Antineoplastic Agents. 487. Synthesis and Biological Evaluation of the Antineoplastic Agent 3,4-Methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene and Derived Amino Acid Amides

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Received May 13, 2002

An efficient synthesis of 3,4-methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene (**1c**) and hydrochloride (**1d**) is reported. The nitrostilbene intermediate **6a** was obtained via a Wittig reaction using phosphonium salt **4** and 3-nitro-4-methoxybenzaldehyde **5**. A one-step reduction using zinc in acetic acid produced the synthetic objective amine **1c**. The coupling of this amine with various Fmoc amino acids, followed by cleavage of the α-amine protecting group, resulted in a series of new cancer cell growth inhibitory amides. Amine **1c**, hydrochloride **1d**, glycine amide **3b**, and tyrosine amide **3f** had the highest level ($GI_{50} = 10^{-2}-10^{-3}$ μg/mL) of activity against a panel of six human and one animal (P388) cancer cell lines. Amine **1c** and its hydrochloride **1d** potently inhibited tubulin polymerization by binding at the colchicine site, while the amides had little activity against purified tubulin. Nevertheless, most of the amides caused a marked increase in the mitotic index of treated cells, indicating that tubulin was their intracellular target.

Introduction

Combretastatin A-2 (**1a**, Chart 1) represents one of the key antineoplastic and cancer vascular targeting stilbenes,[1,2] designated combretastatins A-1 to A-6, that we isolated from the South African bush willow tree *Combretum caffrum*.[3] Initially we selected combretastatin A-4 (**2a**) for preclinical development, based on a spectrum of promising biological properties that were greatly enhanced by a structural modification to sodium combretastatin A-4 phosphate prodrug (**2b**, CA4P).[4,5] For example, **2b** caused a 100-fold decrease in tumor blood flow to the P22 carcinosarcoma in the rat while causing no significant blood flow reduction in normal heart, kidney, and small intestine.[6] Other recent preclinical findings include the antivascular and antitumor effects produced by **2b** against non-small-cell lung cancer in vivo.[7] Most importantly, the initial phase 1 human cancer clinical trials of **2b** have been quite successful.[8−10] Our discovery of combretastatin A-4[11] has prompted the synthesis of many structural variations.[12−14] One important variant replaces the 3′-hydroxyl of the B ring with an amine (**2c**), which subsequently resulted in a water-soluble hydrochloride salt (**2d**) given the code name AC-7739.[15] In 1999, Ohsumi et al. published interesting amino acid amide derivatives of **2c** that showed anticancer activity against murine colon 26 adenocarcinoma cells.[16] Both **2d** and a serine derivative **2e**, code name AC7700, have undergone further evaluation.[17,18] We have extended the early study of the combretastatin A-4 3′-amino derivative to combretastatin A-2 and now report the synthesis of 2,3-methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene (**1c**) and a selection of amino acid amide derivatives (**3a**−**g**) for purposes of an SAR investigation directed at locating promising candidate(s) for preclinical development.

Results and Discussion

Chemistry. The key precursor 3,4-methylenedioxy-5,4′-dimethoxy-3′-nitro-*Z*-stilbene (**6a**) was obtained (**4** → **6**) by employing a Wittig reaction sequence (Scheme 1). The required *cis*-stilbene was obtained in a *Z* to *E* ratio of 1.4:1. Photochemical isomerization[3,19] converted the *trans*-stilbene to the *cis*-stilbene isomer in 62% yield. Reduction of nitrostilbene **6a** with zinc in acetic acid[15] afforded amine **1c**. Following its purification by column chromatography, the 3,4-methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene crystallized (1:9 ethyl acetate/hexane) and was subjected to X-ray crystal structure determination.

Treatment of amine **1c**, in ethyl acetate, with 1 N HCl in diethyl ether yielded the amine hydrochloride (**1d**). Interestingly, this hydrochloride derivative was found to be essentially insoluble in water. Conversion of amine **1c** to a selection of Fmoc-amino acid amides was readily accomplished using PyBroP as the coupling reagent (Scheme 2).[20] All Fmoc deprotection was performed using tris(2-aminoethyl)amine.[21] The acid-sensitive side chain protecting groups on Cys, Ser, Trp, and Tyr were removed with trifluoroactic acid in dichloromethane.

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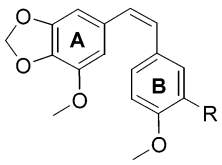
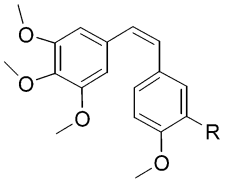
10.1021/jm020204l CCC: $25.00
Published on Web 01/10/2003

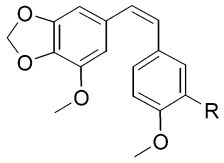
Chart 1



1a, R = OH, Combretastatin A-2
1b, R = OPO_3Na_2, Combretastatin A-2 Prodrug
1c, R = NH_2
1d, R = NH_3Cl



2a, R = OH, Combretastatin A-4
2b, R = OPO_3Na_2, Combretastatin A-4 Prodrug
2c, R = NH_2
2d, R = NH_3Cl
2e, R = NH-Ser HCl salt



3a, R = NH-Cys
3b, R = NH-Gly
3c, R = NH-Phe
3d, R = NH-Ser
3e, R = NH-Trp
3f, R = NH-Tyr
3g, R = NH-Val

