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Antineoplastic Agents. 585. Isolation of *Bridelia ferruginea* Anticancer Podophyllotoxins and Synthesis of 4-Aza-podophyllotoxin Structural Modifications[1]

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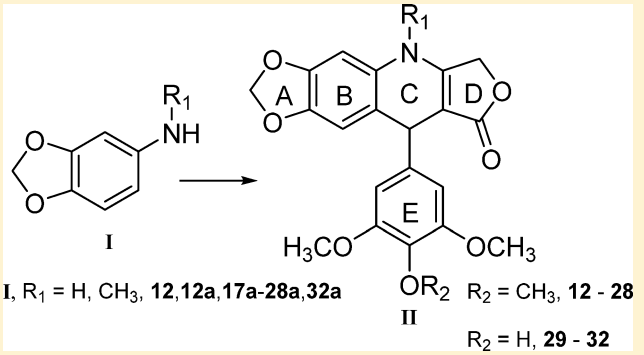
Supporting Information

ABSTRACT: Cytotoxic constituents of the terrestrial plant *Bridelia ferruginea* were isolated using bioactivity-guided fractionation, which revealed the presence of the previously known deoxypodophyllotoxin (**1**), isopicrodeoxypodophyllotoxin (**2**), *β*-peltatin (**3**), *β*-peltatin-5-*O*-*β*-D-glucopyranoside (**3a**), and the indole neoechinulin (**4**). As an extension of previous podophyllotoxin research, SAR studies were undertaken focused on 4-aza-podophyllotoxin structural modifications. A number of such derivatives were synthesized following modifications to the A and E rings. Such structural modifications with alkyl and 4-fluorobenzyl substituents at the 4-aza position provided the most potent cancer cell growth inhibitory activity (GI_{50} 0.1 to <0.03 μg/mL) against a panel of six human cancer cell lines and one murine cancer cell line. Several compounds corresponding to 4′-demethylated modifications were also synthesized and found to be significantly less potent.



The African subtropical medicinal plant *Bridelia ferruginea* Benth. (family Euphorbiaceae) has found use in African traditional medicine for the treatment of diarrhea, dysentery, female sterility, and rheumatic pains. The genus contains some 60 species. One of the early biological studies of this plant showed that crude extracts lowered the fasting blood sugar levels of rats and humans.[2] Later, more extensive studies reported in 1985[3] involving the chemical composition confirmed the presence of several already known terpenoids and flavonoids. This was followed by the discovery of compounds thought to be responsible for the medicinal effects of this plant such as xanthine oxidase inhibition, related to liver disorders, and superoxide-scavenging activity of phenolic compounds possibly related to the rheumatic pain relief[4] associated with the traditional use of *B. ferruginea*. The glucoside flavonoid rutin[5] was also discovered in 1989 and thought to be the source of the hypoglycemic effects.

As a continuation of our long-term research directed at discovery and development of new natural products with anticancer and other biological activities, we undertook an evaluation of the *B. ferruginea* cancer cell growth inhibitory constituents. Initial investigations were limited to only 3.24 g of a dichloromethane−methanol extract but later augmented by a 34.5 kg re-collection of the dry plant. Crude extracts of *B. ferruginea* displayed quite significant in vitro cancer cytostatic activity (ED_{50} 0.02 μg/mL) in the P388 murine lymphocytic leukemia cell line. Those results led us to begin an extensive P388 cell line bioassay-guided fractionation, which early in our research afforded three substantially active cancer cell growth inhibitors (Table 1), but proved to be all previously known compounds, namely, deoxypodophyllotoxin (**1**), *β*-peltatin-5-*O*-*β*-D-glucopyranoside (**3a**), and the indole neoechinulin (**4**).

While Boyd and colleagues[6] had already expertly reported the bioassay-guided fractionation of *B. ferruginea* extracts, the initial sample collections were limited to a relatively small (10−480 g)[6] amount of plant material. On the assumption that a large-scale collection would lead to the isolation of active constituents occurring at very low concentrations in this plant, we proceeded as summarized above with a re-collection of the dry plant. Although no new cancer cell growth inhibitors were found, the scale-up approach did provide isopicrodeoxypodophyllotoxin (**2**), which is a new constituent of *B. ferruginea*. The scale-up results were largely consistent with those reported by the Boyd group[6] in 2000, who summarized the isolation and

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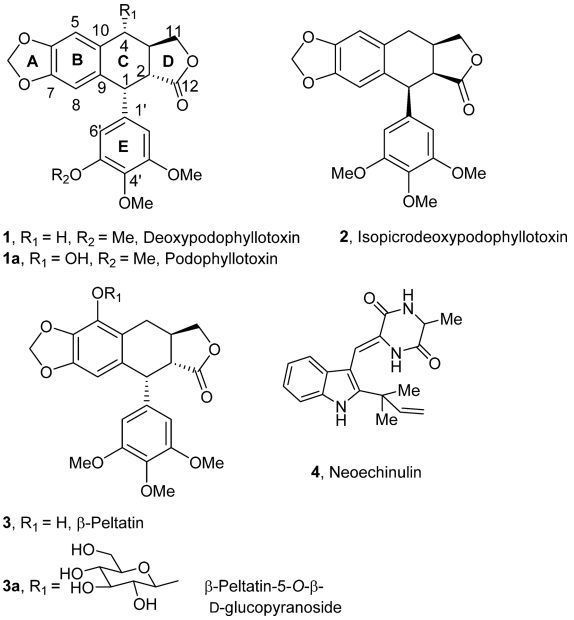
Table 1. Murine P388 Lymphocytic Leukemia Inhibitory Activity (ED_{50} μg/mL) and Human Cancer Cell Line Inhibitory Activity (GI_{50} μg/mL) of Isolated Compounds 1−4

	cell line[a]						
compound no.	P388	BXPC-3	MCF-7	SF268	NCI-H460	KM-20L2	DU-145
1	0.029	0.000 44	0.0027	0.000 56	0.0018	0.0014	0.0017
1a	0.0043	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
2	<0.1	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
3	0.0031	0.0012	0.000 55	0.000 40	0.000 77	0.0076	0.0021
3a	0.21	>1	0.85	0.27	0.28	>1	>1
4	0.21	0.25	0.24	0.21	0.21	0.19	0.27

[a]Cancer cell lines in order: murine lymphocytic leukemia (P388); pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145). [b]ND = not determined.



characterization of deoxypodophyllotoxin (**1**), β-peltatin (**3**), β-peltatin-5-*O*-β-D-glucopyranoside (**3a**), and 5′-demethoxy-β-peltatin-5-*O*-β-D-glucopyranoside. All of the structures of the cancer cell growth inhibitors we isolated from *B. ferruginea* were determined by employing HRMS, 1D NMR experiments (^{1}H, ^{13}C, and APT), and 2D NMR experiments (COSY, HMQC, and HMBC) and verified with literature data.[6−11]

When the U.S. NCI was established by the U.S. Congress in 1937, this prescient action was quickly followed by the rapid appointment of Dr. Jonathan L. Hartwell (the first organic chemist doctoral candidate of Professor L. Fieser at Harvard) among the first few NCI personnel.[12] By about 1955, the early research of Hartwell at the NCI had led to preclinical development of podophyllotoxin (**1a**) from the dried roots and rhizomes of certain *Podophyllum* species, especially the "Mayapple" (*Podophyllum peltatum*) used by the Penobscot Native Americans of Maine.[13−15] These pioneering advances led to the present well-known anticancer drugs and lead compounds ranging from etoposide (**5**) to tenoposide (**6**), **7**, **8**, **9**, and GL-331 (**10**).[16] Indeed, until the development of Taxol (paclitaxel), etoposide, on a worldwide basis, was the most widely prescribed anticancer drug. High-dose etoposide remains a useful drug in combination with other small-molecule anticancer drugs for the treatment of refractory Hodgkin's lymphoma, non-Hodgkin's lymphoma, acute leukemia, and other refractory hematological-type cancers.[17]

The need for producing podophyllotoxin (**1a**)-derived anticancer drugs has stimulated the exploration of other higher plant sources,[18] a number of total synthetic approaches including a recent 12-step route (29% overall),[19a,b] and a microbiological approach utilizing two endophyte fungi from rhizomes of *P. peltatum* that, interestingly, provided podophyllotoxin (**1a**), albeit in low yield.[16] In parallel, a large number of attempts at successful (simple) structural modifications of **1a** have been ongoing over the past nearly 55 years, where some of the most recent appear in ref 20 directed at anticancer,[20a−i] anti-insect,[20j−l] antiparasitic,[20k] DNA damaging,[20l−o] and vascular-disrupting[20p] targets. Two of the earliest arose when our group began to modify the **1a** aromatic system beginning in 1958.[21]

The results of some recent[20p] biological experiments are close to our vascular-disrupting discovery of the combretastatin-type stilbenes and benzophenones, where combretastatins A-4 and A-1 as the phosphate prodrugs are now in advanced human cancer clinical trials.[22] Such considerations combined with the potent (cf., Table 1) cancer cell growth inhibitory activity observed with some of the *B. ferruginea* constituents in our laboratory suggested we should return to our very early objective to discover clinically useful but more readily available structural modifications of the naturally occurring podophyllotoxin lignans. Also, evidence has been reported[23] that the topoisomerase mechanism of action by etoposide does not involve interaction of this enzyme with the glycoside unit, again pointing to the aromatic ring system.

RESULTS AND DISCUSSION

After isolation of the podophyllotoxin analogues **1**−**3** and **3a** from *B. ferruginea*, we shifted our focus to an SAR study of synthetically derived 4-aza-2,3-didehydropodophyllotoxins using the one-step procedure developed by Giorgi-Renault et al.[24a,b] (Scheme 1 and Figure 1). Initially, modifications of the aromatic rings were followed by substitutions at the dihydroquinoline nitrogen. When it became known early in our SAR study that the Giorgi-Renault[24] and Takeya[25] groups were advancing with aromatic ring modifications, we then concentrated on side-chain extensions from the 4-aza position. Since the Giorgi-Renault group first reported the multicomponent reaction (MCR) strategy and several analogues of this type, there has been a large increase in the synthesis of 4-azapodophylotoxins.[20p,24a,b,26] Furthermore, all of the clinically useful podophyllotoxin-derived anticancer drugs (**5** and **6**) and those that have been in advanced clinical trials (GP-11 (**7**),[27] NK-611 (**8**),[28] TOP-53 (**9**),[29] and GL-311 (**10**)[30]) have been substituted at the podophyllotoxin C-4 position.

