Synthesis and Structure−Activity Relationship Study of Lamellarin Derivatives

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Ten derivatives (**3**−**12**) of marine alkaloid lamellarin D (**1**) were synthesized and evaluated for cytotoxicity against a HeLa cell line in an effort to examine their structure−activity relationships. It appeared that the hydroxyl groups at positions C-8 and C-20 of **1** were important structural requirements for cytotoxic activity, while the hydroxyl group at C-14 and the two methoxy groups at C-13 and C-21 were not necessary for the activity.

Lamellarins are a group of polycyclic pyrrole alkaloids that were first isolated from the marine prosobranch mollusc *Lamellaria* sp. in 1985.[1] Since that time, over 30 compounds belonging to this group have been isolated from ascidians.[2−7] Early findings on the biological activities of lamellarins included inhibition of cell division of sea urchin egg,[1] cytotoxicity,[3,5] and immunomodulatory activity.[3] In recent years, two important findings on the biological activity of lamellarins have been reported. First, some lamellarins showed equally good antitumor activity against both multidrug-resistant (MDR) and their corresponding parental cell lines.[8] It was also revealed that lamellarin I reverted multidrug-resistance (MDR) at nontoxic doses by inhibiting P-glycoprotein (P-gp)-mediated drug efflux and that the potency of lamellarin I as an MDR modulator was 9- to 16-fold higher than that of verapamil.[8] Second, the newly isolated lamellarin α 20-sulfate was reported to inhibit HIV-1 integrase in vitro, with IC_{50} values of 16 μM for terminal cleavage activity and 22 μM for strand transfer activity.[7] The same compound was also shown to inhibit replication of the HIV-1 virus in cell cultures (IC_{50}, 8 μM).[7] In this paper, we report on the synthesis and cytotoxicity of 10 derivatives (**3**−**12**) of lamellarin D (**1**) with varying substituents on the aromatic rings and a study of their structure−activity relationships.

Results and Discussion

A synthetic route to the lamellarins of the 5,6-dehydro type by assembly of benzylisoquinoline and 2-methoxymethoxybenzoate was developed earlier in the total synthesis of lamellarins D (**1**) and H (**2**).[9] This method is useful for the synthesis of lamellarin analogues since a variety of compounds can be obtained by the appropriate combination of readily available reagents including benzylisoquiolines and benzoates. The synthesis of lamellarin derivatives is outlined in Scheme 1. Condensation of benzylisoquinolines **13**−**16**, prepared by a standard procedure,[10] with benzoates **17**−**21** in the presence of lithium diisopropylamide (LDA) afforded adducts **22**−**29**. *N*-Alkylation of these compounds with ethyl bromoacetate followed by methanolysis of the methoxymethyl (MOM) protecting group yielded the corresponding quaternary ammonium salts **30**−**37**. The lamellarin ring system (6*H*[1]benzopyrano[4′,3′:4′,5]pyrrolo[2,1-*a*]isoquinolin-6-one) was constructed by *N*-ylide-mediated pyrrole ring formation[11] and subsequent lactonization. Thus, additions of excess triethylamine to methanol solutions of **30**−**37** formed deep purple solutions of the corresponding *N*-ylides, which, on refluxing, underwent dehydrative cyclization with simultaneous aromatization and lactonization to yield compounds **11**, **12**, and **38**−**43**. Finally, lamellarin derivatives **3**−**8** were obtained by hydrogenolysis of the corresponding benzyl ethers over Pearlman's catalyst.[12] The tetrahydroxy derivative **9** was synthesized from **11** by cleavage of the methyl ethers using boron tribromide.

The cytotoxicities of **1** and **11** were measured in terms of the inhibition of colony formation (the colony assay) using two normal (Vero and MDCK) and two tumor (HeLa and XC) cell lines. IC_{50} values of **1** and **11** estimated from dose−response curves are shown in Table 1. Although no selectivity was observed with these cell lines, **1** showed quite high activity with IC_{50} values of 10−23 nM, comparable to that of mitomycin C. We also tested the cytotoxicity of **1** by the MTT tetrazolium cytotoxic assay in MCDK cells; however, it was found to be completely inactive, even at a concentration of 1 mM (data not shown).

The cytotoxicity of lamellarin derivatives evaluated by the colony assay in HeLa cells is shown in Table 2. Most derivatives possessing hydroxyl groups at both the C-8 and C-20 positions (**1**, **3**, **6**, **7**) showed quite high activity, with IC_{50} values of 10.5−70.0 nM. The low toxicity of **2** might be partly due to its low solubility in the assay medium. The hydroxyl functional group at C-20 appears to be an essential prerequisite for activity since the activity of **4** (IC_{50}, 0.85 μM), which lacks the hydroxyl group, decreased markedly compared with that of **1** (IC_{50}, 1.05×10^{-3} μM). The importance of the 20-hydroxyl group for bioactivity is also apparent when comparing the activity of **5** (IC_{50}, 2.5 μM) with those of **11** (IC_{50}, 5.7 μM) and **12** (IC_{50}, >100 μM). The hydroxyl group at C-8 might also be important for the activity since methylation of both hydroxyl groups at C-8 and C-14 of **1** as **5** leads to severe decrease in activity, whereas compound **7**, which has the 8-hydroxy group but lacks the 14-hydroxyl group of **1**, still maintains high activity. The presence of a hydroxyl group at C-14 and methoxy groups at C-13 and C-21 appears not to affect the activity since the 14-dehydroxy, 13-demethoxy, and 21-demethoxy derivatives (**7**, **6**, and **3**, respectively) displayed only a slight decrease in activity relative to their parent compound.

