2-Amino and 2′-Aminocombretastatin Derivatives as Potent Antimitotic Agents

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A novel series of 2-amino and 2′-aminocombretastatin derivatives were synthesized and evaluated for antitumor activity. Several compounds had excellent antiproliferative activity as inhibitors of tubulin polymerization. Compounds **11**, **20**, and **21** with IC_{50} values of 1.6, 1.7, and 1.8 μM, respectively, exhibited more potent inhibition of tubulin polymerization than colchicine and approximately as active as combretastatin A-4. They also displayed antiproliferative activity with an IC_{50} values ranging from 11 to 44 nM in a variety of human cell lines from different organs. Structure activity relationship information suggests that the NH_2 substituent at the 2-position of either ring A or ring B in combretastatin molecular skeleton may play an important role in the bioactivity of this series of compounds.

Introduction

Microtubules are dynamic structures that play a crucial role in cellular division and are recognized as an important target for anticancer therapy.[1] A number of naturally occurring compounds, such as paclitaxel, epothilone A, vinblastine, combretastatin A-4, dolastatin 10, and colchicine, all exhibit their anticancer properties by interfering with the dynamics of tubulin polymerization and depolymerization, resulting in mitotic arrest.[2] Reports that drugs with binding to the colchicine domain are undergoing intensive investigation as vascular disrupting agents for cancer therapy.[3−5] For example, antitubulin agents, **3**, **5**, and **10** act as vascular-disrupting agents, rapidly depolymerizing microtubules of newly formed vasculature to shut down the blood supply to tumors. They are now undergoing human clinical trials for either single use or combination use with chemotherapy drugs[6−8] (Figure 1).

The analysis of the structures of combretastatin A-4 and its derivatives shows that the polar functional group(s) is often located on the B ring,[9] for example, **4**[10] (3′-hydroxyl group), **6**[11] (2′, 3′-dihydroxyl group), and **9**[12] (3′-amino group). So, we synthesized the 2′-aminocombretastatin derivatives by introducing an amino group at the C-2 position on the B-ring of *Z*-stilbenes and then evaluated their bioactivity. Literature reports of SAR studies of combretastatin's ring A indicate that these derivatives mimic combretastatin A-3 by replacement of 3-hydroxyl group on ring A with halides[13] or simulate the resveratrol skeleton with a 3,5-dimethoxyphenyl group on the ring A[14,15] instead of 3,4,5-trimethoxyphenyl group. Because combretastatin compounds have a problem with aqueous solubility, the prodrug approach has been applied for drug candidates, such as **5**, **7**, and **10** utilizing phosphate and amino acids served as promoiety to achieve in vivo antitumor activity.[16−19] Therefore, we also modified the A-ring of *Z*-stilbene by the introduction of 2-amino group, synthesized 2-aminocombretastatins, in an attempt to increase the corresponding structure's polarity without compromising the activity. We herein describe the synthesis and structure−activity relationships of 2-amino and 2′-aminocombretastatins as potent antimitotic agents in continuation of our search for promising anticancer agents (Figure 2).

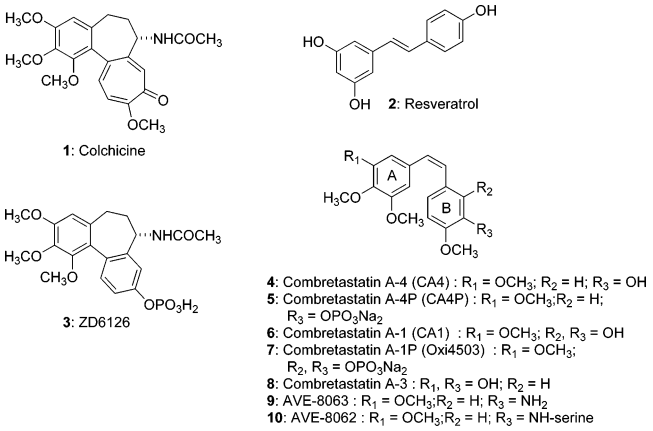

Figure 1.

Results and Discussion

Chemistry. The general methods for the synthesis of 2′-aminocombretastatins and 2-aminocombretastatins are shown in Scheme 1 and Schemes 2 and 3, respectively. The preparation involved a reaction sequence (overall 30−46% yield in two or three steps): (1) Wittig reaction of (3,4,5-trimethoxybenzyl)-phosphonium chloride (Scheme 1), (4-methoxybenzyl)phosphonium bromide (Scheme 2), and (2-nitro-3,4,5-trimethoxybenzyl)phosphonium bromide (Scheme 3) with various substituted benzaldehydes including 2-nitro or 3-nitrobenzaldehydes yielded the corresponding *Z*- and *E*-stilbenes as a ratio of about 3/1. (2) Reduction of the nitro group of *Z*-stilbenes by Zn/AcOH to afford the desired substituted 2-amino and 2′-aminocombretastatins derivatives. Ylide **31** was synthesized from the 2-nitro-3,4,5-trimethoxybenzyl bromide (**30**). The methoxy-substituted benzaldehydes **26**−**29** and **32** are commercially available. The 2-nitrobenzaldehydes **22**, **23**, **24**, **25**, and 3-(*tert*-butyldimethylsilyl)-protected isovanillin **33** were prepared in two-four steps.