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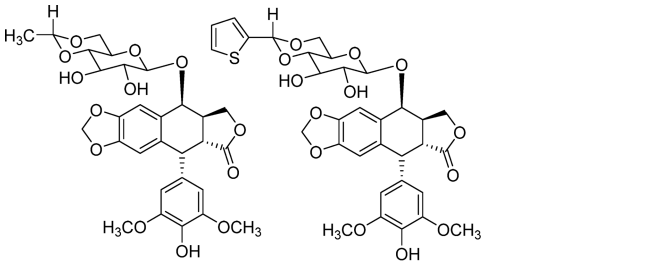
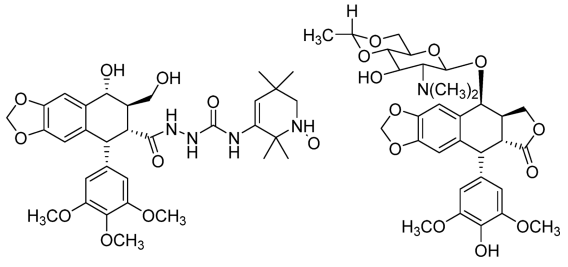
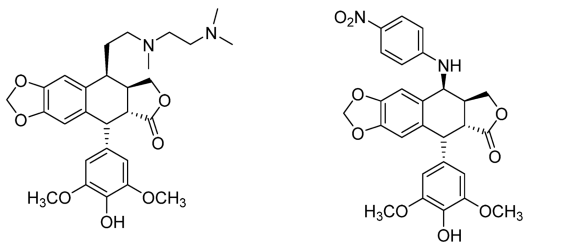


5, Etoposide 6, Tenoposide



7, GP-11 8, NK-611



9, TOP-53 10, GL-331

Initial attempts to directly add substitutions to the N-4-position of the 4-aza-2,3-didehydro-4-deoxypodophyllotoxin skeleton were unsuccessful, a result confirmed by the Giorgi-Renault study. Presumably, this was due to the inactivity of the 4-nitrogen toward electrophilic attack. Indeed, the only reaction explored that allowed the 4-aza-substitution required deprotonation of the nitrogen using butyllithium and subsequent alkylation with iodomethane to yield the *N*-methylated product.

Giorgi-Renault's short synthesis can also be utilized with substituted secondary anilines to yield the N-substituted derivatives, albeit in lower yields. N-Derivatives of 3,4-methylenedioxyaniline were an obvious starting point (Scheme 2), and, for example, 4-aza-alkyl derivatives **13**[31] and **14** were made utilizing this route (Figure 2).

The prealkylated aniline route was successful in producing 4-*N*-alkyl derivatives. However, it was hindered by the necessity of reducing the amide intermediates from acid chlorides or imides from aldehydes and its reliance on the availability of suitable reagents. Reduction of the intermediates (cf., **11** and **11a**) often led to complex mixtures of products from incomplete reduction of both the amide and/or ester groups. Therefore, a new procedure was needed that would simplify reaching the SAR objectives.

The 4-aza substituent syntheses were improved significantly by utilizing phenylboronic acids to arylate amines in the presence of cupric acetate and a tertiary amine base such as triethylamine or pyridine[32] (Scheme 3, path A). The procedure was found useful with a wide variety of reactants. Also, many primary amines and boronic acids are commercially available that can produce a very diverse structural pool of arylated secondary amines. The original report[32] summarizes a variety of substrates that will react by this procedure to form arylamines in yields ranging from 4% to nearly quantitative, with the majority of the reactions providing yields in the 50−60% range. Several side reactions were also experienced leading to phenols and biaryl ethers that resulted from the boronic acid reacting with water, either present in solution or generated in situ by triphenylboroxine formation.[32] The overall reaction procedure chosen for the present investigation is believed to proceed[33,34] by path A instead of the more scope limited variation of path B (Scheme 3). Experimentally, path A has been summarized in Scheme 4.

Employing the synthetic methods outlined in Schemes 2 and 4, a series of 4-aza modifications were synthesized, and their cancer cell growth inhibitory properties were assessed using the murine P388 lymphocytic leukemia and a panel of six human cancer cell lines. From the results recorded in Tables 1 and 2, some structure/activity trends are evident. Podophyllotoxin derivatives with alkyl groups in the 4-position revealed the most potent cancer cell growth inhibitory activity (see substances **16**[24] and **17**−**19**) and were found to be selective for several cell lines, especially breast and CNS. Short linkages between the aza-podophyllotoxin core and aromatic substituents seem to be ideal, as evident in the order of magnitude increase in cytostatic activity of **23** over **25**. Several 4′-demethylated compounds were also synthesized. Although activity remained good overall, it decreased with the removal of the 4′-methyl group in **18** vs **30**; in contrast compounds **25** vs **31** have nearly identical activities in most of the tested cell lines, with compound **31** appearing slightly more potent in a few cases.

The mechanism of the cell growth inhibitory properties of the new active substances described herein needs to be undertaken. Several of these compounds, especially the 4′-demethyl compounds (**29**−**32**), have some of the structural features for topoisomerase II inhibition found in etoposide (**5**) and its derivatives. All possess substituents at the 4-position, and the 4′-methyl is absent. An important structural feature required for topo II inhibition, but absent from our new substances, is the β (epi) configuration at the C-4 position. The nitrogen atom at this site removes this chiral center, and it will be interesting to learn what effect this has on the mechanism of action. The striking differences in the mechanisms of action between podophyllotoxin (acting to arrest cell division at the metaphase by inhibition of tubulin polymerization) and etoposide (topoisomerase II poison) illustrate what very different cellular targets can be realized with subtle changes in structure.

In summary, the cancer cell growth inhibition found for a selection of the SAR products that range to nanomolar levels such as **15d** and **15e**[25] will be considered for linkage to monoclonal antibodies and further development. Other SAR products including **17**, **23**, and **29** are candidates for further evaluation.

EXPERIMENTAL SECTION

General Experimental Procedures. Ether refers to diethyl ether, Ar to argon gas, sgc chromatography to silica gel column chromatography, bac chromatography to basic alumina column chromatography, and rt to room temperature. All solvents were redistilled prior to use. All starting amines, copper II acetate, and 3,4-methylenedioxyphenyl boronic acid were purchased from Sigma-Aldrich Chemical Co. Other reagents were also purchased from either Sigma-Aldrich Chemical Co. or Acros Organics.

Reactions were monitored using thin-layer chromatography using Analtech silica gel GHLF Uniplates visualized with long-wave (366 nm) and/or short-wave (254 nm) UV radiation and using a vapor

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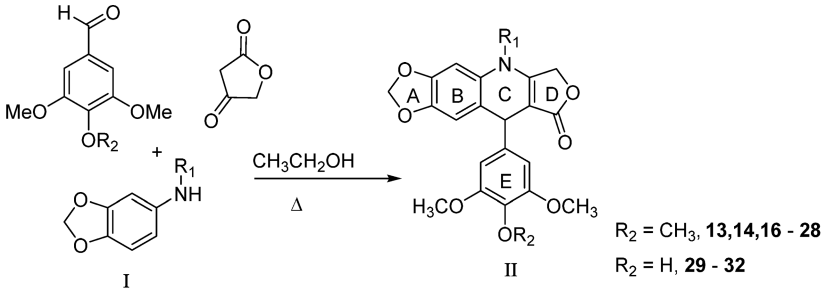
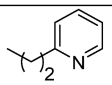
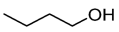
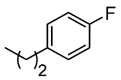
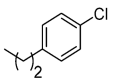
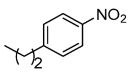
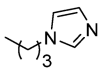
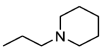
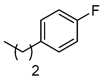
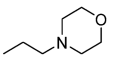
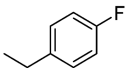
Scheme 1. Efficient Synthesis of 4-Aza-2,3-didehydro-4-deoxypodophyllotoxins



substrate, **I**	R_1	product, **II**	Yield (%)	substrate, **I**	R_1	Product, **II**	Yield (%)
12		**13**	59	**24a**		**24**	39
12a		**14**	38	**25a**		**25**	31
I	CH_3	**16**	53	**26a**		**26**	30
17a		**17**	83	**27a**		**27**	24
18a		**18**	54	**28a**		**28**	10
19a		**19**	43	**I**	H	**29**	87
20a		**20**	52	**18a**		**30**	74
21a		**21**	60	**25a**		**31**	75
22a		**22**	68	**32a**		**32**	85
23a		**23**	46				

chamber. Solvent extracts of aqueous solutions were washed sequentially with brine and dried over anhydrous magnesium sulfate. Where appropriate, silica gel (70−230 mesh ASTM from Merck) or basic alumina (150 mesh ASTM from Sigma-Aldrich) column chromatography was used for separation of products.

Isolation of natural products was performed employing Sephadex LH-20 gel permeation followed by partition column chromatography with decreasing solvent polarities. Reversed-phase HPLC was performed on a Prepex C_{18} column (250 × 10 mm) by Phenomenex with a Waters Delta 600 HPLC with dual λ UV detection.

All products were recrystallized at least once before melting point determination. Melting points are uncorrected and were determined using an Electrothermal 9100 apparatus. The ^{1}H NMR and ^{13}C NMR were recorded on Varian Gemini 300 MHz (^{1}H NMR) and Varian Unity 400 and 500 (^{13}C NMR) instruments using $CDCl_3$, DMSO-d_6, or CD_3OD with the residual solvent signals as internal references. High-resolution mass spectra were obtained using a JEOL LCMate instrument in either FAB or APCI modes.

Plant Material. The plant branches (34.5 kg) were collected in Gabon, West Africa, in 1979 during the month of April. They were identified by Dr. Arthur S. Barclay from the Medicinal Plant Resources Laboratory, Beltsville Agricultural Research Center (BARC)-East, Beltsville, MD.

A herbarium sample was deposited at the Economic Botany Laboratory, Building 265, BARC-East, Beltsville, MD.

