.8 Hz, H-1*β*); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 209.5 (C, C-15), 174.6 (C, C-19), 172.4 (C, C-1′), 166.3 (C, C-5′), 149.1 (C, C-16), 138.6 (C, arom), 133.2 (C, arom), 131.7 (CH, arom), 128.7 (4CH, arom), 127.8 (CH, arom), 127.1 (2CH, arom), 126.9 (2CH, arom), 114.7 (CH_2, C-17), 73.4 (CH, C-2′), 71.3 (CH, C-2), 54.6 (CH, C-3′), 51.9 (C, C-8), 51.5 (CH_3, OMe), 50.4 (CH, C-9), 48.0 (CH, C-5), 43.8 (CH_2, C-1), 43.4 (CH, C-4), 40.8 (C, C-10), 37.9 (CH, C-13), 36.5 (CH_2, C-14), 33.3 (CH_2, C-3), 33.1 (CH_2, C-7), 31.9 (CH_2, C-12), 24.3 (CH_2, C-6), 17.8 (CH_2, C-11), 16.0 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 638 $[M + K]^+$ (17), 622 $[M + Na]^+$ (100), 600 $[M + H]^+$ (25); *anal.* C 72.03%, H 6.90%, N 2.32%, calcd for $C_{36}H_{41}NO_7$, C 72.10%, H 6.89%, N 2.34%.

Synthesis of Compound 10. Compound **3** (550 mg, 1.6 mmol), dissolved in benzene (40 mL), was refluxed in a Dean−Stark apparatus with ethylene glycol (3.5 mL) and *p*-toluenesulfonic acid (454 mg, 2.4 mmol) for 5 h. The reaction was stopped by adding saturated aqueous $NaHCO_3$ and a small amount of Na_2CO_3 and extracted three times with $CHCl_3$ (25 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, 4:1 petroleum ether−EtOAc as eluent) to give 561 mg (93% yield) of compound **10**: amorphous solid; $[\alpha]_D^{25}$ −37.3 (*c* 0.39, $CHCl_3$); IR (film) ν_{max} 2929, 2869, 1724, 1645, 1448, 1265, 1163, 1074, 931, 738, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.95 (1H, brs, H-17a), 5.27 (1H, brs, H-17b), 4.05−3.76 (4H, m, CH_2CH_2), 3.67 (3H, s, OCH_3), 3.06 (1H, m, H-13), 2.70 (1H, m, H-4), 2.58 (1H, ddd, J = 14.0, 2.0, 2.0 Hz, H-3α), 2.42 (1H, d, J = 12.2 Hz, H-14a), 1.94 (1H, dd, J = 13.6, 2.0 Hz, H-1α), 1.26 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 210.0 (C, C-15), 173.7 (C, C-19), 149.1 (C, C-16), 114.4 (CH_2, C-17), 108.1 (C, C-2), 64.1 (CH_2, CH_2O), 62.9 (CH_2, CH_2O), 52.1 (C, C-8), 51.1 (CH_3, OMe), 50.9 (CH, C-9), 48.1 (CH_2, C-1), 47.1 (CH, C-5), 43.1 (CH, C-4), 40.3 (C, C-10), 37.7 (CH, C-13), 36.2 (CH_2, C-3), 34.7 (CH_2, C-14), 33.2 (CH_2, C-7), 31.6 (CH_2, C-12), 24.2 (CH_2, C-6), 18.1 (CH_2, C-11), 16.9 (CH_3, C-20); ESIMS (positive mode) *m*/*z* 397 $[M + Na]^+$ (100), 375 $[M + H]^+$ (38); *anal.* C 70.50%, H 8.05%, calcd for $C_{22}H_{30}O_5$, C 70.56%, H 8.07%.