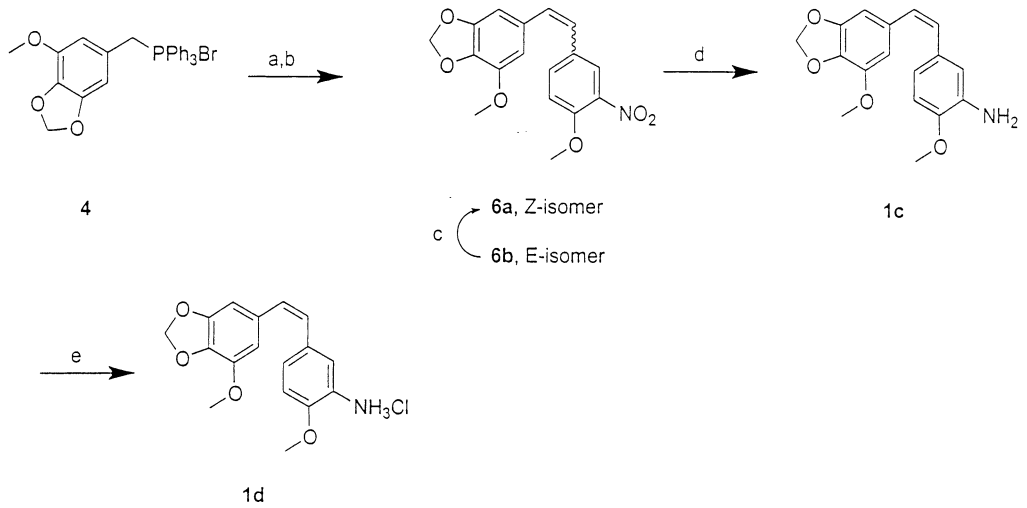
The trityl group on Cys was deprotected in the presence of the triethylsilane.

Biological Evaluation. The cancer cell growth inhibitory activities of the protected amides and related compounds are summarized in Table 1. Amine **1c** was more active than the 3′-alcohol combretastatin A-2.[3] This result mirrors combretastatin A-4 published data reporting increased activity of amine **2c** (IC_{50} = 5.1 nM) over phenol **2a** (IC_{50} = 18.0 nM) in a murine colon 26 adenocarcinoma cell study.[15] Although Oshumi et al. reported the serine derivative **2e** of amine **2c** to be the most active among their amino acid derivatives of combretastatin A-4,[16] we found that our serine derivative **3d** displayed only marginal activity. Against the minipanel of cancer cell lines listed in Table 1, amine **1c**, hydrochloride salt **1d**, glycine amide **3b**, and tyrosine amide **3f** provided the strongest cancer cell line growth inhibition, while the remaining amides were 1.5- to 10-fold less active.

Because of the well-documented interactions of combretastatins A-2 (**1a**) and A-4 (**2a**) with tubulin, the newly synthesized analogues (**1c**,**d**, **3a**−**g**, **6a**,**b**, and **7a**−**g**) were examined for their effects on both tubulin assembly and the binding of [^{3}H]colchicine to tubulin in comparison with the two natural products. Only amine **1c** and its hydrochloride salt **1d** had significant, and essentially identical, effects in either reaction (Table 2; only data for the more cytotoxic amides are shown here; colchicine binding studies with **6a**,**b** and **7a**,**c**−**g** were performed with the compounds at 100 μM). Consistent with their overall cytotoxicity, the effects of **1c** and **1d** on the tubulin-based reactions were between those of the more potent combretastatin A-4 and the less potent combretastatin A-2.

Because the cytotoxic activities of the amides **3a**−**g** in the human tumor cell lines and of **7b** in the murine P388 line were overall not that different from the activities of **1c** and **1d** in these lines, we wondered whether another cellular target might be involved in their activity. As a preliminary approach to this question, we evaluated these amides, the parent compounds **1c** and **1d**, and the combretastatins for their effects on the mitotic index of MCF-7 cells. An increase in the mitotic index generally represents an effect at the tubulin level. As shown in Table 2, like the combretastatins (**1a**, **2a**) and the amine (**1c**) and its salt (**1d**), the amides **3a**−**g** and **7b** caused a marked rise in the

Scheme 1[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, Ar, 0 °C; (b) 3-nitro-4-methoxybenzaldehyde (**5**) in THF, Ar; (c) benzil, benzene, 254 nm lamp, Ar; (d) zinc, acetic acid; (e) 1 M HCl in diethyl ether, ethyl acetate.

Table 1. Human Cancer Cell Line GI_{50} (μg/mL) and Murine P388 Lymphocytic Leukemia Cell Line Inhibitory Activity ED_{50} (μg/mL) of the 3′-Substituted Stilbenes

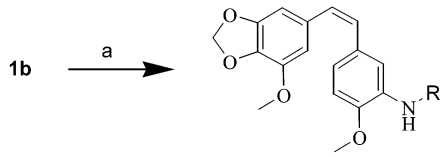
compound	leukemia P388	pancreas BXPC-3	breast MCF-7	CNS SF-295	lung NSC NCI-H460	colon KM2OL2	prostate DU145	mean
6a	0.227	0.38	0.36	0.33	0.35	0.45	0.64	0.39
6b	>10.0	>10.0	>10.0	4.6	>10.0	>10.0	>10.0	
1a	0.016	0.014	0.0042	0.0083	0.043	0.47	0.0054	0.080
1b	0.0250	2.1	0.045	0.042	0.41	3.8	0.053	0.93
1c	0.00189	0.0071	0.0047	0.023	0.0050	0.022	0.028	0.013
1d	0.0388	0.075	0.0042	0.0082	0.0057	0.035	0.0082	0.025
3a	0.332	0.20	0.050	0.037	0.032	0.055	0.0046	0.10
3b	0.0199	0.025	0.014	0.059	0.0042	0.012	0.0028	0.020
3c	0.0585	0.20	0.044	1.7	0.044	0.034	0.040	0.30
3d	0.172	0.027	0.017	0.049	0.042	0.019	0.014	0.49
3e	0.0653	0.044	0.035	0.042	0.053	0.018	0.040	0.042
3f	0.0475	0.0067	0.035	0.0097	0.012	0.022	0.018	0.017
3g	0.306	0.012	0.037	0.055	0.064	0.019	0.036	0.076