Extraction of *Bridelia ferruginea*. The final scale-up extraction and isolation were prepared as follows. Branches were less than 1 in. in diameter accompanied by sawdust and were passed through a wood chipper to increase the surface area for solvent extraction. The chipped plant was then divided in half and placed into two 208 L drums. A 1:1 CH_2Cl_2−CH_3OH solution was added (76 L each) to completely cover the material. The extraction was allowed to proceed for 1 week. To the

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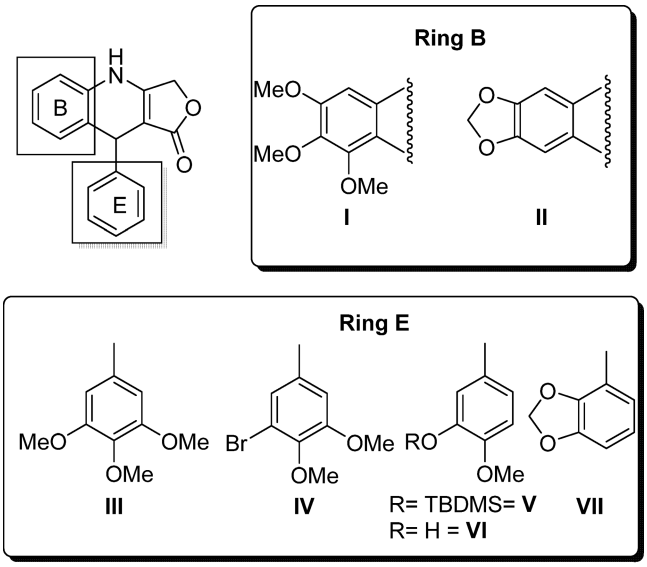


Compound	Ring B	Ring E
15	**I**	**III**
15a	**I**	**IV**
15b	**I**	**V**
15c	**I**	**VI**
15d	**II**	**VII**
15e	**II**	**III**

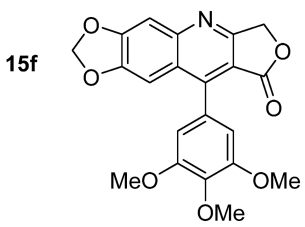
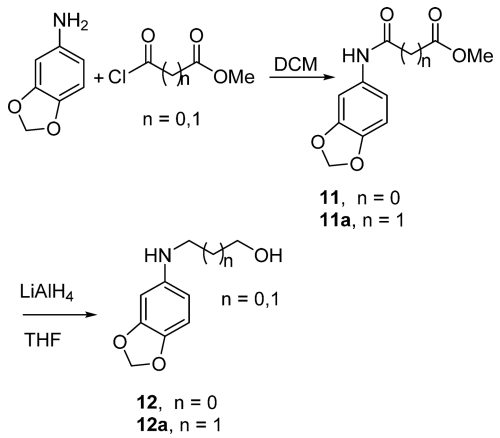


Figure 1. 4-Aza-2,3-didehydropodophyllotoxin analogues (**15**,[25] **15a**–**15d**, and **15e** and **15f**[25]).

Scheme 2. N-Substitution of Anilines



1:1 CH_2Cl_2–CH_3OH extraction solvent (30 L) removed from the drums was added H_2O to make a final concentration of 40% v/v H_2O. The addition of H_2O resulted in a partition forming between CH_2Cl_2–CH_3OH and H_2O–CH_3OH. The CH_2Cl_2 layer was removed, and the solvent concentrated in vacuo, leaving the crude CH_2Cl_2 extract. This procedure was repeated twice for each barrel. The crude CH_2Cl_2 extract was then dissolved in 9:1 CH_3OH–H_2O and partitioned with hexanes (2 L × 3). Enough H_2O was added to the 9:1 CH_3OH–H_2O to make a 3:2 CH_3OH–H_2O solution, which was partitioned exhaustively with CH_2Cl_2 (1.5 L × 5). The solvent from the hexane and CH_2Cl_2 partitions was separately removed, and the crude fractions were collected. The 9:1 CH_3OH–H_2O fraction was also collected. The murine P388 lymphocytic leukemia bioassay of the crude fractions showed the CH_2Cl_2 fraction as the most active (ED_{50} = 0.02 μg/mL).

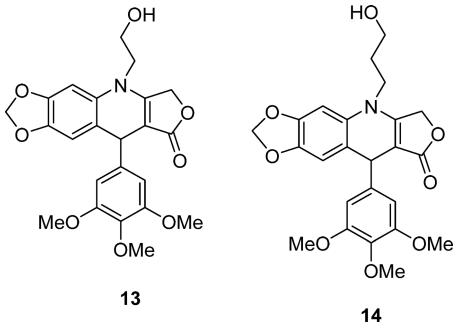


Figure 2. 4-Aza-analogues synthesized using N-substituted anilines.

The P388 bioactivity-guided fractionation of the CH_2Cl_2 fraction (80 g), using a series of Sephadex LH-20 column chromatographic separation steps with decreasing solvent polarities (100% CH_3OH → 3:2 CH_3OH–CH_2Cl_2 → 4:5:1 *n*-hexanes–EtOAc–CH_3OH), led to several active fractions. A highly P388-active fraction was subjected to semipreparative HPLC (gradient 25% CH_3CN–H_2O to 90% CH_3CN–H_2O) to give deoxypodophyllotoxin (**1**, 10.2 mg) and isopicrodeoxypodophyllotoxin (**2**, 10.4 mg) as amorphous powders. Deoxypodophyllotoxin was present in other active fractions, and 400 mg of deoxypodophyllotoxin (**1**) was obtained. β-Peltatin (**3**, 121 mg) was crystallized from another active fraction using CH_3OH–hexanes to yield a colorless crystalline solid. β-Peltatin-5-*O*-β-D-glucopyranoside (**3a**) and the indole derivative neoechinulin (**4**) were isolated from the CH_2Cl_2 partition of a 3.24 g sample of crude *B. ferruginea* extract, employing a series of Sephadex LH-20 column chromatographic separation steps with decreasing solvent polarities. Active fractions were subjected to semipreparative HPLC. All spectroscopic and physical data obtained for compounds **1**–**4** were consistent with literature values.[6–11]

*N-Benzo[1,3]dioxol-5-yl-oxalamic Acid Methyl Ester (**11**, n = 0) and N-Benzo[1,3]dioxol-5-yl-Malonamic Acid Methyl Ester (**11a**, n = 1).* A general procedure for the synthesis of amines **11** and **11a** was used as follows, unless otherwise noted. To a stirred solution of CH_2Cl_2 (100 mL), 3,4-(methylenedioxy) aniline (1 equiv), and triethylamine (3.0 mL) was added methyl chlorooxoacetate (*n* = 0, 1 equiv dropwise) or methyl 3-chloro-3-oxopropionate (*n* = 1, 1 equiv dropwise). The solution was stirred under Ar until TLC analysis showed that no starting material was present (18 h). Next, the reaction mixture was washed with 1 N HCl (3×), the CH_2Cl_2 solution was dried, and the solvent was removed in vacuo. Further product purification was accomplished using sgc chromatography (8:1 CH_2Cl_2–EtOAc), followed by recrystallization from hot EtOAc, to yield esters **11** or **11a** as light yellow needles.

*N-Benzo[1,3]dioxol-5-yl-oxalamic Acid Methyl Ester (**11**, n = 0).* Light yellow needles, 5.17 g (81%). Recrystallization from hot EtOAc: mp 171–173 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.77 (1H, bs) 7.34 (1H, d, *J* = 2.1 Hz), 6.94 (1H, dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz), 6.76 (1H, d, *J* = 8.4 Hz), 5.97 (2H, s), 3.95 (3H, s); (+)-HRFABMS *m/z* 224.0550 $[M + H]^+$ (calcd for $C_{10}H_{10}NO_5$, 224.0559).

*N-Benzo[1,3]dioxol-5-yl-malonamic Acid Methyl Ester (**11a**, n = 1).* Light brown needles, 6.19 g (84%). Recrystallization from hot EtOAc: mp 165–167 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.09 (1H, bs), 7.24 (1H, d, *J* = 2.1 Hz), 6.85 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 6.74 (1H, d, *J* = 8.1 Hz), 5.94 (2H, s), 3.78 (3H, s), 3.45 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.37, 162.61, 147.75, 144.45, 131.62, 113.33, 108.01, 102.91, 101.25, 52.62, 41.12; (+)-HRFABMS *m/z* 237.9864 $[M + H]^+$ (calcd for $C_{11}H_{12}NO_5$, 238.0715).

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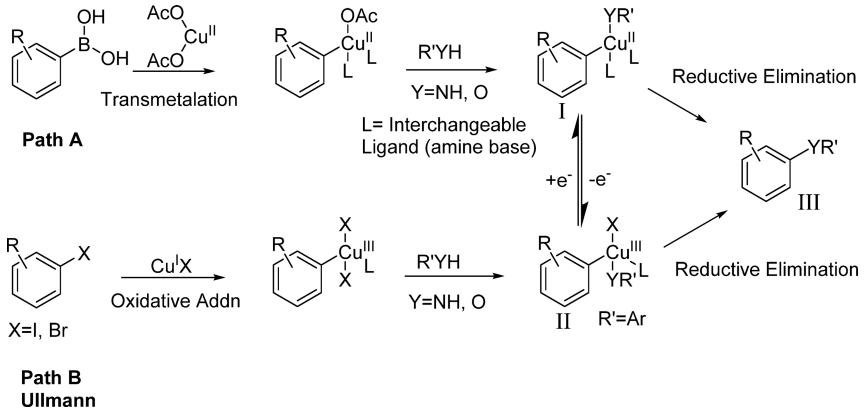
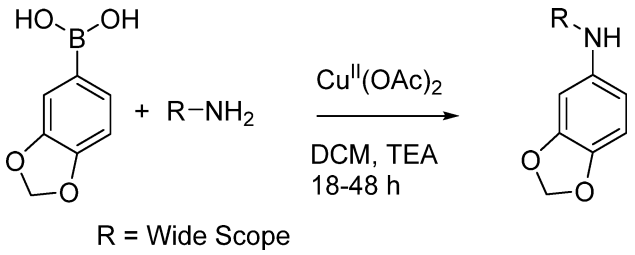
Scheme 3. Proposed Mechanisms for Copper-Mediated Reactions



Scheme 4. General Reaction Scheme for Aryl Amine Formation



*2-(Benzo[1,3]dioxol-5-ylamino)ethanol (**12**) and 3-(Benzo[1,3]-dioxol-5-ylamino)propan-1-ol (**12a**) (**I**, Scheme 2).* General procedure for the synthesis of amines **12** and **12a**, unless otherwise noted. To a stirred solution of anhydrous THF were added slowly ester **11** or **11a** (1 equiv) and $LiAlH_4$ (1.5 equiv). The reaction proceeded for 45 min, at which point TLC revealed that no starting material was present. The reaction was terminated with EtOAc and H_2O and partitioned with EtOAc (3×). The organic layers were combined and extracted with 1 N HCl solution (3×). The latter aqueous extracts were combined, the pH was adjusted to 12 with 2 N KOH, and the solution extracted with CH_2Cl_2 (4×). The CH_2Cl_2 solution was dried, and the solvent was removed in vacuo, leaving a yellow oil. Isolation of the products was achieved with sgc chromatography (3:1 EtOAc−CH_3OH with a gradient to 100%

Table 2. Murine P388 Lymphocytic Leukemia Inhibitory Activity (ED_{50} μg/mL) and Growth Inhibition of Human Cancer Cell Lines (GI_{50} μg/mL) for 4-Aza-2,3-didehydropodophyllotoxin Analogues 13−32

	cell line[a]						
compound	P388	BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
13	2.0	0.60	0.37	0.41	0.58	0.52	1.3
14	0.28	0.48	0.34	0.36	0.33	0.38	0.49
15	1.3	5.6	1.7	3.6	3.6	ND[b]	3.3
15a	0.11	0.56	0.12	0.11	0.35	ND[b]	0.42
15c	0.78	6.5	7.0	7.3	10.1	ND[b]	> 10
15d	0.17	0.065	0.0039	0.0089	0.021	ND[b]	0.041
15e[c]	0.0018	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
15f	3.6	4.1	3.4	2.4	2.8	2.1	2.4
16	0.027	0.049	0.034	0.029	0.032	0.032	0.026
17	0.24	0.040	0.026	0.026	0.028	0.032	0.047
18	15.3	0.083	0.045	0.062	0.16	0.12	0.27
19	3.4	0.17	0.052	0.066	0.27	0.24	0.27
20	0.18	0.43	0.29	0.46	1.0	0.51	2.5
21	1.7	4.4	> 10	> 10	> 10	> 10	> 10
22	2.6	3.5	0.89	2.6	3.0	2.5	2.0
23	1.9	0.046	0.037	0.030	0.031	0.033	0.047
24	2.5	0.38	0.32	0.33	0.34	0.29	0.42
25	3.3	0.90	0.42	0.52	2.3	2.4	2.5
26	2.6	0.54	0.34	0.35	0.30	0.37	0.31
27	34.8	0.39	0.080	0.20	0.30	0.26	0.30
28	2.0	2.9	0.64	2.5	3.0	2.2	2.1
29	0.028	0.028	0.025	0.026	0.027	0.032	0.033
30	30.3	0.53	0.32	0.39	0.33	2.1	0.41
31	0.21	1.4	0.34	0.48	0.36	ND[b]	0.38
32	0.89	1.0	0.054	0.061	0.24	ND[b]	0.39

[a]Cancer cell lines in order: murine lymphocytic leukemia (P388); pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); and prostate (DU-145). [b]ND = not determined; see ref 35. [c]See ref 25.