In conclusion, this study provided basic information regarding the structure−activity relationship of lamellarin D (**1**). The presence of hydroxyl groups at the C-8 and C-20 positions of **1** appears to be essential for cytotoxicity, while

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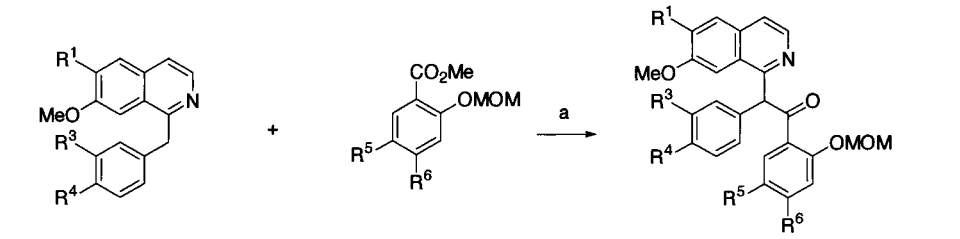
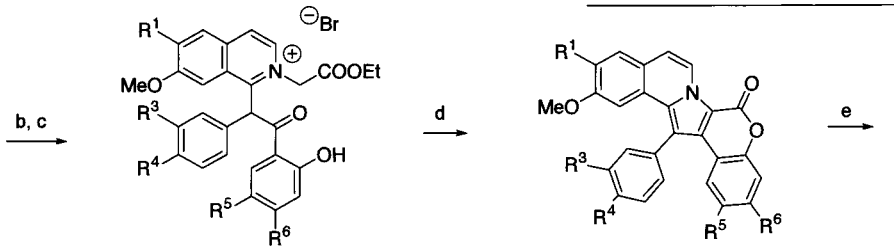
Scheme 1. Synthesis of Lamellarin Derivatives[a]



13: R^1 = OBn, R^3 = OMe, R^4 = OBn
14: R^1 = OBn, R^3 = H, R^4 = OBn
15: R^1 = OBn, R^3 = OMe, R^4 = H
16: R^1 = OMe, R^2 = OMe, R^4 = OMe

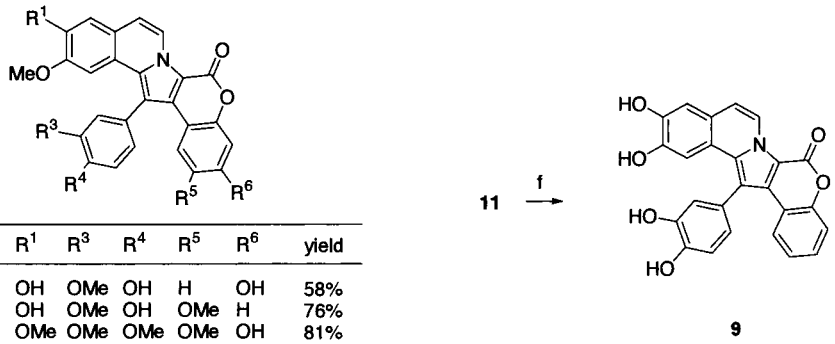
17: R^5 = OMe, R^6 = OBn
18: R^5 = H, R^6 = H
19: R^5 = H, R^6 = OBn
20: R^5 = OMe, R^6 = H
21: R^5,R^6 = -OCH_2O-

	R^1	R^3	R^4	R^5	R^6	yield
22	OBn	OMe	OBn	H	OBn	35%
23	OBn	OMe	OBn	OMe	H	54%
24	OMe	OMe	OMe	OMe	OBn	50%
25	OMe	OMe	OMe	H	H	71%
26	OMe	OMe	OMe	-OCH_2O-		56%
27	OBn	H	OBn	OMe	OBn	12%
28	OBn	OMe	H	OMe	OBn	19%
29	OBn	OMe	OBn	H	H	48%



	R^1	R^3	R^4	R^5	R^6
30	OBn	OMe	OBn	H	OBn
31	OBn	OMe	OBn	OMe	H
32	OMe	OMe	OMe	OMe	OBn
33	OMe	OMe	OMe	H	H
34	OMe	OMe	OMe	-OCH_2O-	
35	OBn	H	OBn	OMe	OBn
36	OBn	OMe	H	OMe	OBn
37	OBn	OMe	OBn	H	H

	R^1	R^3	R^4	R^5	R^6	yield[b]
38	OBn	OMe	OBn	H	OBn	27%
39	OBn	OMe	OBn	OMe	H	33%
40	OMe	OMe	OMe	OMe	OBn	28%
11	OMe	OMe	OMe	H	H	33%
12	OMe	OMe	OMe	-OCH_2O-		28%
41	OBn	H	OBn	OMe	OBn	58%
42	OBn	OMe	H	OMe	OBn	17%
43	OBn	OMe	OBn	H	H	15%



	R^1	R^3	R^4	R^5	R^6	yield
3	OH	OMe	OH	H	OH	58%
4	OH	OMe	OH	OMe	H	76%
5	OMe	OMe	OMe	OMe	OH	81%
6	OH	H	OH	OMe	OH	75%
7	OH	OMe	H	OMe	OH	72%
8	OH	OMe	OH	H	H	50%

[a] Reagents and conditions: (a) LDA, THF, −78 °C; (b) $BrCH_2COOEt$, 70 °C; (c) cat. HCl, MeOH, reflux; (d) Et_3N, MeOH, reflux; (e) H_2, 10% $Pd(OH)_2$−C, EtOAc, rt, 1 atm; (f) BBr_3, CH_2Cl_2, −78 °C then rt, 84% yield. [b]Overall yields from compound **22**−**29**, respectively.

