Synthesis, Anticancer Activity, and Inhibition of Tubulin Polymerization by Conformationally Restricted Analogues of Lavendustin A

Fanrong Mu,[†] Ernest Hamel,[‡] Debbie J. Lee,[‡] Donald E. Pryor,[‡] and Mark Cushman*[,†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702

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Compounds in the lavendustin A series have been shown to inhibit both protein−tyrosine kinases (PTKs) and tubulin polymerization. Since certain lavendustin A derivatives can exist in conformations that resemble both the *trans*-stilbene structure of the PTK inhibitor piceatannol and the *cis*-stilbene structure of the tubulin polymerization inhibitor combretastatin A-4, the possibility exists that the ratio of the two types of activities of the lavendustins could be influenced through the synthesis of conformationally restricted analogues. Accordingly, the benzylaniline structure of a series of pharmacologically active lavendustin A fragments was replaced by either their *cis*- or their *trans*-stilbene relatives, and effects on both inhibition of tubulin polymerization and cytotoxicity in cancer cell cultures were monitored. Both dihydrostilbene and 1,2-diphenylalkyne congeners were also prepared and evaluated biologically. Surprisingly, conformational restriction of the bridge between the two aromatic rings of the lavendustins had no significant effect on biological activity. On the other hand, conversion of the three phenolic hydroxyl groups of the lavendustin A derivatives to their corresponding methyl ethers consistently abolished their ability to inhibit tubulin polymerization and usually decreased cytotoxicity in cancer cell cultures as well, indicating the importance of at least one of the phenolic hydroxyl groups. Further investigation suggested that the phenolic hydroxyl group in the salicylamide ring was required for activity, while the two phenol moieties in the hydroquinone ring could be methylated with retention of activity. Two of the lavendustin A derivatives displayed IC_{50} values of 1.4 μM for inhibition of tubulin polymerization, which ranks them among the most potent of the known tubulin polymerization inhibitors.

Introduction

The *Streptomyces griseolavendus* metabolite lavendustin A (**1**) was originally isolated and characterized as a protein−tyrosine kinase (PTK) inhibitor when tested vs the epidermal growth factor receptor (EGFR) in an A431 cell membrane fraction.[1] Although initially thought to be strictly competitive at the ATP binding site and noncompetitive at the substrate binding site, a more detailed kinetic analysis indicated that lavendustin A is a hyperbolic mixed-type inhibitor with respect to both ATP and the substrate.[2] The first synthesis of lavendustin A passed through the intermediate **2**, which was found to be as active as **1** as a PTK inhibitor.[1] Although the high polarity of **1** precludes it from penetrating cellular membranes, several of its more lipophilic analogues, including the methyl ester **3**[3] and the *N*-*n*-hexyl derivative **4**,[4,5] are effective PTK inhibitors in cellular systems. In addition, a large number of ester and amide derivatives of the active lavendustin A fragment **2** have been synthesized and found to inhibit EGF-stimulated DNA synthesis in ER 22 cells, with the 3-phenyl-*n*-propyl ester **5** being among the best compounds in the series.[6−8] These results led to the synthesis of compound **6**, which displayed strong antiproliferative activity in HaCaT cells but was inactive as a PTK inhibitor when tested on the EGFR tyrosine kinase.[9] The antiproliferative activity of **6** was subsequently found to be due to its antimitotic activity, and it was also determined to be comparable in anticancer activity to mitomycin C when tested in nude mice bearing A431 human vulvar carcinomas.[10] Earlier work with the lavendustin C6 analogue **4** had also suggested that its antiproliferative effects might be related to actions on cellular targets that are unrelated to protein−tyrosine kinases.[4]

Recently, a series of amide derivatives of the pharmacologically active lavendustin A fragment **2** were synthesized for evaluation as EGFR and Syk tyrosine kinase inhibitors.[11] The most cytotoxic compound in the series was the *N*-(4-fluorophenethyl)salicylamide derivative **7**, which yielded a GI_{50} mean-graph midpoint (MGM) value of 0.3 μM when tested in the National Cancer Institute (NCI) cytotoxicity screen of approximately 55 human cancer cell lines. However, a COMPARE analysis[12] of the cytotoxicity profile of **7** revealed that it was likely to be acting on tubulin.[11] When tested for inhibition of tubulin polymerization in a cell-free system, the amide **7** and related amides were in fact determined to be active.[11] Compound **7** and many of the other derivatives were also found to be active against both the nonreceptor PTK Syk and the receptor PTK EGFR. Therefore, both tubulin polymerization inhibi-

* To whom correspondence should be addressed. Phone: 765-494-1465. Fax: 765-494-1414. E-mail: cushman@pharmacy.purdue.edu.
[†] Purdue University.
[‡] National Institutes of Health.

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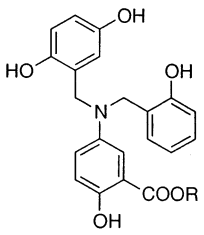
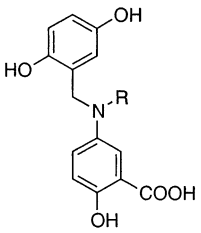
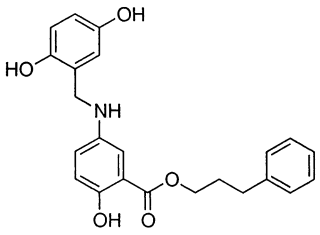


1 R = H
3 R = CH_3



2 R = H
4 R = *n*-C_6H_{13}



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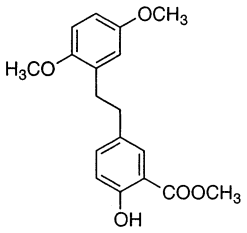

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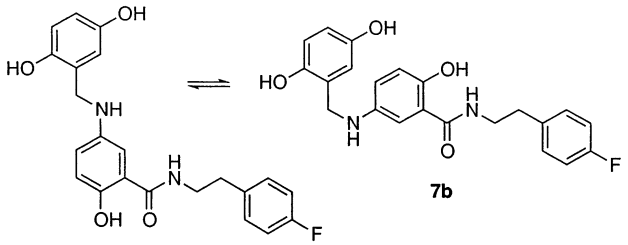
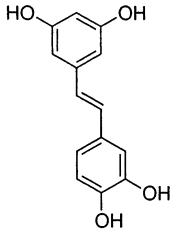


7a

7b



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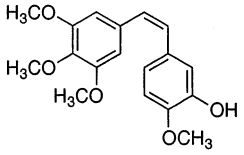

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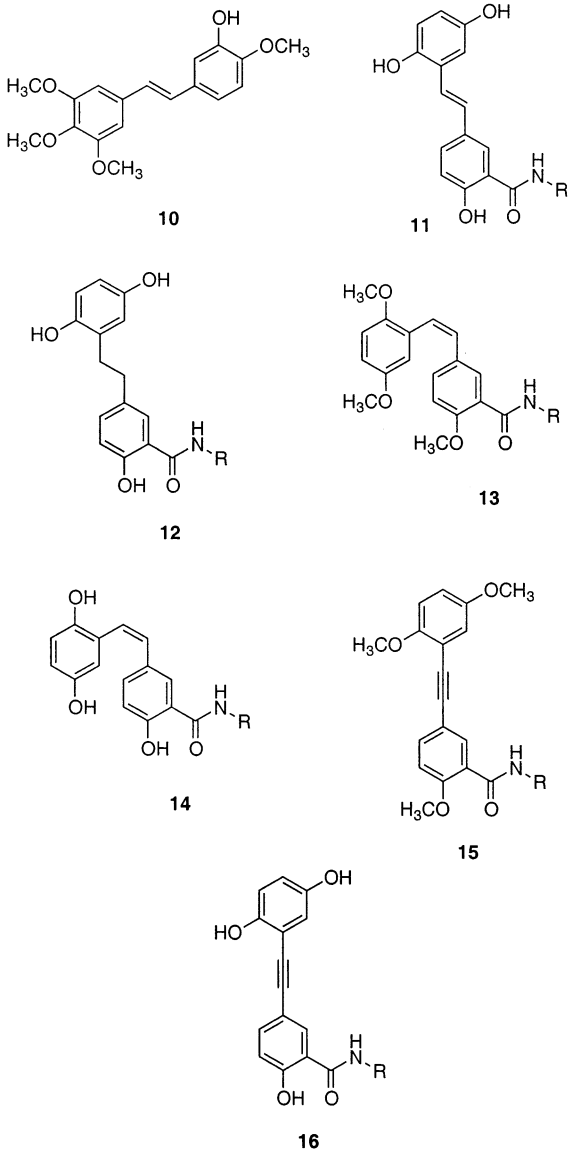
tory activity and PTK inhibitory activity were found to reside in the same molecules. However, it seems unlikely for the following four reasons that the cytotoxicities of these lavendustin A amide analogues are due to protein−tyrosine kinase inhibition: (1) Whereas MCF-10A cells are EGF-dependent while MCF-7 cells are not, lavendustin A amides were equally effective as inhibitors of DNA synthesis in these two breast cancer cell lines.[11] (2) Since almost all of the EGFR tyrosine kinase activity must be inhibited before effects are seen on cell growth,[13] it is unlikely that the potencies of amide **7** and its congeners as EGFR inhibitors could possibly be responsible for the effects seen on cancer cell growth because the IC_{50} values for EGF PTK inhibition are close to their IC_{50} values for cytotoxicity.[11] (3) The fact that lavendustin A (**1**) did not inhibit the PTK activity of the mutant protein $pp60^{F527}$, but nevertheless did exhibit antiproliferative activity, suggests that the antiproliferative effects of **1** could be due to actions on cellular targets downstream from $pp60^{F527}$ or on unrelated receptors.[4] (4) The potencies of amide **7** and its analogues as inhibitors of both the EGF receptor and the nonreceptor protein−tyrosine kinase Syk did not correlate particularly well with their cytotoxic activities against cancer cell cultures.[11]

The activities of the lavendustin A amide derivatives **7** and related compounds as inhibitors of both PTKs and tubulin polymerization are intriguing. Compound **7** does bear some structural resemblance to the PTK inhibitors piceatannol (**8**)[14] and structurally related polyhydroxylated *trans*-stilbenes.[15,16] On the other hand, the structural relationship between the lavendustin A analogues and the tubulin polymerization inhibitor combretastatin A-4 (**9**),[17] as well as the structurally allied polymethoxylated *cis*-stilbene, dihydrostilbene, and benzylaniline tubulin polymerization inhibitors, is apparent.[9,18,19] Whereas combretastatin A-4 (**9**) and a large number of polymethoxylated *cis*-stilbene analogues are potent tubulin polymerization inhibitors that are highly cytotoxic in human cancer cell cultures, their corresponding trans isomers, including *trans*-combretastatin A-4 (**10**), are inactive as tubulin polymerization inhibitors and are less cytotoxic.[18] This suggests that the PTK inhibitory activity of the lavendustin A amide analogues could reside in the anti conformation of the linkage between the two aromatic rings, as displayed in structure **7a**, while the syn conformation, as shown in structure **7b**, might be responsible for their antitubulin effects. Since the cytotoxicity of the lavendustin A amide analogues, including **7**, seems to be due to inhibition of tubulin polymerization and not PTK inhibition, we decided to synthesize a series of conformationally restricted lavendustin A amide analogues and monitor their effects on tubulin polymerization.