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CH_3OH) to give amino alcohol **12** (n = 0, 20%, 0.34 g) or **12a** (n = 1, 43%, 0.41 g).

*2-(Benzo[1,3]dioxol-5-ylamino)ethanol (**12**).* Light yellow oil, 0.34 g (20%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55 (1H, d, J = 8.1 Hz), 6.20 (1H, d, J = 2.1 Hz), 6.00 (1H, dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz), 5.77 (2H, s), 3.72 (2H, t, J = 4.8 Hz), 3.14 (2H, t, J = 5.7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 148.29, 143.51, 140.08, 108.54, 105.12, 100.59, 96.59, 60.99, 47.23; (+)-HRFABMS m/z 182.0818 $[M + H]^+$ (calcd for $C_9H_{12}NO_3$, 182.0817).

*3-(Benzo[1,3]dioxol-5-ylamino)propan-1-ol (**12a**).* Light yellow oil, 0.41 g (43%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55 (1H, d, J = 8.1 Hz), 6.18 (1H, d, J = 2.1 Hz), 5.96 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.76 (2H, s), 3.71 (2H, t, J = 5.7 Hz), 3.12 (2H, t, J = 6 Hz), 1.77 (2H, pent, J = 6 Hz); ^{13}C NMR ($CDCl_3$–CD_3OD, 125 MHz) δ 148.23, 143.97, 139.79, 108.51, 104.93, 100.50, 96.38, 61.38, 43.06, 31.65; (+)-HRAPCIMS m/z 196.0976 $[M + H]^+$ (calcd for $C_{10}H_{14}NO_3$, 196.0974).

*Aryl Amines **17a–32a** (**I**, Scheme 1).* General procedure for the synthesis of component **I** (unless otherwise noted): A solution prepared from CH_2Cl_2 (30 mL), 3,4-methylenedioxyphenylboronic acid (0.65 g, 0.004 mol), and Cu(II)OAc (1.15 g, 0.008 mol) was stirred for a few minutes; then triethylamine (3 mL) and the primary amine (0.004 mol) were added (slowly). The reaction mixture was stirred under Ar for 18–48 h, silica gel or basic alumina was added, and the solvent was removed in vacuo, leaving a dry packed chromatographic substrate. The product was isolated employing either sgc or bac chromatography to yield the necessary aryl amines, **I**, which were obtained as brown oils. Maleate salts were prepared from aryl amines in an attempt to obtain pure compounds by crystallization. General procedure: The aryl amine was dissolved in a small amount of ether; then maleic acid in ether was added dropwise until a cloudy solution formed. The solvent was removed in vacuo, and the residue was recrystallized from either EtOAc–hexanes or CH_2Cl_2–hexanes. Aryl amines **17a–32a** (**I**) were immediately used to yield dihydroquinolines **17–32** (**II**).

*4-Aza-2,3-didehydropodophyllotoxin Modifications **13**,[31] **14**, and **17–32** (**II**, Scheme 1).* General procedure for the synthesis of quinoline **II** (unless otherwise noted): A stirred solution of aniline component **I** (1 equiv) in ethanol (30 mL) was heated to reflux; then 3,4,5-trimethoxybenzaldehyde (1 equiv) and tetronic acid (1 equiv) were added with continued heating at reflux for 20 min to 1.5 h based upon when a TLC plate showed that a bright blue spot was formed when viewed under UV light. The solvent was removed in vacuo, and product (**II**) was isolated by means of sgc chromatography using appropriate solvent systems. Products were often unstable and underwent degradation, as evidenced by the formation of a yellow or yellow-green compound at the baseline when analyzed via TLC. NMR spectroscopy also showed this as an increase of signals in the aliphatic region, not attributable to the desired structure. Unfortunately, late in this research, extenuating circumstances prevented further progress in this SAR study.

*6,7-Methylenedioxy-4-(2-hydroxyethyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**13**).* Purification: The product was recrystallized from CH_2Cl_2–CH_3OH as a colorless powder, 0.485 g (59%): mp 271–273 °C [lit.[31] mp 241–243 °C]; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.15 (1H, d, J = 1.8 Hz), 6.40 (1H, d, J = 5.1 Hz), 6.27 (2H, s), 5.77 (1H, s), 5.75 (s, 1H), 4.83 (1H, s), 4.81 (1H, s), 4.75 (1H, s), 3.96 (2H, m), 3.63 (6H, s), 3.60 (3H, s), 3.50 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.32, 160.66, 152.79, 146.90, 143.23, 143.05, 135.90, 130.70, 119.43, 109.99, 104.47, 101.35, 96.21, 94.79, 65.78, 59.82, 58.05, 55.75, 48.03, 30.68; (+)-HRFABMS m/z 442.1519 $[M + H]^+$ (calcd for $C_{23}H_{23}NO_8$, 442.1502); anal. C 62.41; H 5.58; N 3.23%, calcd for $C_{23}H_{23}NO_8$, C 62.58; H 5.25; N 3.17%.

*6,7-Methylenedioxy-4-(3-hydroxypropyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**14**).* Purification: sgc chromatography (3:1 EtOAc–hexanes → 50:1 EtOAc–CH_3OH) as off-white prisms 0.075 g (38%). Recrystallized from CH_2Cl_2–CH_3OH: mp 269–272 °C (dec); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.60 (1H, s), 6.51 (1H, s), 6.34 (2H, s), 5.89 (1H, s), 5.88 (1H, s), 4.95 (1H, d, J = 15.6 Hz), 4.89 (1H, s), 4.76 (1H, d, J = 15.7 Hz), 3.73 (9H, s), 3.63 (4H, m), 1.95 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.55, 158.87, 153.06, 147.49, 144.10, 141.90, 136.50, 131.16, 118.95, 110.69, 104.92, 101.54, 96.75, 95.44, 65.50, 60.65, 57.87, 55.95, 42.67, 40.74, 29.73; (+)-HRFABMS m/z 456.1657 $[M + H]^+$ (calcd for $C_{24}H_{25}NO_8$, 456.1658); anal. C 63.05; H 5.93; N 3.18%, calcd for $C_{24}H_{25}NO_8$, C 63.29; H 5.53; N 3.08%.

*Benzo[1,3]dioxol-5-yl-cyclobutylamine (**17a**).* Purification: sgc chromatography (1:1 CH_2Cl_2–hexanes) as a light brown oil, 0.144 g (19%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.63 (1H, d, J = 8.1 Hz), 6.18 (1H, d, J = 2.4 Hz), 5.98 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.4 Hz), 5.83 (2H, s), 3.82 (1H, m), 3.57 (1H, bs), 2.38 (2H, m), 1.78 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) maleate δ 168.91, 148.64, 148.10, 135.04, 128.01, 116.65, 108.75, 104.28, 102.11, 31.57, 26.49, 22.64, 14.10; (+)-HRAPCIMS m/z 192.1021 $[M + H]^+$ (calcd for $C_{11}H_{14}NO_2$, 192.1025).

*6,7-Methylenedioxy-4-(cyclobutyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**17**).* Purification: sgc chromatography (1:1 CH_2Cl_2–hexanes) as a colorless powder 0.284 g (83%). Recrystallized from EtOAc–hexanes: mp 224–226 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.60 (1H, s), 6.56 (1H, s), 6.42 (2H, s), 5.95 (1H, s), 5.94 (1H, s), 4.93 (1H, d, J = 15.3 Hz), 4.88 (1H, s), 4.60 (1H, d, J = 15.3 Hz), 4.37 (1H, pent, J = 5.3 Hz), 3.79 (9H, s), 2.69 (1H, m), 2.32 (2H, m), 2.09 (1H, m), 1.87 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.25, 158.59, 153.18, 147.06, 144.15, 140.90, 136.60, 133.28, 119.46, 109.93, 103.98, 101.56, 100.73, 97.06, 66.04, 60.98, 55.96, 52.66, 40.50, 31.59, 29.36, 14.96; (+)-HRAPCIMS m/z 452.1722 $[M + H]^+$ (calcd for $C_{25}H_{25}NO_7$, 452.1709); anal C 66.48; H 5.83; N 3.18%, calcd for $C_{25}H_{25}NO_7$, C 66.51; H 5.58; N 3.10%.

*Benzo[1,3]dioxol-5-yl-cyclopentylamine (**18a**).* Purification: sgc chromatography (10:1 CH_2Cl_2–EtOAc) as light tan oil, 0.314 g (40%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55 (1H, d, J = 8.1 Hz), 6.16 (1H, d, J = 2.1 Hz), 5.96 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.75 (2H, s), 3.61 (1H, pent, J = 5.7 Hz), 3.34 (1H, bs), 1.89 (2H, m), 1.48 (6H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) maleate δ 169.80, 148.50, 147.94, 135.83, 129.87, 116.96, 108.55, 104.63, 101.96, 64.70, 29.29, 23.70; (+)-HRFABMS m/z 206.1175 $[M + H]^+$ (calcd for $C_{12}H_{16}NO_2$, 206.1181).

