Synthesis and Investigation of Conformationally Restricted Analogues of Lavendustin A as Cytotoxic Inhibitors of Tubulin Polymerization

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

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

Received May 28, 2002

A series of conformationally restricted analogues were synthesized in order to elucidate the possible effects of different amide conformations of lavendustin A derivatives on cytotoxicity in cancer cell cultures and on inhibition of tubulin polymerization. The conformationally restricted analogues were based on the oxazinedione and isoindolone ring systems. In addition, the amide bond was replaced by both cis and trans alkene moieties. Surprisingly, the results indicated very little effect of conformational restriction on biological activity. Because all of the compounds synthesized had similar cytotoxicities and potencies as tubulin polymerization inhibitors, the side chain present on the aniline ring system does not appear to be important in the biological effects of the lavendustins. The hydroquinone ring of lavendustin A may be a more important determinant of the biological activity than the structure surrounding the aniline ring.

Introduction

Because the activities of protein-tyrosine kinases (PTKs) are elevated in diseases that are characterized by rapid cellular proliferation, the design and synthesis of PTK inhibitors are a logical strategy for the development of new anticancer drugs.[1−4] Recent examples of clinically useful PTK inhibitors include the Bcr-Abl tyrosine kinase inhibitor Gleevec (imatinib mesylate, STI571)[5,6] and the HER2/neu receptor monoclonal antibody Herceptin (trastuzumab).[7,8] Interest in the *Streptomyces griseolavendus* metabolite lavendustin A (**1**; Chart 1) has been stimulated by its ability to function as a PTK inhibitor.[9] Evaluation of the synthetic intermediate **2** as an inhibitor of the epidermal growth factor (EGF) PTK revealed that it is as potent as the natural product **1**.[9] Lineweaver−Burke plots indicated that inhibition of the EGF PTK by lavendustin A was competitive with respect to adenosine 5′-triphosphate (ATP) and noncompetitive with respect to substrate.[9] However, a subsequent kinetic analysis of the inhibition of the EGF PTK by lavendustin A suggested that it is a hyperbolic mixed type inhibitor with respect to both the substrate and the ATP; therefore, it binds to the kinase domain at a location that is distinct from both the ATP and substrate binding sites.[10]

These initial reports on lavendustin A (**1**) and its biologically active fragment **2** stimulated a great deal of research on the synthesis of **1** and a variety of structural analogues.[11−21] Although it was soon realized that lavendustin A (**1**) was inactive as a PTK inhibitor in cellular systems, the methyl ester of **1** inhibited PTK activity and internalization of the EGF receptor in cell culture.[22] This observation led to the synthesis of a variety of esters, alkoxyamides, and amides of general structure **3** that were found to inhibit PTK activity both in vitro and in cellular systems.[14,15,21,23] Although compounds of general structure **3** are cytotoxic, it seems unlikely for the following four reasons that this cytotoxicity is due to PTK inhibition: (i) Whereas MCF-10A cells are EGF-dependent, while MCF-7 cells are not, a series of amides of general structure **3** were equally effective as inhibitors of DNA synthesis in both breast cancer cell lines.[21] (ii) Because almost all of the EGFR tyrosine kinase activity must be inhibited before effects are seen on cell growth,[24] it is unlikely that the potencies of the amides **3** 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.[21] (iii) 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.[16] (iv) The potencies of amides **3** as inhibitors of both the EGF receptor and the nonreceptor PTK Syk did not correlate particularly well with their cytotoxicities toward cancer cell lines.[21] In view of these considerations, a COMPARE analysis[25] of the cytotoxicity profiles of amides **3** was performed, and the results suggested that the cytotoxicities of these compounds could be due to inhibition of tubulin polymerization.[21] When tested as inhibitors of tubulin polymerization, amides of general structure **3** were in fact found to be active, and their IC_{50} values for both inhibition of tubulin polymerization and for cytotoxicity were relatively close. The most cytotoxic compound in the prior series was the lavendustin A analogue **4**, which dis-

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

10.1021/jm0202270 CCC: $22.00 © 2002 American Chemical Society
Published on Web 09/10/2002

Chart 1

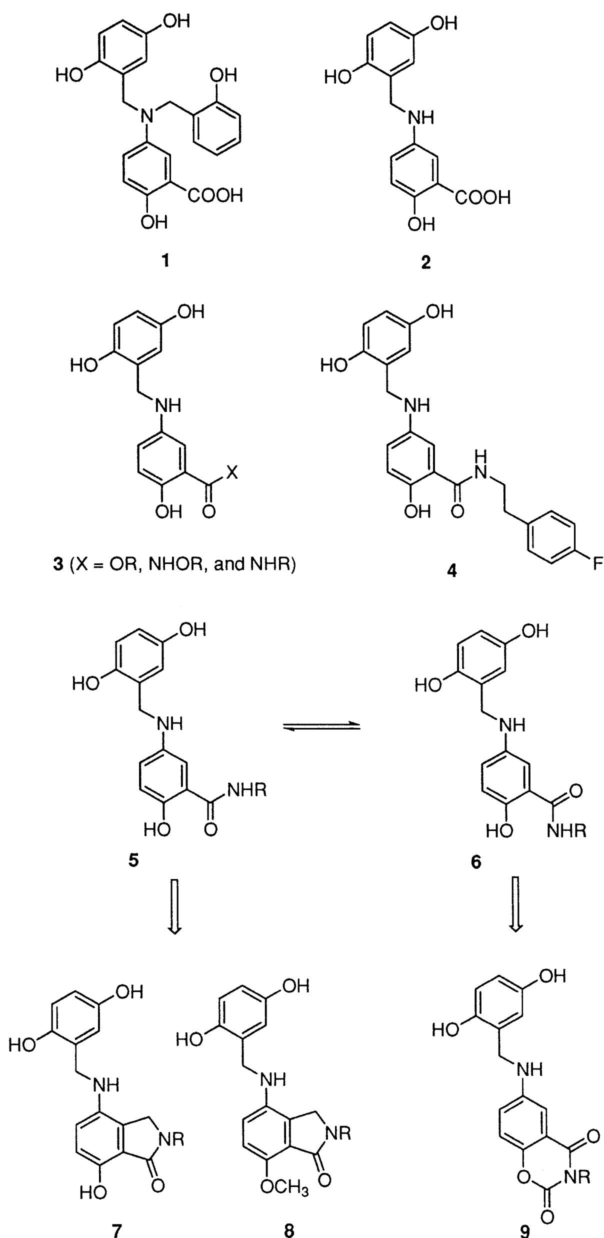
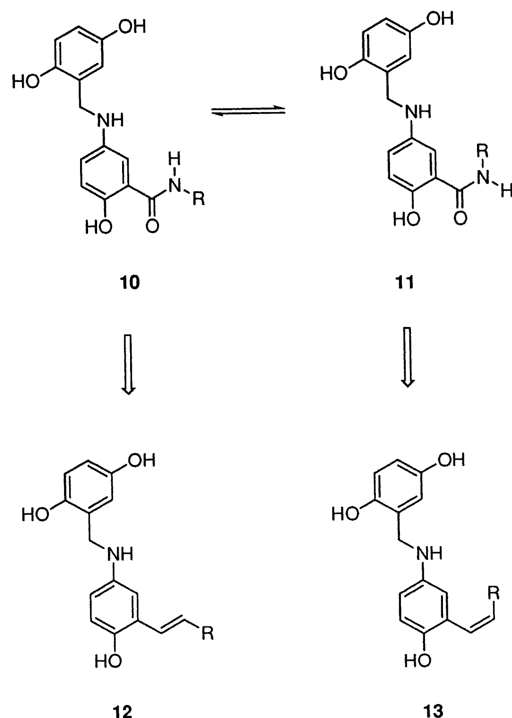


Chart 2



played a mean graph midpoint (MGM) of 0.35 μM in the screen of approximately 55 human cancer cell lines.[21]

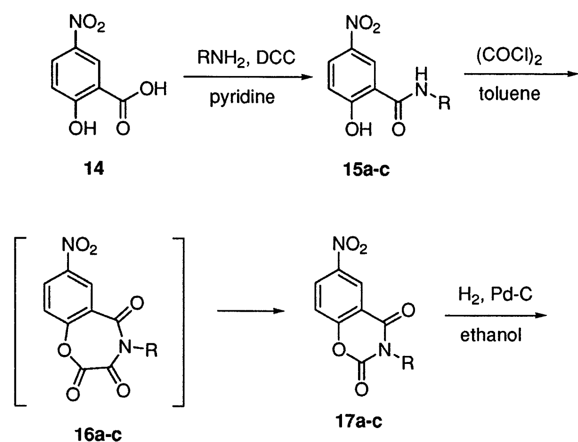
The lavendustin A analogue **4** and related compounds can exist in different conformations resulting from rotation around the bond connecting the amide carbonyl to the aromatic ring (see structures **5** and **6**). Both of these conformations could be stabilized through intramolecular hydrogen bonding between the phenolic hydroxyl group and either the amide nitrogen or the carbonyl. This raises the question of the relative contributions of these two conformers to the observed biological activity. One approach to investigating the general question of conformational effects is to synthesize conformationally restricted analogues. In the present case, the conformation represented by structure **5** could be stabilized by attaching the amide nitrogen to the aromatic ring through a methylene linker, suggesting the isoindolone **7**. On the other hand, the connection of the amide nitrogen to the adjacent phenolic oxygen through insertion of a carbonyl would lead to the oxazinedione structure **9**. The methyl ether **8** was also considered in order to investigate the possibility that differences in activity between the isoindolone **7** and the oxazinedione **9** could be due to the modification of the phenolic hydroxyl group. Two additional conformations of each rotamer **5** and **6** are possible due to rotation about the amide bond (e.g., E and Z conformations **10** and **11** (Chart 2), respectively). To investigate the relative contributions of these conformations to the biological effects, trans alkene analogues **12**, as well as their cis counterparts **13**, were also considered for synthesis as conformationally restricted analogues. The compounds synthesized in the present study were examined for cytotoxicity in the National Cancer Institute (NCI) screen of human cancer cell lines and for inhibition of tubulin polymerization.

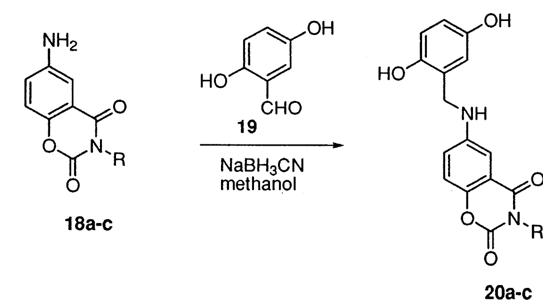
Chemistry

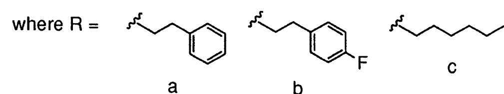
Because in the prior series of lavendustin A amide derivatives maximal activity was observed with the 2-(4-fluorophenyl)ethyl substituent present in compound **4**, it was decided to incorporate that substituent, along with the corresponding defluorinated substituent, in the rigid analogues considered for synthesis.[21] Because the n-hexyl derivative (**5** and **6**, R = n-hexyl) was particularly potent relative to **2** as an EGF receptor tyrosine kinase inhibitor, it was also decided to utilize that substituent in the present series of compounds.[18]

The preparation of the desired conformationally restricted analogues **20a**−**c** is outlined in Scheme 1. The synthesis relies on the ring contraction reaction of the unstable 1,3-benzoxazepine-2,3,5-triones **16a**−**c** to form the desired 1,3-benzoxazine-2,4-dione intermediates **17a**−**c**.[26,27] Conversion of the starting material, 5-nitro-2-hydroxybenzoic acid **14**, to the corresponding amide derivatives **15a**−**c**, was carried out by reaction with the required primary amines in the presence of DCC in