Design of Conformationally Restricted Analogues

To restrict the conformation of the linker connecting the two aromatic rings in the pharmacologically active lavendustin A derivative **7** in an anti conformation to create a series of analogues that would more closely resemble piceatannol (**8**), a series of *trans*-stilbenes **11** was designed. A number of dihydro analogues **12**, which would have more conformational freedom, were also planned, since this would provide insight into the question of whether any difference in activity between **7** and **11** was due to a conformational effect or simply to the absence or presence of the amino group. Methylation of the phenolic hydroxyl groups of **7** and restriction of the linker connecting the aromatic rings in a cis alkene resulted conceptually in the series of analogues represented by the general structure **13**, which bears a closer resemblance to combretastatin A-4 (**9**). Demethylation of the three methoxyl groups of **13** led to series **14**, which would more closely resemble **7** but would approximate the syn conformation **7b**. Finally, replacement of the cis alkene linker of **13** with a linear alkyne linker would result in series **15**, and a similar structural transformation of **14** would provide series **16.** A comparison of the pharmacological activities of the phenolic compounds **11**, **12**, **14**, and **16** should provide a rigorous probe of the optimal spatial orientation of the two aromatic rings, and the methyl ethers **13** and **14** should



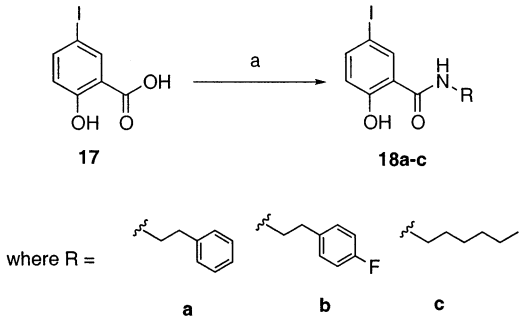
provide insight into the question of whether the tubulin polymerization inhibitory activity of the lavendustins could be maximized by converting them to compounds that more closely resemble combretastatin A-4 (**9**).

Chemistry

In the design of the piceatannol−lavendustin A hybrid molecules **11**, the aminomethylene linkage between the two aromatic rings of the pharmacologically active lavendustin A fragment **7** is replaced by a trans alkene linker, which could possibly be accomplished by a Heck coupling reaction. Aryl iodide coupling partners were constructed as shown in Scheme 1. Treatment of commercially available 5-iodosalicylic acid (**17**) with phenethylamine, 4-fluorophenethylamide, and hexylamine, in the presence of DCC, provided the required synthetic intermediates **18a**−**c**.

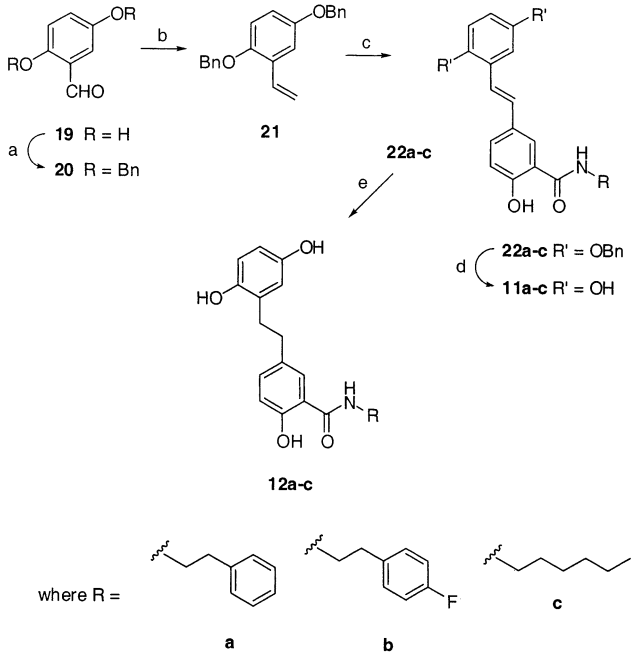
The other coupling partner, 2,5-dibenzyloxystyrene (**21**), was prepared in two steps from 2,5-dihydroxybenzaldehyde as outlined in Scheme 2. First, the two phenolic hydroxyl groups were protected as benzyl ethers by treatment with benzyl bromide in acetone, with potassium carbonate as the base, to yield **20**,[20] which was then converted to **21** through the Wittig

Scheme 1[a]



[a] Reagents and conditions: (a) RNH_2, DCC, pyridine, 70−80 °C (5 h).

Scheme 2[a]

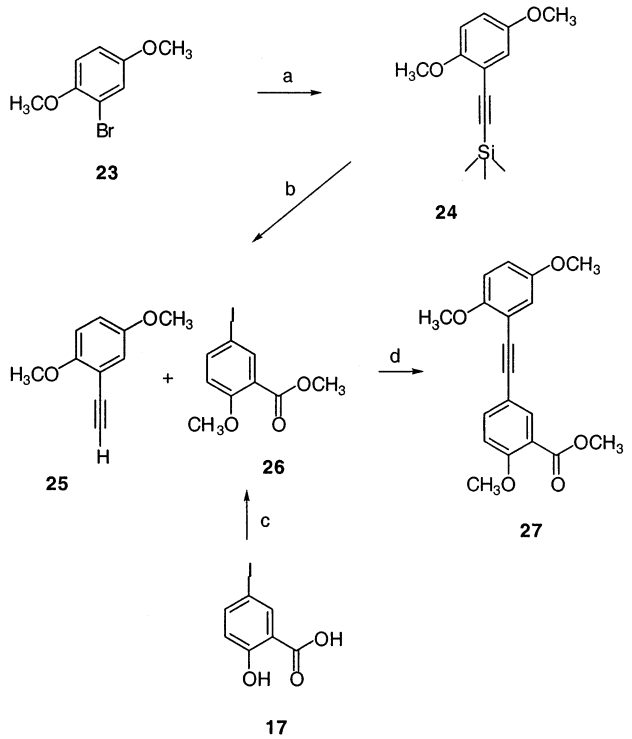


[a] Reagents and conditions: (a) BnBr, Me_2CO, K_2CO_3, reflux (12 h); (b) (1) $MePPh_3Br$, *n*-BuLi, THF 23 °C (1.5 h), (2) **20**, 23 °C (1 h), 70 °C (6 h); (c) **18a**−**c**, $Pd(OAc)_2$, Et_3N, tri-*o*-tolyphosphine, CH_3CN, 100 °C (30 h); (d) BBr_3, CH_2Cl_2, −78 to 23 °C (4 h); (e) H_2, Pd/C, EtOAc (12 h).

reaction. Heck coupling of **21** with the three iodosalicylamides **18a**−**c** was performed at high temperature in acetonitrile in the presence of triethylamine, using $Pd(OAc)_2$ and tri-*o*-tolylphosphine as the catalyst. As expected from the Heck reaction, under these reaction conditions, the trans isomers **22a**−**c** were obtained exclusively. Cleavage of the benzyl groups from **22a**−**c** with boron tribromide at low temperature in methylene chloride afforded the desired *trans*-styrenes **11a**−**c**. The formation of the trans isomers **11a**−**c** was confirmed by the characteristic ^1H NMR coupling constant of 16.5−18.1 Hz observed for the two alkene protons. Catalytic hydrogenation of **22a**−**c** provided the corresponding compounds **12a**−**c** resulting from cleavage of the two benzyl groups and reduction of the double bond.

The alkyne **27** (Scheme 3) was envisaged as an intermediate in the synthesis of the cis alkene systems **13** and **14** as well as the alkynes **15** and **16**, and the Sonogashira coupling was investigated as the key step in its assembly. The required monosubstituted alkyne intermediate **25** was obtained by Sonogashira coupling

Scheme 3[a]

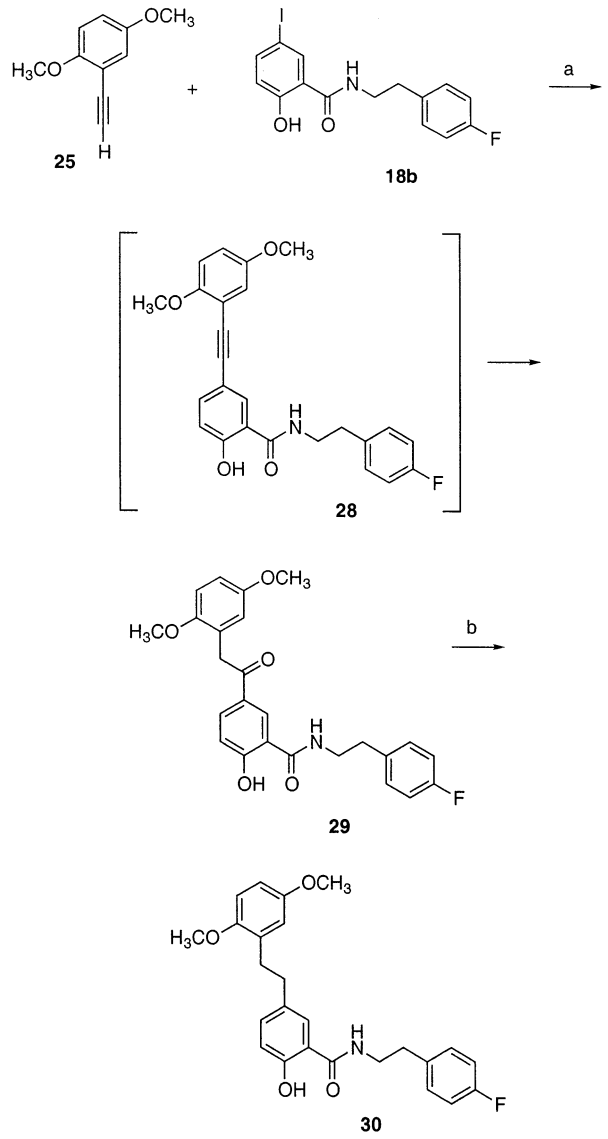


[a] Reagents and conditions: (a) HC≡C−Si$(CH_3)_3$, $PdCl_2$, PPh_3, CuI, piperidine, reflux (24 h); (b) *n*-Bu_4NF, THF, H_2O, 23 °C (3 h); (c) $(CH_3)_2SO_4$, K_2CO_3, Me_2CO, reflux (12 h); (d) $(PPh_3)_2PdCl_2$, CuI, Et_2NH, 50-60 °C (8 h).

of trimethylsilylacetylene with 1-bromo-2,5-dimethoxybenzene (**23**) in the presence of $PdCl_2$, PPh_3, and CuI in refluxing piperidine, followed by desilylation of intermediate **24** with tetra-*n*-butylammonium fluoride in THF. Meanwhile, 5-iodosalicylic acid (**17**) was converted to methyl 5-iodo-2-methoxybenzoate (**26**) with dimethyl sulfate under basic conditions. A second Sonogashira coupling of **25** and **26** under the same reaction conditions afforded the alkyne intermediate **27**.

As shown in Scheme 4, in an iodobenzene system containing a free hydroxyl group, the attempt to use an analogous Sonogashira approach to the synthesis of the alkyne **28** led to an unexpected result. Instead of obtaining the alkyne **28**, another compound was isolated that was assigned the ketone structure **29** on the basis of the ^1H and ^{13}C NMR data, along with chemical ionization mass spectrometry and elemental analysis. The position of the carbonyl group in **29** was subsequently verified by X-ray crystallography, and the resulting ORTEP diagram is displayed in Figure 1.

Scheme 4[a]



[a] Reagents and conditions: (a) $PdCl_2$, PPh_3, CuI, piperidine, reflux (24 h); (b) H_2, Pd/C, EtOAc, 23 °C (4 h).

Hydrogenation of **29** in the presence of palladium on carbon effectively removed the carbonyl group to afford the reduction product **30**.