*6,7-Methylenedioxy-4-(cyclopentyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**18**).* Purification: sgc chromatography (2:1 EtOAc–CH_3OH) as a colorless powder, 0.129 g (54%). Recrystallized from EtOAc–hexanes: mp 221–223 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.64 (1H, s), 6.57 (1H, s), 6.37 (2H, s), 5.93 (1H, s), 5.92 (1H, s), 4.93 (1H, d, J = 15 Hz), 4.88 (1H, s), 4.75 (1H, d, J = 15 Hz), 4.16 (1H, pent., J = 8.5 Hz), 3.76 (6H, s), 3.75 (3H, s), 2.12 (2H, m), 2.00 (4H, m), 1.75 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.58, 158.61, 153.12, 146.77, 143.88, 141.42, 136.58, 131.69, 119.89, 110.57, 104.41, 101.54, 99.56, 97.52, 65.85, 60.66, 60.37, 55.94, 40.68, 28.54, 28.21, 24.81, 24.78; (+)-HRAPCIMS m/z 466.1866 $[M + H]^+$ (calcd for $C_{26}H_{27}NO_7$, 466.1866); anal. C 66.31; H 6.09; N 3.01%, calcd for $C_{29}H_{33}NO_8$, C 66.53; H 6.35; N 2.68%.

*Benzo[1,3]dioxol-5-ylcyclohexylamine Maleate (**19a**).* Purification: sgc chromatography (2:1 hexanes–EtOAc) as light tan oil, 0.184 g (23%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.12 (1H, s), 7.04 (1H, d, J = 8 Hz), 6.75 (1H, d, J = 8 Hz), 6.31 (2H, s), 5.97 (2H, s), 3.29 (1H, m), 2.03 (2H, bd, J = 11.6 Hz), 1.79 (2H, bd, J = 10.4 Hz), 1.63 (1H, m), 1.49 (2H, m), 1.19 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) maleate δ 169.85, 148.43, 148.04, 135.87, 128.02, 117.57, 108.45, 105.12, 101.97, 62.31, 29.02, 24.87, 24.54; (+)-HRAPCIMS m/z 220.1348 $[M + H]^+$ (calcd for $C_{13}H_{18}NO_2$, 220.1338).

*6,7-Methylenedioxy-4-(cyclohexyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**19**).* Purification: sgc chromatography (1:1 EtOAc–hexanes) as light yellow powder 0.132 g (43%). Recrystallized from EtOAc–hexanes: mp 235–237 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.67 (1H, s), 6.44 (1H, s), 6.28 (2H, s), 5.82 (1H, d, J = 1 Hz), 5.81 (1H, d, J = 1 Hz), 4.90 (1H, d, J = 15.3 Hz), 4.76 (1H, s), 4.67 (1H, d, J = 15.3 Hz), 3.65 (9H, s), 1.83 (4H, m), 1.68 (4H, m), 1.30 (2H, m), 1.11 (1H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.35, 158.01, 153.10, 147.14, 143.91, 141.26, 136.59,

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133.51, 119.73, 110.20, 104.38, 101.54, 100.63, 97.31, 66.33, 60.62, 60.25, 55.96, 40.60, 31.63, 30.89, 26.45, 26.20, 25.15; (+)-HRAPCIMS m/z 480.2020 $[M + H]^+$ (calcd for $C_{27}H_{29}NO_7$, 480.2022).

*Benzo[1,3]dioxol-5-yl-N,N-dimethylethane-1,2-diamine (**20a**).* Purification: bac chromatography (1:1 CH_2Cl_2–hexanes) as a light tan oil, 0.066 g (10%): ^1H NMR ($CDCl_3$, 300 MHz) δ 6.56 (1H, d, J = 8.1 Hz), 6.17 (1H, d, J = 2.4 Hz), 6.75 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.4 Hz), 5.74 (2H, s), 5.84 (2H, s), 3.07 (2H, t, J = 6.0 Hz), 2.53 (2H, t, J = 6.0 Hz), 2.24 (6H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 148.33, 142.52, 139.85, 108.58, 104.32, 102.33, 100.51, 96.18, 57.90, 44.54; (+)-HRFABMS m/z 209.1297 $[M + H]^+$ (calcd for $C_{11}H_{16}N_2O_2$, 209.1290).

*6,7-Methylenedioxy-4-(2-dimethyaminoethyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**20**).* Purification: sgc chromatography (15:1 CH_2Cl_2–CH_3OH) as a light brown, amorphous solid 0.06 g (52%). Recrystallized from CH_3OH: mp 251–254 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.57 (1H, s), 6.56 (1H, s), 6.40 (2H, s), 5.95 (1H, s), 5.94 (1H, s), 4.96 (1H, s), 4.90 (2H, s), 3.78 (9H, s), 3.60 (2H, m), 2.60 (2H, m), 2.29 (6H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.73, 158.03, 153.09, 147.53, 144.07, 141.72, 136.72, 131.27, 118.80, 110.85, 105.16, 101.57, 97.36, 95.01, 65.33, 60.66, 56.04, 45.85, 40.75; (+)-HRAPCIMS m/z 469.1976 $[M + H]^+$ (calcd for $C_{25}H_{28}N_2O_7$, 469.1975).

*Benzo[1,3]dioxol-5-yl-(2-piperidin-1-ylethyl)-1,2-diamine (**21a**).* Purification: bac chromatography (5:1 EtOAc–CH_3OH) as brown oil, 0.050 g (10%): ^1H NMR ($CDCl_3$, 300 MHz) δ 6.65 (1H, d, J = 8.1 Hz), 6.17 (1H, d, J = 2.1 Hz), 5.96 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.75 (2H, s), 4.20 (1H, bs), 2.99 (2H, t, J = 6.0 Hz), 2.47 (2H, t, J = 6.0 Hz), 2.31 (4H, m), 1.49 (4H, m), 1.36 (2H, m); (+)-HRFABMS m/z 249.1609 $[M + H]^+$ (calcd for $C_{14}H_{20}N_2O_2$, 249.1603).

*6,7-Methylenedioxy-4-(2-piperidin-1-ylethyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**21**).* Purification: sgc chromatography (15:1 CH_2Cl_2–CH_3OH) as a light brown, amorphous solid, 0.026 g (60%). Recrystallized from EtOAc: mp 170 °C (dec); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.58 (1H, s), 6.56 (1H, s), 6.39 (2H, s), 5.95 (1H, s), 5.94 (1H, s), 4.98 (1H, s), 4.93 (2H, s), 3.79 (9H, s), 3.65 (2H, m), 2.61 (2H, t, J = 6.6 Hz), 2.45 (4H, m), 1.57 (4H, m), 1.43 (2H, m); (+)-HRAPCIMS m/z 509.2293 $[M + H]^+$ (calcd for $C_{28}H_{32}N_2O_7$, 509.2288).

*Benzo[1,3]dioxol-5-yl-(2-morpholin-4-ylethyl)-1,2-diamine (**22a**).* Purification: sgc chromatography (100% CH_2Cl_2), light tan oil, 0.11 g (10%); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.53 (1H, d, J = 8.1 Hz), 6.16 (1H, d, J = 2.1 Hz), 5.94 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.72 (2H, s), 3.60 (4H, t, J = 4.5 Hz), 2.98 (2H, t, J = 5.7 Hz), 2.49 (2H, t, J = 6 Hz), 2.34 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) maleate δ 168.55, 148.35, 141.08, 140.74, 134.02, 108.40, 105.10, 100.64, 96.69, 63.57, 55.59, 52.08, 39.11; (+)-HRAPCIMS m/z 251.1392 $[M + H]^+$ (calcd for $C_{13}H_{18}N_2O_3$, 251.1396).

*6,7-Methylenedioxy-4-(2-morpholin-4-ylethyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**22**).* Purification: sgc chromatography (9:1 EtOAc–CH_3OH) as colorless crystals, 0.14 g (68%). Recrystallized by slow evaporation from CH_2Cl_2–CH_3OH: mp 186–189 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.47 (1H, s), 6.45 (1H, s), 6.27 (2H, s), 5.83 (2H, s), 4.85 (1H, s), 4.80 (2H, bs), 3.67 (9H, s), 3.66 (4H, t, J = 4.8 Hz), 3.61 (2H, m), 2.56 (2H, t, J = 6 Hz) 2.40 (4H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.28, 158.22, 152.93, 147.52, 144.14, 141.63, 136.58, 130.98, 118.62, 110.75, 105.17, 101.54, 97.19, 94.96, 66.53, 65.43, 60.53, 55.92, 55.70, 53.94, 44.34, 40.49; (+)-HRAPCIMS m/z 511.2082 $[M + H]^+$ (calcd for $C_{27}H_{30}N_2O_8$, 511.2080).

*Benzo[1,3]dioxol-5-yl-(4-fluorobenzyl)-1,2-diamine (**23a**).* Purification: sgc chromatography (3:1 CH_2Cl_2–hexanes), light tan oil, 0.14 g (14%); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22 (2H, m), 6.93 (2H, m) 6.55 (1H, d, J = 8.1 Hz), 6.15 (1H, d, J = 2.1 Hz), 5.97 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.75 (2H, s), 4.13 (2H, s), 3.70 (1H, bs); ^{13}C NMR ($CDCl_3$, 125 MHz) maleate δ 172.34, 166.85, 164.87, 151.23, 150.17, 138.09, 135.05, 134.98, 132.60, 129.31, 118.73, 118.60, 118.43, 111.26, 106.49, 104.65, 57.63; (+)-HRFABMS m/z 246.0920 $[M + H]^+$ (calcd for $C_{14}H_{12}NO_2F$, 246.0930).

*6,7-Methylenedioxy-4-(4-fluorobenzyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**23**).* Purification: sgc chromatography (2:1 hexanes–EtOAc → 4:1 EtOAc–hexanes), light yellow crystals, 0.10 g (46%). Recrystallized from EtOAc and from EtOAc–hexanes: mp 246–247 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.23 (2H, m), 7.06 (2H, m), 6.58 (1H, s), 6.43 (2H, s), 6.40 (1H, s), 5.90 (1H, d, J = 1 Hz), 5.88 (1H, d, J = 1 Hz), 5.06 (1H, s), 4.85 (1H, d, J = 15 Hz), 4.77 (2H, s), 4.76 (1H, d, J = 15 Hz), 3.81 (3H, s) 3.79 (6H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.41, 157.60, 153.21, 147.52, 144.37, 141.43, 136.80, 131.77, 130.66, 127.32, 127.25, 118.45, 116.46, 116.29, 110.75, 105.11, 101.61, 98.26, 95.81, 65.05, 60.74, 56.07, 49.08, 40.71; (+)-HRAPCIMS m/z 506.1613 $[M + H]^+$ (calcd for $C_{28}H_{24}NO_7F$, 506.1615); anal. C 66.19; H 5.10; N 2.81%, calcd for $C_{28}H_{24}NO_7F$: C 66.53; H 4.79; N 2.77%.