Table 1. Cytotoxicities of Lamellarin D (**1**) and Compound **11** against Several Cell Lines, IC_{50} (nM)

compound	HeLa	XC	Vero	MDCK
lamellarin D (**1**)	10.5	12.4	10.5	22.5
11	5700	5600	4700	5600
mitomycin C	68.0	ND[a]	ND	ND

[a] ND: not determined.

the hydroxyl group at C-14 and the two methoxy groups at C-13 and C-21 appear to be less important.

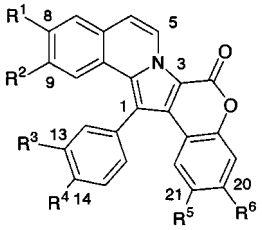
Experimental Section

General Experimental Procedures. Melting points (mp) were uncorrected. ^1H NMR spectra were recorded on Varian Gemini 200, Gemini 300, and UNITY plus 500 instruments at nearly 200, 300, and 500 MHz, respectively. EIMS and HREIMS were recorded on a JEOL JMS-DX303 spectrometer. Tetrahydrofuran (THF) was distilled from sodium metal/benzophenone ketyl under an atmosphere of dry nitrogen prior to use.

Benzylisoquinolines 13−16. Benzylisoquinolines **13**−**16** were prepared via Bishler−Napieralski reactions of corresponding 2-methoxy-2-arylethylamides of aryl acetates (for a detailed procedure, see ref 9). Benzylisoquinoline **16**, papaverine, was obtained from Tokyo Chemical Industry Co. Ltd.

Benzoates 17−21. Benzoates **17**, **18**, **19**, **20**, and **21** were prepared by a standard methoxymethylation (MOMCl, NaH, THF) of methyl 4-benzyloxy-5-methoxy-2-hydroxybenzoate,[9]

Table 2. Cytotoxic Activity of Lamellarin Derivatives on HeLa Cells



compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM)
1	OH	OMe	OMe	OH	OMe	OH	0.0105
2	OH	OH	OH	OH	OH	OH	>100
3	OH	OMe	OMe	OH	H	OH	0.0395
4	OH	OMe	OMe	OH	OMe	H	0.8500
5	OMe	OMe	OMe	OMe	OMe	OH	2.5
6	OH	OMe	H	OH	OMe	OH	0.0380
7	OH	OMe	OMe	H	OMe	OH	0.0700
8	OH	OMe	OMe	OH	H	H	4.0
9	OH	OH	OH	OH	H	H	1.1
10	OAc	OAc	OAc	OAc	OAc	OAc	11.0
11	OMe	OMe	OMe	OMe	H	H	5.7
12	OMe	OMe	OMe	OMe	$-OCH_2O-$		>100

methyl 2-hydroxybenzoate (Tokyo Chemical Industry Co. Ltd.), methyl 4-benzyloxy-2-hydroxybenzoate,[9] methyl 5-methoxy-2-hydroxybenzoate prepared by methylation (MeI, K_2CO_3, acetone) of methyl 2,5-dihydroxybenzoate (Aldrich Chemical Co.), and methyl 2-hydroxy-4,5-methylenedioxybenzoate obtained by MnO_2−KCN−MeOH oxidation of 2-hydroxy-4,5-methylenedioxybenzaldehyde,[13] respectively.

6-Benzyloxy-7-methoxy-1-[1-(4-benzyloxy-3-methoxyphenyl)-2-(4-benzyloxy-2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (22). To a solution of lithium diisopropylamide prepared from diisopropylamine (0.290 mL, 2.06 mmol) and a 1.57 M hexane solution of butyllithium (0.79 mL, 1.24 mmol) in dry THF (8 mL) at −10 °C for 20 min was added dropwise a solution of benzylisoquinoline **13** (405 mg, 0.824 mmol) in THF (10 mL) over 5 min at −78 °C under Ar. The solution was gradually warmed to −10 °C and kept at that temperature for 1 h. A solution of benzoate **19** (375 mg, 1.24 mmol) in THF (3 mL) was then added, and the mixture was stirred at −10 °C for 1 h before being quenched with 10% NH_4-Cl solution (20 mL). The mixture was extracted twice with EtOAc, dried (Na_2SO_4), and concentrated. The oily residue was chromatographed on silica gel eluted with hexanes−EtOAc (2:1−1:2), giving **22** (218 m g, 0.287 mmol) in 35% yield as an yellow amorphous powder: ^{1}H NMR (200 MHz) δ 2.72 (3H, s, OCH_3), 3.76 (3H, s, $ArOCH_3$), 3.84 (3H, s, $ArOCH_3$), 4.48 (1H, d, J = 6.9 Hz, CH_3OCH-O), 4.55 (1H, d, J = 6.9 Hz, CH_3OCH-O), 5.03 (2H, s, $PhCH_2O$), 5.09 (2H, s, $PhCH_2O$), 5.26 (2H, s, $PhCH_2O$), 6.62 (1H, s, H-1′), 6.64 (1H, s, H-5), 6.74−6.88 (3H, m, H-2″, H-5″, H-6″), 7.05 (1H, s, H-8), 7.20−7.50 (18H, m, C_6H_5 × 3, H-4, H-3‴, H-5‴), 8.11 (1H, d, J = 8.4 Hz, H-6‴), 8.23 (1H, d, J = 5.5 Hz, H-3); *anal.* C 75.71%, H 5.89%, N 1.95%, calcd for $C_{48}H_{43}NO_8$, C 75.67%, H 5.69%, N 1.84%.