The formation of **29** most likely involves initial formation of the alkyne, followed by a regioselective palladium-catalyzed hydration. Oxypalladation reactions of alkynes are known,[21,22] including the regioselective hydration of alkynones by palladium catalysis

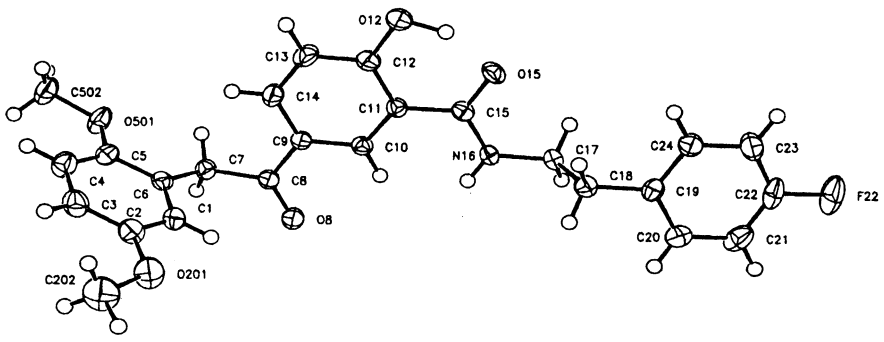
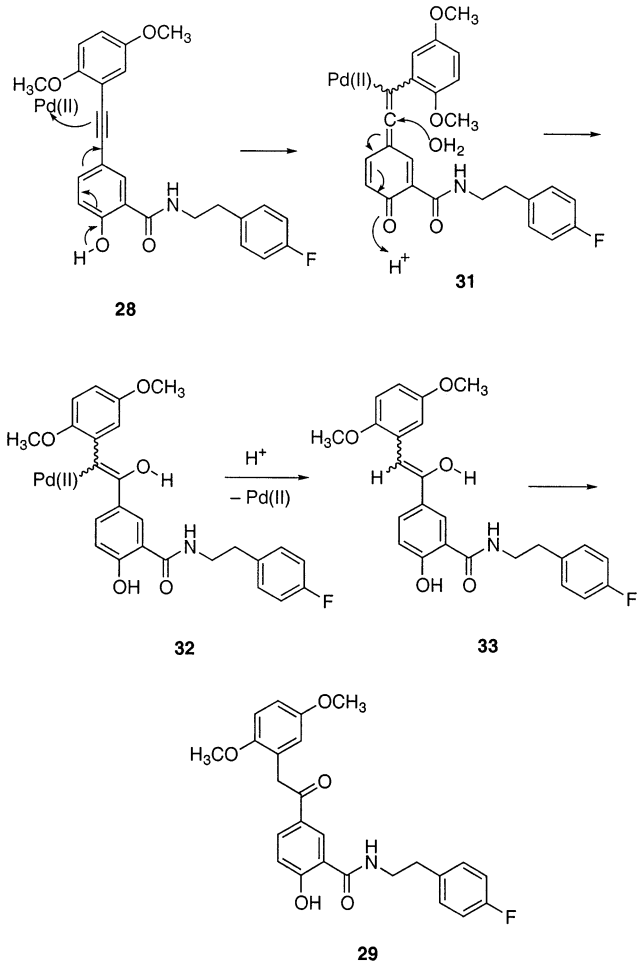


Figure 1. ORTEP diagram resulting from X-ray crystallographic analysis of ketone **29**.

Scheme 5

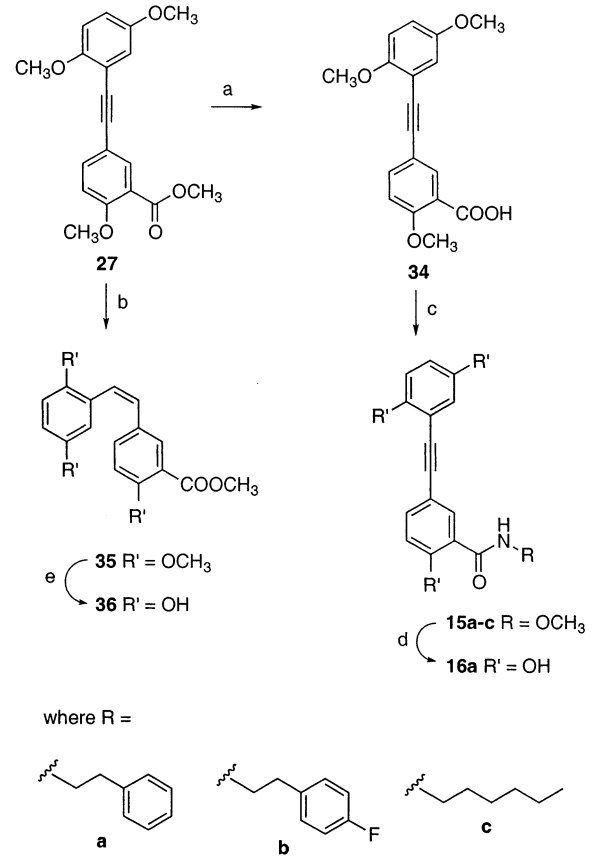


to form ketones.[23] In the present case, the regioselectivity of the reaction is influenced by the substitution pattern on both aromatic rings. As proposed in Scheme 5, this could possibly be explained by the conversion of the initially formed alkyne **28** to the palladium-substituted allene **31**, followed by attack of water on the quinone methide moiety to form **32**.[22] Protonation of **32** and loss of palladium would then generate the enol, which would tautomerize to the observed ketone **29**. The regioselectivity of the reaction is then explained by the participation of the phenol in the formation of the palladium-substituted allene **31**.

After the alkyne **27** was obtained in reasonable quantities, trihydroxylated and trimethoxylated alkynes and cis alkenes were synthesized as outlined in Scheme 6. The methyl ester present in **27** was first hydrolyzed to the acid **34**, which was then converted to the corresponding amide analogues **15a**−**c** by reaction with the required amines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT). Demethylation of **15a** with boron tribromide in methylene chloride then afforded the triphenol **16a**. On the other hand, hydrogenation of the alkyne **27** using Lindlar catalyst (5% Pd on $CaCO_3$, poisoned with Pb) led to the cis alkene **35**, which was demethylated as before to afford the triphenol **36**.

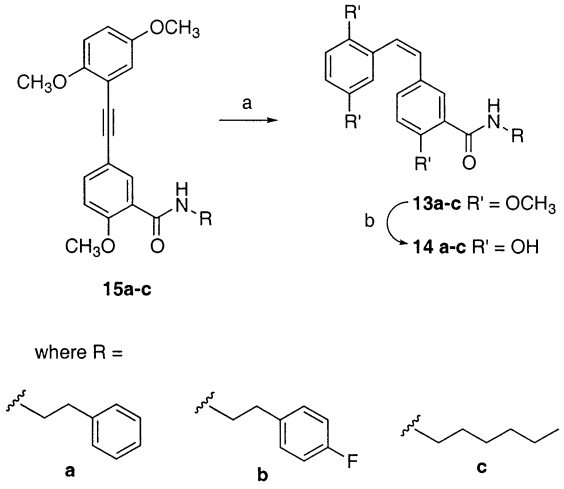
As outlined in Scheme 7, hydrogenation of the alkynes **15a**−**c** using the Lindlar catalyst yielded the three cis

Scheme 6[a]



[a] Reagents and conditions: (a) NaOH, MeOH, 60−70 °C (3 h); (b) H_2, $Pd/CaCO_3$ (poisoned with Pb), EtOH, EtOAc, 23 °C (2 h); (c) RNH_2, Et_3N, EDCI, HOBT, DMF, 23 °C (48 h); (d) BBr_3, CH_2Cl_2, 0 °C (3 h); (e) BBr_3, CH_2Cl_2, −20 °C (2 h).

Scheme 7[a]



[a] Reagents and conditions: (a) H_2, $Pd/CaCO_3$ (poisoned with Pb), EtOAc, EtOH 23 °C (2 h); (b) BBr_3, CH_2Cl_2, −20 °C (2 h).

substituted alkenes **13a**−**c**, which were demethylated to provide the correspinding triphenols **14a**−**c.**

Biological Results and Discussion

The lavendustin A analogues were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute cytotoxicity screen, in which the activity of each compound was evaluated using approximately 55 different cancer cell

Table 1. Cytotoxicities and Antitubulin Activities of Lavendustin A Analogues

	cytotoxicity (GI_{50} in μM)[a]										inhibition of tubulin polymerization[c]
compd	leukemia CCRF-CEM	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	(IC_{50}, μM ± SD)
7	0.29	12	9.2	9.2	7.8	11	7.7	18	13	0.35	6.4 ± 0.7
11a	2.1	9.3	1.8	2.4	2.0	2.9	5.1	10.1	4.0	3.8	4.0 ± 0.6
11b	1.8	8.8	1.9	2.2	2.1	2.7	5.6	11.0	3.8	3.5	2.9 ± 0.1
11c											5.0 ± 0.8
12a		13.9	12.5		11.0	4.4	49.8	14.5	3.9	6.4	5.5 ± 1
12b	0.4	2.6	1.0	1.8	2.8	1.0	2.1	8.0	2.3	2.8	9.7 ± 2
12c	5.1	7.4	3.6	2.2	3.3	2.4	2.2	7.4	3.2	4.4	6.2 ± 0.07
13a	12.3	>100	47.3	>100	16.4	5.0	>100	>100	3.0	38.9	>40
13b	3.4	8.1	3.8	5.9	3.5	4.2	6.4	>100	2.1	4.5	>40
13c	24.7	31.9	21.1	23.6	25.0	16.1	63.1	45.6	10.3	16.6	>40
14a	0.2	3.0	3.5	1.6	2.1	1.0	6.8	1.8	1.7	1.4	5.4 ± 0.6
14b	1.3	6.3	3.8	3.8	2.3	2.1	4.0	15.2	12.9	3.5	4.2 ± 1
14c	1.2	8.3	3.9	3.3	5.8	1.7	11.6	10.5	4.5	4.1	4.2 ± 0.4
15a		>100	12.0	>100	24.4	6.7	>100	>100	2.5	40.7	>40
15b	4.7	4.9	4.7		6.0	3.0	>100	>100	1.2	11.5	>40
15c	7.9	11.8	6.9	>100	56.8	>100	>100	>100	>100	31.6	>40
16a	0.3	10.3	10.5	9.6	1.9	2.8	5.7	12.7	2.9	3.8	3.7 ± 0.08
27	36.8		37.7	81.1	29.3	13.6	37.9	43.6	>100	30.9	>40
29	2.4	1.5	4.0		4.0	1.4	4.3	1.7	0.6	3.6	1.4 ± 0.08
30	3.2	15.0	5.3	3.2	6.6	3.2	15.8	4.2	0.5	6.9	1.4 ± 0.4
34	55.0	25.6	32.3	38.5	22.6	24.4	38.2	26.9	8.8	18.6	>40
35		32.9	20.1	10.6	14.8	4.9	30.2	18.1	10.9	15.5	>40

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. Compounds producing a mean-graph midpoint (MGM) of 10 μM or less on the first determination were tested a second time, and the standard deviations were calculated. Compounds producing a mean-graph midpoint (MGM) greater than 10 μM on the first determination were not evaluated further. [b] Mean-graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c] Minimum of two independent determinations. Tubulin concentration was 1.2 mg/mL (12 μM).

lines of diverse tumor origins. The mean-graph midpoint values (MGMs) listed in Table 1 are based on a calculation of the average GI_{50} values for all of the cancer cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-4}–10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.[24] The cytotoxicity GI_{50} values are also listed in Table 1 for nine representative cell lines, along with the IC_{50} values for inhibition of tubulin polymerization.

The evaluation of the compounds as inhibitors of tubulin polymerization led to some unexpected results. On the basis of the assumption that our lead compound **7** would adopt a syn conformation **7b** in order to more closely resemble the *cis*-stilbene structure of combretastatin A-4 (**9**), one would expect the *cis*-stilbene lavendustin A analogues (**14a**–**c**) to be more potent as tubulin polymerization inhibitors than the *trans*-stilbene lavendustin A analogues (**11a**–**c**). However, the results clearly show that the alkene geometry makes no difference in activity, since all six compounds were active and had IC_{50} values in the 2.9–5.4 μM range. The unimportance of the geometry of the linker connecting the two aromatic rings was corroborated further by the activity of the alkyne **16a** as a tubulin polymerization inhibitor (IC_{50} = 3.7 μM), as well as the activities of the three compounds having a saturated ethylene linker: **12a** (IC_{50} = 5.5 μM), **12b** (IC_{50} = 9.7 μM), and **12c** (IC_{50} = 6.2 μM).