*Benzo[1,3]dioxol-5-yl-(2-pyridin-2-yl-ethyl)-1,2-diamine (**24a**).* Purification: sgc chromatography (4:1 EtOAc–CH_2Cl_2), brown oil, 0.155 g (14%); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.54 (1H, d, J = 4.5 Hz), 7.58 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz), 7.15 (2H, m,), 6.65 (1H, d, J = 8.7 Hz), 6.25 (1H, d, J = 2.1 Hz), 6.07 (1H, dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz), 5.82 (2H, s), 3.93 (1H, bs), 3.45 (2H, t, J = 6.6 Hz), 3.05 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) maleate δ 169.18, 156.71, 148.67, 145.26, 145.13, 141.35, 135.22, 134.58, 125.78, 123.66, 112.00, 108.78, 101.62, 101.10, 48.98, 31.58; (+)-HRFABMS m/z $C_{14}H_{14}N_2O_2$, 243.1115 $[M + H]^+$ (calcd for $C_{14}H_{14}N_2O_2$, 243.1134).

*6,7-Methylenedioxy-4-(2-pyridin-2-ylethyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**24**).* Purification: sgc chromatography (30:1 EtOAc–CH_3OH), light tan crystals, 0.10 g (39%). Recrystallized from EtOAc–CH_3OH: mp 239–241 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.57 (1H, d, J = 3.9 Hz), 7.56 (1H, dd, J_1 = 7.5 Hz, J_2 = 2.1 Hz), 7.17 (1H, m), 7.08 (1H, d, J = 7.5 Hz), 6.77 (1H, s), 6.58 (1H, s), 6.37 (2H, s), 5.97 (1H, d, J = 1.8 Hz), 5.96 (1H, d, J = 1.2 Hz), 4.95 (1H, s), 4.62 (1H, s), 4.61 (1H, s), 4.00 (2H, m), 3.79 (3H, s), 4.77 (6H, s), 3.19 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.76, 157.93, 156.95, 152.95, 149.35, 147.52, 144.05, 141.56, 137.01, 136.65, 131.12, 123.77, 122.29, 118.71, 110.75, 105.21, 101.51, 97.06, 95.26, 65.22, 60.56, 56.01, 45.83, 40.53, 35.55; (+)-HRFABMS m/z 503.1836 $[M + H]^+$ (calcd for $C_{28}H_{26}N_2O_7$, 503.1818).

*Benzo[1,3]dioxol-5-yl-[2-(4-fluorophenyl)ethyl]-1,2-diamine (**25a**).* Purification: sgc chromatography (2:1 CH_2Cl_2–hexanes), light tan oil, 0.28 g (27%); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.06 (2H, m), 6.92 (2H, m), 6.57 (1H, d, J = 8.1 Hz), 6.15 (1H, d, J = 2.1 Hz), 5.96 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz), 5.76 (2H, s), 3.33 (1H, bs), 3.23 (2H, t, J = 6.9 Hz), 2.78 (2H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) maleate δ169.67, 148.76, 148.15, 135.47, 130.37, 130.28, 129.99, 116.28, 115.71, 115.50, 108.81, 103.98, 102.13, 53.99, 31.28; (+)-HRFABMS m/z 260.1077 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_2F$, 260.1087).

*6,7-Methylenedioxy-4-[2-(4-fluorophenyl)ethyl]-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**25**).* Purification: sgc chromatography (4:1 CH_2Cl_2–EtOAc) as light yellow, amorphous solid, 0.07 g (31%). Recrystallized from EtOAc: mp 174–177 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.10 (2H, m), 6.96 (2H, m), 6.66 (1H, s), 6.59 (1H, s), 6.41 (2H, s), 5.98 (1H, s), 5.97 (1H, s), 4.99 (1H, s), 4.35 (1H, d, J = 15.2 Hz), 4.30 (1H, d, J = 15.2 Hz), 3.79 (9H, s), 3.65 (2H, m), 2.99 (2H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.55, 157.54, 153.16, 147.70, 144.24, 136.97, 133.08, 133.05, 131.18, 130.30, 130.23, 130.14, 118.70, 115.73, 115.64, 111.14, 105.55, 101.69, 97.28, 95.06, 64.98, 60.74, 56.24, 48.35, 40.59, 33.19; (+)-HRFABMS m/z 520.1808 $[M + H]^+$ (calcd for $C_{29}H_{26}NO_7F$, 520.1772).

*Benzo[1,3]dioxol-5-yl-[2-(4-chlorophenyl)ethyl]-1,2-diamine (**26a**).* Purification: sgc chromatography (100% CH_2Cl_2), light tan oil, 0.063 g (10%); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.26 (2H, m), 7.13 (2H, m), 6.65 (1H, d, J = 8.4 Hz), 6.23 (1H, d, J = 2.1 Hz), 5.96 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz), 5.85 (2H, s), 3.44 (1H, bs), 3.31 (2H, t, J = 6.6 Hz), 2.86 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) maleate δ 170.61, 151.44, 150.77, 138.22, 137.38, 135.67, 132.85, 132.78, 131.58, 118.87, 111.50, 106.61, 104.81, 56.37, 34.18;

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(+)-HRAPCIMS m/z 276.0795 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_2{}^{35}Cl$, 276.0791).

*6,7-Methylenedioxy-4-[2-(4-chlorophenyl)ethyl]-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**26**).* Purification: sgc chromatography (15:1 CH_2Cl_2–CH_3OH), pale yellow, amorphous solid, 0.03 g (30%). Recrystallized from CH_2Cl_2–hexanes: mp 170–173 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.26 (2H, m), 7.08 (2H, m), 6.65 (1H, s), 6.60 (1H, s), 6.41 (2H, s), 5.98 (1H, s), 5.97 (1H, s), 4.99 (1H, s), 4.39 (1H, d, J = 15.4 Hz), 4.33 (1H, d, J = 15.4 Hz), 3.80 (9H, s), 3.66 (2H, m), 2.99 (2H, t, J = 7.2 Hz); (+)-HRFABMS m/z 538.1442 $[M + H]^+$ (calcd for $C_{29}H_{26}NO_7{}^{37}Cl$, 538.1447).

*Benzo[1,3]dioxol-5-yl-[2-(4-nitrophenyl)ethyl]-1,2-diamine (**27a**).* Purification: bac chromatography (100% CH_2Cl_2), light tan oil, 0.166 g (14%); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.06 (2H, d, J = 9 Hz), 7.26 (2H, d, J = 9 Hz), 6.65 (1H, d, J = 8.4 Hz), 6.13 (1H, d, J = 2.4 Hz), 5.94 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz), 5.76 (2H, s), 3.29 (2H, t, J = 6.9 Hz), 2.90 (2H, t, J = 6.9 Hz); (+)-HRAPCIMS m/z 287.1021 $[M + H]^+$ (calcd for $C_{15}H_{14}N_2O_4$, 287.1032).

*6,7-Methylenedioxy-4-[2-(4-nitrophenyl)ethyl]-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**27**).* Purification: bac chromatography (1:1 CH_2Cl_2–EtOAc), light yellow, amorphous solid, 0.076 g (24%). Recrystallized from EtOAc: mp 188–190 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.94 (2H, d, J = 9 Hz), 7.18 (2H, d, J = 9 Hz), 6.50 (1H, s), 6.40 (1H, s), 6.20 (2H, s), 5.79 (1H, s), 5.78 (1H, s), 4.77 (1H, s), 4.34 (1H, d, J = 15.5 Hz), 4.30 (1H, d, J = 15.5 Hz), 3.68 (2H, m), 3.59 (6H, s), 3.58 (3H, s), 2.96 (2H, t, J = 7.2 Hz); (+)-HRFABMS m/z 547.1703 $[M + H]^+$ (calcd for $C_{29}H_{26}N_2O_9$, 547.1716).

*Benzo[1,3]dioxol-5-yl-(3-imidazol-1-ylpropyl)-1,2-diamine (**28a**).* Purification: sgc chromatography (1:1 CH_2Cl_2–hexanes), light tan oil, 0.12 g (12%); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36 (1H, s), 6.96 (1H, s), 6.81 (1H, s), 6.53 (1H, d, J = 8.1 Hz), 6.10 (1H, d, J = 1.8 Hz), 5.94 (1H, dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz), 5.74 (2H, s), 3.96 (2H, t, J = 6.9 Hz), 2.95 (2H, t, J = 6.9 Hz), 1.95 (2H, sept, J = 7.2 Hz); (+)-HRAPCIMS m/z 246.1240 $[M + H]^+$ (calcd for $C_{13}H_{15}N_3O_2$, 246.1243).

*6,7-Methylenedioxy-4-(3-imidazol-1-ylpropyl)-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**28**).* Purification: sgc chromatography (3:1 EtOAc–CH_3OH) as yellow glass, 0.043 g (10%). Recrystallized from hot EtOAc: mp 206 °C (dec); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.56 (1H, bs), 7.15 (1H, bs), 6.92 (1H, bs), 6.54 (1H, s), 6.35 (2H, s), 6.34 (1H, s), 5.94 (1H, s), 5.93 (1H, s), 4.93 (1H, s), 4.55 (2H, s), 4.04 (2H, t, J = 7.2 Hz), 3.76 (3H, s), 3.74 (6H, s), 3.46 (2H, m), 2.19 (2H, m); (+)-HRAPCIMS m/z 506.1937 $[M + H]^+$ (calcd for $C_{27}H_{27}N_3O_7$, 506.1927).

*Benzo[1,3]dioxol-5-yl-(furan-2-ylmethyl)-1,2-diamine (**32a**).* Purification: sgc chromatography (7:1 hexanes–EtOAc → 5:1 hexanes–EtOAc), tan oil, 0.22 g (24%); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.37 (1H, bs), 6.67 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 6.34 (1H, d, J = 0.9 Hz), 6.32 (1H, d, J = 2.4 Hz), 6.23 (1H, d, J = 0.9 Hz), 6.13 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.18 Hz), 5.86 (1H, s), 5.85 (1H, s), 3.96, 4.26 (2H, s); (+)-HRAPCIMS m/z 218.0817 $[M + H]^+$ (calcd for $C_{12}H_{11}NO_3$, 218.0817).

*6,7-Methylenedioxy-9-(4-hydroxy-3,5-dimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**29**).* A stirred solution of ethanol (35 mL), syringaldehyde (0.51 g, 0.0027 mol), tetronic acid (270 mg, 0.0027 mol), and 3,4-methylenedioxyaniline (380 mg, 0.0027 mol) were heated at reflux for 45 min. The product crystallized from the solution upon cooling. After collection and recrystallization from hot CH_3OH–acetone (~1 L) was performed, the product was again quickly recovered from cooling to provide a light tan powder (87%, 0.90 g): mp 275 °C (dec); ^1H NMR (DMSO-d_6, 300 MHz) δ 9.67 (1H, s), 8.02 (1H, bs), 6.53 (1H, s), 6.40 (1H, s), 6.32 (2H, s), 5.83 (1H, s), 5.78 (1H, s), 4.85 (1H, d, J = 15.3 Hz), 4.70 (1H, d, J = 15.3 Hz), 4.68 (1H, s), 3.57 (6H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.14, 158.21, 147.86, 146.32, 143.15, 137.41, 134.27, 130.23, 117.02, 109.55, 105.18, 101.10, 97.20, 94.36, 64.85, 56.03; (+)-HRFABMS m/z 384.0888 $[M + H]^+$ (calcd for $C_{20}H_{17}NO_7$, 384.1083).