Scheme 1







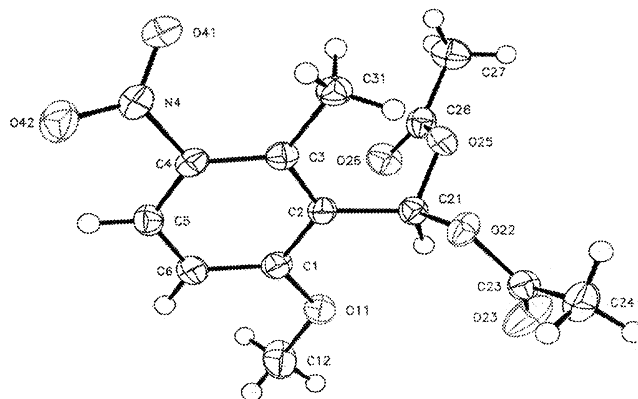
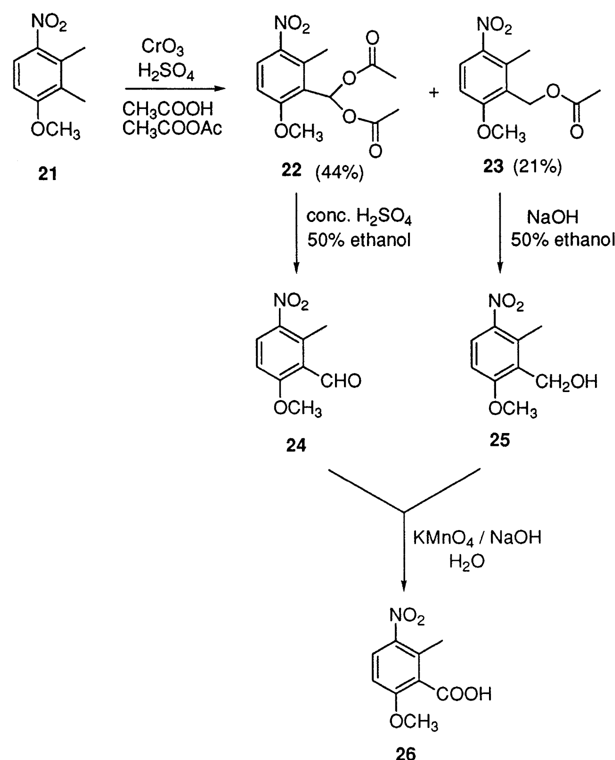


Figure 1. ORTEP plot of the X-ray crystal structure of diacetate **22**.

Scheme 2

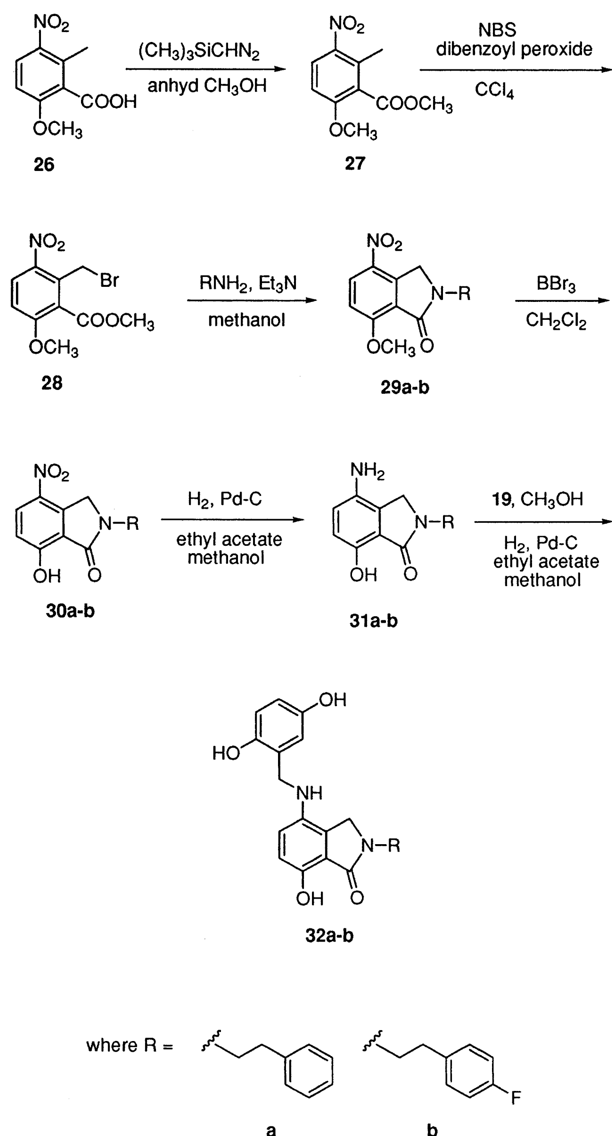


pyridine. Reaction of these compounds **15a–c** with oxalyl chloride in refluxing toluene afforded the required 1,3-benzoxazine-2,4-diones **17a–c**. Catalytic reduction of the nitro group present in **17a–c** was performed in the presence of hydrogen and 10% activated palladium on carbon to provide the amines **18a–c**. A reductive amination reaction involving 2,5-dihydroxybenzaldehyde (**19**) and the three amines **18a–c** afforded the target compounds **20a–c**.

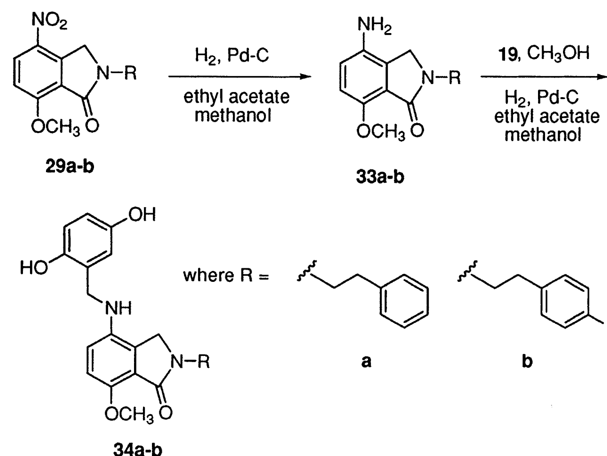
It was anticipated that the conformationally restricted analogues of general type **7** could be synthesized if the substituted benzoic acid derivative **26** was available. In considering possible routes for the synthesis of **26**, we decided to try to exploit the fact that the electron-attracting nitro group in nitrotoluenes inhibits the oxidation of the methyl group, and the effect is maximal in *o*-nitrotoluenes and decreases in the order o > m > p.[28,29] To take advantage of this fact, 2,3-dimethyl-4-nitroanisole (**21**) was chosen as the starting material (Scheme 2). Subjection of **21** to the Thiele reagent (CrO_3–Ac_2O–H_2SO_4) resulted in oxidative attack on the methyl group that was more remote from the nitro group, resulting in the formation of the diacetate **22** as the major product as well as the monoacetate **23** as the minor product. To be absolutely sure about the regiochemistry of the oxidation reaction, an X-ray structure was obtained on a crystalline sample of the diacetate **22**. The resulting ORTEP drawing, which is displayed in Figure 1, proves that selective oxidation of the methyl that is meta to the nitro group had in fact taken place. After it was separated by column chromatography, the diacetate **22** was converted to the aldehyde **24** under acidic conditions.[30] Oxidation of the aldehyde with permanganate in aqueous sodium hydroxide at reflux afforded the desired intermediate **26**. To avoid wasting any of the material, an attempt was also made to convert the minor product **23** to **26**. Hydrolysis of the acetate **23** with sodium hydroxide in ethanol afforded the corresponding primary alcohol **25**, which was then oxidized with permanganate to afford **26**.

The conversion of intermediate **26** to the conformationally restricted target compounds **32a,b** is depicted in Scheme 3. The acid **26** was converted to the expected methyl ester **27** by treatment with (trimethylsilyl)-diazomethane in anhydrous methanol at room temperature. Benzylic bromination of **27** under Wohl–Ziegler conditions (*N*-bromosuccinimide (NBS), dibenzoyl peroxide) yielded the anticipated intermediate **28**, which was converted to the isoindolones **29a,b** when heated with *β*-phenethylamine or *p*-fluoro-*β*-phenethylamine, respectively, in methanol in the presence of triethyl-

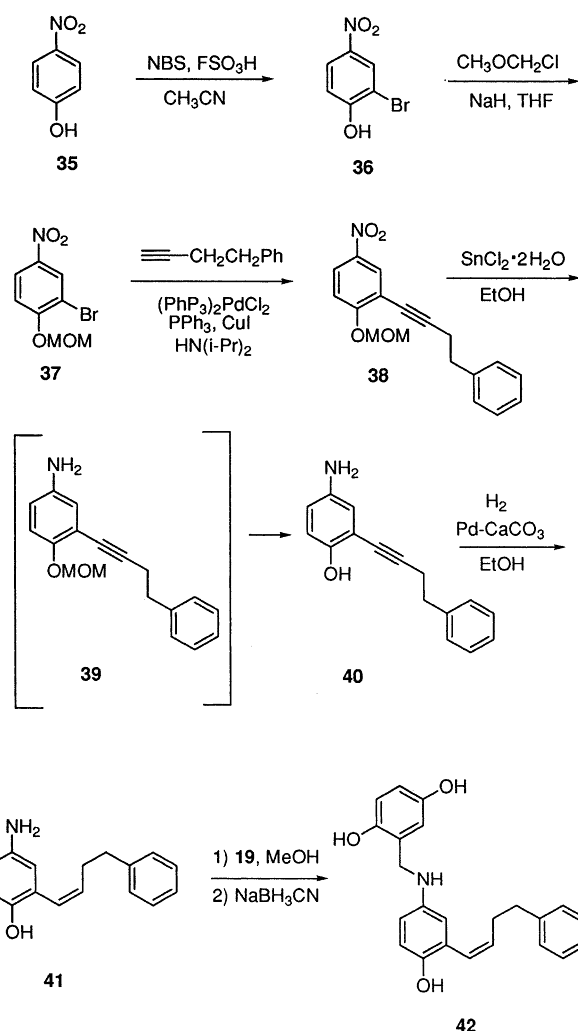
Scheme 3



Scheme 4



Scheme 5

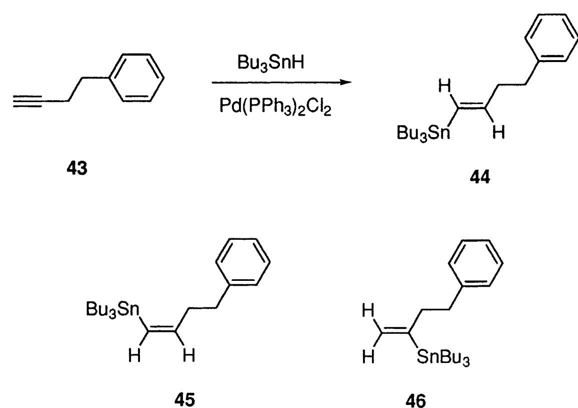


amine. Cleavage of the methyl ethers present in **29a**,**b** with boron tribromide provided the phenols **30a**,**b**. Catalytic hydrogenation over palladium on carbon led to the amines **31a**,**b**. Reaction of the amines **31a**,**b** with the aldehyde **19** furnished the Schiff bases, which were subjected to catalytic hydrogenation over palladium on carbon to yield the target compounds **32a**,**b**.

The synthesis of the compounds **34a**,**b**, having a methyl ether present in the isoindolone ring system in place of the phenol located in **32a**,**b**, is outlined in Scheme 4. Catalytic reduction of the nitro compounds **29a**,**b** afforded the aniline derivatives **33a**,**b**. Reaction of **33a**,**b** with the aldehyde **19** resulted in the expected Schiff bases, which were reduced to yield the required rigid analogues **34a**,**b**.