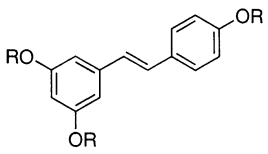
Comparison of the tubulin polymerization inhibitory activities of the amine **7** (IC_{50} = 6.4 μM), the hydrocarbon **12b** (IC_{50} = 9.7 μM), and the ketone **29** (IC_{50} = 1.4 μM) provides some insight into the importance of the amine nitrogen in the benzylamine linker chain of the lavendustins. The evidence indicates that the basic nitrogen atom is not necessary for activity, and the ketone **29** is 4- to 5-fold more active than the lead compound **7**.

By examination of the relative potencies of similar compounds that only vary by having different substituents on the amide nitrogen, it is possible to draw conclusions about how the phenethyl (substituent "a"), 4-fluorophenethyl (substituent "b"), and *n*-hexyl (substituent "c") groups on the amide influence activity. The results in Table 1 reveal that the amide substituent has very little, if any, real effect on the abilities of these compounds to inhibit tubulin polymerization. Also, with the exception of **13b**, the amide substituent has no significant effect on cytotoxicity in human cancer cell cultures.

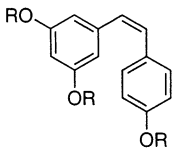
Methylation of the three phenolic hydroxyl groups in **14a**–**c** might be expected to make these compounds closer in structure to combretastatin A-4 (**9**) and conceivably increase their potencies as tubulin polymerization inhibitors, but the results show that the trimethoxylated intermediates **13a**–**c** were completely inactive as tubulin polymerization inhibitors. These results indicate the importance of the phenolic hydroxyl groups for activity. In agreement with this conclusion, while the triphenolic alkyne **16a** is active, the corresponding trimethoxylated derivative **15a** is inactive, as are **15b** and **15c**. The additional inactive methyl ethers include alkynes **27** and **34**. However, it should be noted that compounds **29** and **30**, which bear two methoxyl groups in the benzyl aromatic ring and one phenolic hydroxyl group in the salicylamide aromatic ring, are the two most potent compounds in the series as inhibitors of tubulin polymerization, which indicates that only the free phenolic hydroxyl group in the "lower" ring is critical for activity. The IC_{50} values for **29** and **30** as tubulin polymerization inhibitors (both 1.4 μM) rank them with combretastatin A-4 (IC_{50} = 1.9 μM), podo-

phyllotoxin (2.1 μM), and thiocolchicine (1.4 μM).[18] (In experiments contemporaneous with those in which these lavendustin analogues were examined, combretastatin A-4 (**9**) yielded a lower IC_{50} value, 0.94 μM, for inhibition of tubulin polymerization.)

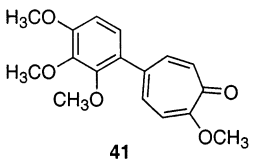
With the exception of analogue **13b**, the cytotoxicities of the compounds appear to correlate fairly well with their abilities to inhibit tubulin polymerization. In general, compounds having MGM values of less than 10 μM also had IC_{50} values of less than 10 μM. On the other hand, except for **13b**, all of the compounds with IC_{50} values greater than 40 μM for inhibition of tubulin polymerization were less inhibitory for cell growth. These results indicate that the observed cytotoxicities of the compounds in this series may in fact be related to inhibition of tubulin polymerization. Furthermore, the MGM values of the *trans*-stilbenes **11a** (MGM = 4.0 μM) and **11b** (MGM = 2.9 μM) vs their cis isomers **14a** (MGM = 5.4 μM) and **14b** (MGM = 4.2 μM) agree with the antitubulin data in indicating a lack of geometrical effects on the biological activity. These data contrast with those reported recently by Pettit et al. for resveratrol **37** and its cis isomer **38**, which demonstrated that the trans isomer is more cytotoxic than the cis isomer.[25] The same study also documented the fact that the cis trimethyl ether derivative **39** of *cis*-resveratrol was significantly more cytotoxic than resveratrol trimethyl ether (**40**). The greater potency of *cis*-resveratrol trimethyl ether (**39**) vs the trans isomer **40** is in line with what one would expect from the known structure–activity data for combretastatin A-4 (**9**) and its trans isomer **10**.[18,26] These results contrast with those of the present series of stilbenes, which showed no effect of alkene geometry on biological activity. The unusual lack of a geometrical effect in the present series may relate to the presence of the salicylamide moiety.



37 R = H
40 R = CH_3



38 R = H
39 R = CH_3



41

To summarize the tubulin polymerization results, when the molecules in the present lavendustin series were constrained in ways that should have made them more potent on the basis of the assumption that their SAR would mimic the SAR observed with combretastatin A-4 and its analogues, either no change in activity or a large decrease in potency occurred. Especially surprising was the lack of an effect resulting from structural changes in the hydrocarbon linker connecting the two aromatic rings. This is in contrast to the isomerization of the cis double bond in combretastatin A-4 (**9**) and related stilbenes to their trans isomers, which abolishes tubulin polymerization inhibitory activity and also makes them much less cytotoxic.[18] These observations lead to the conclusion that the present lavendustin derivatives may bind to tubulin in a way that is structurally distinct from their combretastatin A-4 relatives. This possibility was explored by further examining the interaction of lavendustin analogues with tubulin. The most potent inhibitors of assembly described here (compounds **11ab**, **14bc**, **16a**, **29**, and **30**) were examined for inhibitory effects on the binding of [^{3}H]colchicine to tubulin, and compound **29** was the most active. However, like **7**,[11] **29** was a relatively weak inhibitor in that significant inhibition required at least a 10-fold excess of **29** over the [^{3}H]colchicine. In contrast, 50% inhibition of colchicine binding by combretastatin A-4 (**9**) is readily demonstrated even when the concentration of **9** is less than the colchicine concentration.[27]

The binding of colchicine, as well as other colchicinoids such as thiocolchicine, to tubulin has a number of unusual properties (for a review, see ref 28).[28] Two features of the drug–tubulin interaction are especially notable. First, binding is negligible at 0 °C, and even at higher temperatures, depending on the precise reaction conditions, it can take hours to fully saturate tubulin with the drug. Second, once bound, colchicine and thiocolchicine dissociate very slowly from tubulin, with the half-lives of these tubulin–drug complexes being at least 24 h at 37 °C.[29] As a consequence, in practical terms, formation of the tubulin–colchicine complex is generally considered an irreversible reaction.

In contrast, in our experience, binding of most colchicine site compounds to tubulin is substantially more rapid and reversible than that of colchicinoids. Maximum inhibition of tubulin assembly by colchicine and its analogues is enhanced (i.e., a substantially lower IC_{50} value obtained) by a drug–tubulin preincubation prior to addition of the GTP required for assembly (for example, see ref 30).[30] The IC_{50} values obtained for **29**, as well as for combretastatin A-4 (**9**), changed relatively little if the preincubation step used in the experiments presented in Table 1 was omitted (data not presented).

The reversibility of binding of **29** at the colchicine site was evaluated in a more complex experiment. Because compound **41**, as well as colchicine, fluoresces more intensely upon binding to tubulin,[28] its interaction with the protein has been studied extensively, with its association and dissociation parameters well-characterized.[28] Besides binding rapidly to tubulin, compound **41** dissociates rapidly from the protein. One can therefore obtain good insight into the behavior of a new colchicine site drug by comparing its inhibitory effect on colchicine binding as a function of time to the rapidly dissociating **41** and to the stably bound thiocolchicine.

Such a study is shown in Figure 2. In this experiment, inhibitors and tubulin were preincubated prior to addition of [^{3}H]colchicine, and the extent of inhibition of colchicine binding was followed over time. If a drug binds reversibly at the colchicine site, the extent of inhibition "decays" over time, with the exact pattern observed reflecting the combined effects of the inability of [^{3}H]colchicine to dissociate significantly once bound, the rate of dissociation of the inhibitor, the binding rate of colchicine, and the rebinding rate of the inhibitor. The

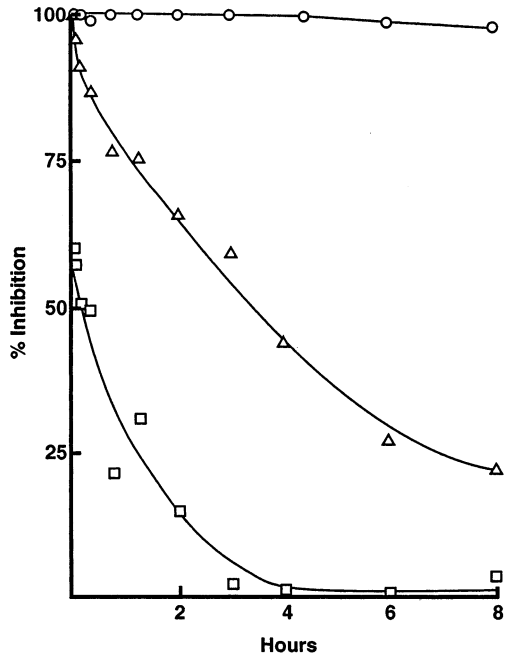


Figure 2. The experiment was performed essentially as described previously.[36] Reaction mixtures (3.0 mL) were prepared containing tubulin at 0.1 mg/mL (1.0 μM), either no inhibitor or the indicated inhibitor at 20 μM with 5% (v/v) dimethyl sulfoxide (final concentration) as solvent for the inhibitors, 1.0 M monosodium glutamate (pH 6.6 with HCl), 0.1 M glucose 1-phosphate, 1 mM $MgCl_2$, 1 mM GTP, and 0.5 mg/mL bovine serum albumin. The reaction mixtures were warmed to 37 °C for 45 min and then chilled on ice. Aliquots (0.1 mL each) of each reaction mixture were distributed into test tubes, and a total of 10 μL containing 200 pmol of [^{3}H]-colchicine was added to each tube. The samples were incubated for the indicated times at 30 °C. Duplicate data points were taken for each sample at each time point. For each time point, reaction mixtures containing each inhibitory drug were compared with the control reaction mixture without inhibitor, with the data expressed as a percentage of inhibition of colchicine binding. Symbols represent the following: ○, thiocolchicine; △, compound **41**; □, compound **29**.

precise pattern obtained also depends on the inhibitor/colchicine/tubulin ratio used in the experiment.

In the experiment of Figure 2, the total inhibition of colchicine binding by thiocolchicine persisted throughout the experiment, reflecting the tighter binding of thiocolchicine than colchicine (both faster k_f and slower k_r),[29] as well as the 20:1 inhibitor/colchicine ratio used in this experiment.

Compound **41**, in contrast, was totally inhibitory only at the earlier time points, and at the final time point, it inhibited colchicine binding by only about 15%. The half-life of this decay process was about 3 h under these experimental conditions.

Compound **29** was not totally inhibitory even at the earliest time point examined. At 2.5 min after colchicine addition, the amount of [^{3}H]colchicine bound was reduced by 62% in the presence of a 20-fold excess of **29**. The loss of inhibitory effect of **29** was even more rapid than that of compound **41**. The half-life of inhibition by **29** was only 50−60 min, about 3-fold faster than that of **41**. Compound **29** therefore must dissociate from tubulin even more rapidly than compound **41**. In contrast, combretastatin A-4 dissociates from tubulin more slowly than does compound **41**.[31]

Inhibition of tubulin assembly by **9** and **41** does not differ greatly from inhibition by **29** (IC_{50} values of 0.94 and 1.5 μM versus 1.4 μM for **29**). If we make the assumption that similar effects on assembly reflect similar rapid binding rates, then the dissociation rates may be roughly proportional to the inhibition decay half-lives. Since the k_f for **41** is 0.04−0.07 s^{-1} ($t_{1/2}$ = 15−25 s),[32−34] we can estimate the dissociation rate for **29** as 3-fold higher, or about 0.15 s^{-1} ($t_{1/2}$ = 7 s). It would also be reasonable to conclude that the relatively weak inhibitory effects of **29** and other lavendustin analogues on colchicine binding are derived from rapid dissociation from the colchicine site.