Biological Evaluation. (A) In Vitro Cell Growth Inhibitory Activity. The synthesized *Z*-stilbenes **11**−**21** were evaluated for their antiproliferative activities against five types of human cancer cell lines, oral epidermoid carcinoma KB cells, colorectal

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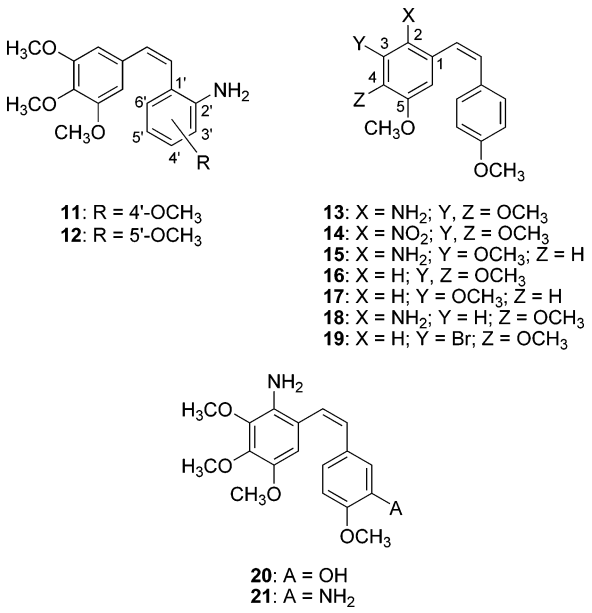


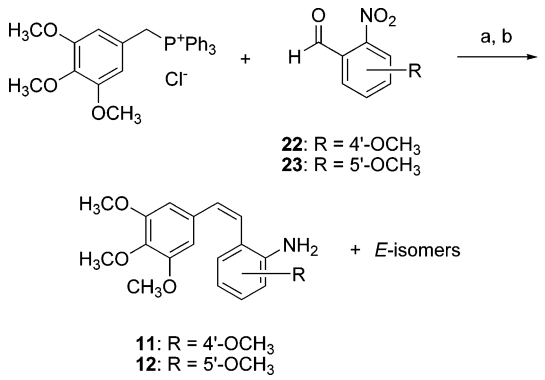
Figure 2.

carcinoma HT29 cells, nonsmall cell lung carcinoma H460 cells, and two stomach carcinoma TSGH, MKN45 cells, as well as one type of MDR-positive cell line: KB-VIN10 cells, overexpressed P-gp 170/MDR (Table 1).

First, we evaluated the cytotoxic effect of an amino group at the C-2′ position of *Z*-stilbenes. Synthesized combretastatin A-4 derivative **11**[20,21] with an amino group at C-2′ position on the B-ring exhibited potent cell growth inhibitory activity with a mean IC_{50} of 17 nM against six human cell lines. Changing the position of the methoxy group on the B ring from C-4 to C-5, as in compound **12**, resulted in a drastic loss in activity. This revealed that the position effect of methoxy substitutions at the pivotal C-4′ position of the B-ring in combretastatins significantly influences their antiproliferative activities. This is consistent with observations in the literature reports[14] on other CA-4 derivatives, which suggest that the methoxy group of the B-ring in the CA-4 family is located at the C-4′ site for maximal activity.