A conformationally restricted analogue **42**, which has a cis alkene in place of the amide linkage in the side chain, was designed and prepared as outlined in Scheme 5. The coupling partner **37** for the Sonogashira reaction was made by methoxymethyl (MOM) protection of 2-bromo-4-nitrophenol (**36**), which was prepared by selective monobromination of 4-nitrophenol (**35**) with NBS in the presence of FSO_3H in acetonitrile.[31] The palladium-catalyzed Sonogashira coupling reaction of **37** with 4-phenyl-1-butyne gave the disubstituted alkyne **38** in quantitative yield.[32,33] Reduction of the nitro group of **38** to the amine **39** was carried out with stannous chloride ($SnCl_2$) in ethanol.[34] Stannous chloride was chosen as the reducing agent because it is selective in the catalytic reduction of aromatic nitro compounds containing groups capable of undergoing catalytic hydrogenation (the triple bond in our molecule). In addition, the MOM protecting group was removed under acidic condition during the workup process of the $SnCl_2$

Scheme 6

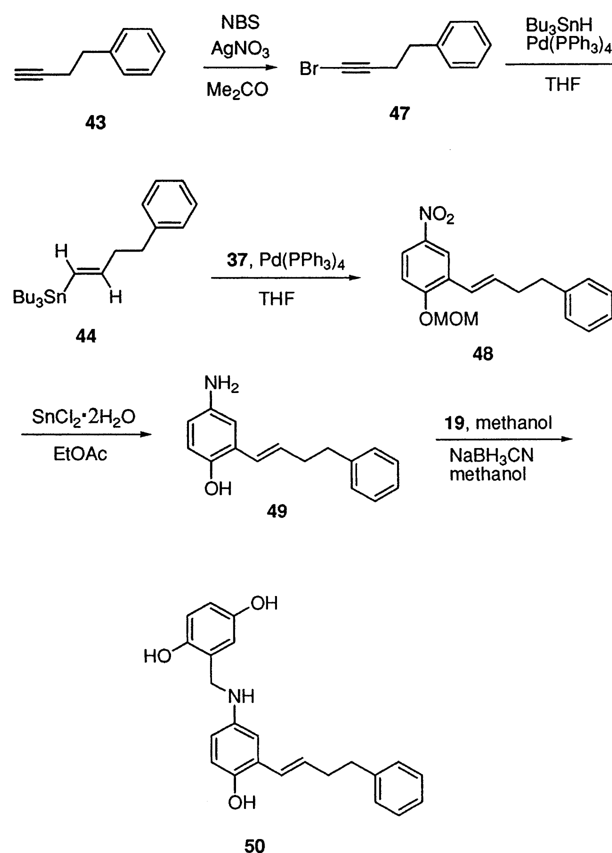


reduction to furnish **40** without isolation of intermediate **39**. Reduction of the alkyne **40** to the cis-substituted alkene **41** was performed using the Lindlar catalyst (Pd–$CaCO_3$, poisoned with lead). The reaction was closely monitored by thin-layer chromatography (TLC) in order to avoid the reduction of the cis double bond. Finally, the reductive amination with 2,5-dihydroxybenzaldehyde (**19**) and $NaBH_3CN$ reduction afforded analogue **42**.

To synthesize the counterpart of **42** having a trans double bond, a Stille coupling reaction of the vinylstannane **44** with the aryl bromide **37** was considered. As shown in Scheme 6, the formation of the *E*-vinylstannane **44** was first attempted using palladium-catalyzed hydrostannylation of 4-phenyl-1-butyne (**43**).[35–37] Although the product gave one spot on TLC, the ^1H NMR spectrum indicated the formation of a mixture of both regio- and stereoisomers **44**–**46**. Separation of this mixture by column chromatography failed.

In view of the difficulties encountered in the hydrostannylation of the alkyne **43**, alternative methods were sought for the synthesis of the desired *E*-vinylstannane **44**. Zhang et al. previously revealed that 1-bromoalkynes can be converted into (*E*)-1-vinylstannanes in a regioselective manner using 2 equiv of tributylstannane in the presence of $PdCl_2(PPh_3)_2$.[38] This method was successfully applied in our synthesis of analogue **50** (Scheme 7). The 1-bromoalkyne **47** was easily prepared from the corresponding 4-phenyl-1-butyne (**43**) by reaction with NBS in the presence of a catalytic amount of silver nitrate.[39] When solutions of the 1-bromoalkyne **47** in tetrahydrofuran (THF) were treated with 2 equiv of tributylstannane at room temperature in the presence of a catalytic amount of Pd(PPh_3)$_4$, it was smoothly converted into the corresponding (*E*)-1-vinylstannane (**44**). The ^1H NMR spectrum of **44** displayed a doublet at δ 5.95 ppm with $J = 18.93$ Hz and a doublet of triplets at δ 6.03 ppm with $J = 18.84$ and 5.71 Hz, suggesting the formation of the *E*-isomer. Because **44** was not separable from the byproduct bis(tributyltin), an in situ Stille coupling of the crude vinylstannane **44** with aryl bromide **37** was carried out, and the desired product **48** was obtained in 76% yield in two steps.[40] Next, following the procedure described above, the nitro group in **48** was reduced with stannous chloride, and the MOM protecting group was removed to yield **49**, which then underwent the reductive amination with 2,5-dihydroxybenzaldehyde (**19**) to form the desired analogue **50**.

Scheme 7



Biological Results and Discussion

The lavendustin A analogues were examined for antiproliferative activity against the human cancer cell lines in the NCI cytotoxicity screen, in which the activity of each compound was evaluated using approximately 55 different cancer cell lines of diverse tumor origins. The GI_{50} values, which are the concentrations of the compounds producing 50% growth inhibition, are listed in Table 1 for nine representative cell lines. The MGM values 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} to 10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.[41] Also listed in Table 1 are the IC_{50} values for inhibition of the polymerization of purified bovine brain tubulin. In addition to the new compounds synthesized in the present study, data for the previously prepared analogues **4**, **51**, and **52** (Chart 3) are also included in Table 1 for comparison purposes.[21]

It is apparent from the cytotoxicity data presented in Table 1 that all of the lavendustin A analogues were more active vs the leukemia cell line CCRF-CEM than for the other cell lines investigated. This selectivity of the lavendustin A analogues extended generally to all of the other leukemia cell lines that were tested, including HL-60(TB), K-562, RPMI-8226, MOLT-4, and SR (data not shown).

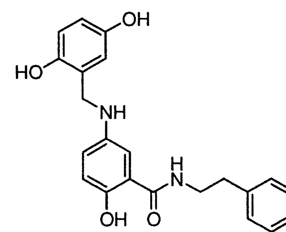
The conformationally restricted oxazinediones **20a** (MGM 5.5 μM) and **20c** (MGM 3.0 μM) were modestly more potent than their ring-opened counterparts **51** (MGM 14.8 μM) and **52** (MGM 8.7 μM). On the other

Table 1. Cytotoxicities and Antitubulin Activities of Lavendustin A Analogues

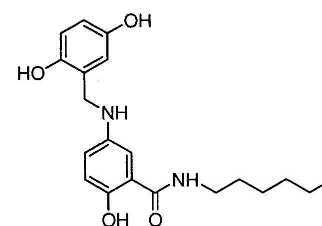
no.	cytotoxicity (GI_{50} in μM)[a] leukemia CCRF-CEM	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	inhibition of tubulin polymerization IC_{50}[c] (IC_{50}, μM ± SD)
4	0.22 ± 0.07	0.28 ± 0.10	0.33 ± 0.01	0.32 ± 0.11	0.41 ± 0.03	0.15 ± 0.05	0.58 ± 0.25	0.27 ± 0.04	0.047 ± 0.021	0.35 ± 0.05	4.0 ± 0.4
20a	1.3 ± 0.4	21.4 ± 4.8	4.6 ± 0.3	8.9 ± 4.1	6.0 ± 0.6	3.6 ±0.4	9.6 ± 2.2	15.3 ± 0	5.6 ± 0.3	5.5 ± 0.4	8.3 ± 1
20b	0.78 ± 0.4	17.2 ± 3.0	4.2 ± 0.4	5.1 ± 3.0	7.0 ± 3.4	2.8 ± 0.4	6.6 ± 0.2	17.7 ± 2.8	4.9 ± 0.9	4.7 ± 0.7	6.8 ± 0.1
20c	0.27 ± 0	14.8 ± 2.2	3.2 ± 0.2	2.4 ± 0.05	3.2 ± 0.2	1.7 ± 0.1	4.2 ± 1.6	13.8 ± 0.2	3.9 ± 0.4	3.0 ± 0.2	6.0 ± 0.6
32a	2.3	26.8	19.9	23.1	10.1	6.2	12.9	14.5	14.8	12.0	7.4 ± 1
32b	1.8 ± 0	19.6 ± 1.5	18.7 ± 3.8	14.4 ± 0.4	13.3 ± 1.0	8.8 ± 5.4	16.0 ± 0.4	24.6 ± 0.05	15.6 ± 0.05	12.4 ± 6.6	5.9 ± 1
34a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.5 ± 4
34b	3.4	31.4	20.4	14.4	19.1	8.4	20.4	16.1	14.0	20.4	5.9 ± 0.2
42	0.70	20.5	19.3	11.6	15.9	21.9	17.8	34.0	17.1	13.1	3.7 ± 0.4
50	1.1	35.4	30.4	16.3	18.0	20.4	21.4	18.7	5.7	19.5	7.2 ± 1
51	2.7	20	18	17	14	7.7	18	16	19	14.8 ± 1.4	3.6 ± 1
52	0.56	19	17	2.3	12	4.0	6.1	15	15	8.7 ± 0.4	3.6 ± 0.7

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. Compounds producing an MGM of 10 μM or less on the first determination were tested a second time, and the standard deviations were calculated. Compounds producing an MGM greater than 10 μM on the first determination were not further evaluated. [b] MGM for growth inhibition of all human cancer cell lines successfully tested. [c] Minimum of two independent determinations. Tubulin concentration was 1.0 mg/mL (10 μM).

Chart 3



51



52

hand, the fluorinated ring-opened compound **4** (MGM 0.35 μM) proved to be more active than its conformationally restricted oxazinedione analogue **20b** (MGM 4.7 μM).

Considering the isoindolones **32a** (MGM 12.0 μM) and **32b** (MGM 12.4 μM), both of these compounds were slightly less active than the corresponding oxazinediones **20a** (MGM 5.5 μM) and **20b** (MGM 4.7 μM). The effect of methylation of the phenolic hydroxyl group in the isoindolone ring of **32b**, resulting in **34b** (MGM 20.4 μM), was to slightly decrease potency.

Turning to the cis and trans alkenes **42** (MGM 13.1 μM) and **50** (MGM 19.5 μM), respectively, it is apparent that the cis alkene is modestly more active than the trans isomer, but the effect is very small. Both of the alkenes were about as active as their amide counterpart **51** (MGM 14.8 μM). This reinforces the conclusion that the conformation of the amide in lavendustin A amide analogues is unimportant as far as their biological activities are concerned, and it demonstrates that both *E* and *Z* alkenes are biologically effective replacements of the amides.

All of the compounds synthesized in this investigation were relatively good inhibitors of tubulin polymerization ($IC_{50} < 10$ μM). The striking aspect of their potencies is how similar they actually are. The total range of IC_{50} values for inhibition of tubulin polymerization is 3.6–9.5 μM. Although in general, the range of cytotoxicities observed in the cancer cell cultures is also relatively narrow, there is more variability in the MGM values listed in Table 1 than there is in the IC_{50} values for inhibition of tubulin polymerization. For example, at the extreme, compound **51** is 1.1 times more potent than **4** as an inhibitor of tubulin polymerization, but it is 42 times less cytotoxic as judged by the corresponding MGM values. This reflects the fact that the values for inhibition of tubulin polymerization are determined in a cell-free system, whereas the cytotoxicity values are established in cellular systems, in which additional factors such as cellular uptake, metabolism, and additional targets may exert significant effects.

Overall, the results indicate that the conformation of the amide side chain in the lead compounds **4**, **51**, **52**, and related lavendustin A analogues does not exert a significant effect on their biological activities.

Experimental Section

Melting points were determined with a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) 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 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. Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI).