In conclusion, the present series of compounds has yielded at least two representatives, **29** and **30**, that are potent tubulin polymerization inhibitors, despite an apparently weak binding interaction with tubulin at the colchicine site. The observed cytotoxicities of these compounds in human cancer cell cultures warrant their further investigation as potential anticancer agents. Both compounds are structurally related to the antimitotic agent **6**, which has displayed in vivo tumor growth inhibition that is comparable to 5-fluorouracil and mitomycin C in nude mice.[10]

Experimental Section

Melting points were determined with a Mel-Temp capillary melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker VXR-300S or DRX-500 spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. Column chromatography was carried out using Merck silica gel (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on silica gel GF (Analtech) glass-coated plates (2.5 cm × 10 cm with 250 μm layer and prescored), and spots were visualized with UV light at 254 nm. Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). The authors thank Drs. A. Brossi (National Institute of Diabetes and Digestive and Kidney Diseases) and M. G. Banwell (Australian National University) for gifts of thiocolchicine and compound **41**, respectively.

Tubulin Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[35] The tubulin polymerization and colchicine binding assays were performed as described previously[27,36] except that Beckman DU7400/7500 spectrophotometers equipped with "high-performance" temperature controllers were used in the former assay. Unlike the manual control possible with the previously used Gilford spectrophotometers, the polymerization assays required use of programs provided by MDB Analytical Associates, South Plainfield, NJ, since the Beckman instruments are microprocessor-controlled. Temperature changes were more rapid than in the Gilford instruments with the jump from 0 to 30 °C taking about 20 s and the reverse jump taking about 100 s.

NIH Cytotoxicity Screen.[24,37] The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40 000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two wells of each cell line were fixed in situ with TCA to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional 4-fold, 10-fold, or $^1/_2$ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After the samples were stained, the unbound dye was removed by washing five times with 1% acetic acid, and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (T_z), control growth (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as

$$[(T_i - T_z)/(C - T_z)] \times 100$$

for concentrations for which $T_i \geq T_z$ and as

$$[(T_i - T_z)/T_z] \times 100$$

for concentrations for which $T_i < T_z$.

Three dose−response parameters were calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment compared to that at the beginning), indicating a net loss of cells following treatment, was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

5-[2-(2,5-Dihydroxyphenyl)vinyl]-*N*-(2-phenethyl)salicylamide (11a). BBr_3 (3 mL, 1 M solution in CH_2Cl_2, 3 mmol) was added dropwise via syringe to compound **22a** (0.30 g, 0.54 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C under argon. The solution was warmed to room temperature and stirred for 4 h. After the mixture was recooled to −78 °C, H_2O (10 mL) was added, followed by ethyl acetate (20 mL), and the solution was warmed to room temperature. The resulting solution was extracted with ethyl acetate (3 × 50 mL), the combined organic layer was washed with brine (10 mL) and dried over $MgSO_4$, and the solvent was removed to afford a crude oily residue, which was further purified by flash chromatography (silica gel 85 g, ethyl acetate/hexane 1:2). The product **11a** (99 mg, 49%) was isolated as a purple crystalline solid: mp 237−239 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.39 (bs, 1 H, OH), 9.11 (s, 1 H, OH), 9.02 (t, J = 5.13 Hz, 1 H, NH), 8.80 (s, 1 H, OH), 7.93 (d, J = 1.50 Hz, 1 H), 7.56 (dd, J = 8.64, 1.87 Hz, 1 H), 7.30−7.13 (m, 6 H), 7.00 (d, J = 16.50 Hz, 1 H), 6.90 (d, J = 2.41 Hz, 1 H), 6.85 (d, J = 8.68 Hz, 1 H), 6.67 (d, J = 8.61 Hz, 1 H), 6.51 (dd, J = 8.61, 2.73 Hz, 1 H), 3.51 (q, J = 7.07 Hz, 2 H), 2.84 (t, J = 7.31 Hz, 2 H); CIMS m/z 376 (MH^+). Anal. ($C_{23}H_{21}NO_4$) C, H, N.

5-[2-(2,5-Dihydroxyphenyl)vinyl]-*N*-[2-(4-fluorophenyl)-ethyl]salicylamide (11b). From compound **22b** (0.3 g, 0.52 mmol) and BBr_3 (3 mL, 1 M solution in CH_2Cl_2, 3 mmol), **11b** was prepared by a procedure similar to that described for **11a**. Compound **11b** (0.145 g, 71%) was a dark-brown solid: mp 232−234 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.59 (s, 1 H, OH), 9.03 (s, 1 H, OH), 9.01 (t, J = 3.73 Hz, 1 H, NH), 8.71 (s, 1 H, OH), 8.01 (d, J = 1.33 Hz, 1 H), 7.57 (dd, J = 8.50, 1.52 Hz, 1 H), 7.30 (m, 2 H), 7.24 (d, J = 18.11 Hz, 1 H), 7.12 (t, J = 8.93 Hz, 2 H), 6.98 (d, J = 16.54 Hz, 1 H), 6.88 (m, 2 H), 6.68 (d, J = 8.49 Hz, 1 H), 6.52 (dd, J = 8.54, 2.64 Hz, 1 H), 3.54 (q, J = 6.29 Hz, 2 H), 2.87 (t, J = 7.34 Hz, 2 H); CIMS m/z 394 (MH^+). Anal. ($C_{23}H_{20}FNO$) C, H, F, N.

5-[2-(2,5-Dihydroxyphenyl)vinyl]-*N*-(*n*-hexyl)salicylamide (11c). From compound **22c** (0.35 g, 0.65 mmol) and BBr_3 (3 mL, 1 M solution in CH_2Cl_2, 3 mmol), **11c** was prepared by a procedure similar to that described for **11a**. Compound **11c** (0.11 g, 51%) was a purple crystalline solid: mp 218−220 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.56 (s 1 H, OH), 9.04 (s, 1 H, OH), 8.95 (bs, 1 H, NH), 8.72 (s, 1 H, OH), 8.05 (s, 1 H), 7.59 (d, J = 8.61 Hz, 1 H), 7.28 (d, J = 16.52 Hz, 1 H), 7.03 (d, J = 16.46 Hz, 1 H), 6.89 (m, 2 H), 6.70 (d, J = 8.52 Hz, 1 H), 6.52 (dd, J = 8.95, 2.73 Hz, 1 H), 3.32 (m, 2 H), 1.56 (m, 2 H), 1.30 (m, 6 H), 0.87 (t, J = 6.98 Hz, 3 H); CIMS m/z 356 (MH^+). Anal. ($C_{21}H_{25}NO_4$) C, H, N.

5-[2-(2,5-Dihydroxyphenyl)ethyl]-*N*-(2-phenethyl)salicylamide (12a). Compound **22a** (0.26 g, 0.46 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.05 g) in ethyl acetate (15 mL) at room temperature and atmospheric pressure for 12 h. The mixture was filtered through Celite and washed with ethyl acetate (10 mL). The combined filtrate was evaporated on a rotary evaporator to give the crude product, which was recrystallized from ethyl acetate/hexane. Compound **12a** was obtained as an off-white solid (0.15 g, 86%): mp 160−161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.33 (s, 1 H, OH), 8.88 (t, J = 5.42 Hz, 1 H, NH), 8.59 (s, 1 H, OH), 8.53 (bs, 1 H, OH), 7.69 (d, J = 1.74 Hz, 1 H), 7.32−7.17 (m, 6 H), 6.80 (d, J = 8.38 Hz, 1 H), 6.58 (d, J = 8.45 Hz, 1 H), 6.46 (d, J = 2.84 Hz, 1 H), 6.40 (dd, J = 8.42, 2.88 Hz, 1 H), 3.51 (q, bs, 2 H), 2.85 (t, J = 7.68 Hz, 2 H), 2.69 (s, 4 H); CIMS m/z 378 (MH^+). Anal. ($C_{23}H_{23}NO_4$) C, H, N.

5-[2-(2,5-Dihydroxyphenyl)ethyl]-*N*-[2-(4-fluorophenyl)-ethyl]salicylamide (12b). From compound **22b** (0.2 g, 0.35 mmol), H_2, and palladium on activated carbon (wet, contains 50% water, 0.05 g), **12b** was prepared by a procedure similar to that described for **12a**. Compound **12b** was a white solid (99.5 mg, 72%): mp 173−174 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.29 (s, 1 H, OH), 8.85 (t, J = 5.38 Hz, 1 H, NH), 8.55 (s, 1 H, OH), 8.50 (s, 1 H, OH), 7.68 (s, 1 H), 7.27 (m, 2 H), 7.21 (d, J = 8.32 Hz, 1 H), 7.10 (t, J = 8.68 Hz, 2 H), 6.78 (d, J = 8.33 Hz, 1 H), 6.57 (d, J = 8.46 Hz, 1 H), 6.40 (d, J = 2.78 Hz, 1 H), 6.39 (dd, J = 8.33, 2.87 Hz, 1 H), 3.49 (q, J = 6.55 Hz, 2 H), 2.84 (t, J = 7.24 Hz, 2 H), 2.69 (s, 4 H); CIMS m/z 396 (MH^+). Anal. ($C_{23}H_{22}FNO_4$) C, H, F, N.

5-[2-(2,5-Dihydroxyphenyl)ethyl]-*N*-(*n*-hexyl)salicylamide (12c). From compound **22c** (0.20 g, 0.37 mmol), H_2, and palladium on activated carbon (wet, contains 50% water, 0.05 g), **12c** was prepared by a similar procedure as that described for **12a**. Compound **12c** was an off-white crystalline solid: mp 150−151 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1 H, OH), 8.82 (t, J = 4.50 Hz, 1 H, NH), 8.64 (s, 1 H, OH), 8.49 (s, 1 H, OH), 7.75 (s, 1 H), 7.19 (d, J = 8.55 Hz, 1 H), 6.80 (d, J = 8.41 Hz, 1 H), 6.57 (d, J = 8.82 Hz, 1 H), 6.37 (m, 2 H), 3.27 (m, 4 H), 1.53 (m, 2 H), 1.46 (d, J = 7.18 Hz, 2 H), 1.28

(m, 6 H), 0.86 (t, J = 6.77 Hz, 3 H); CIMS m/z 358 (MH^+). Anal. ($C_{21}H_{27}NO_4$) C, H, N.

5-[(*Z*)-2-(2,5-Dimethoxyphenyl)vinyl]-2-methoxy-*N*-(2-phenethyl)benzamide (13a). Compound **15a** (0.41 g, 0.99 mmol) was hydrogenated over 5% palladium on calcium carbonate, poisoned with lead (30 mg), in 3:1 ethanol/ethyl acetate (40 mL) at room temperature and atmospheric pressure for 2 h. The mixture was filtered through Celite and washed with ethyl acetate (20 mL). The combined filtrate was evaporated on a rotary evaporator to give the crude product. Purification by flash chromatography (ethyl acetate/hexane 1:4) led to the recovery of the starting material (0.21 g) and afforded the product **13a** (0.20 g, 51%) as a white solid: mp 93−94 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 2.25 Hz, 1 H), 7.78 (bs, 1 H, NH), 7.33−7.31 (m, 1 H), 7.29−7.22 (m, 5 H), 6.80 (d, J = 8.50 Hz, 1 H), 6.76 (d, J = 2.91 Hz, 1 H), 6.72 (dd, J = 7.41, 2.84 Hz, 1 H), 6.68 (d, J = 8.66 Hz, 1 H), 6.63 (d, J = 12.41 Hz, 1 H), 6.59 (d, J = 12.34 Hz, 1 H), 3.80 (s, 3 H), 3.75 (t, J = 6.70 Hz, 2 H), 3.70 (s, 3 H), 3.56 (s, 3 H), 2.92 (t, J = 6.72 Hz, 2 H); ESIMS 418 (MH^+), 440 ($M + Na^+$). Anal. ($C_{26}H_{27}NO_4$) C, H. N.