Synthesis of Compound 11. Compound **10** (486 mg, 1.3 mmol), dissolved in MeOH (60 mL), was stirred at room temperature with $NaBH_4$ (77.3 mg, 2 mmol). After 10 min, the reaction was stopped by adding water (50 mL) and extracted three times with CH_2Cl_2 (25 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to dryness, leaving a residue, which was purified by chromatography (Si gel, 7:3 petroleum ether−EtOAc as eluent) to give 440 mg (90% yield) of compound **11**: amorphous solid; $[\alpha]_D^{25}$ −50.0 (*c* 1.12, $CHCl_3$); IR (film) ν_{max} 3444, 2922, 1718, 1661, 1465, 1377, 1263, 1193, 1070, 972, 948, 827, 709 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.09 (1H, brs, H-17a), 4.96 (1H, brs, H-17b), 4.04−3.76 (4H, m, CH_2-CH_2), 3.76 (1H, m, H-15), 3.65 (3H, s, OCH_3), 2.65 (1H, m, H-13), 2.65 (1H, m, H-4), 2.58 (1H, ddd, J = 14.0, 2.0, 2.0 Hz, H-3α), 2.05 (1H, d, J = 12.0 Hz, H-14a), 2.00 (1H, dd, J = 13.6, 2.0 Hz, H-1α), 1.27 (1H, d, J = 13.6 Hz, H-1*β*), 1.12 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 174.2 (C, C-19), 158.2 (C, C-16), 108.5 (C, C-2), 104.9 (CH_2, C-17), 84.2 (CH, C-15), 64.3 (CH_2, CH_2O), 62.9 (CH_2, CH_2O), 51.1 (CH_3, OMe), 48.9 (CH_2, C-1), 47.4 (CH, C-5), 45.6 (CH, C-9), 45.5 (C, C-8), 43.3 (CH, C-4), 39.9 (CH, C-13), 39.6 (C, C-10), 38.5 (CH_2, C-7), 36.1 (CH_2, C-3), 34.7 (CH_2, C-14), 32.6 (CH_2, C-12), 25.7 (CH_2, C-6), 18.3 (CH_2, C-11), 17.2 (CH_3, C-20); ESIMS *m*/*z* 377 $[M + H]^+$ (100); *anal.* C 70.15%, H 8.60%, calcd for $C_{22}H_{32}O_5$, C 70.18%, H 8.57%.

Synthesis of Compound 12. Compound **11** (390 mg, 1.0 mmol), dissolved in a 1:1 mixture of MeOH−H_2O (40 mL), was stirred at room temperature with *p*-toluenesulfonic acid (107 mg, 0.5 mmol) for 2 h. The reaction was stopped by adding saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (25 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to dryness, leaving a residue, which was crystallized to give 289 mg (87% yield) of compound **12**: amorphous solid; $C_{20}H_{28}O_4$; $[\alpha]_D^{25}$ −96.0 (*c* 0.92, $CHCl_3$); IR (film) ν_{max} 3555, 2933, 1713, 1664, 1433, 1257, 1197, 1074, 954 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.10 (1H, brs, H-17a), 4.99 (1H, brs, H-17b), 3.82 (1H, m, H-15), 3.65 (3H, s, OCH_3), 3.02 (1H, m, H-4), 2.87 (1H, ddd, J = 14.4, 2.0, 2.0 Hz, H-3α), 2.68 (1H, m, H-13), 2.65 (1H, dd, J = 14.4, 2.0 Hz, H-1α), 2.39 (1H, dd, J = 14.4, 7.6 Hz, H-3*β*), 1.92 (1H, d, J = 14.4 Hz, H-1*β*), 1.87 (1H, d, J = 12.0 Hz, H-14a), 0.89 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 208.8 (C, C-2), 173.9 (C, C-19), 157.7 (C, C-16), 105.3 (CH_2, C-17), 82.1

(CH, C-15), 56.0 (CH_2, C-1), 51.7 (CH_3, OMe), 47.8 (CH, C-5), 45.3 (CH, C-4), 45.2 (C, C-8), 44.5 (CH, C-9), 42.9 (CH_2, C-3), 42.5 (C, C-10), 39.8 (CH, C-13), 37.9 (CH_2, C-7), 35.6 (CH_2, C-14), 32.6 (CH_2, C-12), 25.4 (CH_2, C-6), 18.0 (CH_2, C-11), 16.7 (CH_3, C-20); ESIMS (positive mode) *m/z* 687 $[2M + Na]^+$ (100), 371 $[M + K]^+$ (4), 355 $[M + Na]^+$ (60), 333 $[M + H]^+$ (4); *anal.* C 72.24%, H 8.52%, calcd for $C_{20}H_{28}O_4$, C 72.26%, H 8.49%.