Table 2. Effects of Combretastatin A-2 Derivatives on Tubulin and on Mitosis in MCF-7 Breast Cancer Cells

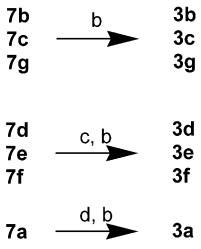
		inhibition of colchicine binding[b] (% inhibition)				
compound	inhibition of tubulin assembly[a] ($IC_{50} \pm$ SD, μM)	2 μM inhibitor concentration	5 μM inhibitor concentration	20 μM inhibitor concentration	100 μM inhibitor concentration	mitotic index[c] (% mitotic cells ± SD)
combretastatin A-4	2.1 ± 0.1	96	98			57 ± 6
combretastatin A-2	4.0 ± 0.2	79	92			57 ± 4
1c	3.1 ± 0.05	84	94			48 ± 5
1d	2.9 ± 0.05	87	95			47 ± 6
3a	>40				15	42 ± 4
3b	>40			31	66	52 ± 7
3c	>40				16	52 ± 4
3d	>40			22	64	35 ± 7
3e	>40				16	57 ± 12
3f	>40				19	44 ± 1
3g	>40				14	48 ± 1
7b	>40				8	56 ± 11

[a] Reaction mixtures contained 10 μM tubulin (1.0 mg/mL) and varying drug concentrations. A drug/tubulin preincubation in the absence of GTP preceded the assembly reaction.[24] [b] Reaction mixtures contained tubulin at 1.0 μM, [^{3}H]colchicine at 5.0 μM, and the inhibitory compounds at the indicated concentrations.[24] [c] MCF-7 cells were treated for 12 h with 10 times the GI_{50} concentrations shown in Table 2 except that the concentrations of combretastatins A-4 and A-2 and of **7b** were 50 nM, 1.0 μM, and 1.0 μM, respectively. Cells with condensed chromosomes were quantitated as mitotic cells. The mitotic index in untreated cells was 3.6%. See text for further details.

Scheme 2[a]



7a, R = N^{α}-Fmoc-Cys(STrt)
7b, R = N^{α}-Fmoc-Gly
7c, R = N^{α}-Fmoc-Phe
7d, R = N^{α}-Fmoc-Ser(OBu^t)
7e, R = N^{α}-Fmoc-Trp(NBoc)
7f, R = N^{α}-Fmoc-Tyr(OBu^t)
7g, R = N^{α}-Fmoc-Val



[a] Reagents and conditions: (a) Fmoc-amino acid, PyBrOP, DIPEA, Ar; (b) TAEA, DCM; (c) TFA, DCM; (d) TFA, triethylsilane, DCM.

mitotic index of the MCF-7 cells (data shown for drug treatment for 12 h). The values obtained ranged from 35% to 57% mitotic cells, defined as those displaying condensed chromosomes, compared with 4% in untreated cultures. This suggests either that there is a markedly enhanced uptake of these amides by the cell or that the amides are reconverted to **1c** by an extracellular or intracellular amidase.

All of the new substances were screened against the bacteria *Stenotrophomonas maltophilia*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, and the fungi *Candida albicans* and *Cryptococcus neoformans* according to established broth microdilution susceptibility assays.[22,23] In broth microdilution assays, glycine amide **3b** exhibited the broadest antimicrobial spectrum, targeting pathogenic yeasts and bacteria.

Conclusions

Present evidence indicates that the 3′-amino counterpart (**1c**) of combretastatin A-2 and derived glycine amide (**3b**) and tyrosine amide (**3f**) correspond to new stilbenes with a very high level of inhibition against a minipanel of cancer cell lines. The potential here was further supported by the potent inhibition of tubulin polymerization exhibited by amine **1c**. Selected members of the corresponding amides (**3a**−**g**), such as **3b** and **3f**, are being further developed.

Experimental Section

Materials and Methods. Ether refers to diethyl ether and Ar to argon gas. All solvents were redistilled, and 3-nitro-4-methoxybenzaldehyde was obtained from Alfa Aesar (Ward Hill, MA). Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), *O*-Boc-N^{α}-Fmoc-L-tryptophan, *O*-*tert*-butyl-N^{α}-Fmoc-L-tyrosine, N^{α}-Fmoc-glycine, and *S*-trityl-N^{α}-Fmoc-L-cysteine were obtained from Calbiochem-Novabiochem Corporation (San Diego, CA). Acetic acid, *N*-butyllithium (2.5 M solution in hexanes), diisopropylethylamine (DIPEA), triethylsilane (TES), and trifluoroacetic acid (TFA) were obtained from Acros Organics (Fisher Scientific, Pittsburgh, PA). All other reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI).

Reactions were monitored by thin-layer chromatography using Analtech silica gel GHLF uniplates visualized under long-wave and short-wave UV irradiation. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Where appropriate, the crude products were separated by column chromatography, flash (230−400 mesh ASTM) or gravity (70−230 mesh ASTM) silica from E. Merck.