5-[(*Z*)-2-(2,5-Dimethoxyphenyl)vinyl]-*N*-[(4-fluorophenyl)ethyl]-2-methoxybenzamide (13b). From compound **15b** (0.4 g, 0.9 mmol), H_2, and 5% palladium on calcium carbonate poisoned with lead (10 mg), **13b** was prepared by a procedure similar to that described for **13a**. Compound **13b** (0.38 g, 97%) was a yellowish liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, J = 1.54 Hz, 1 H), 7.80 (bs, 1 H, NH), 7.32 (dd, J = 1.57, 8.59 Hz, 1 H), 7.23 (m, 2 H), 7.03 (t, J = 8.51 Hz, 2 H), 6.84 (d, J = 8.72 Hz, 1 H), 6.80−6.75 (m, 2 H), 6.71 (d, J = 8.60 Hz, 1 H), 6.66 (d, J = 12.32 Hz, 1 H), 6.60 (d, J = 12.34 Hz, 1 H), 3.80 (s, 3 H), 3.76 (s, 3 H), 3.72 (q, J = 6.40 Hz, 2 H), 3.58 (s, 3 H), 2.91 (t, J = 6.92 Hz, 2 H); ESIMS 436 (MH^+), 458 ($M + Na^+$). Anal. ($C_{26}H_{26}FNO_4$) C, H, F, N.

5-[(*Z*)-2-(2,5-Dimethoxyphenyl)vinyl]-2-methoxy-*N*-(*n*-hexyl)benzamide (13c). From compound **15c** (0.43 g, 1.09 mmol), H_2, and 5% palladium on calcium carbonate poisoned with lead (10 mg), **13c** was prepared by procedure similar to that described for **13a**. Compound **13c** (0.38 g, 97%) was a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 2.37 Hz, 1 H), 7.72 (bs, 1 H, NH), 7.25 (dd, J = 8.63, 2.42 Hz, 1 H), 6.77−6.68 (m, 4 H), 6.57 (s, 2 H), 3.84 (s, 3 H), 3.73 (s, 3 H), 3.54 (s, 3 H), 3.38 (q, J = 6.96 Hz, 2 H), 1.55 (quint, J = 7.21 Hz, 2 H), 1.28 (m, 6 H), 0.85 (t, J = 7.02 Hz, 3 H); EIMS m/z 397 (M^+); CIMS m/z 398 $(MH)^+$. Anal. ($C_{24}H_{31}NO_4$) C, H, N.

5-[(*Z*)-2-(2,5-Dihydroxyphenyl)vinyl]-*N*-(2-phenethyl)-salicylamide (14a). BBr_3 (2.0 mL, 1 M solution in CH_2Cl_2, 2.0 mmol) was added dropwise via syringe to compound **13a** (0.18 g, 0.43 mmol) in anhydrous CH_2Cl_2 (30 mL) at −78 °C under argon. The solution was warmed at −20 °C and stirred for 2 h. After the mixture was recooled to −78 °C, H_2O (10 mL) was added, followed by ethyl acetate (30 mL), and the solution was warmed to room temperature. The resulting solution was extracted with ethyl acetate (3 × 50 mL), and the combined organic layer was washed with brine (10 mL) and dried over $MgSO_4$. Removal of the solvent gave a crude oily residue, which was further purified by flash chromatography (silica gel 80 g, ethyl acetate/hexane 1:5). The product **14a** (0.079 g, 49%) was isolated as a solid: mp 179−180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.60 (s, 1 H, OH), 12.32 (s, 1 H, OH), 9.03 (bs, 1 H, NH), 9.00 (s, 1 H, OH), 8.01 (d, J = 1.60, 1 H), 7.56 (dd, J = 8.55, 1.74 Hz, 1 H), 7.13−7.19 (m, 6 H), 6.97 (d, J = 16.47 Hz, 1 H), 6.68 (m, 2 H), 6.66 (d, J = 8.59 Hz, 1 H), 6.51 (dd, J = 8.55, 2.78 Hz, 1 H), 3.53 (q, J = 6.69 Hz, 2 H), 2.88 (t, J = 7.62 Hz, 2 H); CIMS m/z 376 (MH^+). Anal. ($C_{23}H_{21}NO_4$) C, H, N.

5-[(*Z*)-2-(2,5-Dihydroxyphenyl)vinyl]-*N*-[(4-fluorophenyl)ethyl]salicylamide (14b). From compound **13b** (0.20 g, 0.46 mmol) and BBr_3 (3.0 mL, 1 M solution in CH_2Cl_2, 3.0 mmol), **14b** was prepared by a similar procedure as that described for **14a**. Compound **14b** (0.094 g, 52%) was a solid: mp 181−182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.73 (s, 1 H, OH), 8.11 (bs, 1 H, NH), 7.69 (s, 1 H, OH), 7.64 (s, 1 H), 7.61 (s, 1 H, OH), 7.33 (d, J = 8.58 Hz, 1 H), 7.29−7.26 (m, 2 H), 7.02 (t, J = 8.68 Hz, 2 H), 6.74 (d, J = 8.44 Hz, 1 H), 6.73 (d, J = 8.34 Hz, 1 H), 6.63 (d, J = 2.14 Hz, 1 H), 6.60 (m, 1 H), 6.57 (d, J = 11.72 Hz, 1 H), 6.44 (d, J = 12.24 Hz, 1 H), 3.62 (q, J = 6.65 Hz, 2 H), 2.91 (t, J = 7.21 Hz, 2 H); positive ion ESIMS m/z 394 (MH^+); negative ion ESIMS m/z 392 $(M - H)^-$. Anal. ($C_{23}H_{20}FNO_4$) C, H, F, N.

5-[(*Z*)-2-(2,5-Dihydroxyphenyl)vinyl]-*N*-(*n*-hexyl)salicylamide (14c). From compound **13c** (0.22 g, 0.55 mmol) and BBr_3 (2.5 mL, 1 M solution in CH_2Cl_2, 2.5 mmol), **14c** was prepared by a similar procedure as that described for **14a**. Compound **14c** (0.13 g, 67%) was a white solid: mp 180−181 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 10.68 (s, 1 H, OH), 7.96 (bs, 1 H, NH), 7.69 (d, J = 1.42 Hz, 1 H), 7.68 (s, 1 H, OH), 7.60 (s, 1 H, OH), 7.32 (dd, J = 8.55, 1.74 Hz, 1 H), 6.72 (d, J = 8.50 Hz, 1 H), 6.70 (d, J = 8.62 Hz, 1 H), 6.63 (d, J = 2.75 Hz, 1 H), 6.58 (dd, J = 8.53, 3.00 Hz, 1 H), 6.56 (d, J = 12.61 Hz, 1 H), 6.46 (d, J = 12.23 Hz, 1 H), 3.39 (q, J = 2.95 Hz, 2 H), 1.60 (quint, J = 7.10 Hz, 2 H), 1.34 (m, 6 H), 0.88 (t, J = 6.52 Hz, 3 H); positive ion ESIMS m/z 356 (MH^+), negative ion ESIMS m/z 354 $(M - H)^-$. Anal. ($C_{21}H_{25}NO_4$) C, H, N.

5-[2-(2,5-Dimethoxyphenyl)ethynyl]-2-methoxy-*N*-(2-phenethyl)benzamide (15a). To a solution of **34** (0.45 g, 1.44 mmol) in dry DMF (30 mL) was added EDCI (0.40 g, 2.1 mmol), HOBT (0.28 g, 2.1 mmol), and Et_3N (0.89 mL, 6.3 mmol). After the mixture was stirred at room temperature for 1 h, 2-phenethylamine (0.87 mL, 6.4 mmol) was added dropwise, and the reaction continued for 48 h at room temperature under argon. Water (200 mL) was then added, and the product was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (1 × 100 mL), dried over sodium sulfate, and filtered, and the solvent was removed. Purification was achieved by flash chromatography, eluting with ethyl acetate/hexane (1:1 by volume) to yield pure **15a** (0.43 g, 73%) as a white crystalline solid: mp 112−113 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (d, J = 1.97 Hz, 1 H), 7.72 (bs, 1 H, NH), 7.53 (dd, J = 8.50, 2.04 Hz, 1 H), 7.34−7.19 (m, 5 H), 6.97 (d, J = 2.67 Hz, 1 H), 6.80 (d, J = 8.54 Hz, 1 H), 6.78 (dd, J = 8.60, 2.85 Hz, 1 H), 6.75 (d, J = 8.64 Hz, 1 H), 3.78 (s, 3 H), 3.65−3.76 (m, 8 H), 2.78 (t, J = 6.71 Hz, 2 H); ESIMS m/z 416 (MH^+). Anal. ($C_{26}H_{25}NO_4$) C, H, N.

5-[2-(2,5-Dimethoxyphenyl)ethynyl]-*N*-[(4-fluorophenyl)ethyl]-2-methoxybenzamide (15b). From compound **33** (0.45 g, 1.44 mmol), EDCI (0.40 g, 2.08 mmol), HOBT (0.28 g, 2.08 mmol), and Et_3N (0.87 mL, 6.3 mmol), **15b** was prepared by a procedure similar to that described for **15a**. Compound **15b** was a white crystalline solid (0.45 g, 73%): mp 105−106 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (d, J = 2.15 Hz, 1 H), 7.75 (bs, 1 H, NH), 7.62 (dd, J = 8.50, 2.17 Hz, 1 H), 7.24 (m, 2 H), 7.03 (m, 3 H), 6.92 (d, J = 8.59 Hz, 1 H), 6.85 (dd, J = 8.96, 2.88 Hz, 1 H), 6.85 (d, J = 9.00 Hz, 1 H), 3.88 (s, 3 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.74 (q, J = 5.96 Hz, 2 H), 2.93 (t, J = 6.71 Hz, 2 H); EI m/z 433 (M^+); CIMS m/z 434 (MH^+). Anal. ($C_{26}H_{24}FNO_4$) C, H, F, N.

5-[2-(2,5-Dimethoxyphenyl)ethynyl]-2-methoxy-*N*-(*n*-hexyl)benzamide (15c). From compound **33** (0.48 g, 1.53 mmol), EDCI (0.60 g, 3.14 mmol), HOBT (0.42 g, 3.14 mmol), and Et_3N (0.87 mL, 6.3 mmol), **15c** was prepared by a procedure similar to that described for **15a**. Compound **15c** was a white crystalline solid (0.57 g, 92%): mp 90−91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, J = 2.26 Hz, 1 H), 7.71 (bs, 1 H, NH), 7.59 (dd, J = 2.27, 8.55 Hz, 1 H), 7.01 (d, J = 2.58 Hz, 1 H), 6.92 (d, J = 8.62 Hz, 1 H), 6.84−6.77 (m, 2 H), 3.95 (s, 3 H), 3.84 (s, 3 H), 3.75 (s, 3 H), 3.42 (q, J = 7.02 Hz, 2 H), 1.58 (quint, J = 7.32 Hz, 2 H), 1.33 (m, 6 H), 0.89 (t, J = 6.89 Hz, 3 H); CIMS m/z 396 (MH^+). Anal. ($C_{24}H_{29}NO_4$) C, H, N.