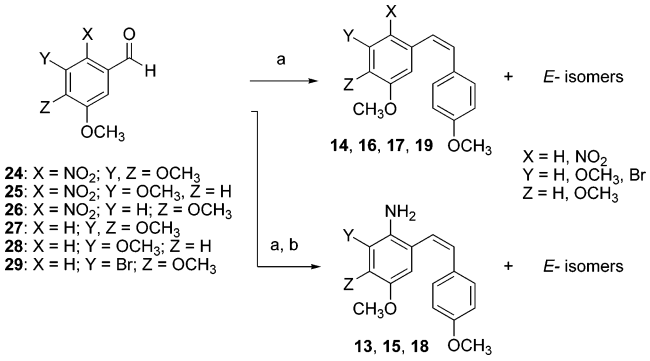
Demonstration of strong cytotoxic activity by analogues with the amino substitution at C-2′ position of *Z*-stilbenes (2′-aminocombretastatins) sparked us to construct the 2-aminocombretastatins series by introducing an amino group at the C-2 position on the A-ring. The compound **13**, namely, 2-amino-3,4,4′,5-tetramethoxy-*Z*-stilbenes, exhibited cytotoxicity activity against six cancer cell lines, with an average IC_{50} of 44 nM. An investigation of the electronic effects on the A-ring of *Z*-stilbenes by introduction of an electron-withdrawing nitro group at C-2 position (e.g., compound **14**) resulted in a significant loss of activities to micromolar range in the cell growth inhibitory assay for six human cancer cell lines. The replacement of 2-amino-3,4,5-trimethoxyphenyl moiety on the A-ring by a 2-amino-3,5-dimethoxyphenyl group (**15**) resulted in a slight decrease in cytotoxicity against several lines in comparison to compound **13** by a mean IC_{50} of 133 nM. A comparison of the substituent effect of the 2-amino group in the A-ring of *Z*-stilbenes (**13** vs **16**,[22] **15** vs **17**[14]) indicated that the 2-amino group was more tolerated in 3,5-dimethoxy-4′-methoxy-*Z*-stilbenes than in 3,4,5-trimethoxy-4′-methoxy-*Z*-stilbenes. The C3-methoxy group on A-ring in combretastatins skeleton is thought to play an important role in potency.[14] The removal of C3-methoxy from compound **13**, to synthesize compound **18**, resulted in complete loss in activity. Replacement

Scheme 1[a]



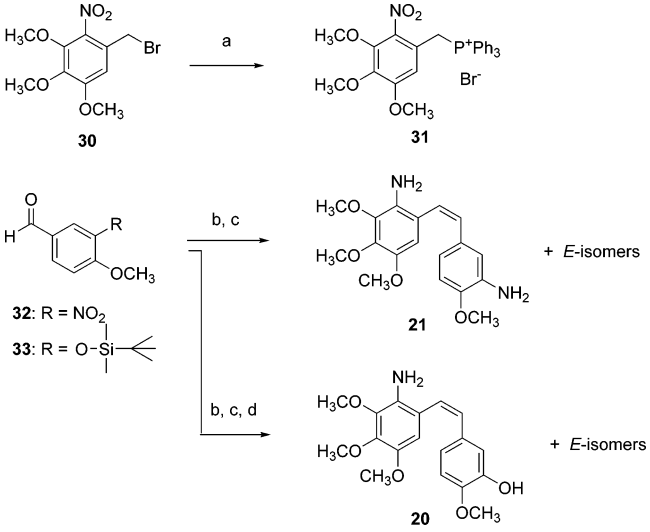
[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) Zn, AcOH, rt.

Scheme 2[a]



[a] Reagents and conditions: (a) 4-methoxybenzyl-triphenylphosphonium bromide, *n*-BuLi, THF, −78 °C; (b) Zn, AcOH, rt.

Scheme 3[a]



[a] Reagents and conditions: (a) PPh_3, toluene, reflux; (b) **31**, *n*-BuLi, THF, −78 °C; (c) Zn, AcOH, rt.; (d) tetra-*n*-butylammonium fluoride, THF, rt.

of the C3-methoxy group of compound **16** to give 3-bromo-4,4′,5-triimethoxy-*Z*-stilbenes (**19**), however, resulted in a substantial activity in the cell growth inhibitory assay with a mean IC_{50} of 76 nM against six cancer cell lines. This observation is in agreement with other reports of 3-halo-*Z*-stilbenes combretastatin derivatives, indicating that the C3-methoxy group of combretastatins could be replaced by the halogen substituents.[13] As the addition of a C2-amino group to the A ring could apparently retain the cellular growth inhibitory

Table 1. IC_{50} Values (nM ± SD[a]) of Compounds **4**, **9**, and 2-Amino and 2′-Aminocombretastatins (**11**−**21**)

	cell type (IC_{50} nM ± SD)[a]					
compd	KB	KB-vin10	H460	HT29	TSGH	MKN45
4	1.5 ± 0.4	1.5 ± 0.4	1.9 ± 0.1	54 ± 7	38 ± 13	52 ± 8
9	7 ± 1.6	4.8 ± 0.4	9 ± 2.5	5 ± 0	7 ± 4.5	5.1 ± 0.1
11	14 ± 2.1	14 ± 1.4	19 ± 0.7	20 ± 0.7	19.5 ± 2.1	11 ± 0.1
12	>10 000	8000 ± 850	>10 000	>10 000	>10 000	6300 ± 245
13	50 ± 2	35 ± 4	54 ± 1	33 ± 6	55 ± 1	34 ± 3
14	4300 ± 128	4100 ± 78	4900 ± 210	4200 ± 680	5300 ± 396	3800 ± 265
15	154 ± 35	96 ± 15	156 ± 29	125 ± 19	186 ± 41	82 ± 11
16[22]	16 ± 4.2	11.9 ± 1.2	18.8 ± 0.3	13 ± 1.6	19.9 ± 1.2	16.6 ± 6.4
17[14]	280 ± 66	223 ± 58	330 ± 74	253 ± 51	324 ± 119	218 ± 4
18	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
19	74 ± 25	68 ± 8	94 ± 6	70 ± 29	83 ± 32	72 ± 33
20	27 ± 3	20 ± 1	37 ±1	36 ± 5	39 ± 13	19 ± 4
21	44 ± 1	30 ± 4	41 ± 6	40 ± 2	43 ± 3	30 ± 3