Synthesis of Compound 13. Compound **12** [50 mg, 0.15 mmol, dissolved in benzene (25 mL)] was refluxed in a Dean−Stark apparatus with ethanedithiol (25 μL) and *p*-toluenesulfonic acid (34 mg, 0.17 mmol) for 2 h. The reaction was stopped by adding saturated aqueous $NaHCO_3$ and extracted with EtOAc (25 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness, leaving a residue, which was purified by chromatography (Si gel, 4:1 petroleum ether−EtOAc as eluent) to give 23 mg (47% yield) of compound **13**: amorphous solid; $[\alpha]_D^{25}$ −115.2 (*c* 0.55, $CHCl_3$); IR (film) ν_{max} 2931, 1728, 1438, 1251, 1190, 1167, 1070, 953 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 3.65 (3H, s, OCH_3), 3.03 (1H, m, H-4), 2.85 (1H, ddd, J = 14.7, 1.8, 1.8 Hz, H-3α), 2.54 (1H, dd, J = 13.9, 1.8 Hz, H-1α), 2.39 (1H, dd, J = 14.7, 7.6 Hz, H-3β), 2.29 (1H, d, J = 12.4 Hz, H-14a), 2.22 (1H, quint., J = 6.9 Hz, H-16), 1.09 (3H, d, J = 6.9 Hz, Me-17), 0.91 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 223.7 (C, C-15), 207.8 (C, C-2), 173.7 (C, C-19), 55.2 (CH_2, C-1), 51.8 (C, C-8), 51.6 (CH_3, OMe), 50.1 (CH, C-9), 47.6 (CH, C-5), 47.5 (CH, C-16), 45.2 (CH, C-4), 43.0 (CH_2, C-3), 42.6 (C, C-10), 36.6 (CH_2, C-7), 34.7 (CH, C-13), 33.3 (CH_2, C-12), 24.3 (CH_2, C-6), 24.2 (CH_2, C-14), 17.9 (CH_2, C-11), 16.6 (CH_3, C-20), 9.9 (CH_3, C-17); ESIMS *m/z* 687 $[2M + Na]^+$ (100), 371 $[M + K]^+$ (18), 355 $[M + Na]^+$ (64), 333 $[M + H]^+$ (4); *anal.* C 72.29%, H 8.45%, calcd for $C_{20}H_{28}O_4$, C 72.26%, H 8.49%.

Catalytic Reduction of Atractyligenin Methyl Ester (3). Compound **3** (300 mg, 0.9 mmol), dissolved in MeOH (150 mL), was reduced in a Parr apparatus with Pd/C (410 mg) and H_2 (1 atm) for 1 h. The reaction was stopped and the solvent evaporated, leaving a residue, which was purified by chromatography (Si gel, 3:7 petroleum ether−EtOAc as eluent) to give, in order of increasing polarity, 50 mg (17% yield) of compound **15** and 230 mg (77% yield) of compound **14**.

Compound 14: amorphous solid; $[\alpha]_D^{25}$ −66.2 (*c* 0.23, $CHCl_3$); IR (film) ν_{max} 3380, 2925, 2870, 1722, 1450, 1263, 1192, 1178, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 4.27 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 3.67 (3H, s, OCH_3), 3.26 (1H, d, J = 4.2 Hz, H-15), 2.67 (1H, m, H-4), 2.45 (1H, m, H-3α), 2.23 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 1.89 (1H, d, J = 12.0 Hz, H-14a), 1.14 (3H, d, J = 7.1 Hz, Me-17), 0.90 (3H, s, Me-20), 0.70 (1H, dd, J = 11.8, 11.8 Hz, H-1β); ESIMS *m/z* 695 $[2M + Na]^+$ (60), 375 $[M + K]^+$ (7), 359 $[M + Na]^+$ (100), 337 $[M + H]^+$ (2); *anal.* C 71.38%, H 9.61%, calcd for $C_{20}H_{32}O_4$, C 71.39%, H 9.59%.