6-Benzyloxy-7-methoxy-1-[1-(4-benzyloxy-3-methoxyphenyl)-2-(5-methoxy-2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (23). The title compound was synthesized from **13** and **20** in 54% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (300 MHz) δ 2.85 (3H, s, OCH_3), 3.77 (3H, s, $ArOCH_3$), 3.78 (3H, s, $ArOCH_3$), 3.85 (3H, s, $ArOCH_3$), 4.52 (2H, s, OCH_2OCH_3), 5.11 (2H, s, $PhCH_2O$), 5.26 (2H, s, $PhCH_2O$), 6.68 (1H, s, H-1′), 6.81 (1H, s, H-5), 6.82−6.89 (3H, m, H-2″, H-5″, H-6″), 7.05 (1H, s, H-8), 7.24−7.48 (12H, m, C_6H_5 x 2, H-4, H-3‴, H-4‴), 7.58 (1H, d, J = 2.8 Hz, H-6‴), 8.23 (1H, d, J = 5.3 Hz, H-3). *anal.* C 73.51%, H 5.84%, N 2.16%, calcd for $C_{42}H_{39}NO_8$, C 73.56%, H 5.73%, N 2.04%.

6,7-Dimethoxy-1-[1-(3,4-dimethoxyphenyl)-2-(4-benzyloxy-5-methoxy-2-methoxymethoxyphenyl)-2-oxoethyl]-isoquinoline (24). The title compound was synthesized from **16** and **17** in 50% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (300 MHz) δ 2.73 (3H, s, OCH_3), 3.75 (3H, s, $ArOCH_3$), 3.82 (3H, s, $ArOCH_3$), 3.87 (6H, s, $ArOCH_3$ × 2), 4.00 (3H, s, $ArOCH_3$), 4.28 (1H, d, J = 7.7 Hz, CH_3OCH-O), 4.33 (1H, d, J = 7.7 Hz, CH_3OCH-O), 5.10 (1H, d, J = 11.0 Hz, $PhCH$-O−), 5.13 (1H, d, J = 11.0 Hz, $PhCH$-O−), 6.63 (1H, s, H-1′), 6.67 (1H, s, H-5), 6.77−6.88 (3H, m, H-2″, H-5″, H-6″), 7.03 (1H, s, H-8), 7.25−7.42 (7H, m, C_6H_5-CH_2O, H-4, H-3‴), 7.73 (1H, s, H-6‴), 8.26 (1H, d, J = 7.6 Hz, H-3); *anal.* C 68.99%, H 5.89%, N 2.15%, calcd for $C_{37}H_{37}NO_9$, C 69.47%, H 5.83%, N 2.19%.

6,7-Dimethoxy-1-[1-(3,4-dimethoxyphenyl)-2-(2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (25). The title compound was synthesized from **16** and **18** in 71% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (500 MHz) δ 2.92 (3H, s, OCH_3), 3.76 (3H, s, $ArOCH_3$), 3.83 (3H, s, $ArOCH_3$), 3.88 (3H, s, $ArOCH_3$), 3.99 (3H, s, $ArOCH_3$), 4.64 (1H, d, J = 6.9 Hz, CH_3OCH-O), 4.66 (1H, d, J = 6.9 Hz, CH_3OCH-O), 6.68 (1H, s, H-1′), 6.80 (1H, d, J = 8.0 Hz, H-5″), 6.83−6.90 (2H, m, H-2″, H-6″), 7.02−7.06 (2H, m, H-3‴, H-5‴), 7.03 (1H, s, H-5), 7.33−7.37 (1H, m, H-4‴), 7.35 (1H, d, J = 5.7 Hz, H-4), 7.39 (1H, s, H-8), 8.02 (1H, dd, J = 7.8, 1.6 Hz, H-6‴), 8.26 (1H, d, J = 5.7 Hz, H-3); *anal.* C 68.96%, H 5.92%, N 2.79%, calcd for $C_{29}H_{29}NO_7$, C 69.17%, H 5.81%, N 2.78%.

6,7-Dimethoxy-1-[1-(3,4-dimethoxyphenyl)-2-(2-methoxymethoxy-4,5-methylenedioxyphenyl)-2-oxo]ethylisoquinoline (26). The title compound was synthesized from **16** and **21** in 56% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (200 MHz) δ 2.79 (3H, s, OCH_3), 3.76 (3H, s, $ArOCH_3$), 3.82 (3H, s, $ArOCH_3$), 3.89 (3H, s, $ArOCH_3$), 4.00 (3H, s, $ArOCH_3$), 4.43 (2H, s, CH_3OCH_2-O), 5.96 (2H, s, OCH_2O), 6.62 (1H, s, H-1′), 6.63 (1H, s, H-3‴), 6.76−6.90 (3H, m, H-2″, H-5″, H-6″), 7.03 (1H, s, H-5), 7.32−7.40 (2H, m, H-4, H-6‴), 7.59 (1H, s, H-8), 8.27 (1H, d, J = 5.6 Hz, H-3); *anal.* C 65.38%, H 5.37%, N 2.75%, calcd for $C_{30}H_{29}NO_8$, C 65.81%, H 5.34%, N 2.56%.