5-[2-(2,5-Dihydroxyphenyl)ethynyl]-2-hydroxy-*N*-(2-phenethyl)benzamide (16a). BBr_3 (4.5 mL, 1 M solution in CH_2Cl_2, 4.5 mmol) was added dropwise via syringe to compound **15a** (0.37 g, 0.89 mmol) in anhydrous CH_2Cl_2 (30 mL) at −78 °C under argon. The solution was warmed at 0 °C and stirred for 3 h. After the mixture was recooled to −78 °C, H_2O (10 mL) was added, followed by ethyl acetate (50 mL), and the solution was warmed to room temperature. The resulting solution was extracted with ethyl acetate (3 × 50 mL), and

the combined organic layer was washed with brine (10 mL) and dried over $MgSO_4$. Removal of the solvent gave a crude oily residue, which was further purified by flash chromatography (silica gel 95 g, ethyl acetate/hexane 1:1). The product **16a** (0.16 g, 48%) was isolated as a solid: mp 192−194 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.88 (s, 1 H, OH), 9.19 (s, 1 H, OH), 9.03 (s, 1 H, NH), 8.90 (s, 1 H, OH), 8.02 (d, J = 1.59, 1 H), 7.50 (dd, J = 8.55, 1.64 Hz, 1 H), 7.28−7.23 (m, 5 H), 6.91 (d, J = 8.51 Hz, 1 H), 6.70 (m, 2 H), 6.63 (dd, J = 8.54, 2.58 Hz, 1 H), 3.51 (q, J = 6.70 Hz, 2 H), 2.86 (t, J = 7.62 Hz, 2 H); CIMS m/z 374 (MH^+). Anal. ($C_{23}H_{19}NO_4$) C, H, N.

5-Iodo-*N*-(2-phenethyl)salicylamide (18a). To a solution of 96% 5-iodosalicylic acid (**17**, 1.37 g, 5.0 mmol) in dry pyridine (50 mL) was added DCC (1.24 g, 6.0 mmol) and β-phenethylamine (0.63 mL, 5.0 mmol). The mixture was heated at 70−80 °C for 5 h. On cooling, the precipitate was removed by filtration. The pyridine was removed on a rotary evaporator. The residue was treated with 10% HCl (50 mL), and the mixture was stirred for 30 min. The yellow precipitate was removed by filtration, washed with water (2 × 50 mL), and dried in vacuo. Purification was achieved by flash chromatography (ethyl acetate/hexane 1:4) to yield **18a** as a white crystalline solid (1.19 g, 63%): mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 12.20 (s, 1 H, OH), 7.64 (dd, J = 8.71, 2.01 Hz, 1 H), 7.50 (d, J = 2.09 Hz, 1 H), 7.40 (t, J = 1.98, 1 H, NH), 7.31−7.24 (m, 5 H), 6.79 (d, J = 8.75 Hz, 1 H), 3.71 (q, J = 6.95 Hz, 2 H), 2.95 (t, J = 7.00 Hz, 2 H); CIMS m/z 368 (MH^+). Anal. ($C_{15}H_{14}INO_2$) C, H, I, N.

5-Iodo-*N*-[(4-fluorophenyl)ethyl]salicylamide (18b). From compound **17** (1.38 g, 5.0 mmol), DCC (1.24 g, 6.0 mmol), and 4-fluoro-2-phenethylamine (0.70 g, 5.0 mmol), **18b** was prepared by a procedure similar to that described for **18a**. Compound **18b** was a light-yellow crystalline solid (1.18 g, 61%): mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 12.24 (s, 1 H, OH), 7.63 (dd, J = 8.77, 2.01 Hz, 1 H), 7.49 (d, J = 2.11 Hz, 1 H), 7.19 (m, 2 H), 7.03 (m, 2 H), 6.78 (d, J = 8.76 Hz, 1 H), 6.51 (bs, 1 H, NH), 3.67 (q, J = 6.22 Hz, 2 H), 2.91 (t, J = 7.08 Hz, 2 H); CIMS m/z 386 (MH^+). Anal. ($C_{15}H_{13}FINO_2$) C, H, F, I, N.

5-Iodo-*N*-(*n*-hexyl)salicylamide (18c). From compound **17** (1.38 g, 5.0 mmol), DCC (1.24 g, 6.0 mmol), and *n*-hexylamine (0.66 mL, 5.0 mmol), **18c** was prepared by a procedure similar to that described for **18a**. Compound **18c** was a light-yellow crystalline solid (0.85 g, 49%): mp 58−60 °C; ^{1}H NMR ($CDCl_3$) δ 12.37 (s, 1 H, OH), 7.63 (d, J = 2.03 Hz, 1 H), 7.60 (s, 1 H), 6.78 (d, J = 8.52 Hz, 1 H), 6.20 (bs, 1 H, NH), 3.42 (q, J = 6.31 Hz, 2 H), 1.65 (quint, J = 6.47 Hz, 2 H), 1.37 (m, 6 H), 0.90 (t, J = 6.61 Hz, 3 H); CIMS m/z 348 (MH^+). Anal. ($C_{13}H_{18}INO_2$) C, H, I, N.

2,5-Dibenzyloxybenzaldehyde (20). K_2CO_3 (1.4 g, 10 mmol) and benzyl bromide (2.0 mL, 16.8 mmol) were added to a solution of 2,5-dihydroxybenzaldehyde (**19**) (1.0 g, 7.1 mmol) in acetone (12 mL). The mixture was heated to reflux under argon overnight, after which time TLC (silica gel, ethyl acetate/hexane, 1:2) showed that the reaction was complete. The potassium carbonate was removed by filtration, and the acetone was evaporated in vacuo. The solid product **20** (2.05 g, 91%) was recrystallized from diethyl ether/hexane: mp 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 10.51 (s, 1 H), 7.44 (d, J = 3.03 Hz, 1 H), 7.42−7.33 (m, 10 H), 7.18 (dd, J = 9.05, 3.26 Hz, 1 H), 7.01 (d, J = 9.07 Hz, 1 H), 5.15 (s, 2 H), 5.05 (s, 2 H); CIMS m/z 319 (MH^+), 291, 227, 213. Anal. ($C_{21}H_{18}O_3$) C, H.

2,5-Dibenzyloxystyrene (21). Methyltriphenylphosphonium bromide (3.03 g, 8.1 mmol) was placed in a flask fitted with a dropping funnel and a reflux condenser. The air in the flask was replaced by argon, and dry, degassed THF (25 mL) was added to the flask. *n*-Butyllithium (3.2 mL of a 2.5 M solution in hexane, 8.0 mmol) was added dropwise to the suspension, and the mixture was stirred for 1.5 h at room temperature. Compound **20** (2.5 g, 7.8 mmol) in dry THF (10 mL) was then added, and the resulting mixture was stirred for 1 h at room temperature. After the solution was stirred for another 6 h at 70 °C, it was allowed to cool to room temperature. Addition of diethyl ether (60 mL) and hexane (60 mL) produced a precipitate, which was removed by filtration. The filtrate was concentrated in vacuo to afford an oily residue, which was purified by flash chromatography (ethyl acetate/hexane 1:5) to obtain a light-yellowish solid **21** (2.33 g, 94%): mp 34−35 °C; ^{1}H NMR ($CDCl_3$) δ 7.45−7.30 (m, 10 H), 7.16 (d, J = 2.37 Hz, 1 H), 7.12 (d, J = 6.64 Hz, 1 H), 6.87−6.80 (m, 2 H), 5.73 (dd, J = 17.71, 1.33 Hz, 1 H), 5.27 (dd, J = 11.10, 1.35 Hz, 1 H), 5.04 (s, 4 H); CIMS m/z 317 (MH^+), 207, 117. Anal. ($C_{22}H_{20}O_2$) C, H.

5-[2-(2,5-Dibenzyloxyphenyl)vinyl]-*N*-(2-phenethyl)-salicylamide (22a). A solution of **21** (0.63 g, 1.99 mmol), **18a** (0.62 g, 1.69 mmol), 98% palladium acetate (0.004 g, 0.018 mmol), 97% tri-*o*-tolylphosphine (0.023 g, 0.073 mmol), and Et_3N (1 mL, 7.2 mmol) in acetonitrile (10 mL) was heated at 100 °C under argon for 30 h. After the mixture was cooled to room temperature, H_2O (200 mL) was added and the mixture was stirred for 10 min. It was then extracted with methylene chloride (2 × 50 mL), and the combined organic layer was washed with brine (10 mL), dried over $MgSO_4$, and concentrated to furnish the crude product, which was further purified by flash chromatography (silica gel 100 g, ethyl acetate/hexane 1:5). The product **22a** (0.31 g, 33%) was isolated as a slightly yellow crystalline solid: mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 12.36 (s, 1 H, OH), 7.55 (dd, J = 8.69, 1.98 Hz, 1 H), 7.45−7.22 (m, 17 H), 7.18 (d, J = 2.80 Hz, 1 H), 6.95 (d, J = 5.20 Hz, 1 H), 6.94 (d, J = 2.50 Hz, 1 H), 6.87 (d, J = 8.86 Hz, 1 H), 6.81 (dd, J = 8.96, 2.84 Hz, 1 H), 5.08 (s, 2 H), 5.05 (s, 2 H), 3.71 (q, J = 6.15 Hz, 2 H), 2.93 (t, J = 6.93 Hz, 2 H); CIMS m/z 556 (MH^+). Anal. ($C_{37}H_{33}NO_4$) C, H, N.

5-[2-(2,5-Dibenzyloxyphenyl)vinyl]-*N*-[(4-fluorophenyl)-ethyl]salicylamide (22b). From **21** (0.85 g, 2.7 mmol), **18b** (1.1 g, 2.8 mmol), 98% palladium acetate (0.010 g, 0.044 mmol), 97% tri-*o*-tolylphosphine (0.050 g, 0.18 mmol), and Et_3N (1.5 mL, 10.8 mmol), **22b** was prepared by a procedure similar to that described for **22a**. Compound **22b** (0.64 g, 40%) was a slightly yellow crystalline solid: mp 145−147 °C; ^{1}H NMR ($CDCl_3$) δ 12.35 (s, 1 H, OH), 7.59 (dd, J = 8.66, 1.94 Hz, 1 H), 7.49−7.34 (m, 12 H), 7.27 (d, J = 1.96 Hz, 1 H), 7.24−7.19 (m, 3 H), 7.06 (d, J = 8.64 Hz, 1 H), 7.01 (d, J = 5.93 Hz, 1 H), 6.98 (d, J = 2.06 Hz, 1 H), 6.92 (d, J = 8.89 Hz, 1 H), 6.84 (dd, J = 8.87, 2.76 Hz, 1 H), 6.40 (t, J = 3.05 Hz, 1 H, NH), 5.12 (s, 2 H), 5.08 (s, 2 H), 3.71 (q, J = 6.26 Hz, 2 H), 2.93 (t, J = 7.07 Hz, 2 H). Anal. ($C_{37}H_{32}FNO_4$) C, H, F, N.

5-[2-(2,5-Dibenzyloxyphenyl)vinyl]-*N*-(*n*-hexyl)salicyl-amide (22c). From **21** (1.45 g, 4.6 mmol), **18c** (1.4 g 4.0 mmol), 98% palladium acetate (0.01 g, 0.04 mmol), 97% tri-*o*-tolylphosphine (0.05 g, 0.18 mmol), and Et_3N (1.5 mL, 10.8 mmol), **22c** was prepared by a procedure similar to that described for **22a**. Compound **22c** (0.6 g, 30%) was a white crystalline solid: mp 98−100 °C; ^{1}H NMR ($CDCl_3$) δ 12.44 (s, 1 H, OH), 7.56 (dd, J = 8.66, 1.79 Hz, 1 H), 7.45−7.33 (m, 10 H), 7.26 (d, J = 8.43 Hz, 1 H), 7.20 (d, J = 2.79 Hz, 1 H), 7.03 (d, J = 16.48 Hz, 1 H), 6.97 (d, J = 8.63 Hz, 1 H), 6.88 (d, J = 8.93 Hz, 1 H), 6.80 (dd, J = 8.84, 2.88 Hz, 1 H), 6.30 (t, J = 5.29 Hz, 1 H, NH), 5.08 (s, 2 H), 5.04 (s, 2 H), 3.45 (q, J = 6.32 Hz, 2 H), 1.62 (quint, J = 7.10 Hz, 2 H), 1.37 (m, 6 H), 0.89 (t, J = 6.58 Hz, 3 H); CIMS m/z 536 (MH^+). Anal. ($C_{35}H_{37}NO_4$) C, H, N.