5-Nitro-*N*-(2-phenethyl)salicylamide (15a). DCC (1.24 g, 6.0 mmol) and 2-phenethylamine (0.63 mL, 5.0 mmol) were added to a solution of 2-hydroxy-5-nitrobenzoic acid **(14)** (0.91 g, 5.0 mmol) in dry pyridine (50 mL). The mixture was heated at 90−100 °C for 2 h. After it was cooled, the precipitate was removed by filtration. The pyridine was removed on a rotary evaporator. The residue was treated with 10% HCl (50 mL) and stirred for 1 h. The yellow precipitate was filtered, washed with water (2 × 50 mL), and dried in vacuo. Purification was achieved by recrystallization from ethanol to yield pure **15a** as a yellow crystalline solid (1.31 g, 83%); mp 196−198 °C. ^{1}H NMR ($CDCl_3$): δ 13.50 (s, 1 H, OH), 8.86 (bs, 1 H, NH), 8.70 (d, J = 2.66 Hz, 1 H), 8.30 (dd, J = 9.18, 2.67 Hz, 1 H), 7.32−7.18 (m, 5 H), 7.10 (d, J = 9.18 Hz, 1 H), 3.71 (q, J = 7.81 Hz, 2 H), 2.98 (t, J = 7.69 Hz, 2 H). CIMS m/z 287 (MH^+). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

5-Nitro-*N*-[2-(4-fluorophenyl)ethyl]salicylamide (15b). From compounds **14** (0.91 g, 5.0 mmol), DCC (1.24 g, 6.0 mmol), and 4-fluoro-2-phenethylamine (0.65 mL, 5.0 mmol), a similar procedure to that described for **15a** yielded pure **15b** as a yellow crystalline solid (1.35 g, 89%); mp 206−208 °C. ^{1}H NMR ($CDCl_3$): δ 13.49 (s, 1 H, OH), 8.32 (d, J = 2.50 Hz, 1 H), 8.27 (dd, J = 9.04, 2.57 Hz, 1 H), 7.22 (m, 2 H), 7.07 (t, J = 8.74, 2 H), 7.01 (d, J = 8.68 Hz, 1 H), 6.66 (bs, 1 H, NH), 3.75 (q, J = 6.82 Hz, 2 H), 2.96 (t, J = 7.19 Hz, 2 H). CIMS m/z 305 (MH^+). Anal. ($C_{15}H_{13}FN_2O_4$) C, H, F, N.

5-Nitro-*N*-(hexyl)salicylamide (15c). From compound **14** (0.91 g, 5.0 mmol), DCC (1.24 g, 6.0 mmol), and hexylamine (0.66 mL, 5.0 mmol), a similar procedure to that described for **15a** yielded product **15c** as a yellow crystalline solid (1.04 g, 78%); mp 94−95 °C. ^{1}H NMR ($CDCl_3$): δ 13.51 (s, 1 H, OH), 8.42 (d, J = 2.61 Hz, 1 H), 8.28 (dd, J = 9.16, 2.67 Hz, 1 H), 7.08 (d, J = 9.13 Hz, 1 H), 6.67 (bs, 1 H, NH), 3.50 (q, J = 7.02 Hz, 2 H), 1.67 (quint, J = 7.44 Hz, 2 H), 0.49 (m, 6 H), 0.90 (t, J = 6.94 Hz, 3 H). CIMS m/z 348 (MH^+). Anal. ($C_{13}H_{18}INO_2$) C, H, I, N.

6-Nitro-3-phenethyl-benzo[*e*][1,3]oxazine-2,4-dione (17a). A solution of **15a** (0.71 g, 2.5 mmol) in toluene (10 mL) was stirred at room temperature for 10 min. Oxalyl chloride (0.32 g, 2.5 mmol) in toluene (2.5 mL) was added, and the reaction mixture was heated at reflux under argon for 6 h. After it was cooled, the precipitate that appeared was removed by filtration and **17a** was recrystallized from ethanol (0.43 g, 55%); mp 180−181 °C. ^{1}H NMR ($CDCl_3$): δ 8.96 (d, J = 2.53 Hz, 1 H), 8.55 (dd, J = 9.02, 2.53 Hz, 1 H), 7.46 (d, J = 9.03 Hz, 1 H), 7.38−7.22 (m, 5 H), 4.29 (t, J = 8.31 Hz, 2 H), 3.03 (t, J = 7.95 Hz, 2 H). CIMS m/z 313 (MH^+). Anal. ($C_{16}H_{12}N_2O_5$) C, H, N.

3-[2-(4-Fluoro-phenyl)ethyl]-6-nitro-benzo[*e*][1,3]-oxazine-2,4-dione (17b). From compound **15b** (0.50 g, 1.64 mmol) and oxalyl chloride (0.15 mL, 1.8 mmol), a similar procedure as that described for **17a** gave **17b** as a white crystalline solid (0.42 g, 77%); mp 170−172 °C. ^{1}H NMR ($CDCl_3$): δ 8.96 (d, J = 2.50 Hz, 1 H), 8.58 (dd, J = 9.07, 2.76 Hz, 1 H), 7.48 (d, J = 9.16 Hz, 1 H), 7.23 (q, J = 5.42 Hz, 2 H), 7.00 (t, J = 8.56 Hz, 2 H), 4.26 (t, J = 8.10 Hz, 2 H), 3.01 (t, J = 7.75 Hz, 2 H). CIMS m/z 331 (MH^+). Anal. ($C_{14}H_{11}FN_2O_5$) C, H, F, N.

3-Hexyl-6-nitro-benzo[*e*][1,3]oxazine-2,4-dione (17c). From compounds **15c** (0.50 g, 1.90 mmol) and oxalyl chloride (0.19 mL, 2.2 mmol), a similar procedure as that described for **17a** gave **17c** as a white crystalline solid (0.50 g, 90%); mp 94−96 °C. ^{1}H NMR ($CDCl_3$): δ 8.98 (d, J = 2.76 Hz, 1 H), 8.56 (dd, J = 8.98, 2.64 Hz, 1 H), 7.47 (d, J = 9.01 Hz, 1 H), 4.05 (t, J = 7.52 Hz, 2 H), 1.72 (quint, J = 7.42 Hz, 2 H), 1.34 (m, 6 H), 0.25 (t, J = 6.82 Hz, 3 H). CIMS m/z 293 (MH^+). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

6-Amino-3-phenethyl-benzo[*e*][1,3]oxazine-2,4-dione (18a). Compound **17a** (0.38 g, 1.2 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.5 g, 0.23 mmol) in ethanol (20 mL) at atmospheric pressure for 10 h. The mixture was then filtered through Celite and washed with acetone (50 mL). The combined filtrate was evaporated on a rotary evaporator to give compound **18a** as a white solid (0.30 g, 89%); mp 193−195 °C. ^{1}H NMR (DMSO-d_6): δ 7.35−7.17 (m, 5 H), 7.12 (d, J = 8.85 Hz, 1 H), 7.06 (d, J = 2.65 Hz, 1 H), 6.98 (dd, J = 8.85, 2.65 Hz, 1 H), 5.47 (s, 2 H, NH_2), 4.05 (t, J = 7.96 Hz, 2 H), 2.89 (t, J = 7.96 Hz, 2 H). CIMS m/z 283 (MH^+). Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

6-Amino-3-[2-(4-fluoro-phenyl)ethyl]benzo[*e*][1,3]-oxazine-2,4-dione (18b). Compound **17b** (0.38 g, 1.15 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.05 g) in ethyl acetate (20 mL) at atmospheric pressure for 4 h. 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 compound **18b** as a white solid (0.28 g, 81%); mp 212−213 °C. ^{1}H NMR (DMSO-d_6): δ 7.26 (m, 2 H), 7.14−7.08 (m, 3 H), 7.06 (d, J = 2.71 Hz, 1 H), 6.99 (dd, J = 8.77, 2.76 Hz, 1 H), 5.47 (s, 2 H, NH_2), 4.04 (t, J = 7.65 Hz, 2 H), 2.89 (t, J = 7.43 Hz, 2 H). Anal. ($C_{16}H_{13}FN_2O_3$) C, H, F, N.

6-Amino-3-hexyl-benzo[*e*][1,3]oxazine-2,4-dione (18c). Compound **17c** (0.38 g, 1.15 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.3 g) in ethyl acetate (25 mL) at atmospheric pressure for 3 h. 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 purified by recrystallization from ethyl acetate−hexane. Pure **18c** was obtained as a white solid (0.40 g, 89%); mp 113−115 °C. ^{1}H NMR (DMSO-d_6): δ 7.11 (d, J = 9.19, 1 H), 7.07 (d, J = 2.67 Hz, 1 H), 6.98 (dd, J = 8.77, 2.78 Hz, 1 H), 5.45 (s, 2 H), 3.82 (t, J = 7.3 Hz, 2 H), 1.57 (quint, J = 7.09 Hz, 2 H), 1.27 (m, 6 H), 0.85 (t, J = 6.50 Hz, 3 H). Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

6-[*N*-(2,5-Dihydroxyphenyl)methyl]amino]-3-phenethyl-benzo[*e*][1,3]oxazine-2,4-dione (20a). 2,5-Dihydroxybenzaldehyde (**19**, 0.15 g, 1.06 mmol) was added to **18a** (0.28 g, 0.99 mmol) in methanol (30 mL), and the mixture was heated to reflux under argon for 6 h. The reaction mixture was then cooled to room temperature, and the yellow precipitate was removed by filtration and dried to obtain the imine (0.39 g), which was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.02 g) in methanol (20 mL) at atmospheric pressure for 15 min. The mixture was then filtered through Celite and washed with ethyl acetate (10 mL). The combined filtrate was evaporated on a rotary evaporator to furnish the crude product, which was further purified by flash chromatography (ethyl acetate−hexane 1:2). The product **20a** (0.37 g, 92%) was isolated as an off-white solid; mp 201−203 °C. ^{1}H NMR (DMSO-d_6): δ 8.86 (s, 1 H, OH), 8.57 (s, 1 H, OH), 7.33−7.21 (m, 5 H), 7.16 (d, J = 8.94 Hz, 1 H), 7.05 (dd, J = 8.87, 2.84 Hz, 1 H), 6.91 (d, J = 2.53 Hz, 1 H), 6.65 (d, J = 8.50 Hz, 1 H), 6.59 (d, J = 2.63 Hz, 1 H), 6.54 (t, J = 5.90

Hz, 1 H, NH), 6.45 (dd, $J = 8.56$, 2.65 Hz, 1 H), 4.16 (d, $J = 4.78$ Hz, 2 H), 4.03 (t, $J = 7.57$ Hz, 2 H), 2.87 (t, $J = 7.94$ Hz, 2 H). CIMS *m/z* 405 (MH^+). Anal. ($C_{23}H_{20}N_2O_5$) C, H, N.

6-[*N*-(2,5-Dihydroxyphenyl)methyl]amino]-3-[2-(4-fluoro-phenyl)ethyl]benzo[*e*][1,3]oxazine-2,4-dione (20b). From compounds **19** (0.07 g, 0.5 mmol), **18b** (0.15 g, 0.5 mmol), H_2, and 10% Pd−C (wet, contains 50% water, 0.02 g), a similar procedure as that described for **20a** gave pure **20b** (0.18 g, 89%) as a light yellow solid; mp 199−200 °C. ^1H NMR (DMSO-d_6): δ 8.85 (s, 1 H, OH), 8.57 (s, 1 H, OH), 7.25 (q, $J = 5.77$, 2 H), 7.18 (d, $J = 8.94$ Hz, 1 H), 7.11 (t, $J = 8.89$ Hz, 2 H), 7.05 (dd, $J = 9.01$, 2.81 Hz, 1 H), 6.91 (d, $J = 2.64$ Hz, 1 H), 6.64 (d, $J = 8.52$ Hz, 1 H), 6.59 (d, $J = 2.74$ Hz, 1 H), 6.53 (t, $J = 5.92$ Hz, 1 H, NH), 6.43 (dd, $J = 8.50$, 2.85 Hz, 1 H), 4.17 (d, $J = 4.99$ Hz, 2 H), 4.02 (t, $J = 7.76$ Hz, 2 H), 2.87 (t, $J = 7.90$ Hz, 2 H). CIMS *m/z* 423 (MH^+). ESIMS *m/z* 421 ($M - H)^-$. Anal. ($C_{23}H_{19}FN_2O_5$) C, H, F, N.