6-Benzyloxy-7-methoxy-1-[1-(4-benzyloxyphenyl)-2-(4-benzyloxy-5-methoxy-2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (27). The title compound was synthesized from **14** and **17** in 12% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (200 MHz) δ 2.68 (3H, s, OCH_3), 3.86 (3H, s, $ArOCH_3$), 3.81 (3H, s, $ArOCH_3$), 4.31 (2H, s, $PhCH_2O$), 4.99 (2H, s, CH_3OCH_2O), 5.11 (2H, s, $PhCH_2O$), 5.26 (2H, s, $PhCH_2O$), 6.63 (1H, s, H-1′), 6.66 (1H, s, H-5), 6.90 (2H, d, J = 8.7 Hz, H-3″, H-5″), 7.04 (1H, s, H-6‴), 7.21 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.20−7.50 (18H, m, C_6H_5 × 3, H-4, H-8, H-3‴), 8.27 (1H, d, J = 5.6 Hz, H-3); *anal.* C 75.73%, H 5.68%, N 2.03%, calcd for $C_{48}H_{43}NO_8$, C 75.67%, H 5.69%, N 1.84%.

6-Benzyloxy-7-methoxy-1-[1-(3-methoxyphenyl)-2-(4-benzyloxy-5-methoxy-2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (28). The title compound was synthesized from **15** and **17** in 19% yield by the same procedure as that for **22**: yellow amorphous powder; ^{1}H NMR (300 MHz) δ 2.96 (3H, s, OCH_3), 3.71 (3H, s, $ArOCH_3$), 3.87 (3H, d, $ArOCH_3$), 3.88 (3H, d, ArOCH3), 4.32 (2H, s, CH_3OCH_2O), 5.13 (2H, s, $PhCH_2O$), 5.27 (2H, s, $PhCH_2O$), 6.66 (1H, s, H-1′), 6.68 (1H, s, H-5), 6.73−6.90 (3H, m, H-2″, H-4″, H-6″), 7.20−7.50 (14H, C_6H_5 × 2, H-4, H-5″, H-3‴, H-6‴), 7.73 (1H, s, H-8), 8.24 (1H, d, J = 5.7 Hz, H-3).

6-Benzyloxy-7-methoxy-1-[1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxymethoxyphenyl)-2-oxoethyl]isoquinoline (29). The title compound was synthesized from **13** and **18** in 48% yield by the same procedure as that for **22**: pale crystals; mp 147−150 °C; ^{1}H NMR (300 MHz) δ 2.86 (3H, s, OCH_3), 3.77 (3H, s, $ArOCH_3$), 3.86 (3H, s, $ArOCH_3$), 4.62 (1H, d, J = 7.5 Hz, CH_3OCH-O), 4.68 (1H, d, J = 7.5 Hz, CH_3OCH-O), 5.11 (2H, s, $PhCH_2O$), 5.26 (2H, s, $PhCH_2O$), 6.66 (1H, s, H-1′), 6.81 (1H, s, H-5), 6.84 (1H, d, J = 14 Hz, H-5″), 6.95−7.08 (2H, m, H-3‴, H-5‴), 7.05 (1H, s, H-8), 7.24−7.49 (14H, m, C_6H_5 × 2, H-4, H-2″, H-6″, H-4‴), 8.02 (1H, dd, J = 7.9, 2.0 Hz, H-6‴), 8.23 (1H, d, J = 5.6 Hz, H-3); *anal.* C 74.64%, H 5.84%, N 5.01%, calcd for $C_{41}H_{37}NO_7$, C 75.10%, H 5.69%, N 2.14%.

3,11-Bis(benzyloxy)-12-methoxy-14-(4-benzyloxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (38). A mixture of **22** (201.7 mg, 0.2647 mmol) was heated together with ethyl bromoacetate (1 mL) at 70 °C for 22 h under Ar. The mixture was poured into dry ether, and the precipitated quaternary ammonium salt was collected by filtration. The salt was dissolved in MeOH (20 mL) containing 0.2 mL of concentrated HCl and heated under reflux for 30 min. After the mixture had been cooled to room temperature, Et_3N (1.0 mL) was added and the resulting deep purple solution was heated under reflux for 2 h, at which time white crystals of **38** appeared. The mixture was then concentrated, and the residue was chromatographed on silica gel eluted with CH_2Cl_2−EtOAc (1:1) to give **38** (53.5 mg, 0.0723 mmol) in 27% yield as white crystals: mp 227−228 °C; ^{1}H NMR (300 MHz) δ 3.31 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.09 (2H, s, $PhCH_2O$), 5.24 (2H, s, $PhCH_2O$), 5.28 (1H, d, J = 12.6 Hz, PhC*H*-O), 5.38 (1H, d, J = 12.6 Hz, PhC*H*-O), 6.71 (1H, dd, J = 2.6, 8.8 Hz, H-6′), 6.97 (1H, d, J = 7.4 Hz, H-1), 7.01 (1H, d, J = 2.6 Hz, H-2′), 7.05 (1H, s, H-10), 7.08 (1H, s, H-13), 7.15 (1H, dd, J = 6.3, 8.9 Hz, H-5), 7.30−7.55 (18H, m, C_6H_5 × 3, H-4, H-9, H-5′), 9.19 (1H, d, J = 7.4 Hz, H-8); EIMS *m*/*z* 739 (M^+, 1.7), 648 (1.1), 557 (4.1), 483 (1.9), 453 (2.6), 354 (4.1), 278 (3.1), 105 (2.4), 91 (7.2), 69 (3.8), 57(4.5), 55 (4.1) 44 (100), 43 (9.0); HREIMS *m*/*z* 739.2620 (calcd for $C_{48}H_{37}NO_7$, 739.2570).