Melting points are uncorrected and were determined on an Electrothermal 9100 apparatus. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. The $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The IR spectra were obtained using a Mattson Instruments 2020 Galaxy series FT-IR. The ^{1}H NMR and ^{13}C NMR spectra were recorded employing Varian Gemini 300, Varian Unity 400, and Varian Unity 500 instruments using a deuterated solvent and were referenced either to TMS or the solvent. HRMS data were recorded with a JEOL LCmate mass spectrometer. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-nitrostilbene, *Z* and *E* Isomers (6a and 6b). The phosphonium bromide **4** (20.1 g, 39.6 mmol) was placed in a flame-dried flask under Ar and suspended in THF (300 mL). After being stirred for 45 min at room temperature, the solution was cooled to 0 °C and *n*-butyllithium (15.9 mL, 39.8 mmol) was added. This resulted in the reaction mixture turning deep-red. Stirring continued for 4 h at room temperature. 3-Nitro-4-methoxybenzaldehyde (**5**, 7.20 g, 3.97 mmol) was dissolved in THF (100 mL), and the solution was added dropwise to the reacting mixture via an addition funnel. The solution turned from deep-red to yellow-green. After being stirred 18 h, the reaction mixture was cooled to 0 °C. The reaction was terminated with ethyl acetate, and the solution was filtered and concentrated under vacuum to yield a dark-green oil. The product was separated by gravity column chromatography on silica gel (4:1 hexane/ethyl acetate) and recrystallized (hexane/acetone) to yield the *Z*-stilbene **6a** (4.09 g, 31%) as a yellow-green solid: mp 109−110 °C; R_f = 0.29 (4:1 hexane/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.96 (2H, s, $-CH_2-$), 6.41 (3H, m, vinyl H, 2 × ArH), 6.54 (1H, d, J = 12.4 Hz, vinyl H), 6.94 (1H, d, J = 8.4 Hz, ArH), 7.42 (1H, dd, J = 8.8, 2.0 Hz, ArH), 7.76 (1H, d, J = 2.0 Hz, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 151.7, 148.9, 143.6, 139.6, 134.9, 134.5, 131.1, 130.7, 129.7, 126.6, 126.0, 113.2, 108.5, 102.8, 101.5, 56.5, 56.5; HRMS calcd for $C_{17}H_{16}NO_6$ $[M + H]^+$ 330.0978, found 330.0947. Anal. ($C_{17}H_{15}NO_6$) C, H, N.

The *E*-stilbene **6b** was isolated from the aforementioned column and recrystallized (hexane/acetone) as an orange solid (2.90 g, 22%): mp 165.5−167 °C; R_f = 0.18 (4:1 hexane/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.97 (2H, s, $-CH_2-$), 6.62 (1H, s, ArH), 6.70 (1H, s, ArH), 6.81 (1H, d, J = 16 Hz, vinyl H), 6.90 (1H, d, J = 16 Hz, vinyl H), 7.03 (1H, d, J = 9.0 Hz, ArH), 7.58 (1H, dd, J = 9.0, 2.0 Hz, ArH), 7.92 (1H, d, J = 2.0 Hz, ArH); ^{13}C NMR (500 MHz, $CDCl_3$) δ 152.0, 149.3, 143.6, 139.7, 135.4, 131.7, 131.6, 130.3, 129.2, 124.4, 122.9, 113.7, 107.1, 101.6, 99.8, 56.6, 56.6; HRMS calcd for $C_{17}H_{16}NO_6$ $[M + H]^+$ 330.0978, found 330.0869. Anal. ($C_{17}H_{15}NO_6$) C, H, N.

Photochemical Isomerization of *E*-Stilbene 6b to *Z*-Stilbene (6a). To a stirred solution of the *E*-stilbene **6b** (2.9 g, 8.8 mmol) in benzene (550 mL) was added benzil (9.5 g, 45 mmol, 5.1 equiv). After flushing the reaction flask with Ar, the reaction mixture was stirred overnight. The mixture was then irradiated with a 254 nm UV lamp for 5 h. Upon removal of the benzene in vacuo, the product was separated by gravity column chromatography (4:1 hexane/ethyl acetate) to afford unreacted starting material **6b** (0.92 g, 32%) and the desired *Z*-stilbene **6a** (1.8 g, 62%).

3,4-Methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene (1c). To a stirred solution of nitrostilbene **6a** (1.4 g, 4.3 mmol) in acetic acid (350 mL) was added zinc dust (60 g, <10 μm diameter). After 1.5 h, the solution was filtered under vacuum through Celite and the filtrate was concentrataed under vacuum. The product was separated by flash column chromatography (4:1 hexane/ethyl acetate) and recrystallized (∼9:1 hexane/ethyl acetate) to afford colorless crystals of **1c** (1.0 g, 77%): mp 93.5−94.5 °C; R_f = 0.17 (4:1 hexane/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.25−4.45 (2H, br, NH_2), 5.93 (2H, s, $-CH_2-$), 6.34 (1H, d, J = 12.0 Hz, vinyl H), 6.41 (1H, d, J = 12.0 Hz, vinyl H), 6.48 (1H, s, ArH), 6.51 (1H, s, ArH), 6.68 (2H, m, 2 × ArH), 6.72 (1H, s, ArH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 148.5, 146.6, 143.2, 135.5, 134.1, 132.0, 130.0, 129.6, 129.2, 119.5, 115.4, 110.1, 108.3, 103.0, 101.3, 56.3, 55.4; HRMS calcd for $C_{17}H_{18}NO_4$ $[M + H]^+$ 300.1236, found 330.1250. Anal. ($C_{17}H_{17}NO_4$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-amino-*Z*-stilbene Hydrochloride (1d). To a stirred solution of amine **1c** (40 mg, 0.13 mmol) in ethyl acetate (1 mL) was added ethereal HC1 (1 M) in excess. A white solid immediately formed, and this was collected and washed with ethyl acetate followed by ether to yield a colorless powder **1d** (45 mg, quantitative): mp 179.5−181 °C. Anal. ($C_{17}H_{18}NO_4Cl$) C, H, N.