Compound 15: amorphous solid; $[\alpha]_D^{25}$ −82.1 (*c* 0.52, $CHCl_3$); IR (film) ν_{max} 3374, 2932, 2862, 1718, 1448, 1264, 1194, 1176, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 4.26 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 3.67 (3H, s, OCH_3), 3.62 (1H, d, J = 7.6 Hz, H-15), 2.67 (1H, m, H-4), 2.45 (1H, m, H-3α), 2.22 (1H, dd, J = 11.8, 4.3 Hz, H-1α), 0.96 (3H, d, J = 7.6 Hz, Me-17), 0.89 (3H, s, Me-20), 0.72 (1H, dd, J = 11.8, 11.8 Hz, H-1β); ESIMS *m/z* 695 $[2M + Na]^+$ (58), 375 $[M + K]^+$ (8), 359 $[M + Na]^+$ (100), 337 $[M + H]^+$ (2); *anal.* C 71.35%, H 9.56%, calcd for $C_{20}H_{32}O_4$, C 71.39%, H 9.59%.

Oxidation of Compound 14. Compound **14** (60 mg, 0.18 mmol), dissolved in CH_2Cl_2 (12 mL), was stirred at room temperature with PCC (108 mg) for 1 h. The reaction was stopped by filtering over Florisil (CH_2Cl_2 and EtOAc as eluents), and the residue was purified by chromatography (Si gel, 3:2 petroleum ether−EtOAc as eluent) to give 40 mg (67% yield) of compound **13**.

Synthesis of Compound 16. Compound **4** (50 mg. 0.15 mmol) was dissolved in dry pyridine (5 mL), added to 43 mg of *p*-toluenesulfonyl chloride (0.22 mmol), and allowed to stand for 7 days at room temperature. The reaction was stopped by evaporation in vacuo of the solvent with toluene, and the residue was purified by preparative TLC (4:1 petroleum ether−EtOAc as eluent) to give 49 mg (66% yield) of compound **16**: amorphous solid; $[\alpha]_D^{25}$ −43.3 (*c* 0.68, $CHCl_3$); IR (film) ν_{max} 2927, 2861, 1725, 1644, 1598, 1448, 1360, 1257, 1188, 1188, 1012, 933 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 7.84 (2H, d, J = 9.9 Hz, H-2′ and H-6′), 7.35 (2H, d, J = 9.9 Hz, H-3 and H-5′), 5.96 (1H, brs, H-17a), 5.28 (1H, brs, H-17b), 5.10 (1H, dddd, J = 11.8, 11.8, 4.3, 4.3 Hz, H-2), 3.64 (3H, s, OCH_3), 3.06 (1H, m, H-13), 2.65 (1H, m, H-4), 2.45 (3H, s, Me-7′), 2.30 (1H, d, J = 13.1 Hz, H-14a), 0.92 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 209.7 (C, C-15), 174.2 (C, C-19), 148.9 (C, C-16), 144.4 (C, C-4′), 134.7 (C, C-1′), 129.7 (2CH, C-3′ and C-5′), 127.8 (2CH, C-2′ and C-6′), 115.0 (CH_2, C-17), 77.5 (CH, C-2), 51.9 (C, C-8), 51.5 (CH_3, OMe), 50.6 (CH, C-9), 47.8 (CH, C-5), 45.6 (CH_2, C-1), 43.5 (CH, C-4), 41.0 (C, C-10), 37.9 (CH, C-13), 36.5 (CH_2, C-14), 34.2 (CH_2, C-3), 33.0 (CH_2, C-7), 31.8 (CH_2, C-12), 24.1 (CH_2, C-6), 21.6 (CH_3, C-7′), 18.1 (CH_2, C-11), 15.9 (CH_3, C-20); ESIMS (positive mode) *m/z* 525 $[M + K]^+$ (15), 509 $[M + Na]^+$ (100), 487 $[M + H]^+$ (4); *anal.* C 66.60%, H 7.00%, S 6.55% calcd for $C_{27}H_{34}O_6S$, C 66.64%, H 7.04%, S 6.59%.