11-(Benzyloxy)-2,12-dimethoxy-14-(4-benzyloxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (39): 33% yield from **23**; white crystals; mp 239−241 °C; ^{1}H NMR (300 MHz) δ 3.35 (3H, s, OCH_3), 3.44 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.23 (1H, d, J = 3.8 Hz, PhC*H*-O), 5.24 (2H, s, $PhCH_2O$), 5.32 (1H, d, J = 3.8 Hz, PhC*H*-O), 6.77 (1H, d, J = 3.0 Hz, H-1), 6.91 (1H, dd, J = 3.0, 7.4 Hz, H-3), 7.50 (1H, d, J = 7.4 Hz, H-4), 7.10−7.20 (3H, m, H-2′, H-5′, H-6′), 7.13 (1H, s, H-10), 7.250−7.65 (12H, m, C_6H_5 × 2, H-9, H-13), 9.24 (1H, d, J = 7.6 Hz, H-8); EIMS *m*/*z* 664 [(M + 1)$^+$, 9], 663 (M^+, 19), 572 (12), 481 (5), 354 (8), 272 (9), 207 (5), 149 (6), 91 (31), 58 (26), 44 (100), 43 (77); HREIMS *m*/*z* 663.2279 (calcd for $C_{42}H_{33}NO_7$, 663.2257).

3-Benzyloxy-14-(3,4-dimethoxyphenyl)-2,11,12-trimethoxy-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (40): 28% yield from **24**; white crystals; mp 253−254 °C; ^{1}H NMR (300 MHz) δ 3.48 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 5.04 (2H, s, $PhCH_2O$), 6.75 (1H, s, H-4), 6.81 (1H, s, H-1), 6.95−7.05 (3H, s, H-2′, H-5′, H-6′), 7.12−7.43 (8H, m, C_6H_5, H-9, H-10, H-13), 9.12 (1H, d, J = 8.2 Hz, H-8); *anal*. C 71.93%, H 5.30%, N 2.15%, calcd for $C_{37}H_{31}NO_8$, C 71.85%, H 5.06%, N 2.27%.

11,12-Dimethoxy-14-(3,4-dimethoxyphenyl)-6*H*[1]benzopyrano-[4′,3′;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (11): 33% yield from **25**; white crystals; mp 254−256 °C; ^{1}H NMR (300 MHz) δ 3.47 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 7.03−7.20 (6H, m, H-9, H-10, H-13, H-2″, H-5″, H-6″), 7.26−7.46 (4H, m, H-1, H-2, H-3, H-4), 9.28 (d, 1H, J = 7.4 Hz, H-8); EI-MS *m*/*z* 482 [(M + 1)$^+$, 33%], 481 (M^+, 100%); *anal*. C 71.59%, H 4.82%, N 2.93%, calcd for $C_{29}H_{23}NO_6$, C 72.34%, H 4.81%, N 2.91%.

11,12-Dimethoxy-14-(3,4-dimethoxyphenyl)-2,3-methylenedioxy-6*H*[1]benzopyrano[4′,3′;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (12): 28% yield from **26**; white crystals; mp 267−268 °C; ^{1}H NMR (200 MHz) δ 3.45 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 5.97 (2H, s, OCH_2O), 6.69 (1H, s, H-4), 6.94−7.15 (7H, m, H-1, H-9, H-10, H-13, H-2′, H-5′, H-6′), 9.24 (1H, d, J = 7.24, H-8); *anal*. C 67.88%, H 4.44%, N 2.68%, calcd for $C_{30}H_{23}NO_8$, C 68.57%, H 4.41%, N 2.67%.

3,11-Bis(benzyloxy)-2,12-dimethoxy-14-(4-benzyloxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (41): 58% yield from **27**; white crystals; mp 198 °C; ^{1}H NMR (200 MHz) δ 3.37 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 5.19 (2H, s, $PhCH_2O$), 5.23 (2H, s, $PhCH_2O$), 5.25 (2H, s, $PhCH_2O$), 6.68 (1H, s, H-4), 6.94 (1H, s, H-1), 6.96 (2H, d, J = 7.0 Hz, H-3, H-5), 7.10 (2H, d, J = 7.0 Hz, H-2, H-6), 7.40−7.57 (18H, m, C_6H_5 × 3, H-9, H-10, H-13), 9.18 (1H, d, J = 7.2 Hz, H-8); *anal*. C 78.23%, H 5.15%, N 2.01%, calcd for $C_{48}H_{37}NO_7$, C 77.93%, H 5.04%, N 1.89%.

3,11-Bis(benzyloxy)-2,12-dimethoxy-14-(3-methoxyphenyl)-6*H*[1]-benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (42): 17% yield from **28**; white crystals; mp 270−271 °C; ^{1}H NMR (300 MHz) δ 3.44 (3H, s, OCH_3), 3.46, 3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.20 (2H, s, $PhCH_2O$), 5.26 (2H, s, $PhCH_2O$), 6.72 (1H, s, H-4), 6.95 (1H, s, H-1), 6.99 (1H, dd, J = 7.5, 0.7 Hz, H-4′), 7.11 (1H, s, H-10), 7.09−7.18 (3H, m, H-13, H-2′, H-6′), 7.22−7.48 (11H, m, C_6H_5 × 2, H-9, H-10), 7.57 (1H, t, J = 8.1 Hz, H-5′), 9.20 (1H, d, J = 7.2 Hz, H-8); EIMS *m*/*z* 663 (M^+, 10), 572 (9), 105 (28), 91 (31), 77 (18), 69 (10), 57 (11), 55 (14), 51 (8), 44 (100), 43 (21); HREIMS *m*/*z* 663.2220 (calcd for $C_{42}H_{33}NO_7$, 663.2257).