(2,5-Dimethoxyphenyl)trimethylsilylethyne (24). 1-Bromo-2,5-dimethoxybenzene (**23**, 2.0 g, 9.2 mmol), (trimethylsilyl)acetylene (2.6 mL, 18.4 mmol), PPh_3 (0.048 g, 0.18 mmol), and CuI (0.018 g, 0.009 mmol) were dissolved in piperidine (35 mL). Palladium chloride (0.032 g, 0.18 mmol) was added, and the resulting mixture was heated at reflux for 24 h. The reaction mixture was concentrated in vacuo, and saturated $NaHCO_3$ (100 mL) was added. The product was extracted with hexane (3 × 70 mL). The combined hexane extracts were washed with brine (1 × 20 mL), dried over $MgSO_4$, and concentrated. The crude product was subjected to column chromatography (silica gel 50 g, ethyl acetate/hexane 1:6) to give **24** (1.92 g, 89%) as a brownish liquid, which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 2.26 Hz, 1 H), 6.83 (dd, J = 8.98, 2.76 Hz, 1 H), 6.77 (d, J = 9.00 Hz, 1 H), 3.91 (s, 3 H), 3.76 (s, 3 H), 0.32 (s, 9 H).

(2,5-Dimethoxy)phenylacetylene (25). Compound **24** (1.92 g, 8.2 mmol) was dissolved in a mixture of THF (30 mL) and H_2O (2.5 mL). A 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (10 mL, 10 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred for 1 h at 0 °C and 3 h at room temperature. The reaction mixture was concentrated, and H_2O (100 mL) was added. The products were extracted with ethyl acetate (2 × 50 mL), and the combined extracts were dried over $MgSO_4$ and concentrated. The product was purified via column chromatography on silica gel (120 g). The compound was eluted with ethyl acetate/hexane (1:8). The solvent was evaporated to provide **25** (1.2 g, 90%) as a yellowish liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, J = 2.97 Hz, 1 H), 6.92 (dd, J = 9.04, 2.97 Hz, 1 H), 6.84 (d, J = 9.10 Hz, 1 H), 3.89 (s, 3 H), 3.79 (s, 3 H), 3.34 (s, 1 H). Anal. ($C_{10}H_{10}O_2$) C, H.

Methyl 5-Iodo-2-methoxybenzoate (26). To a solution of 5-iodosalicylic acid (**17**, 2.0 g, 7.27 mmol) in acetone (50 mL) was added anhydrous K_2CO_3 (4.5 g, 32.5 mmol). The mixture was cooled in an ice bath, and $(CH_3)_2SO_4$ (1.4 mL, 15.27 mmol) was added dropwise via syringe. The mixture was heated to reflux and stirred overnight. After the mixture was cooled to room temperature, water (200 mL) was added and the mixture was stirred for 1 h. Acetone was removed on a rotary evaporator, and the aqueous solution was extracted with ethyl acetate (3 × 50 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, yielding a yellow liquid, which was purified by flash chromatography (silica gel 120 g, ethyl acetate/hexane 1:5) to afford **26** (1.68 g, 79%) as white crystalline solid: mp 48−50 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 2.32 Hz, 1 H), 7.72 (dd, J = 8.78, 2.33 Hz, 1 H), 6.75 (d, J = 8.81 Hz, 1 H), 3.86 (s, 6 H); CIMS m/z 293 (MH^+), 261 (M − MeOH). Anal. ($C_9H_9IO_3$) C, H, I.

Methyl 5-[2-(2,5-Dimethoxyphenyl)ethynyl]-2-methoxybenzoate (27). Compound **25** (0.70 g, 4.3 mmol), compound **26** (0.63 g, 2.15 mmol), bis(triphenylphosphine)palladium(II) chloride (0.005 g, 0.007 mmol), and CuI (0.0041 g, 0.02 mmol) were dissolved in diethylamine (50 mL). The mixture was heated at 50−60 °C for 8 h. The reaction mixture was then concentrated in vacuo, and saturated $NaHCO_3$ (100 mL) was added. The product was extracted with ethyl acetate (3 × 80 mL). The combined organic extracts were washed with brine (1 × 50 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (silica gel 150 g, ethyl acetate/hexane 1:2) to give **27** (0.66 g, 95%) as a yellowish crystalline solid: mp 99−101 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.66 (dd, J = 8.75, 2.23 Hz, 1 H), 7.28 (d, J = 2.22 Hz, 1 H), 7.18 (d, J = 8.68 Hz, 1 H), 7.03 (d, J = 2.90 Hz, 1 H), 6.96 (d, J = 8.92 Hz, 1 H), 6.91 (dd, J = 9.05, 2.90 Hz, 1 H), 3.91 (s, 3 H), 3.84 (s, 6 H), 3.76 (s, 3 H); ^{13}C NMR (300 MHz, acetone-d_6) δ 166.00, 159.52, 155.44, 154.28, 136.87, 134.78, 122.01, 118.66, 116.44, 116.12, 113.60, 113.26, 92.51, 86.12, 56.61, 56.44, 55.99, 52.22; CIMS m/z 327 (MH^+). Anal. ($C_{19}H_{18}O_5$) C, H.

5-[2-(2,5-Dimethoxyphenyl)acetyl]-*N*-[(4-fluorophenyl)ethyl]salicylamide (29). Compound **25** (0.98 g, 2.5 mmol), compound **18b** (0.83 g, 5.1 mmol), PPh_3 (0.027 g, 0.1 mmol), and CuI (0.01 g, 0.05 mmol) were dissolved in piperidine (30 mL). Palladium chloride (0.018 g, 0.02 mmol) was added, and the resulting mixture was heated at reflux for 24 h. The reaction mixture was then concentrated in vacuo, and saturated $NaHCO_3$ (100 mL) was added. The product was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (1 × 20 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (silica gel 80 g, ethyl acetate/hexane 2:3) to give **29** (1.92 g, 89%) as a yellowish liquid. ^{1}H NMR (300 MHz, acetone-d_6) δ 12.95 (s, 1 H, OH), 8.10 (d, J = 1.96 Hz, 1 H), 7.17 (m, 2 H), 7.14 (dd, J = 8.70, 1.92 Hz, 1 H), 6.99 (m, 3 H), 6.75 (d, J = 1.59 Hz, 1 H), 6.73 (dd, J = 6.29, 2.36 Hz, 1 H), 6.69 (t, J = 5.32 Hz, 1 H, NH), 4.13 (s, 2 H), 3.71 (s, 3 H), 3.67 (s, 3 H), 3.63 (q, J = 6.23 Hz, 2 H), 2.88 (t, J = 7.14 Hz, 2 H); ^{13}C NMR (300 MHz, acetone-d_6) δ 199.90, 168.20, 165.12, 158.77, 156.82, 140.46, 139.11, 135.73, 135.61, 133.39, 132.82, 130.46, 122.96, 122.55, 120.27, 120.00, 117.15, 116.68, 60.58, 60.00, 46.15, 46.03, 44.39, 39.42; CIMS m/z 438 (MH^+). Anal. ($C_{25}H_{24}FNO_5$) C, H, F, N.

5-[2-(2,5-Dimethoxyphenyl)ethyl]-*N*-[(4-fluorophenyl)ethyl]salicylamide (30). Compound **29** (0.50 g, 1.2 mmol) was hydrogenated for 4 h over 10% palladium on activated carbon (wet, contains 50% water, 0.1 g) in ethyl acetate (10 mL) at room temperature and atmospheric pressure. The mixture was then filtered through Celite and washed with ethyl acetate (10 mL). The combined filtrate was evaporated on a rotary evaporator to give the crude product, which was then recrystallized from ethyl acetate/hexane. Compound **30** (0.39 g, 77%) was obtained as a white crystalline solid: mp 110−112 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.99 (s, 1 H, OH), 10.34 (m, 2 H), 7.22−7.15 (m, 3 H), 6.88 (d, J = 8.45 Hz, 1 H), 6.83 (d, J = 1.90 Hz, 1 H), 6.74 (d, J = 8.81 Hz, 1 H), 6.68 (dd, J = 8.82, 2.90 Hz, 1 H), 6.59 (d, J = 2.91 Hz, 1 H), 6.20 (bs, 1 H, NH), 3.72 (s, 3 H), 3.70 (s, 3 H), 3.62 (q, J = 6.26 Hz, 2 H), 2.88 (t, J = 7.01 Hz, 2 H), 2.78 (m, 4 H); CIMS m/z 424 (MH^+). Anal. ($C_{25}H_{26}FNO_4$) C, H, F, N.

5-[2-(2,5-Dimethoxyphenyl)ethynyl]-2-methoxybenzoic Acid (34). Compound **27** (0.50 g, 1.53 mmol) was added to a solution of 10% aqueous NaOH (20 mL) and methanol (10 mL). The mixture was heated at 60−70 °C for 3 h. After the mixture was cooled to room temperature, the organic solvent was removed on a rotary evaporator. The reaction mixture was acidified with 1 N HCl, and the resulting precipitate was extracted with CH_2Cl_2 (25 mL × 2), washed with water, and dried over anhydrous $MgSO_4$. Evaporation of the solvent yielded **34** (0.48 g, 100%) as a yellowish solid: mp 139−140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, J = 2.18 Hz, 1 H), 7.71 (dd, J = 2.20, 8.69 Hz, 1 H), 7.01 (d, J = 8.62 Hz, 1 H), 7.00 (d, J = 2.78 Hz, 1 H), 6.86 (dd, J = 2.69, 8.93 Hz, 1 H), 6.81 (d, J = 9.00 Hz, 1 H), 4.05 (s, 3 H), 3.85 (s, 3 H), 3.76 (s, 3 H); CIMS m/z 313 (MH^+). Anal. ($C_{18}H_{16}O_5$) C, H.

Methyl 5-[(*Z*)-2-(2,5-Dimethoxyphenyl)vinyl]-2-methoxybenzoate (35). From compound **27** (0.5 g, 1.53 mmol), H_2, and 5% palladium on calcium carbonate poisoned with lead (30 mg), **35** was prepared by a procedure similar to that described for **13a**, Compound **35** (0.46 g, 92%) was a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, J = 2.30 Hz, 1 H), 7.39 (dd, J = 2.33, 8.65 Hz, 1 H), 7.00 (d, J = 8.71 Hz, 1 H), 6.94 (d, J = 8.87 Hz, 1 H), 6.81 (dd, J = 3.08, 8.88 Hz, 1 H), 6.75 (d, J = 3.05 Hz, 1 H), 6.61 (d, J = 12.30 Hz, 1 H), 6.55 (d, J = 12.29 Hz, 1 H), 3.84 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.56 (s, 3 H); CIMS m/z 329 (MH^+), 328 (M^+); ESIMS m/z 329 (MH^+), 351 (MNa^+). Anal. ($C_{19}H_{20}O_5$) C, H.

Methyl 5-[(*Z*)-2-(2,5-Dihydroxyphenyl)vinyl]-2-hydroxybenzoate (36). From compound **35** (0.06 g, 0.18 mmol) and BBr_3, **36** was prepared by a procedure similar to that described for **14a**. Compound **36** (0.025 g, 48%) was an off-white crystalline solid: mp 173−175 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.68 (s, 1 H, OH), 7.80 (d, J = 2.10, 1 H), 7.67 (s, 1 H, OH), 7.58 (s, 1 H, OH), 7.44 (dd, J = 8.75, 2.45 Hz, 1 H), 6.81 (d, J = 8.65 Hz, 1 H), 6.74 (d, J = 8.23 Hz, 1 H), 6.72 (m, 3 H), 6.53 (d, J = 12.26 Hz, 1 H), 3.90 (s, 3 H); ESIMS m/z 287 (MH^+), 309 (MNa^+). Anal. ($C_{16}H_{14}O_5$) C, H.

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