TBDMS-Protected Syringaldehyde. To a stirred solution of CH_2Cl_2 (75 mL) were added slowly syringaldehyde (2.65 g, 0.0143 mol), imidazole (4.43 g, 0.06 mol), and TBDMS-Cl (6.59 g, 0.044 mol) in CH_2Cl_2 (20 mL), and the solution turned cloudy over 16 h. The CH_2Cl_2 phase was washed with water and dried, and the solvent was removed in vacuo to afford a light oil. The oil was separated by sgc chromatography (2:1 EtOAc–hexanes), yielding a colorless, amorphous solid upon standing at rt (93%, 3.95 g): mp 70–72 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.74 (1H, s), 7.00 (2H, s), 3.77 (6H, s), 0.915 (9H, s), 0.062 (6H, s), consistent with the literature.[27]

*6,7-Methylenedioxy-4-(cyclopentyl)-9-(4-hydroxy-3,5-dimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**30**) and 4′-TBDMS-Protected **30**.* This was prepared from a stirred solution of ethanol (15 mL), and amine **18a** (0.31g, 0.0015 mol) was reacted with tetronic acid (163 mg, 0.001 mol) and TBDMS-protected syringaldehyde (0.44 mg, 0.0015 mol). The product was isolated using sgc chromatography (1:10 EtOAc–CH_2Cl_2) to yield TBDMS-protected **30** as a colorless, amorphous powder, 0.22 g (25%): ^1H NMR ($CDCl_3$, 300 MHz) δ 6.63 (1H, s), 6.57 (1H, s), 6.33 (2H, s), 5.93 (1H, s), 5.92 (1H, s), 4.93 (1H, d, J = 14.7 Hz), 4.85 (1H, s), 4.74 (1H, d, J = 14.7 Hz), 4.16 (1H, sept, J = 8.7 Hz), 3.69 (6H, s), 2.00 (6H, m), 1.74 (2H, m), 0.97 (9H, s), 0.08 (6H, s). Cleavage of the silyl protecting group was achieved using a stirred solution of THF (20 mL), 4′-TBDMS-protected **30** (0.22 g, 0.00038 mol), and TBAF (0.5 mL added dropwise) with the solution stirred under Ar for 35 min. TLC showed no starting material, and so the reaction mixture was diluted with H_2O (3 × 15 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic phase was dried, and the solvent was removed in vacuo. By means of sgc chromatography (10:1 CH_2Cl_2–EtOAc → 5:1 CH_2Cl_2–EtOAc) separation, the product **30** was isolated as light yellow crystals, 0.127 g (74%). Recrystallized from CH_2Cl_2–CH_3OH: mp 221–223 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.55 (1H, s), 6.48 (1H, s), 6.29 (2H, s), 5.85 (1H, s), 5.83 (1H, s), 5.33 (1H, bs), 4.83 (1H, d, J = 15.3 Hz), 4.77 (1H, s), 4.65 (1H, d, J = 15.3 Hz), 4.07 (1H, p, J = 9 Hz), 3.70 (6H, s), 1.91 (6H, m), 1.67 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.65, 158.46, 146.94, 146.76, 143.91, 137.14, 133.50, 131.75, 120.16, 110.67, 104.24, 101.56, 99.86, 97.51, 65.88, 60.41, 56.22, 40.49, 28.59, 28.27, 24.84, 24.86; (+)-HRFABMS m/z 452.1698 $[M + H]^+$ (calcd for $C_{25}H_{25}NO_7$, 452.1709); anal. C 63.87; H 6.06; N 2.84%, calcd for $C_{25}H_{25}NO_7{+}H_2O$, C 63.96; H 5.80; N 2.98%.

*6,7-Methylenedioxy-4-[2-(4-fluorophenyl)ethyl]-9-(4-hydroxy-3,5-dimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**31**) and 4′-TBDMS-Protected **31**.* The preceding reaction sequence was repeated with ethanol (15 mL), amine **25a** (0.220g, 0.00085 mol), tetronic acid (91 mg, 0.00085 mol), and TBDMS-protected syringaldehyde (0.31 mg, 0.00085 mol). In this case, the solution was heated at reflux for 2 h. Upon cooling, the product crystallized from solution and was recrystallized from hot CH_3OH, yielding colorless crystals of 4′-TBDMS-protected **31** (0.165 g, 30%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.10 (2H, m), 6.96 (2H, m), 6.65 (1H, s), 6.60 (1H, s), 6.38 (2H, s), 5.97 (2H, s), 4.97 (1H, s), 4.37 (1H, d, J = 14.7 Hz), 4.23 (1H, d, J = 14.7 Hz), 3.73 (6H, s), 3.60 (2H, m), 2.99 (2H, t, J = 6.6 Hz), 0.99 (9H, s), 0.01 (6H, s). Cleavage of the silyl protecting group was again conducted with anhydrous THF (15 mL), TBDMS-protected **31** (0.16 g, 0.00027 mol), and TBAF (0.5 mL). After stirring under Ar for 0.5 h, TLC showed no starting material. The product was isolated as per **30** (above) followed by sgc chromatography (30:1 CH_2Cl_2–EtOAc → 10:1 CH_2Cl_2–EtOAc → 3:2 CH_2Cl_2–EtOAc) and obtained as an off-white powder, 0.101 g (75%). Recrystallized from hot CH_3OH: mp 198–199 °C (dec); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.12 (2H, m), 6.96 (2H, m), 6.67 (1H, s), 6.60 (1H, s), 6.44 (2H, s), 5.98 (1H, s), 5.97 (1H, s), 4.99 (1H, s), 4.37 (1H, d, J = 15 Hz), 4.29 (1H, d, J = 15 Hz), 3.83 (6H, s), 3.70 (2H, m), 3.00 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.78, 158.42, 147.32, 147.17, 143.97, 136.73, 133.63, 133.13, 130.44, 130.09, 130.00118.70, 115.31, 115.10, 110.39, 105.01, 101.27, 96.05, 94.96, 65.06, 55.67, 39.85, 32.52, 29.93; (+)-HRAPCIMS m/z 506.1613 $[M + H]^+$ (calcd for $C_{28}H_{24}NO_7F$, 506.1615).

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*6,7-Methylenedioxy-4-(furan-2-ylmethyl)-9-(4-hydroxy-3,5-dimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**32**) and 4′-TBDMS-Protected **32***. The preceding reaction sequence was repeated with ethanol (15 mL), **32a** (0.17g, 0.00078 mol), tetronic acid (100 mg, 0.00078 mol), and TBDMS-protected syringaldehyde (0.28 mg, 0.00078 mol). The solution was heated at reflux for 1.5 h, and the solvent was removed in vacuo to afford a yellow solid. The solid was separated by sgc chromatography (30:1 CH_2Cl_2−EtOAc → 400:15 CH_2Cl_2−EtOAc), and the resulting yellow powder recrystallized from hot hexanes−EtOAc, yielding 4′-TBDMS-protected **32** as an off-white powder (0.19 g, 43%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (1H, d, J = 1.5 Hz), 6.67 (1H, s), 6.54 (1H, s), 6.25 (1H, s), 6.24 (2H, s), 6.23 (1H, s), 5.84 (1H, s), 5.83 (1H, s), 4.97 (1H, s), 4.95 (1H, s), 4.92 (1H, s), 4.76 (1H, d, J = 15.1 Hz), 4.63 (1H, d, J = 15.1 Hz), 3.68 (6H, s), 0.89 (9H, s), 0.01 (6H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.64, 159.23, 151.90, 149.08, 147.89, 144.90, 143.44, 139.43, 133.35, 131.69, 119.75, 111.11, 110.94, 109.49, 105.50, 102.10, 98.46, 96.18, 66.25, 55.94, 43.46, 41.04, 26.01, 19.00, 0.00; (+)-HRAPCIMS m/z 578.2212 $[M + H]^+$ (calcd for $C_{31}H_{35}NO_8Si$, 578.2210); anal. C 64.30; H 6.48; N 2.55%, calcd for $C_{31}H_{35}NO_8Si$, C 64.45; H 6.11; N 2.42%. Cleavage of the silyl protecting group was again conducted with anhydrous THF (15 mL), TBDMS-protected **32** (0.19 g, 0.00033 mol), and TBAF (1.0 mL). After 0.5 h under Ar, TLC showed no starting material. The product was isolated as per **31** followed by sgc chromatography (2:1 CH_2Cl_2−EtOAc) and was recovered as a colorless powder, 0.132 g (85%). Recrystallized from hot CH_3OH−hexanes: mp 223−225 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (1H, d, J = 1.5 Hz), 6.68 (1H, s), 6.53 (1H, s), 6.28 (2H, s), 6.35 (1H, s), 6.34 (1H, s), 5.91 (1H, d, J = 1.8 Hz), 5.90 (1H, d, J = 1.8 Hz), 4.97 (1H, s), 4.96 (1H, s), 4.94 (1H, s), 4.74 (1H, d, J = 15.3 Hz), 4.68 (1H, d, J = 15.3 Hz), 3.78 (6H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.78, 158.37, 148.20, 147.13, 146.94, 143.97, 142.47, 136.78, 133.37, 130.66, 118.85, 109.91, 109.88, 108.53, 104.38, 101.14, 97.41, 95.26, 65.33, 55.39, 42.45, 40.01; (+)-HRAPCIMS m/z 464.1351 $[M + H]^+$ (calcd for $C_{25}H_{21}NO_8$, 464.1345); anal. C 64.51; H 4.97; N 3.04%, calcd for $C_{25}H_{21}NO_8$, C 64.79; H 4.57; N 3.06%.

General Procedure for the Synthesis of 4-Aza-podophyllotoxin Structural Modifications: B and E Ring Substitutions 15,[25] 15a−d, 15e,f[2] (Figure 1). The general synthetic procedure now follows for all compounds listed in Figure 1 unless otherwise noted: A stirred solution of ethanol, substituted aniline (**I** or **II**, 1 equiv), tetronic acid (1 equiv), and benzaldehyde (**III**−**VII**, 1 equiv) was heated at reflux and monitored by TLC. The formation of the product was usually evident (after 10 to 60 min) by the appearance of a bright blue spot when viewed under UV. The solvent was then removed in vacuo, and the product was either recrystallized from or purified using sgc chromatography, with appropriate solvent systems.

*6,7,8-Trimethoxy-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15**)*. Purification: recrystallized from CH_3OH as colorless, amorphous crystals, 0.535 g (45%); mp 137−139 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (1H, s), 6.47 (2H, s), 6.29 (1H, s), 5.16 (1H, s), 4.69 (1H, d, J = 16 Hz), 4.61 (1H, d, J = 16 Hz), 3.82 (3H, s), 3.78 (3H, s), 3.76 (3H, s), 3.74 (6H, s), 3.48(3H, s); (+)-HRFABMS m/z 444.1659 $[M + H]^+$ (calcd for $C_{23}H_{25}NO_8$, 444.1658).