Reduction of Compound 16. Compound **16** (40 mg. 0.08 mmol) was dissolved in dry THF (5 mL), added to 10 mg of $LiAlH_4$ (0.24 mmol), and allowed to stir for 2 h at room temperature. The reaction was stopped by adding saturated NH_4Cl solution (5 mL). The residue was filtered off, and the solution was extracted three times with EtOAc (10 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to dryness. TLC analysis of the residue showed an unresolvable complex mixture of products.

Synthesis of Compound 17. Compound **12** (210 mg, 0.63 mmol), dissolved in EtOH (3 mL), was heated at 70 °C with *p*-toluenesulfonyl hydrazine (140 mg, 0.75 mmol) for 5 h. The reaction was stopped by removal of the solvent in vacuo, and the residue was purified by preparative TLC (7:3 petroleum ether−EtOAc as eluent) to give 305 mg (97% yield) of compound **17**: amorphous solid; $[\alpha]_D^{25}$ 54.1 (*c* 0.39, $CHCl_3$); IR (film) ν_{max} 3435, 3217, 2930, 1703, 1643, 1599, 1450, 1337, 1267, 1167, 1091, 925 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 8.62 (1H, brs, NH), 7.89 (2H, d, J = 8.2 Hz, H-2′ and H-6′), 7.26 (2H, d, J = 8.2 Hz, H-3′ and H-5′), 5.10 (1H, brs, H-17a), 4.98 (1H, brs, H-17b), 3.77 (1H, brs, H-15), 3.55 (3H, s, OCH_3), 3.09 (1H, ddd, 13.7, 1.6, 1.6 Hz, H-3α), 2.79 (1H, m, H-4), 2.65 (1H, m, H-13), 2.47 (1H, dd, J = 12.1, 1.6 Hz, H-1α), 2.41 (3H, s, Me-Ar), 0.34 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 175.7 (C, C-19), 158.1 (C, C-2), 157.4 (C, C-16), 143.1 (C, C-1′), 135.3 (C, C-4′), 128.7 (CH, C-3′ and C-5′), 128.1 (CH, C-2′ and C-6′), 104.9 (CH_2, C-17), 81.8 (CH, C-15), 51.6 (CH_3, OMe), 49.5 (CH_2, C-1), 49.0 (CH, C-5), 45.3 (CH, C-4), 45.2 (C, C-8), 44.3 (CH, C-9), 42.3 (C, C-10), 39.7 (CH, C-13), 37.8 (CH_2, C-7), 35.8 (CH_2, C-14), 32.6 (CH_2, C-12), 29.8 (CH_2, C-3), 25.5 (CH_2, C-6), 21.3 (CH_3, Me-Ar), 16.1 (CH_2, C-11), 14.2 (CH_3, C-20); ESIMS *m/z* 539 $[M + K]^+$ (11), 523 $[M + Na]^+$ (57), 501 $[M + H]^+$ (100); *anal.* C 64.72%, H 7.22%, N 5.58%, S 6.42%, calcd for $C_{27}H_{36}N_2O_5S$, C 64.77%, H 7.25%, N 5.60%, S 6.40%.