X-ray Crystal Structure Determination of Amine 1c. A single plate-shaped X-ray sample (∼0.40 mm × 0.10 mm × 0.10 mm) of **1c** was obtained by cleavage from a pale-yellow crystalline cluster grown from a hexane/ethyl acetate solution and was mounted on the tip of a glass fiber. Initial cell constants were calculated from reflections collected from three sets of 60 frames at 298(2) K on a Bruker 6000 diffractometer. Cell parameters indicated a monoclinic space group. Subsequent data collection, using 15 s scans/frame and 0.396° steps in ω, was conducted in such a manner to completely survey a complete hemisphere of reflections. This resulted in >93% coverage of the total reflections possible to a resolution of 0.83 Å. A total of 7211 reflections were harvested from the total data collection, and final cell constants were calculated from a set of 332 strong, unique reflections from these data. Subsequent statistical analysis of the complete reflection data set using the XPREP[25] program indicated that the space group was $P2_1$. Crystal data for $C_{17}H_{17}N_1O_4$: a = 11.5714(3) Å, b = 5.3425(2) Å, c = 12.5632(3) Å, β = 105.605(1)°, V = 748.03(4) Å^3, λ(Cu Kα) = 1.541 78 Å, μ(Cu Kα) = 0.783 mm^{-1}, P_c = 1.329 g cm^{-3} for Z = 2 and M_r = 299.32, F(000) = 316.

After data reduction and merging of equivalent reflections and rejection of systematic absences, 2310 unique observed reflections remained (R_{int} = 0.1867) and these were used in the subsequent structure solution and refinement. An absorption correction was applied to the data with SADBS.[26] Direct methods structure determination and refinement were accomplished with the SHELXTL NT, version V5.10[25] suite of programs. All non-hydrogen atoms for amine **1c** were located using the default settings of that program. Hydrogen atom coordinates were calculated at optimum positions and forced to ride the atom to which they were attached. Anisotropic refinement of the model shown in Figure 1 resulted in a final residual value of 0.0778 for the observed data (0.0878 for all data). The difference Fourier map showed insignificant residual electron density, the largest difference peak and hole being +0.324 and −0.286 e/Å^3, respectively. Final bond distances and angles were all within acceptable limits.

*Unless otherwise noted, the following general procedure was employed for synthesis of the Fmoc-protected amino acid amides of amine **1c**.*

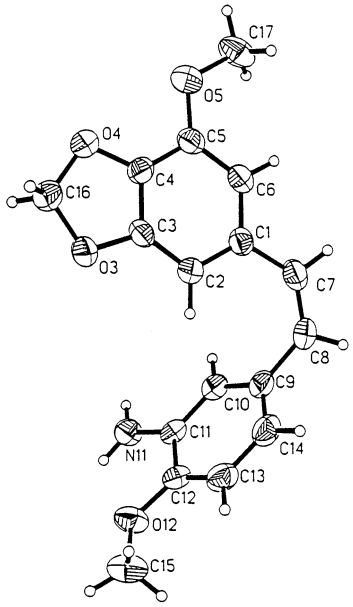


Figure 1. ORTEP molecular structure of amine (**1c**) with the numbering scheme and thermal ellipsoids drawn at the 40% probability level.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*S*-Trt-*N*$^{\alpha}$-Fmoc-L-Cys)amido-*Z*-stilbene (7a). To a stirred mixture of amine **1c** (57 mg, 0.19 mmol), *S*-Trt-*N*$^{\alpha}$-Fmoc-L-Cys (0.15 g, 0.26 mmol, 1.4 equiv), and PyBroP (127 mg, 0.27 mmol, 1.4 equiv) in DCM (1 mL) at 0 °C under Ar was added DIPEA (0.075 mL, 0.43 mmol, 2.3 equiv). The reaction mixture was stirred for 1 h at room temperature. Ethyl acetate was added, and the solvents were removed in vacuo, leaving a white foam. The product was obtained by flash column chromatography (8:1 DCM/ethyl acetate) as a white foam **7a** (0.14 g, 85%): R_f = 0.81 (8:1 DCM/ethyl acetate); $[\alpha]^{22}_D$ −11.5° (*c* 0.87, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.71 (1H, m), 2.80 (1H, m), 3.71 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.93 (1H, m), 4.21 (1H, t, J = 7.0 Hz, Fmoc), 4.38 (2H, d, J = 7.0, Fmoc), 5.88 (2H, s, $-CH_2-$), 6.39 (1H, d, J = 12.0 Hz, vinyl H), 6.44 (1H, s, ArH), 6.44 (1H, d, J = 12.5 Hz, vinyl H), 6.47 (1H, s, ArH), 6.66 (1H, d, J = 9.0 Hz, ArH), 6.98 (1H, dd, J = 8.0, 1.5 Hz, ArH), 7.27 (2H, m, Fmoc), 7.36 (2H, m, Fmoc), 7.58 (2H, d, J = 6.5 Hz, Fmoc), 7.74 (2H, m, Fmoc), 8.23 (1H, d, J = 2.0 Hz, ArH); ^{13}C NMR (500 MHz, $CDCl_3$) δ 167.8, 155.8, 148.5, 147.1, 147.1, 144.3, 143.7, 143.6, 143.3, 141.3, 134.3, 131.8, 130.0, 129.5, 129.3, 129.0, 128.1, 127.9, 127.8, 127.1, 126.9, 125.0, 124.6, 120.8, 120.7, 120.0, 120.0, 109.6, 108.4, 103.0, 101.3, 67.4, 67.1, 56.3, 55.7, 53.4, 47.1, 33.9. Anal. ($C_{54}H_{46}N_2O_7S$) C, H, N.