11-(Benzyloxy)-12-methoxy-14-(4-benzyloxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (43): 15% yield from **29**; white crystals; mp 223−225 °C; ^{1}H NMR (300 MHz) δ 3.32 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.23 (2H, s, $PhCH_2O$), 5.28 (1H, d, J = 12.5 Hz, PhC*H*-O), 5.39 (1H, d, J = 12.5 Hz, PhC*H*-O), 6.97 (1H, d, J = 7.5 Hz, H-5′), 7.02−7.18 (7H, m, Ar*H*s), 7.25−7.58 (12H, m, Ar*H*s), 9.22 (1H, d, J = 7.3 Hz, H-8); *anal*. C 77.96%, H 5.14%, N 2.13%, calcd for $C_{41}H_{31}NO_6$, C 77.71%, H 4.93%, N, 2.21%.

Lamellarin D (1), Lamellarin H (2), and Hexaacetate of 2 (10). See ref 9.

3,11-Dihydroxy-12-methoxy-14-(4-benzyloxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (3). Compound **38** (48.5 mg, 0.0656 mmol) was hydrogenated over 20% $Pd(OH)_2$−C (20 mg) in EtOAc (80 mL) at room temperature at atmospheric pressure for 8 h. The mixture was then filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel eluted with hexanes−EtOAc (1:2) to give **3** (17.8 mg, 0.0379 mmol) in 58% yield as a light gray amorphous powder: ^{1}H NMR (300 MHz, acetone-d_6) δ 3.47 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.64 (1H, dd, J = 2.4, 8.7 Hz, H-6′), 6.86 (1H, d, J = 2.4 Hz, H-2′), 7.03−7.28 (7H, m, Ar*H*s), 8.01 (1H, br s, OH), 8.36 (1H, br s, OH), 8.98 (lH, br s, OH), 9.14 (1H, d, J = 7.4 Hz, H-8); EIMS *m*/*z* 469 (M^+, 1.2), 199 (2.5), 111 (1.6), 98 (2.5), 97 (2.8), 95 (2.1), 85 (2.4), 84 (2.1), 83 (3.4), 81 (2.2), 71 (3.4), 69 (5.6), 57 (6.6), 55 (5.4), 45 (3.3), 44 (100), 43 (7.6); HREIMS *m*/*z* 469.1174 (calcd for $C_{27}H_{19}NO_7$, 469.1162).

11-Hydroxy-2,12-dimethoxy-14-(4-hydroxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (4): 76% yield from **39**; light gray amorphous powder; ^{1}H NMR (300 MHz, acetone-d_6) δ 3.49 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.89 (1H, d, J = 3.0 Hz, H-1), 6.99 (1H, dd, J = 9.0, 3.0 Hz, H-3), 7.02−7.28 (6H, m, H-9, H-10, H-13 and 14-Ar*H*s), 8.02 (1H, s, OH), 8.37 (1H, s, OH), 9.19 (1H, d, J = 7.2 Hz); EIMS *m*/*z* 484 [(M + 1)$^+$, 28], 483 (M^+, 86), 86 (26), 84 (41), 58 (23), 49 (41), 44 (100), 43 (54), 42 (23); HREIMS *m*/*z* 483.1313 (calcd for $C_{28}H_{21}NO_7$, 483.1318).

3-Hydroxy-14-(3,4-dimethoxyphenyl)-2,11,12-trimethoxy-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-a]isoquinoline-6-one (5): 81% yield from **40**; pale yellow amorphous powder; ^{1}H NMR (300 MHz, acetone-d_6) δ 3.42 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.78 (1H, s, H-4), 6.88 (1H, s, H-1), 7.20 (1H, s, H-10), 7.20−7.35 (4H, m, 14-Ar*H*s and H-9), 7.36 (1H, s, H-13), 8.25 (1H, br s, OH), 9.16 (1H, d, J = 7.4 Hz, H-8); *anal*. C 68.00%, H 5.09%, N 2.39%, calcd for $C_{30}H_{25}NO_8$, C 68.30%, H 4.78%, N 2.66%.

3,11-(Dihydroxy)-2,12-dimethoxy-14-(4-hydroxyphenyl)-6*H*[1]benzopyrano-[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (6): 75% yield from **41**; light gray amorphous powder; ^{1}H NMR (200 MHz, acetone-d_6) δ 3.48 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 6.79 (1H, s, H-4), 6.88 (1H, s, H-1), 7.17 (1H, d, J = 7.4 Hz, H-9), 7.20 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 7.22 (1H, s, H-10), 7.25 (1H, s, H-13), 7.49 (2H, d, J = 8.6 Hz, H-2′ and H-6′), 8.26 (1H, br s, OH), 8.35 (1H, br s, OH), 8.76 (1H, br s, OH), 9.13 (1H, d, J = 7.4 Hz, H-8); EIMS *m*/*z* 470 [(M + 1)$^+$, 7], 469 (M^+, 20), 97 (8), 83 (9), 78 (48), 73 (8), 71 (9), 69 (12), 63 (54), 44 (100); HREIMS *m*/*z* 469.1158 (calcd for $C_{27}H_{19}NO_7$, 469.1162).