6-[*N*-(2,5-Dihydroxyphenyl)methyl]amino]-3-hexylbenzo[*e*][1,3]oxazine-2,4-dione (20c). From compounds **19** (0.17 g, 1.2 mmol), **18c** (0.30 g, 1.14 mmol), and $NaBH_3CN$ (0.28 g, 4.5 mmol), a similar procedure as that described for **20a** gave **20c** (0.33 g, 75%) as a light yellow solid; mp 152−153 °C. ^1H NMR (DMSO-d_6): δ 8.85 (s, 1 H, OH), 8.56 (s, 1 H, OH), 7.16 (d, $J = 8.94$ Hz, 1 H), 7.06 (dd, $J = 8.94$, 2.82 Hz, 1 H), 6.91 (d, $J = 2.64$ Hz, 1 H), 6.63 (d, $J = 8.56$ Hz, 1 H), 6.59 (d, $J = 2.76$ Hz, 1 H), 6.52 (t, $J = 5.79$ Hz, 1 H, NH), 6.43 (dd, $J = 8.48$, 2.91 Hz, 1 H), 4.17 (d, $J = 4.24$ Hz, 2 H), 3.81 (t, $J = 7.13$ Hz, 2 H), 1.56 (quint, $J = 7.42$ Hz, 2 H), 1.27 (m, 6 H), 0.85 (t, $J = 6.45$ Hz, 3 H). CIMS *m/z* 263 (MH^+). Anal. ($C_{19}H_{24}N_2O_5$) C, H, N.

6-Methoxy-2-methyl-3-nitrobenzylidene Diacetate (22) and 6-Methoxy-2-methyl-3-nitrobenzyl Acetate (23). Concentrated H_2SO_4 (8.5 mL) was added to a cooled solution of 2,3-dimethyl-4-nitroanisole **(21)** (7.0 g, 38.6 mmol) in acetic anhydride (45 mL) and glacial acetic acid (55 mL). The mixture was cooled to 0 °C, and a solution of chromium trioxide (10 g, 65.8 mmol) in acetic anhydride (20 mL) was added to the stirred solution at a rate such that the temperature was maintained at 5−10 °C. Stirring was continued for a further 2 h at below 10 °C, and the mixture was then poured into ice (500−700 g) and set aside overnight. The precipitate was removed by filtration, washed with water until the washings were no longer colored, and then dried in a vacuum at room temperature to give a pale yellow solid, which showed two spots on a TLC plate (SiO_2, EtOAc−hexane 1:3, R_f **(22)** = 0.41, R_f **(23)** = 0.55). Separation and purification were accomplished by silica gel column chromatography (ethyl acetate−hexane 1:4). Compound **23** was eluted first, followed by compound **22**. Evaporation of the solvent gave **23** (1.95 g, 21%) as a white crystalline solid; mp 98−100 °C. ^1H NMR ($CDCl_3$): δ 7.97 (d, $J = 9.13$ Hz, 1 H), 6.84 (d, $J = 9.14$ Hz, 1 H), 5.26 (s, 2 H), 3.91 (s, 3 H), 2.52 (s, 3 H), 2.07 (s, 3 H). CIMS *m/z* 240 (MH^+). Anal. ($C_{11}H_{13}NO_5$) C, H, N. Compound **22** (5.1 g, 44%) was isolated as a light yellow crystalline solid; mp 132−134 °C. ^1H NMR ($CDCl_3$): δ 8.35 (s, 1 H), 7.91 (d, $J = 9.12$ Hz, 1 H), 6.84 (d, $J = 9.14$ Hz, 1 H), 3.94 (s, 3 H), 2.68 (s, 3 H), 2.09 (s, 6 H). CIMS *m/z* 298 (MH^+). Anal. ($C_{13}H_{15}NO_7$) C, H, N.

6-Methoxy-2-methyl-3-nitrobenzaldehyde (24). Compound **22** (3.0 g, 10.0 mmol) was added to a solution of 50% ethanol (80 mL) and concentrated H_2SO_4 (1.5 mL). The mixture was heated to reflux for 2 h. After it was cooled, the ethanol was removed on a rotary evaporator, and the precipitate was removed by filtration, washed with water until the washings were no longer acidic, and dried in vacuo at room temperature. The product **24** (1.87 g, 96%) was obtained as a light yellow solid; mp 120−121 °C. ^1H NMR ($CDCl_3$): δ 10.57 (s, 1 H), 8.08 (d, $J = 9.15$ Hz, 1 H), 6.94 (d, $J = 9.16$ Hz, 1 H), 3.99 (s, 3 H), 2.66 (s, 3 H). CIMS *m/z* 196 (MH^+). Anal. ($C_9H_9NO_4$) C, H, N.

6-Methoxy-2-methyl-3-nitrobenzyl Alcohol (25). Compound **23** (3.0 g, 12.5 mmol) was added to a solution of 50% ethanol (50 mL) containing NaOH (0.5 g, 12.5 mmol). The mixture was heated to reflux for 2 h. After it was cooled to room temperature, it was extracted with diethyl ether (2 × 60 mL). The combined ether layers were washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a yellow solid, which was recrystallized from ethyl acetate−hexane. Compound **25** (2.16 g, 87%) was obtained as a yellow crystalline solid; mp 94−95 °C. ^1H NMR ($CDCl_3$): δ 7.90 (d, $J = 9.09$ Hz, 1 H), 6.82 (d, $J = 9.09$ Hz, 1 H), 4.80 (d, $J = 6.33$ Hz, 2 H), 3.92 (s, 3 H), 2.57 (s, 3 H), 1.83 (t, $J = 6.36$ Hz, 1 H, OH). CIMS *m/z* 198 (MH^+). Anal. ($C_9H_{11}NO_4$) C, H, N.

5-Methoxy-2-methyl-3-nitrobenzoic Acid (26) from 24. Compound **24** (1.32 g, 6.8 mmol) was added to a solution of potassium permanganate (2.14 g, 13.6 mmol) and NaOH (0.5 g, 12.5 mmol) in water (20 mL). The mixture was heated to reflux for 2 h. After it was cooled, the mixture was acidified with 3 N HCl and extracted with ethyl acetate (4 × 50 mL). The combined ethyl acetate solution was dried over $MgSO_4$, and removal of the solvent furnished **26** (1.2 g, 84%) as a light yellow solid; mp 152−154 °C.

5-Methoxy-2-methyl-3-nitrobenzoic Acid (26) from 25. Compound **25** (2.15 g, 10.9 mmol) was added to a solution of potassium permanganate (3.4 g, 21.5 mmol) and NaOH (1.0 g, 25 mmol) in water (50 mL). The mixture was heated to reflux for 2 h. After it was cooled, the mixture was acidified with 3 N HCl and extracted with ethyl acetate (4 × 80 mL). The combined ethyl acetate solution was dried over $MgSO_4$, and removal of the solvent furnished **26** (2.20 g, 96%) as a light yellow solid; mp 152−154 °C. ^1H NMR (DMSO-d_6): δ 13.5 (bs, 1 H, OH), 8.11 (d, $J = 9.14$ Hz, 1 H), 7.17 (d, $J = 9.14$ Hz, 1 H), 3.90 (s, 3 H), 2.40 (s, 3 H). CIMS *m/z* 212 (MH^+). Anal. ($C_9H_9NO_5$) C, H, N.

Methyl 6-Methoxy-2-methyl-3-nitrobenzoate (27). A 2 M solution of (trimethylsilyl)diazomethane in hexane (10 mL) was added dropwise to a solution of **26** (2.45 g, 11.6 mmol) in methanol (40 mL) until the solution stayed yellow. After it was stirred for 30 min at room temperature, the reaction mixture was poured into ice−water (50 g) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$, and the solvent was removed to furnish **27** (2.49 g, 95%) as a white crystalline solid; mp 45−46 °C. ^1H NMR (DMSO-d_6): δ 8.06 (d, $J = 9.17$ Hz, 1 H), 6.84 (d, $J = 9.16$ Hz, 1 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 2.46 (s, 3 H). Anal. ($C_{10}H_{11}NO_5$) C, H, N.

Methyl 2-(Bromomethyl)-6-methoxy-3-nitrobenzoate (28). A mixture of **27** (1.5 g, 6.6 mmol) and NBS (1.32 g, 3.7 mmol) in CCl_4 (30 mL) was preheated to 50 °C, and dibenzoyl peroxide (0.2 g, 0.8 mmol) was then added. The mixture was stirred and heated at reflux for 3 h, and then, another portion of dibenzoyl peroxide (0.05 g, 0.2 mmol) was added. The mixture was stirred and heated at reflux for another 10 h. After it was cooled to room temperature, the precipitate was removed by filtration, and the filtrate was washed with cold diethyl ether (2 × 30 mL). The combined filtrate and washings were concentrated on a rotary evaporator without the application of heat to yield a brown oily liquid. TLC indicated the presence of both product and starting material. Column chromatography (silica gel, ethyl acetate−hexane 1:3) yielded starting material **27** (0.38 g) and pure **28** (0.81 g, 53%) as a light yellowish oil. ^1H NMR ($CDCl_3$): δ 8.12 (d, $J = 9.20$ Hz, 1 H), 6.92 (d, $J = 9.22$ Hz, 1 H), 4.70 (s, 2 H), 3.92 (s, 3 H), 3.87 (s, 3 H). ESIMS *m/z* 304 (MH^+). Anal. ($C_{10}H_{10}BrNO_5$) C, H, N, Br.

7-Methoxy-4-nitro-2-phenethyl-2,3-dihydroisoindol-1-one (29a). β-Phenethylamine (0.085 mL, 0.66 mmol) and Et_3N (0.2 mL, 1.2 mmol) were added to a solution of **28** (0.2 g, 0.66 mmol) in methanol (10 mL). The mixture was heated at 80 °C under argon for 24 h. After it was cooled to room temperature, the solvent was removed. The solid was dissolved in methylene chloride (50 mL) and washed with 0.1 N HCl (2 × 50 mL). The organic phase was isolated, washed with brine (30 mL), and dried over $MgSO_4$. Removal of the solvent gave an oily residue, which was recrystallized from ethyl acetate−hexane (1:1). Compound **29a** (0.19 g, 93%) was obtained as a yellowish solid; mp 173−175 °C. ^1H NMR ($CDCl_3$): δ 8.35 (d, $J = 9.13$ Hz, 1 H), 7.28−7.19 (m, 5 H), 7.00 (d, $J = 9.12$ Hz, 1 H), 4.59

(s, 2 H), 4.06 (s, 3 H), 3.84 (t, J = 7.47 Hz, 2 H), 2.99 (t, J = 7.53 Hz, 2 H). CIMS m/z 313 (MH^+). Anal. ($C_{17}H_{16}N_2O_4$) C, H, N.

2-[2-(4-Fluorophenyl)ethyl]-7-methoxy-4-nitro-2,3-dihydroisoindol-1-one (29b). From 4-fluoro-2-phenethylamine (0.25 mL, 1.9 mmol), Et_3N (0.44 mL, 3.3 mmol), and **28** (0.5 g, 1.64 mmol), a similar procedure as that described for **29a** gave **29b** (0.43 g, 84%) as a yellowish solid; mp 209−211 °C. ^1H NMR (DMSO-d_6): δ 8.41 (d, J = 9.16 Hz, 1 H), 7.31 (m, 3 H), 7.11 (t, J = 8.88 Hz, 2 H), 4.77 (s, 2 H), 4.00 (s, 3 H), 3.73 (t, J = 7.21 Hz, 2 H), 2.92 (t, J = 7.19 Hz, 2 H). Anal. ($C_{17}H_{15}FN_2O_4$) C, H, F, N.