Synthesis of 3′-L-Cys-amide-*Z*-stilbene **3a** provides the general procedure (except for use of trifluoroacetic acid (TFA) and triethylsilane (TES) with Cys Trt cleavage) for cleavage of the Fmoc-amino acid protecting group

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Cys)amido-*Z*-stilbene (3a). To a stirred solution of 3′-L-Cys-amido-*Z*-stilbene (**7a**, 42 mg, 0.048 mmol) in DCM (1 mL) was added TES (0.5 mL) and TFA (1.5 mL). The reaction mixture was stirred for 20 min, and the solution was concentrated under vacuum. The residue was dissolved in DCM (1 mL), and tris-(2-aminoethyl)amine (TAEA, 0.5 mL) was added. Fifteen minutes later, DCM (10 mL) was added and the mixture was washed with brine (10 mL). The organic solvent was removed in vacuo to yield an oil that was subjected to gravity column chromatography (8:1 DCM/ethyl acetate followed by 9:1 DCM/CH_3OH). Product **3a** was obtained as a colorless foam (5.0 mg, 26%): R_f = 0.87 (9:1 DCM/CH_3OH); $[\alpha]^{28}_D$ −97.3° (*c* 0.44, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (1H, m), 3.36 (1H, m), 3.72 (3H, s, OCH_3), 3.83 (1H, m), 3.86 (3H, s, OCH_3), 5.91 (2H, s, $-CH_2-$), 6.38 (1H, d, J = 12.3 Hz, vinyl H), 6.45 (1H, s, ArH), 6.47 (1H, s, ArH), 6.48 (1H, d, J = 12.6 Hz, vinyl H), 6.72 (1H, d, J = 8.7 Hz, ArH), 6.98 (1H, dd, J = 8.7, 1.8 Hz, ArH), 8.33 (1H, d, J = 1.5 Hz, ArH), 9.90 (1H, s); ^{13}C NMR (500 MHz, $CDCl_3$) δ 170.9, 148.5, 147.6, 143.3, 134.2, 131.9, 130.0, 129.4, 128.8, 127.1, 124.3, 120.4, 109.7, 108.4, 103.0, 101.3, 56.5, 56.3, 55.8, 38.2. Anal. ($C_{20}H_{22}N_2O_5S$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*N*$^{\alpha}$-Fmoc-L-Gly)-amido-*Z*-stilbene (7b). Following the general procedure, to *N*$^{\alpha}$-Fmoc-Gly (82 mg, 0.28 mmol, 1.9 equiv) in DCM (1 mL) at 0 °C under Ar was added DIPEA (0.075 mL, 0.43 mmol, 2.9 equiv). Next, PyBroP (0.123 g, 0.26 mmol, 1.8 equiv) and amine **1c** (44 mg, 0.15 mmol) were added. The product was crystallized from hexanes/ethyl acetate (75 mg, 88%): mp 67−69 °C (dec); R_f = 0.40 (8:1 DCM/ethyl acetate). Anal. ($C_{34}H_{30}N_2O_7$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Gly)amido-*Z*-stilbene (3b). Following the general procedure, amide **7b** (75 mg, 0.13 mmol) in chloroform (6 mL) was treated with TAEA (0.4 mL, 2.7 mmol, 21 equiv). After the reaction mixture was stirred for 15 min, additional TAEA (0.4 mL, 2.7 mmol, 21 equiv) was added. The product was obtained as a colorless oil **3b** (38 mg, 83%): R_f = 0.38 (9:1 DCM/CH_3OH); HRMS calcd for $C_{19}H_{21}N_2O_5$ $[M + H]^+$ 357.1450, found 357.1489. Anal. ($C_{19}H_{20}N_2O_5 \cdot 0.5H_2O$) C, H. N: calcd 7.67; found 8.19.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*N*$^{\alpha}$-Fmoc-L-Phe)-amido-*Z*-stilbene (7c). Amine **1c** (0.16 g, 0.53 mmol) was mixed with *N*$^{\alpha}$-Fmoc-Phe (0.31 g, 0.80 mmol, 1.5 equiv) in DCM (3 mL), DIPEA (0.23 mL, 1.3 mmol, 2.5 equiv), and PyBroP (0.36 g, 0.77 mmol, 1.5 equiv). After 40 min, a white precipitate was collected and recrystallized from DCM/ethyl acetate to afford the product as a colorless solid (**7c**, 270 mg, 75%): mp 85−87 °C; R_f = 0.42 (2:1 hexane/ethyl acetate); $[\alpha]^{25}_D$ −29.4° (*c* 1.00, $CHCl_3$). Anal. ($C_{41}H_{36}N_2O_7 \cdot 0.5H_2O$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Phe)amido-*Z*-stilbene (3c). Cleavage of amide **7c** (0.12 g, 0.18 mmol) was achieved in chloroform (3 mL) and TAEA (0.60 mL, 4.0 mmol, 22 equiv). After 20 min, DCM (12 mL) was added to the reaction mixture and the solvent was successively washed with brine (4 mL) and phosphate buffer (pH 5.5, 2 × 6 mL). The organic phase was concentrated under vacuum, and the residue was subjected to gravity column chromatography (1:1 hexane/DCM followed by 95:5 DCM/CH_3OH) to yield a colorless oil **3c** (74 mg, 94%): R_f = 0.76 (95:5 DCM/CH_3OH); $[\alpha]^{25}$ −67.0° (*c* 1.00, $CHCl_3$). Anal. ($C_{26}H_{26}N_2O_5$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*O*-Bu^t-*N*$^{\alpha}$-Fmoc-L-Ser)amido-*Z*-stilbene (7d). To a stirred mixture of **1c** (0.13 g, 0.43 mmol) and *O*-Bu^t-*N*$^{\alpha}$-Fmoc-Ser (0.25 g, 0.66 mmol, 1.5 equiv) in DCM (2 mL) at 0 °C under Ar was added DIPEA (0.180 mL, 1.0 mml, 2.3 equiv) followed by PyBroP (0.29 g, 0.63 mmol, 1.5 equiv). The mixture was stirred at room temperature for 1 h. It was then washed with aqueous citric acid (10% by weight), dried with magnesium sulfate, and concentrated under vacuum. DCM and ethyl acetate were added to the residue, and the resulting off-white precipitate was collected. The solvents were removed in vacuo, and the