3,11-(Dihydroxy)-2,12-dimethoxy-14-(3-methoxyphenyl)-6*H*[1]-benzopyrano[4,3;4,5]pyrrolo[2,1-a]isoquinoline-6-one (7): 72% yield from **42**; light gray amorphous powder; ^{1}H NMR (200 MHz, acetone-d_6) δ 3.42. (6H, s, $OCH_3 \times 2$), 3.78 (3H, s, OCH_3), 5.72 (1H, br s, OH), 5.81 (1H, br s, OH), 6.60 (1H, s, H-4), 6.95 (1H, s, H-1), 6.90−7.16 (4H, m, 14-Ar*H*s), 7.03 (1H, s, H-10), 7.12 (1H, s, H-13), 7.48 (1H, d, J = 7.4 Hz, H-9), 9.15 (1H, d, J = 7.4 Hz, H-8); EIMS m/z 484 [$(M + 1)^+$, 24], 483 (M^+, 74), 264 (10), 256 (9), 236 (7), 129 (11), 111 (11), 97 (21), 83 (26), 81 (21), 69 (40), 57 (46), 55 (31), 44 (100), 43 (56), 41 (55); HREIMS m/z 483.1329 (calcd for $C_{28}H_{21}NO_7$, 483.1318).

11-Hydroxy-12-methoxy-14-(4-hydroxy-3-methoxyphenyl)-6*H*[1]benzopyrano[4,3;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (8): 50% yield from **43**; light gray crystals; mp 300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.38 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 6.95−7.50 (10H, m, Ar*H*s), 9.08 (1H, d, J = 7.5 Hz, H-8), 9.37 (1H, s,OH), 9.94 (1H, s, OH); EIMS m/z 454 [$(M + 1)^+$, 16], 453 (M^+, 41), 86 (17), 84 (26), 58 (13), 51 (12), 49 (30), 44 (100), 43 (43); HREIMS m/z 453.1218 (calcd for $C_{27}H_{19}NO_6$, 453.1213).

11,12-Dihydroxy-14-(3,4-dihydroxyphenyl)-6*H*[1]-benzopyrano-[4′,3′;4,5]pyrrolo[2,1-*a*]isoquinoline-6-one (9). To a solution of **11** (206 mg, 0.426 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise a solution of BBr_3 (0.162 mL, 1.71 mmol) in CH_2Cl_2 (5 mL) at −10 °C. The mixture was gradually warmed to room temperature and stirred for 4 h. The reaction was quenched with a 4.7 M NH_4OH solution (3 mL), and the aqueous layer was made acidic (pH = 5) with a 2 M HCl solution. The mixture was extracted three times with a mixture of EtOAc and THF (30 mL × 3), dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel eluted with hexane−THF (1:2) to give 213 mg of crude **9**, which was recrystallized from THF−CH_2Cl_2 to give **9** (152 mg, 0.358 mmol, 84%) as pale yellow crystals: mp not determined (over 300 °C); ^{1}H NMR (acetone-d_6, 300 MHz) δ 6.91 (1H, dd, J = 8.1, 1.8 Hz, H-6′), 7.04 (1H, d, J = 1.8 Hz, H-2′), 7.04−7.11 (2H, m, Ar*H*s), 7.12 (1H, d, J = 8.1 Hz, H-5′), 7.17 (1H, d, J = 7.5 Hz, H-9), 7.26 (1H, s, H-10), 7.29 (1H, s, H-13), 7.36−7.40 (2H, m, Ar*H*s), 8.24 (1H, br s, OH), 8.36 (1H, br s, OH), 8.67 (1H, br s, OH), 8.74 (1H, br s, OH), 9.13 (1H, d, J = 7.5 Hz, H-8). An analytically pure sample was obtained as its tetraacetate by a standard acetylation (Ac_2O, pyridine) followed by recrystallization from aqueous EtOH: *anal*. C 66.38%, H 4.25%, N 2.32%, calcd for $C_{33}H_{23}NO_{10}$, C 66.78%, H 3.91%, N, 2.32%.

Cytotoxicity Assay. The cytotoxicity of test compounds was determined by the inhibitory activity of colony formation of HeLa cells as described previously.[14] In brief, 100−150 cells per well in a 24-well plate were cultured with varying concentrations of each test compound in the growth medium (MEM containing 10% fetal calf serum) for 5−8 days at 37 °C under 5% CO_2/95% air. The number of colonies formed was counted after staining with 1% methylene blue in 50% methanol. Clusters of 40 or more cells were considered as colonies.

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References and Notes

(1) Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492−5495.
(2) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570−4574.
(3) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 489−501.
(4) Urban, S.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 711−713.
(5) Reddy, M. V. R.; Faulkner, D. J.; Venkateswarlu, Y.; Rao, M. R. *Tetrahedron* **1997**, *53*, 3457−3466.
(6) Davis, R. A.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 419−424.
(7) Reddy, M. V. R.; Rao, M. R.; Rhodes, D.; Hansen, M. S. T.; Rubins, K.; Bushman, F. D.; Venkateswarlu, Y.; Faulkner, D. J. *J. Med. Chem.* **1999**, *42*, 1901−1907.
(8) Quesada, A. R.; García Grávalos, M. D.; Fernández Puentes, J. L. *Br. J. Cancer* **1996**, *74*, 677−682.
(9) Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951−5962.
(10) For example: Chen, C.-H.; Soine, T. O.; Lee, K.-H. *J. Pharm. Sci.* **1971**, *60*, 1634−1638.
(11) Iwao, M.; Kuraishi, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 297−298.
(12) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663−1664.
(13) Fukui, K.; Nakayama, M. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 1321.
(14) Oda, T.; Tsuru, M.; Hatakeyama, T.; Nagatomo, H.; Muramatsu, T.; Yamasaki, N. *J. Biochem.* **1997**, *121*, 560−567.

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