*6,7,8-Trimethoxy-9-(3-bromo-4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15a**)*. Purification: sgc chromatography (4:2:1 hexanes−EtOAc−CH_3OH) obtained as colorless crystals, 0.584 g (50%). Recrystallized from acetone−CH_3OH: mp 175−177 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.41 (1H, s), 6.93 (1H, s), 6.81 (1H, s), 5.16 (1H, s), 4.75 (1H, d, J = 15.8 Hz), 4.66 (1H, d, J = 15.8 Hz), 3.88 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 3.80 (3H, s), 3.53 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.90, 157.69, 153.59, 152.38, 144.69, 143.66, 138.65, 132.66, 123.72, 117.29, 111.96, 109.83, 97.26, 95.70, 65.67, 60.88, 60.59, 60.40, 56.16, 56.05, 35.48; (+)-HRAPCIMS m/z 494.0628 $[M + H]^+$ (calcd for $C_{22}H_{22}NO_7{}^{81}Br$, 494.0637); anal. C 53.22; H 4.74; N 2.86%, calcd for $C_{22}H_{22}NO_7{}^{81}Br$, C 53.67; H 4.50; N 2.85%.

3-(TBDMS-O)-4-methoxybenzaldehyde. A solution of CH_2Cl_2 (30 mL), TBDMSCl (1.38 g, 0.008 mol), and imidazole (0.61 g, 0.008 mol) was stirred for 5 min. The solution turned cloudy, and 3-hydroxy-4-methoxybenzaldehyde was added slowly. The reaction proceeded under Ar for 3 h and was terminated with H_2O (30 mL). The CH_2Cl_2 layer was dried, the solvent was removed in vacuo, and sgc chromatography (4:1 hexanes−EtOAc) was used to isolate the product, 3-(TBDMS-*O*)-4-methoxybenzaldehyde[24,25] as a light yellow oil, 1.76 g (83%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.68 (1H, s); 7.31 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz), 7.25 (1H, d, J = 2.4 Hz), 6.80 (1H, d, J = 8.4 Hz), 3.73 (3H, s), 0.89 (9H, s), 0.05 (6H, s).

*6,7,8-Trimethoxy-9-(3-OTBDMS-4-methoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15b**)*. A stirred solution of 3-(TBDMS-*O*)-4-methoxybenzaldehyde (1.99 g, 0.0075 mol) and ethanol (30 mL) was heated to reflux. Next, 3,4,5-trimethoxyaniline (1.39 g, 0.0075 mol) and tetronic acid (0.76 g, 0.0075 mol) were added, and heating at reflux continued for 1.5 h. TLC showed that a bright blue UV-absorbing spot formed, and the product (**15b**) was isolated with sgc chromatography (6:1 CH_2Cl_2−EtOAc) as a yellow oil that solidified at room temperature to yield 6,7,8-trimethoxy-9-(3-OTBDMS-4-methoxyphenyl)-4,9-dihydro-3*H*-furo[3,4-*b*]quinolin-1-one (**15b**) as an amorphous, light yellow solid, 2.23 g (58%). Recrystallized from EtOAc−hexanes: mp 169−171 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (1H, s), 6.63 (3H, m), 6.16 (1H, s), 4.98 (1H, s), 4.54 (1H, s), 4.53 (1H, s), 3.73 (3H, s), 3.65 (3H, s), 3.60 (3H, s), 3.31 (3H, s), 0.82 (9H, s), 0.00 (6H, s); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.71, 162.10, 157.55, 156.44, 153.53, 148.43, 144.48, 142.40, 137.89, 125.24, 124.78, 116.87, 115.06, 100.91, 100.32, 69.59, 65.08, 64.70, 60.57, 60.25, 39.17, 30.34, 22.88, 0.07; (+)-HRAPCIMS m/z 514.2280 $[M + H]^+$ (calcd for $C_{27}H_{36}NO_7Si$, 514.2261); anal. C 62.99; H 6.79; N 2.99%, calcd for $C_{27}H_{36}NO_7Si$, C 63.13; H 6.87; N 2.73%.

*6,7,8-Trimethoxy-9-(3-hydroxy-4-methoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15c**)*. TBAF (1 M solution in THF, 3.3 mL) was added slowly to a stirred solution of silyl ether **15b** (1.55 g, 0.0030 mol) in THF (50 mL), and the reaction was allowed to proceed for 1 h, at which point TLC showed no starting material present. The reaction was terminated with H_2O (30 mL), and the aqueous portion was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extractions were partitioned with 2 N KOH (3 × 30 mL). The base extracts were combined and brought to pH 7 with 1 N HCl, and this solution was extracted with EtOAc (3 × 35 mL). The organic extracts were combined and dried, and the solvent was removed in vacuo, leaving a yellowish-white solid that was recrystallized from hot EtOAc to yield colorless crystals, 0.95 g (79%). Recrystallized from EtOAc: mp 297−299 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.54 (1H, s), 6.68 (1H, d, J = 8.4 Hz), 6.64 (2H, s), 6.55 (1H, d, J = 8.1 Hz), 6.19 (1H, s), 5.69 (1H, bs), 4.97 (1H, s), 4.45 (2H, s), 3.71 (3H, s), 3.65 (3H, s), 3.61 (3H, s), 3.34 (3H, s); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.46, 157.91, 153.09, 152.18, 146.46, 146.35, 140.37, 137.99, 133.60, 118.54, 115.55, 112.27, 110.94, 96.64, 96.02, 65.28, 60.80, 60.45, 56.17, 56.09, 35.05; (+)-HRAPCIMS m/z 400.1424 $[M + H]^+$ (calcd for $C_{21}H_{22}NO_7$, 400.1396).

*6,7-Methylenedioxy-9-(2,3-methylenedioxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15d**)*. Purification: recrystallized from acetone−CH_3OH as a light brown powder, 0.55 g (62%); mp 324−327 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.75 (1H, s), 6.30 (1H, d, J = 1.2 Hz), 6.50 (1H, d, J = 4.5 Hz), 6.46 (1H, s), 6.38 (1H, s, 1H), 5.90 (2H, d, J = 18.6 Hz), 5.80 (2H, d, J = 18 Hz), 4.95 (1H, s), 4.83 (1H, d, J = 15.9 Hz), 4.73 (1H, d, J = 15.9 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.39, 168.52, 156.39, 156.12, 153.42, 152.87, 139.85, 137.47, 125.46, 118.47, 118.39, 116.12, 110.71, 110.15, 106.91, 106.82, 101.92, 74.47, 43.24; (+)-HRFABMS m/z 352.0843 $[M + H]^+$ (calcd for $C_{19}H_{14}NO_6$, 352.0821); anal. C 65.05; H 4.08; 3.99%, calcd for $C_{19}H_{14}NO_6$, C 64.96; H 3.73; N 3.99%.

*6,7-Methylenedioxy-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**15e**)*.[25] Purification: recrystallized from acetone−CH_3OH as a colorless powder, 8.32 g (72%), mp 273−275 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.82 (1H, s), 6.65 (1H, s), 6.51 (1H, s), 6.47 (2H, s), 5.84 (1H, s), 5.79 (1H, s), 4.85 (1H, d, J = 15.9

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Hz), 4.75 (1H, s), 4.74 (1H, d, J = 15.9 Hz), 3.59 (6H, s), 3.49 (3H, s), consistent with literature data.[24,25]

*6,7-Dimethoxy-9-(3,4,5-trimethoxyphenyl)-3H-furo[3,4-b]-quinolin-1-one (**15f**).*[25] A stirred mixture of toluene, olefin **15e** (2.0 g), and 10% Pd/C (1.0 g) was heated at reflux as air was bubbled through the solution. After oxidation for 72 h, the catalyst was collected and the solvent was removed in vacuo, leaving a white solid, which recrystallized from EtOAc−CH_3OH as a colorless, amorphous solid, 1.76 g (88%); mp dec 150 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.50 (1H, s), 7.04 (1H, s), 6.70 (2H, s), 6.26 (2H, s), 5.39 (2H, s), 3.76 (3H, s), 3.74 (6H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.35, 163.38, 153.59, 153.14, 150.50, 149.14, 148.25, 138.28, 128.13, 123.91, 112.47, 107.69, 105.43, 103.59, 102.22, 69.42, 60.62, 56.56; (+)-HRAPCIMS m/z 396.1016 $[M + H]^+$ (calcd for $C_{21}H_{17}NO_7$, 396.1083).

*6,7-Methylenedioxy-4-methyl-9-(3,4,5-trimethoxyphenyl)-4,9-dihydro-3H-furo[3,4-b]quinolin-1-one (**16**).* To a stirred solution of anhydrous THF (10 mL) and secondary amine **15e** (0.30 g, 0.0008 mol) was added BuLi (0.4 mL, 0.0008 mol, 2 M in THF). The solution turned deep red in color, and iodomethane (0.05 mL, 0.0008 mol) was added dropwise. After 1.5 h under Ar, the reaction was terminated by slowly adding H_2O (20 mL). The aqueous phase was partitioned with CH_2Cl_2 (3 × 30 mL). The combined organic extract was dried, and the solvent removed in vacuo. The *N*-methyl derivative **16** was obtained by sgc (4:1 CH_2Cl_2−EtOAc) and recrystallized from hot EtOAc as a light yellow solid, 0.162 g (53%): mp 224−226 °C [lit.[24] mp 226 °C]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.58 (1H, s), 6.57 (1H, s), 6.40 (2H, s), 5.95 (2H, d, J = 1.5 Hz), 5.00 (1H, s), 4.86 (1H, d, J = 15.9 Hz), 4.81 (1H, d, J = 15.9 Hz), 3.79 (3H, s), 3.78 (6H, s); ^{13}C NMR (CDCl3, 125 MHz) δ 172.65, 158.01, 153.16, 147.52, 144.16, 141.51, 136.82, 133.08, 118.26, 110.68, 105.23, 101.62, 97.42, 94.96, 65.00, 60.73, 56.17, 56.08, 40.67, 33.61; (+)-HRAPCIMS m/z 412.1399 $[M + H]^+$ (calcd for $C_{22}H_{21}NO_7$, 412.1396), consistent with literature data.[24]

Cancer Cell Line Procedures. The human cancer cell growth inhibition data were determined using the standard sulforhodamine B assay of the US National Cancer Institute as previously described.[35] In short, cells in a 5% fetal bovine serum/RPMI1640 medium were inoculated in 96-well plates and incubated for 24 h. Next, serial dilutions of the samples were added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50}, or the drug concentration causing a 50% reduction in the net protein increase) was determined from optical density data employing Immunosoft software.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00873.

NMR and MS spectra of compounds **14**, **15a,c,d**, **17**−**28**, and **30**−**32** (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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