7-Hydroxy-4-nitro-2-phenethyl-2,3-dihydroisoindol-1-one (30a). Compound **29a** (0.45 g, 1.45 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and cooled to −78 °C. BBr_3 (2.0 mL, 1 M solution in CH_2Cl_2, 2.0 mmol) was added to the reaction mixture under argon. The solution was warmed to 0−10 °C and stirred for 2 h. After it was recooled to −78 °C, H_2O (20 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 $MgSO_4$. The solvent was removed, and the crude oily residue was further purified by flash chromatography (ethyl acetate−hexane 1:1). The product **30a** (0.38 g, 88%) was isolated as an off-white crystalline solid; mp 135−137 °C. ^1H NMR (DMSO-d_6): δ 9.50 (bs, 1 H, OH), 8.27 (d, J = 9.05 Hz, 1 H), 7.30−7.21 (m, 5 H), 7.05 (d, J = 9.03 Hz, 1 H), 4.76 (s, 2 H), 3.75 (t, J = 7.34 Hz, 2 H), 2.94 (t, J = 7.47 Hz, 2 H). Anal. ($C_{16}H_{14}N_2O_4$) C, H, N.

2-[2-(4-Fluorophenyl)ethyl]-7-hydroxy-4-nitro-2,3-dihydroisoindol-1-one (30b). From compounds **29b** (0.50 g, 1.60 mmol) and BBr_3 (3.0 mL, 1 M solution in CH_2Cl_2, 3.0 mmol), a similar procedure as that described for **30a** gave product **30b** (0.41 g, 86%) as a yellowish crystalline solid; mp 188−190 °C. ^1H NMR (DMSO-d_6): δ 11.31 (bs, 1 H, OH), 8.27 (d, J = 9.04 Hz, 1 H), 7.39 (m, 2 H), 7.11 (t, J = 8.80 Hz, 2 H), 7.04 (d, J = 9.03 Hz, 1 H), 4.77 (s, 2 H), 3.73 (t, J = 7.27 Hz, 2 H), 2.93 (t, J = 7.28 Hz, 2 H). Anal. ($C_{16}H_{13}FN_2O_4$) C, H, F, N.

4-Amino-7-hydroxy-2-phenethyl-2,3-dihydroisoindol-1-one (31a). Compound **30a** (0.35 g, 1.2 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.05 g) in methanol (50 mL) at room temperature and atmospheric pressure for 30 min. The mixture was then filtered through Celite and washed with methanol (10 mL). The combined filtrate was evaporated on a rotary evaporator to give **31a** (0.29 g, 90%) as an oily residue, which was used without further purification. ^1H NMR (DMSO-d_6): δ 8.37 (s, 1 H, OH), 7.30−7.21 (m, 5 H), 6.64 (d, J = 8.30 Hz, 1 H), 6.56 (d, J = 8.39 Hz, 1 H), 4.65 (bs, 2 H, NH_2), 4.15 (s, 2 H), 3.71 (t, J = 7.12 Hz, 2 H), 2.93 (t, J = 7.09 Hz, 2 H).

4-Amino-2-[2-(4-fluorophenyl)ethyl]-7-hydroxy-2,3-dihydroisoindol-1-one (31b). From compound **30b** (0.40 g, 1.3 mmol), H_2, and Pd−C (wet, contains 50% water, 0.05 g), a similar procedure as that described for **31a** afforded **31b** (0.34 g, 89%) as a dark yellow solid; mp 78−80 °C. ^1H NMR (DMSO-d_6): δ 8.37 (s, 1 H, OH), 7.28 (m, 2 H), 7.11 (t, J = 8.84 Hz, 2 H), 6.66 (d, J = 8.33 Hz, 1 H), 6.57 (d, J = 8.41 Hz, 1 H), 4.72 (bs, 2 H, NH_2), 4.15 (s, 2 H), 3.70 (t, J = 7.12 Hz, 2 H), 2.90 (t, J = 7.13 Hz, 2 H). Anal. ($C_{16}H_{15}FN_2O_2$) C, H, F, N.

4-[*N*-[(2,5-Dihydroxyphenyl)methyl]amino]-7-hydroxy-2-phenethyl-2,3-dihydroisoindol-1-one (32a). 2,5-Dihydroxybenzaldehyde **19** (0.25 g, 1.80 mmol) was added to **31a** (0.29 g, 1.1 mmol) in anhydrous methanol (40 mL), and the mixture was heated to reflux under argon for 6 h. After it was cooled to 0 °C, the precipitate was removed by filtration, washed with cold methanol (5 mL), and dried. The solid was dissolved in a mixture of ethyl acetate (100 mL) and methanol (100 mL) and hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.1 g) at room temperature and atmospheric pressure for 10 min. The mixture was then filtered through Celite and washed with methanol (40 mL). The combined filtrate was evaporated on a rotary evaporator to give a solid, which was purified by column chromatography (ethyl acetate as elute). Compound **32a** (0.27 g, 63%) was obtained as a yellowish solid; mp 201−203 °C. ^1H NMR (DMSO-d_6): δ 8.77 (s, 1 H, OH), 8.53 (s, 1 H, OH), 8.42 (bs, 1 H, OH), 7.32−7.20 (m, 5 H), 6.60 (m, 3 H), 6.49 (d, J = 8.37 Hz, 1 H), 6.44 (dd, J = 8.38, 2.43 Hz, 1 H), 5.44 (bs, 1 H, NH), 4.27 (s, 2 H), 4.15 (s, 2 H), 3.73 (t, J = 7.10 Hz, 2 H), 2.93 (t, J = 7.20 Hz, 2 H). ESIMS m/z 391 (MH^+), 389 ($M - H^-$). Anal. ($C_{23}H_{22}N_2O_4$) C, H, N.

4-[*N*-[(2,5-Dihydroxyphenyl)methyl]amino]-2-[2-(4-fluorophenyl)ethyl]-7-hydroxy-2,3-dihydroisoindol-1-one (32b). From compounds **19** (0.18 g, 1.30 mmol), **31b** (0.30 g, 1.04 mmol), H_2, and 10% Pd−C (wet, contains 50% water, 0.1 g), a similar procedure as that described for **32a** gave compound **32b** (0.32 g, 76%) as a white crystalline solid; mp 178−180 °C. ^1H NMR (DMSO-d_6): δ 8.77 (s, 1 H, OH), 8.53 (s, 1 H, OH), 8.42 (bs, 1 H, OH), 7.28 (m, 2 H), 7.12 (t, J = 8.87 Hz, 2 H), 6.60 (m, 3 H), 6.50 (d, J = 8.17 Hz, 1 H), 6.44 (dd, J = 8.34, 2.33 Hz, 1 H), 5.40 (bs, 1 H, NH), 4.27 (s, 2 H), 4.23 (s, 2 H), 3.72 (t, J = 7.09 Hz, 2 H), 2.92 (t, J = 7.04 Hz, 2 H). ESIMS m/z 409 (MH^+). Anal. ($C_{23}H_{21}FN_2O_4$) C, H, F, N.

4-Amino-7-methoxy-2-(2-phenethyl)-2,3-dihydroisoindol-1-one (33a). Compound **29a** (0.15 g, 0.48 mmol) was hydrogenated over 10% palladium on activated carbon (wet, contains 50% water, 0.05 g) in ethanol (20 mL) at atmospheric pressure for 3 h. The mixture was then filtered through Celite and washed with ethanol (10 mL). The combined filtrate was evaporated on a rotary evaporator to give **33a** (0.12 g, 91%) as an off-white solid; mp 188−190 °C. ^1H NMR (DMSO-d_6): δ 7.30−7.19 (m, 5 H), 6.76 (d, J = 8.44 Hz, 1 H), 6.71 (d, J = 8.49 Hz, 1 H), 4.82 (bs, 2 H, NH_2), 4.10 (s, 2 H), 3.68 (m, 5 H), 2.88 (t, J = 7.16 Hz, 2 H). Anal. ($C_{17}H_{18}N_2O_2$) C, H, N.

4-Amino-2-[2-(4-fluorophenyl)ethyl]-7-methoxy-2,3-dihydroisoindol-1-one (33b). From compound **29b** (0.20 g, 0.6 mmol), H_2, and 10% Pd−C (wet, contains 50% water, 0.05 g), a similar procedure as that described for **33a** gave pure **33b** (0.16 g, 87%) as a yellowish solid; mp 198−200 °C. ^1H NMR (DMSO-d_6): δ 7.25 (m, 2 H), 7.08 (t, J = 8.76 Hz, 2 H), 6.76 (d, J = 8.53 Hz, 1 H), 6.73 (d, J = 8.51 Hz, 1 H), 5.05 (bs, 2 H, NH_2), 4.08 (s, 2 H), 3.69 (s, 3 H), 3.66 (t, J = 7.17 Hz, 2 H), 2.86 (t, J = 7.06 Hz, 2 H). Anal. ($C_{17}H_{17}FN_2O_2$) C, H, F, N.

4-[*N*-[(2,5-Dihydroxyphenyl)methyl]amino]-7-methoxy-2-(2-phenylethyl)-2,3-dihydroisoindol-1-one (34a). From compounds **19** (0.23 g, 1.70 mmol), **33a** (0.48 g, 1.70 mmol), H_2, and 10% Pd−C (wet, contains 50% water, 0.1 g), a similar procedure as that described for **32a** yielded compound **34a** (0.22 g, 87%) as an off-white solid; mp 172−174 °C. ^1H NMR (DMSO-d_6): δ 8.76 (s, 1 H, OH), 8.51 (s, 1 H, OH), 7.30−7.18 (m, 5 H), 6.76 (d, J = 8.65 Hz, 1 H), 6.60 (d, J = 8.54 Hz, 1 H), 6.57 (d, J = 2.05 Hz, 1 H), 6.49 (d, J = 8.67 Hz, 1 H), 6.40 (dd, J = 8.36, 2.42 Hz, 1 H), 5.53 (t, J = 5.80 Hz, 1 H, NH), 4.19 (s, 2 H), 4.16 (d, J = 5.73 Hz, 2 H), 3.71 (q, J = 7.80 Hz, 2 H), 3.69 (s, 3 H), 2.89 (t, J = 7.30 Hz, 2 H). CIMS 404 (MH^+), 283. Anal. ($C_{23}H_{24}N_2O_3 \cdot 2.2\ H_2O$) C, H, N.

4-{[(2,5-Dihydroxyphenyl)methyl]amino}-2-[2-(4-fluorophenyl)ethyl]-7-methoxy-2,3-dihydroisoindol-1-one (34b). From compounds **19** (0.16 g, 1.16 mmol), **33b** (0.35 g, 1.16 mmol), H_2, and 10% Pd−C (wet, contains 50% water, 0.05 g), a similar procedure as that described for **32a** afforded compound **34b** (0.33 g, 69%) as an off-white solid; mp 205−207 °C. ^1H NMR (DMSO-d_6): δ 8.75 (s, 1 H, OH), 8.50 (s, 1 H, OH), 7.26 (m, 2 H), 7.10 (t, J = 8.85 Hz, 2 H), 6.76 (d, J = 8.65 Hz, 1 H), 6.60 (d, J = 8.52 Hz, 1 H), 6.57 (d, J = 2.55 Hz, 1 H), 6.49 (d, J = 8.69 Hz, 1 H), 6.41 (dd, J = 8.45, 2.46 Hz, 1 H), 5.53 (t, J = 5.75 Hz, 1 H, NH), 4.19 (s, 2 H), 4.16 (d, J = 5.73 Hz, 2 H), 3.71 (q, J = 7.80 Hz, 2 H), 3.69 (s, 3 H), 2.88 (t, J = 7.03 Hz, 2 H). ESIMS m/z 423 (MH^+). Anal. ($C_{24}H_{23}FN_2O_4$) C, H, F, N.