residue was subjected to gravity column chromatography (1:1, DCM/ethyl acetate) to yield a pale-yellow oil. The product was precipitated from hexanes/ethyl acetate to afford a colorless powder **7d** (0.21 g, 73%): mp 107.5−109.5 °C; R_f = 0.80 (1:1 DCM/ethyl acetate); $[\alpha]^{24}_D$ −3.4° (*c* 1.01, $CHCl_3$). Anal. ($C_{39}H_{40}N_2O_8$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Ser)amido-*Z*-stilbene (3d). To a stirred solution of **7d** (110 mg, 0.16 mmol) in DCM (1 mL) at 0 °C under Ar was added trifluoracetic acid (1 mL). The mixture immediately turned magenta. After being stirred for 1 h, the solution was concentrated under vacuum. The residue was taken up in DCM (2 mL). TAEA (1 mL) was added, and stirring continued for 10 min. DCM (15 mL) was added, and the solution was washed successively with brine (6 mL) and phosphate buffer (pH 5.5, 15 mL). After back-extracting the phosphate buffer with DCM, the organic extracts were concentrated under vacuum and subjected to gravity column chromatography (1:1 DCM/ethyl acetate followed by 95:5 DCM/CH_3OH) to afford an oil **3d** (25 mg, 41%): R_f = 0.33 (95:5 DCM/CH_3OH); $[\alpha]^{26}_D$ −8.6° (*c* 0.70, $CHCl_3$). Anal. ($C_{20}H_{22}N_2O_6 \cdot 0.5H_2O$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*N*-Boc-N^{α}-Fmoc-L-Trp)amido-*Z*-stilbene (7e). Following the general method, amine **1c** (51 mg, 0.17 mmol), N^{α}-Fmoc-Trp(Boc) (0.13 g, 0.25 mmol, 1.5 equiv), PyBroP (0.12 g, 0.26 mmol, 1.5 equiv) in DCM (1 mL), and DIPEA (0.075 mL, 0.43 mmol, 2.5 equiv) were used to obtain amide **7e** as a colorless solid (0.13 g, 93%, reprecipitated from hexanes/ethyl acetate): mp 118−120 °C; R_f = 0.71 (8:1 DCM/ethyl acetate); $[\alpha]^{22}_D$ −22.8° (*c* 0.65, $CHCl_3$). Anal. ($C_{48}H_{45}N_3O_9$) N. C, H: calcd 71.36, 5.61; found 71.84, 6.03.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Trp)amido-*Z*-stilbene (3e). Amide **7e** (90 mg, 0.11 mmol), DCM (0.2 mL), and TFA (1.8 mL) were mixed and stirred for 1 h at 0 °C and then condensed in vacuo. By use of the general Fmoc deprotection procedure, the residue was taken up in DCM (2 mL), heated with TAEA (1 mL), and stirred for 20 min to yield an oil (**3e**, 11 mg, 20%): R_f = 0.31 (8:1 DCM/ethyl acetate); $[\alpha]^{29}_D$ −87.5° (*c* 0.44, $CHCl_3$); HRMS calcd for $C_{28}H_{28}N_3O_5$ $[M + H]^+$ 486.2029, found 486.2008. Anal. ($C_{28}H_{27}N_3O_5 \cdot H_2O$) H, N. C: calcd 66.79; found 66.31.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(*O*-Bu^t-N^{α}-Fmoc-L-Tyr)amido-*Z*-stilbene (7f). Amine **1c** (0.13 g, 0.43 mmol), *O*-Bu^t-N^{α}-Fmoc-Tyr (0.29 g, 0.63 mmol, 1.5 equiv), DCM (2 mL), DIPEA (0.180 mL, 1.0 mmol, 2.3 equiv), and PyBroP (0.29 g, 0.63 mmol, 1.5 equiv) were mixed to yield stilbene **7f**. The product was isolated by gravity column chromatography (1:1 DCM/ethyl acetate) as a slightly yellow oil. Stilbene **7f** was then precipitated from hexanes/ethyl acetate as a colorless powder (**7f**, 0.29 g, 91%): mp 95−97 °C; R_f = 0.78 (1:1 DCM/ethyl acetate); $[\alpha]^{24}_D$ −9.2° (*c* 1.15, $CHCl_3$). Anal. ($C_{45}H_{44}N_2O_8$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Tyr)amido-*Z*-stilbene (3f). To a stirred solution of **7f** (0.63 g, 0.85 mmol) in DCM (4 mL) was added TFA (4 mL). The mixture was stirred for 20 min. Solvent was removed under vacuum, and the mixture was subjected to gravity column chromatography (4:1 DCM/ethyl acetate). The phenol obtained was dissolved in DCM (4.5 mL) to which TAEA (2.7 mL) was added. The mixture was stirred for 20 min, washed with brine, and dried with magnesium sulfate. Solvent was removed under vacuum, and the product was purified by gravity column chromatography (4:1 DCM/ethyl acetate followed by 9:1 DCM/CH_3OH). The product was obtained as a colorless oil (**3f**, 0.35 g, 90%): R_f = 0.65 (9:1 DCM/CH_3OH); $[\alpha]^{24}_D$ −94.9° (*c* 1.02, CH_2Cl_2). Anal. ($C_{26}H_{26}N_2O_6 \cdot H_2O$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(N^{α}-Fmoc-L-Val)-amido-*Z*-stilbene (7g). To a stirred mixture of amine **1c** (49 mg, 0.16 mmol), N^{α}-Fmoc-Val (93 mg, 0.27 mmol, 1.7 equiv), and PyBroP (114 mg, 0.25 mmol, 1.5 equiv) in DCM (1 mL at 0 °C under Ar) was added DIPEA (0.075 mL, 0.43 mmol, 2.6 equiv). The mixture was stirred for 1 h at room temperature, and the solvent was removed under vacuum to yield an oil. The product was precipitated from ether and collected as a colorless solid **7g** (93 mg, 91%): mp 203−204.5 °C; R_f = 0.28 (2:1 *n*-hexane/ethyl acetate); $[\alpha]^{23}$ −36.6° (*c* 1.01, $CHCl_3$). Anal. ($C_{37}H_{36}N_2O_7 \cdot H_2O$) C, H, N.