Reduction of Compound 17. Compound **17** (160 mg, 0.31 mmol), dissolved in a 1:1 mixture of DMF/sulfolane (14 mL), was heated at 120 °C with *p*-toluenesulfonic acid (6 mg) and $NaBH_3CN$ (80 mg, 1.3 mmol) for 24 h. The reaction was stopped by adding saturated aqueous NaCl and extracted three times with Et_2O (25 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, 4:1 petroleum ether−EtOAc as eluent) to give 48 mg (48% yield) of compound **18**: amorphous solid; $[\alpha]_D^{25}$ −116.3 (*c* 0.55, $CHCl_3$); IR (film) ν_{max} 3480, 3054, 2930, 1722, 1447, 1265, 1192, 1002, 896 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.10 (1H, brs, H-17a), 4.97 (1H, brs, H-17b), 3.76 (1H, brs, H-15), 3.66 (3H, s, OCH_3), 2.67 (1H, m, H-13), 2.47 (1H, m, H-4), 2.12 (1H, brd, 12.2 Hz, H-3α), 1.98 (1H, d, 12.0 Hz, H-14), 0.89 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 176.0 (C, C-19), 158.4 (C, C-16), 104.9 (CH_2, C-17), 82.6 (CH, C-15), 51.4 (CH_3, OMe), 49.1 (CH, C-5), 46.0 (C, C-8), 45.2 (CH, C-9), 43.2 (CH, C-4), 40.3 (CH_2, C-1), 40.1 (CH, C-13), 38.7 (C, C-10), 37.5 (CH_2, C-7), 36.3 (CH_2, C-14), 33.0 (CH_2, C-12), 28.5 (CH_2, C-3), 26.7 (CH_2, C-6), 18.7 (CH_2, C-2), 18.1 (CH_2, C-11), 15.2 (CH_3, C-20); ESIMS *m/z* 659 $[2M + Na]^+$ (89), 357 $[M + K]^+$ (32), 341 $[M + Na]^+$ (100), 319 $[M + H]^+$ (32); *anal.* C 75.45%, H 9.47%, calcd for $C_{20}H_{30}O_3$, C 75.43%, H 9.50%.

Oxidation of Compound 18. Compound **18** (30 mg, 0.09 mmol), dissolved CH_2Cl_2 (6 mL), was stirred at room temperature with PCC (54 mg, 0.25 mmol). The reaction was stopped after 1 h by filtering over Florisil with EtOAc as eluent. The solvent was evaporated to dryness, leaving a residue, which was purified by chromatography (Si gel, 4:1 petroleum ether−EtOAc as eluent) to give 20 mg (67% yield) of compound **19**: amorphous solid; $[\alpha]_D^{25}$ −157.8 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 2927, 2855, 1727, 1644, 1447, 1255, 1197, 1173, 1038, 945 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.94 (1H, brs, H-17a), 5.24 (1H, brs, H-17b), 3.66 (3H, s, OCH_3), 3.04 (1H, m, H-13), 2.49 (1H, m, H-4), 2.39 (1H, d, 12.0 Hz, H-14), 2.15 (1H, brd, 12.2 Hz, H-3α), 0.94 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 62.7 MHz) δ 210.5 (CH, C-15),

175.8 (C, C-19), 149.5 (C, C-16), 114.5 (CH_2, C-17), 52.6 (C, C-8), 51.2 (CH, C-9), 51.1 (CH_3, OMe), 48.8 (CH, C-5), 43.0 (CH, C-4), 39.7 (C, C-10), 39.6 (CH_2, C-1), 38.1 (CH, C-13), 36.5 (CH_2, C-14), 33.3 (CH_2, C-7), 32.1 (CH_2, C-12), 28.3 (CH_2, C-3), 25.3 (CH_2, C-6), 18.5 (CH_2, C-2), 18.1 (CH_2, C-11), 15.1 (CH_3, C-20); ESIMS *m*/*z* 355 $[M + K]^+$ (32), 339 $[M + Na]^+$ (100), 317 $[M + H]^+$ (28); *anal.* C 75.96%, H 8.90%, calcd for $C_{20}H_{28}O_3$, C 75.91%, H 8.92%.

In Vitro Cytotoxicity Assay. The sulforhodamine B assay was used according to the procedures developed and validated at NCI.[10] Doxorubicin was used as the positive control antitumor drug. The in vitro anticancer activities are expressed as EC_{50} values, which is the test compound concentration (μM) that reduced the cell number by 50% after 72 h of continuous treatment. The values were interpolated from dose−response data. Each test was performed in triplicate with variation less than 5%. The EC_{50} values determined in each of independent tests varied less than 10%. Compound stock solutions were prepared in DMSO with the final solvent concentration ≤1% DMSO (v/v), a concentration without effect on cell replication. The cells were cultured at 37 °C in RPMI-1640 supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum, and 100 μg/mL kanamycin in a humidified atmosphere containing 5% CO_2.

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