2-Bromo-4-nitrophenol (36). Fluorosulfonic acid (3.16 g, 31.6 mmol) and NBS (6.16 g, 34.4 mmol) were slowly added to a cold (−30 °C) solution of 4-nitrophenol (**35**) (4 g, 28.8 mmol) in CH_3CN (40 mL) under argon at a rate that kept the temperature below −20 °C. After it was added, the reaction mixture was allowed to warm to room temperature and stirred

for 48 h. $NaHSO_3$ (38%, 20 mL) was added, and the reaction mixture was extracted with diethyl ether (2 × 100 mL). The combined ether layer was washed with H_2O (3 × 50 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was subjected to flash chromatography (silica gel 60, ethyl acetate−hexane 1:4). The product **36** (5.97 g, 95%) was isolated as a yellow solid; mp 114−115 °C (lit.[31] mp 112−114 °C). ^{1}H NMR ($CDCl_3$): δ 8.44 (d, J = 2.31 Hz, 1 H), 8.17 (dd, J = 9.01, 2.37 Hz, 1 H), 7.13 (d, J = 9.01 Hz, 1 H), 6.24 (bs, 1 H, OH).

2-Bromo-1-(methoxymethoxy)-4-nitrobenzene (37). A suspension of **36** (2.0 g, 9.17 mmol) and NaH (0.44 g, 18.3 mmol) in dry THF (50 mL) was stirred for 1 h. Chloromethyl methyl ether (1.38 mL, 18.3 mmol) was added to the mixture, and the reaction mixture was stirred for 24 h at room temperature. Water (50 mL) and saturated NH_4Cl were added to adjust the pH to 7.0, and the solvent was evaporated. The residue was extracted with diethyl ether (3 × 50 mL), and the organic phase was washed with water (50 mL) and brine (20 mL). The ether layer was dried over $MgSO_4$ and evaporated to afford the MOM ether **37** (2.16 g, 90%) as a yellow crystalline solid; mp 86−88 °C. ^{1}H NMR ($CDCl_3$): δ 8.47 (d, J = 2.67 Hz, 1 H), 8.17 (dd, J = 9.14, 2.68 Hz, 1 H), 7.25 (d, J = 9.15 Hz, 1 H), 5.35 (s, 2 H), 3.53 (s, 3 H). CIMS m/z 262, 264 (MH^+). Anal. ($C_8H_8BrNO_4$) C, H, Br, N.

1-(2-Methoxymethoxy-5-nitro)phenyl-4-phenylbutyne (38). 2-Bromo-1-(methoxymethoxy)-4-nitrobenzene (**37**) (1 g, 3.8 mmol), bis(triphenylphosphine) palladium (II) chloride (0.08 g, 0.11 mmol), triphenylphosphine (0.06 g, 0.23 mmol), and copper(I) iodide (0.015 g, 0.076 mmol) were added to a dry round-bottomed flask, which was then sparged with argon and charged with diethylamine (30 mL). 4-Phenyl-1-butyne (1.07 mL, 7.6 mmol) was added via syringe. The stirred reaction mixture was heated at 50−60 °C for 6 h. During the reaction, precipitation of $[H_2N(i\text{-}Pr)_2]Br$ was observed. After it was cooled to room temperature, the reaction mixture was diluted with EtOAc (30 mL), filtered through a small pad of silica gel with EtOAc rinsings, concentrated in vacuo, and purified by flash chromatography (silica gel 180 g, ethyl acetate−hexane 1:6) to give **38** (1.20 g, 100%) as a dark brown liquid. ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J = 2.78 Hz, 1 H), 7.99 (dd, J = 9.18, 2.80 Hz, 1 H), 7.25−7.15 (m, 5 H), 7.09 (d, J = 9.19 Hz, 1 H), 5.20 (s, 2 H), 3.41 (s, 3 H), 2.87 (t, J = 7.37, 2 H), 2.69 (t, J = 7.34 Hz, 2 H). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

1-(5-Amino-2-hydroxy)phenyl-4-phenylbutyne (40). A mixture of **38** (0.5 g, 1.6 mmol) and $SnCl_2{\cdot}2H_2O$ (1.8 g, 8.0 mmol) in absolute ethanol (10 mL) was heated at 70 °C under argon for 2 h. The reaction mixture was allowed to cool to room temperature and then poured into ice. The pH was adjusted to 7−8 by addition of 5% aqueous $NaHCO_3$, and the mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (silica gel, ethyl acetate−hexane 1:2) to give **40** (0.20 g, 52%) as a solid; mp 96−98 °C. ^{1}H NMR ($CDCl_3$): δ 9.98 (s, 1 H, OH), 7.28−7.18 (m, 5 H), 6.63 (d, J = 8.59 Hz, 1 H), 6.53 (d, J = 2.62 Hz, 1 H), 6.48 (dd, J = 8.61, 2.73 Hz, 1 H), 3.60 (bs, 2 H, NH_2), 2.85 (t, J = 7.19 Hz, 2 H), 2.70 (t, J = 7.11 Hz, 2 H). ESIMS m/z 238 (MH^+); high-resolution ESI m/z calcd, 238.1232; found, 238.1237. Anal. ($C_{16}H_{15}NO$) C, H, N.

***cis*-1-(5-Amino-2-hydroxy)phenyl-4-phenylbutene (41).** Compound **40** (0.13 g, 0.55 mmol) was hydrogenated over 5 wt % palladium on calcium carbonate, poisoned with lead (8 mg), in ethanol (15 mL) at room temperature and atmospheric pressure. The reaction was traced by TLC every 10 min until the transformation was completed. The mixture was then filtered through Celite and washed with ethanol (5 mL). The combined filtrate was used directly in the next step. For the purpose of identification, a small portion of the above combined filtrate was evaporated on a rotary evaporator to give the crude product, which was then purified by flash chromatography (silica gel 80 g, ethyl acetate: hexane 1:4). The purified product was isolated as a dark brown oily residue. ^{1}H NMR ($CDCl_3$): δ 10.50 (s, 1 H, OH), 7.22−7.11 (m, 5 H), 6.59 (d, J = 8.47 Hz, 1 H), 6.44 (dd, J = 8.43, 2.70 Hz, 1 H), 6.27 (d, J = 11.29 Hz, 1 H), 6.18 (d, J = 2.58 Hz, 1 H), 5.78 (dt, J = 11.29, 7.31 Hz, 1 H), 3.96 (bs, 2 H, NH_2), 2.65 (t, J = 7.39 Hz, 2 H), 2.40 (q, J = 7.36 Hz, 2 H).

***cis*-1-{5-[*N*-[(2,5-Dihydroxyphenyl)methyl]amino]-2-hydroxy}phenyl-4-phenylbutene (42).** 2,5-Dihydroxybenzaldehyde **19** (0.11 g, 0.82 mmol) was added to **41** in ethanol (20 mL), and the mixture was stirred at room temperature under argon for 6 h. While it was stirred, $NaBH_3CN$ (0.13 g, 2.06 mmol) was added, and the reaction mixture was stirred for another 1 h. The reaction mixture was concentrated, and H_2O (100 mL) was added to it. The mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and concentrated to furnish the crude product, which was further purified by flash chromatography (silica gel 60 g, ethyl acetate−hexane 1:2). The product **42** (0.11 g, 58% in two steps) was isolated as a brownish liquid. ^{1}H NMR (DMSO-d_6): δ 8.72 (s, 1 H, OH), 8.51 (s, 1 H, OH), 8.38 (s, 1 H, OH), 7.20−7.04 (m, 5 H), 6.67 (d, J = 8.44 Hz, 1 H), 6.66 (d, J = 8.42 Hz, 1 H), 6.59 (m, 2 H), 6.52 (d, J = 2.25 Hz, 1 H), 6.30 (d, J = 2.20 Hz, 1 H), 6.25 (d, J = 11.24 Hz, 1 H), 5.80 (dt, J = 11.59, 7.33 Hz, 1 H), 4.50 (bs, 1 H, NH), 4.17 (s, 2 H), 2.62 (t, J = 7.21 Hz, 2 H), 2.31 (q, J = 7.17 Hz, 2 H). ESIMS m/z 362 (MH^+). Anal. ($C_{23}H_{23}NO_3{\cdot}CH_3COOCH_2CH_3$) C, H, N.

1-Bromo-4-phenyl-1-butyne (47). A solution of 4-phenyl-1-butyne (**43**, 0.4 g, 3.0 mmol) in acetone (20 mL) was treated at room temperature with NBS (0.62 g, 3.5 mmol) and $AgNO_3$ (50 mg). The mixture was stirred at room temperature for 4 h. The precipitate was removed by filtration, and the solvent was evaporated. The residue was redissolved in hexane (10 mL), and the precipitate was again removed by filtration. Evaporation of the solvent gave product **47** (0.62 g, 99%) as a colorless oily liquid, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 7.32−7.20 (m, 5 H), 2.83 (t, J = 7.47 Hz, 2 H), 2.49 (t, J = 7.53 Hz, 2 H).

1-Methoxymethoxy-2-[(1*E*)-4-phenylbut-1-enyl]-4-nitrobenzene (48). Tributyltin hydride (1.89 g, 6.5 mmol) was added dropwise over 30 min to a stirred solution of the 1-bromoalkyne **47** (0.68 g, 3.25 mmol) and tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.017 mmol) in THF (10 mL) under argon at room temperature. The mixture was stirred at room temperature for a further 2 h. The crude vinylstannane product **44** was then treated with **37** (0.6 g, 2.3 mmol) and additional tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.017 mmol). The mixture was heated to reflux for 3 h and then cooled and diluted with an equal volume of saturated aqueous KF. The two phase mixture was stirred vigorously overnight. Diethyl ether (30 mL) was added, the separated organic layer was washed with saturated ammonium chloride (50 mL) and brine (50 mL), and the solvent was removed by evaporation. The residue was purified by column chromatography (silica gel 180 g, ethyl acetate−hexane 1:5) to give compound **48** (0.55 g, 76%) as light yellow oil. ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 2.73 Hz, 1 H), 8.08 (dd, J = 9.09, 2.76 Hz, 1 H), 7.35−7.23 (m, 5 H), 7.18 (d, J = 9.10 Hz, 1 H), 6.72 (d, J = 15.97 Hz, 1 H), 6.43 (dt, J = 15.94, 6.86 Hz, 1 H), 5.31 (s, 2 H), 3.52 (s, 3 H), 2.86 (t, J = 7.35 Hz, 2 H), 2.64 (q, J = 7.27 Hz, 2 H). Anal. ($C_{18}H_{19}NO_4$) C, H, N.

2-[(1*E*)-4-Phenylbut-1-enyl]-4-aminophenol (49). A mixture of **48** (0.5 g, 1.6 mmol) and $SnCl_2{\cdot}2H_2O$ (1.8 g, 8.0 mmol) in absolute ethanol (10 mL) was heated at 70 °C under argon for 2 h. The reaction mixture was allowed to cool to room temperature and then poured into ice and stirred for 30 min. The pH was adjusted to 7−8 by addition of 5% aqueous $NaHCO_3$, and the reaction mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The crude product was subjected to column chromatography (silica gel 80 g, ethyl acetate−hexane 1:2) to give **49** (0.21 g, 55%) as an oily residue, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 9.98 (s, 1 H, OH), 7.31−7.17 (m, 5 H), 6.72 (d, J = 8.45 Hz, 1 H), 6.55 (dd, J = 8.41, 2.39 Hz, 1 H), 6.36 (d, J = 11.21 Hz, 1 H), 6.25 (d, J = 2.47 Hz, 1 H), 5.90

(dt, $J = 11.21, 7.34$ Hz, 1 H), 3.40 (bs, 2 H, NH_2), 2.74 (t, $J = 7.39$ Hz, 2 H), 2.48 (q, $J = 7.33$ Hz, 2 H).