3,4-Methylenedioxy-5,4′-dimethoxy-3′-(L-Val)amido-*Z*-stilbene (3g). Following the general method, amide **7g** (93 mg, 0.15 mmol) in DCM (5 mL) and TAEA (0.75 mL, 5.0 mmol, 33 equiv) were mixed to yield an oil, which was subjected to gravity column chromatography (4:1 DCM/ethyl acetate). The product was obtained as a colorless oil (**3g**, 59 mg, 98%): R_f = 0.26 (4:1 DCM/ethyl acetate); $[\alpha]^{25}$ −39.5° (*c* 0.43, $CHCl_3$). Anal. ($C_{22}H_{26}N_2O_5 \cdot 0.5\ H_2O$) C, H, N.

Assays for evaluation of tubulin polymerization and the binding of [^{3}H]colchicine to tubulin were performed as described previously.[24] The mitotic index of drug-treated MCF-7 cells was obtained by examining cells grown on Lab-Tek II chamber slides obtained from Nalge Nunc International. The cells were maintained at 37 °C and 5% CO_2 in RPMI medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cells were seeded at 5000/chamber the day before exposure to drugs and grown an additional 12 h (final dimethyl sulfoxide concentration, 1%). The cells were fixed in a solution containing 8% formaldehyde, 50 mM 1,4-piperazineethanesulfonate (pH 6.9 with NaOH), 5 mM $MgC1_2$, and 5% dimethyl sulfoxide for 45 min. The slide was washed twice with phosphate-buffered saline (pH 7.4), and the DNA was fluorescently labeled with 2.5 μM 4′,6-diamidino-2-phenylindole. Coverslips were mounted on the Citifluor AF1 antifade agent obtained from Marivac, Ltd. The slides were examined with a Nikon E800 epifluorescence microscope equipped with an appropriate filter, and cells with condensed chromosomes were quantitated.

Acknowledgment. We are pleased to thank the following for financial assistance: Division of Cancer Treatment, National Cancer Institute, DHHS, for awarding an Outstanding Investigator Grant CA44344-05-12; the Arizona Disease Control Research Commission; the Caitlin Robb Foundation; Diane Cummings Halle; Rod J. and Hazel V. McMullin; Gary L. and Diane Tooker; Lottie Flugel; Polly J. Trautman; John and Edit Reyno; Dr. John C. Budzinski; the Ladies Auxiliary to the Veterans of Foreign Wars; and the Robert B. Dalton Endowment Fund. In addition, we thank Dr. Gerald Boland for conducting some preliminary experiments. Other very helpful assistance was provided by Drs. Fiona Hogan, John C. Knight, Jean M. Schmidt, Jean-Charles Chapuis, Ms. Bridget Fakoury, and Mr. Michael J. Dodson. This work was supported in part by NCI, National Institutes of Health Contract NO1-CO-1240.

Supporting Information Available: X-ray supporting material for amine **1c**, murine P388 lymphocytic leukemia cell line ED_{50} (μg/mL) evaluation of N^{α}-Fmoc-amino acid amides **7a**−**g**, and antimicrobial activities of protected amides and related compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM020204L