***trans*-1-{5-[*N*-[(2,5-Dihydroxyphenyl)methyl]amino]-2-hydroxy}phenyl-4-phenyl-1-butene (50).** 2,5-Dihydroxybenzaldehyde **19** (0.2 g, 1.5 mmol) was added to **49** (0.36 g, 1.5 mmol) in methanol (30 mL), and the mixture was stirred at room temperature under argon overnight. While stirring, $NaBH_3CN$ (0.26 g, 4.12 mmol) was added and the reaction mixture was stirred for another 3 h at room temperature. The reaction mixture was then concentrated, and H_2O (100 mL) was added to it. The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, and concentrated to furnish the crude product, which was further purified by flash chromatography (silica gel 150 g, ethyl acetate−hexane 1:2). The product **49** (0.26 g, 48%) was isolated as a brown liquid. ^{1}H NMR (DMSO-d_6): δ 8.99 (s, 1 H, OH), 8.72 (s, 1 H, OH), 8.53 (s, 1 H, OH), 7.26−7.22 (m, 5 H), 6.69 (d, $J = 8.28$ Hz, 1 H), 6.65−6.52 (m, 5 H), 6.31 (dd, $J = 8.49, 2.30$ Hz, 1 H), 6.06 (dt, $J = 15.92, 6.77$ Hz, 1 H), 5.30 (s, 1 H, NH), 4.02 (d, $J = 7.03$ Hz, 2 H), 2.70 (t, $J = 7.34$ Hz, 2 H), 2.44 (q, $J = 7.27$ Hz, 2 H). CIMS *m/z* 362 (MH^+). Anal. ($C_{23}H_{23}NO_3 \cdot 3/4H_2O$) C, H, N.

Acknowledgment. This research was made possible by grants from the Showalter Trust and Purdue Research Foundation.

References

(1) Levitski, A. Protein Tyrosine Kinase Inhibitors as Therapeutic Agents. *Top. Curr. Chem.* **2001**, *211*, 1−15.
(2) Traxler, P.; Furet, R. Strategies Toward the Design of Novel and Selective Protein Tyrosine Kinase Inhibitors. *Pharmacol. Ther.* **1999**, *82*, 195−206.
(3) Hamby, J. M.; Showalter, H. D. Small Molecule Inhibitors of Tumor-Promoted Angiogenesis, Including Protein Tyrosine Kinase Inhibitors. *Pharmacol. Ther.* **1999**, *82*, 169−193.
(4) Al-Obeidi, F. A.; Lam, K. S. Development of Inhibitors for Protein Tyrosine Kinases. *Oncogene* **2000**, *19*, 5690−5701.
(5) Traxler, P. Tyrosine Kinase Inhibitors in Cancer Treatment (Part II). *Exp. Opin. Ther. Pat.* **1998**, *8*, 1599−1625.
(6) Buchdunger, E.; Cioffi, C. L.; Law, N.; Stover, D.; Ohno-Jones, S.; Druker, B. J.; Lydon, N. B. Abl Protein-Tyrosine Kinase Inhibitor STI571 Inhibits In Vitro Signal Transduction Mediated by c-Kit and Platelet-Derived Growth Factor. Receptors *J. Pharmacol. Exp. Ther.* **2000**, *295*, 139−145.
(7) Bange, J.; Zwick, E.; Ullrich, A. Molecular Targets for Breast Cancer Therapy and Prevention. *Nat. Med.* **2001**, *7*, 548−525.
(8) Lu, Y.; Zi, X.; Zhao, Y.; Mascarenhas, D.; Pollack, M. Insulin-Like Growth Factor-I Receptor Signaling and Resistance to Trastuzumab (Herceptin). *J. Natl. Cancer Inst.* **2001**, *93*, 1852−1857.
(9) Onoda, T.; Iinuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Naganawa, H.; Takeuchi, T. Isolation of a Novel Tyrosine Kinase Inhibitor, Lavendustin A, from *Streptomyces griseolavendus*. *J. Nat. Prod.* **1989**, *52*, 1252−1257.
(10) Hsu, C.-Y. J.; Persons, P. E.; Spada, A. P.; Bednar, R. A.; Levitzki, A.; Zilberstein, A. Kinetic Analysis of the Inhibition of the Epidermal Growth Factor Receptor Tyrosine Kinase by Lavendustin A and Its Analogue. *J. Biol. Chem.* **1991**, *266*, 21105−21112.
(11) Imoto, M.; Sujikai, I.; Ui, H.; Umezawa, K. Involvement of Tyrosine Kinase in Growth Factor-Induced Phospholipase C Activation in NIH3T3 Cells. *Biochim. Biophys. Acta* **1993**, *1166*, 188−192.
(12) Smyth, M. S.; Stefanova, I.; Hartman, F.; Horad, I. D.; Osherov, N.; Levitzki, A.; Burke, T. R. J. Non-Amine Based Analogues of Lavendustin A as Protein-Tyrosine Kinase Inhibitors. *J. Med. Chem.* **1993**, *36*, 3010−3014.
(13) Smyth, M. S.; Stefanova, I.; Horak, I. D.; Burke, T. R., Jr. Hydroxylated 2-(5′-Salicyl)naphthalenes as Protein-Tyrosine Kinase Inhibitors. *J. Med. Chem.* **1993**, *36*, 3015−3020.
(14) Chen, H.; Boiziau, J.; Parker, F.; Maroun, R.; Tocque, B.; Roques, B. P.; Garbay-Jaureguiberry, C. Synthesis and Structure−Activity Studies of a Series of [(Hydroxybenzyl)amino]salicylates as Inhibitors of EGF Receptor-Associated Tyrosine Kinase Activity. *J. Med. Chem.* **1993**, *36*, 4094−4098.
(15) Chen, H.; Boiziau, J.; Parker, F.; Mailliet, P.; Commerçon, A.; Tocque, B.; Le Pecq, J.-B.; Roques, B.-P.; Garbay, C. Structure−Activity Relationships in a Series of 5-[(2,5-Dihydroxybenzyl)-amino]salicylate Inhibitors of EGF-Receptor-Associated Tyrosine Kinase: Importance of Additional Hydrophobic Aromatic Interactions. *J. Med. Chem.* **1994**, *37*, 845−859.
(16) Agbotounou, W. K.; Umezawa, K.; Jacuemin-Sablon, A.; Pierre, J. Inhibition by Two Lavendustins of the Tyrosine Kinase Activity of pp60^{F527} In Vitro and in Intact Cells. *Eur. J. Pharmacol.* **1994**, *269*, 1−8.
(17) Nussbaumer, P.; Winiski, A. P.; Cammisuli, S.; Hiestand, P.; Weckbecker, G.; Stütz, A. Novel Antiproliferative Agents Derived from Lavendustin A. *J. Med. Chem.* **1994**, *37*, 4079−4084.
(18) Liu, T.; Shirai, R.; Matsui, T.; Umezawa, K.; Iwasaki, S. Synthesis and Biological Activity of 5-[2,5-Dihydroxybenzyl)-amino]salicylic Acid Analogues as Inhibitors of EGF Receptor-Associated Protein Tyrosine Kinase. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 365−368.
(19) Green, J. Solid-Phase Synthesis of Lavendustin A and Analogues. *J. Org. Chem.* **1995**, *60*, 4287−4290.
(20) Devraj, R.; Cushman, M. A Versatile Solid-Phase Synthesis of Lavendustin A and Certain Biologically Active Analogues. *J. Org. Chem.* **1996**, *61*, 9368−9373.
(21) Mu, F.; Coffing, S. L.; Riese, D. J. I.; Geahlen, R. L.; Verdier-Pinnard, P.; Hamel, E.; Johnson, J.; Cushman, M. Design, Synthesis, and Biological Evaluation of a Series of Lavendustin A Analogues That Inhibit EGFR and Syk Tyrosine Kinases, as Well as Tubulin Polymerization. *J. Med. Chem.* **2001**, *44*, 441−452.
(22) Onoda, T.; Isshiki, K.; Takeuchi, T.; Tatsuta, K.; Umezawa, K. Inhibition of Tyrosine Kinase and Epiderman Growth Factor Internalization by Lavendustin A Methyl Ester in Cultured A431 Cells. *Drugs Exptl. Clin. Res.* **1990**, *16*, 249−253.
(23) Schweitzer, B. A.; Kool, E. Aromatic Nonpolar Nucleosides as Hydrophobic Isosteres of Pyrimidine and Purine Nucleosides. *J. Org. Chem.* **1994**, *59*, 7238−7242.
(24) Riese, D. J. I.; van Raaij, T. M.; Plowman, G. D.; Andrews, G. C.; Stern, D. F. The Cellular Response to Neuregulins is Governed by Complex Interactions of the ErbB Receptor Family. *Mol. Cell. Biol.* **1995**, *15*, 5770−5776.
(25) Paull, K. D.; Hamel, E.; Malspeis, L. *Prediction of Biochemical Mechanism of Action from the In Vitro Antitumor Screen of the National Cancer Institute.*; American Chemical Society: Washington, DC, 1995.
(26) Zaleska, B.; Lis, S. An Efficient and Novel Synthesis of 1,3-Benzoxazine-2,4-dione and Its Derivatives. *Pharmazie* **1995**, *50*, 150−151.
(27) Stenseth, R. E.; Baker, J. W.; Roman, D. P. Substituted 3-Phenyl-1,3-benzoxazine-2,4-diones and Their Bacteriostatic Activity. *J. Med. Chem.* **1963**, *6*, 212−213.
(28) Cullis, C. F.; Ladbury, J. W. Kinetic Studies of the Oxidation of Aromatic Compounds by Potassium Permanganate. Part II. Substituted Toluenes. *J. Chem. Soc.* **1955**, 1407−1410.
(29) Askam, V.; Deeks, R. H. L. Oxidation and Claisen Condensation Products of 3-Nitro-*o*-xylene. *J. Chem. Soc.* **1969**, 1935−1936.
(30) Visentin, S.; Amiel, P.; Fruttero, R.; Boschi, D.; Roussel, C.; Giusta, L.; Carbone, E.; Gasco, A. Synthesis and Voltage-Clamp Studies of Methyl 1,4-Dihydro-2,6-dimethyl-5-nitro-4-(benzofurazanyl)pyridine-3-carboxylate. *J. Med. Chem.* **1999**, *42*, 1422−1427.
(31) Oberhauser, T. A New Bromination Method for Phenols and Anisoles: NBS/$HBF_4 \cdot Et_2O$ in CH_3CN. *J. Org. Chem.* **1997**, *62*, 4504−4506.
(32) Sonogashira, K.; Tohda, Y.; Hagihara, N. A Convenient Synthesis of Acetylenes: Catalytic Substitution of Acetylenic Hydrogen with Bromoalkenes, Iodoarenes, and Bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4470.
(33) Hundermark, T.; Littke, A. F.; Buchwald, S. L.; Fu, G. C. Pd-$(PhCN)_2Cl_2$/P(*t*-Bu)$_3$: A Versatile Catalyst for Sonogashira Reactions of Aryl Bromides at Room Temperature *Org. Lett.* **2000**, *2*, 1729−1731.
(34) Bellamy, F. D.; Ou, K. Selective Reduction of Aromatic Nitro Compounds with Stannous Chloride in Non Acidic Medium. *Tetrahedron Lett.* **1984**, *25*, 839−842.
(35) Kikukawa, K.; Umekawa, H.; Wada, F.; Matsuda, T. Regioselective Hydrostannation of Terminal Acetylenes Under Transition Metal Catalysis. *Chem Lett.* **1988**, 881−884.
(36) Ichinose, Y.; Oda, H.; Oshima, K.; Utimoto, K. Palladium Catalyzed Hydrostannylation and Hydrogermylation of Acetylenes. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3468−3470.
(37) Hibino, J.; Matsubara, S.; Morizawa, Y.; Oshima, K.; Nozaki, H. Regioselective Stannylmetalation of Acetylenes in the Presence of Transition-Metal Catalysis. *Tetrahedron Lett.* **1984**, *25*, 2151−2154.
(38) Zhang, H. X.; Guibé, F.; Balavoine, G. Palladium- and Molybdenum-Catalyzed Hydrostannation of Alkynes. A Novel Access to Regio- and Stereodefined Vinylstannanes. *J. Org. Chem.* **1990**, *55*, 1857−1867.
(39) Laudano, A. P.; Buchanan, J. M. Phosphorylation of Tyrosine in the Carboxyl-Terminal Tryptic Peptide of pp60$^{c-src.}$ *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 892−896.

(40) Boden, C. D. J.; Pattenden, G.; Ye, T. Palladium-Catalyzed Hydrostannylations of 1-Bromoalkynes, a Practical Synthesis of (*E*)-1-Stannylalk-1-enes. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2417–2419.

(41) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91–109.

JM0202270