[a] SD: standard deviation. All experiments were independently performed at least three times.

Table 2. Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds **11**−**21**, Colchicine, and Combretastatin A-4

compd	tubulin[a] IC_{50} ± SD (μM)	colchicine binding[b] (% ± SD)
11	1.6 ± 0.2	87 ± 3
12	>10	19 ± 7
13	2.1 ± 0.3	75 ± 6
14	>10	23 ± 5
15	2.4 ± 0.3	84 ± 2
16	1.5 ± 0.2	89 ± 3
17	3.5 ± 0.4	82 ± 3
18	>10	17 ± 5
19	2.1 ± 0.2	86 ± 2
20	1.7 ± 0.2	83 ± 4
21	1.8 ± 0.1	80 ± 4
colchicine	3.2 ± 0.3	
combretastatin A-4	1.4 ± 0.2	92 ± 2

[a] Inhibition of tubulin polymerization. [b] Inhibition of [^{3}H]colchicine binding. Tubulin was at 1 μM; both [^{3}H]colchicine and inhibitor were at 5 μM.

activity, we further studied the C2-amino-substituted derivatives of combretastatin A-4 and **9**, compounds **20** and **21**, respectively. 2-Aminocombretastatins, namely, 2-amino-3′-hydroxy-3,4,4′,5-tetramethoxy-*Z*-stilbenes (**20**) and 2,3′-diamino-3,4,4′,5-tetramethoxy-*Z*-stilbenes (**21**), showed potent antiproliferative activity by a mean IC_{50} of 29 and 38 nM, respectively, in all six human cancer cell lines.

A comparison between cellular growth inhibitory activity of **9**, **11**, **13**, **15**, **20**, **21**, and combretastatin A-4 revealed that the introduction of an amino group at the C-2 or C-3 position on the B-ring and at the C-2 position on the A-ring is beneficial for potency. Hence, it may be assumed that an amino group located at the C-2 position, either on the A ring or B ring, and the C-3 position on the B ring, seems to perform in a requisite role of the inhibition of cellular growth. Furthermore, newly synthesized 2-aminocombretastatins **13**, **15**, **20**−**21**, and 2′-aminocombretastatins **11** overcome MDR-positive resistant cell line (KB-VIN10), indicating 2-amino and 2′-aminocombretastatin derivatives are not substrate for efflux pump.

(B) Inhibition of Tubulin Polymerization and Colchicine Binding Activity. To investigate whether the activities of these 2-amino and 2′-amino-*Z*-stilbenes compounds were related to interactions with microtubulin system, all compounds **11**−**21** and reference compounds (colchicine and combretastatin A-4) were evaluated for in vitro tubulin polymerization inhibitory activities and colchicine binding activities (Table 2). The results showed that compound cytotoxicities correlated with the inhibition of tubulin polymerization and colchicine binding affinity. As shown in Table 2, **11**, **20**, and **21** were effective in inhibiting tubulin assembly, with IC_{50} of 1.6, 1.7, and 1.8 μM, respectively. These values were slightly inferior or comparable to combretastatin A-4 (IC_{50} = 1.4 μM) and superior to the IC_{50} values of 3.2 μM for colchicine. In the colchicine binding assay, 2-amino and 2′-amino-*Z*-stilbenes derivatives were bound to the colchicine binding site, which suggested that 2-amino and 2′-aminocombretastatins displayed antiproliferative activity resulting from effective inhibition of tubulin polymerization at the colchicine binding site.

Conclusion

We have synthesized a series of 2-amino and 2′-aminocombretastatins, which compounds **12**, **13**−**15**, and **18**−**21** are new combretastatin derivatives. The synthesized 2-amino and 2′-aminocombretastatins, compounds **11**, **13**, **20**, and **21**, are potent cytotoxic agents and inhibitors of tubulin polymerization through the colchicine binding site on microtubules. The compounds **11**, **13**, **20**, and **21** display antiproliferative activity, with IC_{50} values ranging from 11 to 55 nM in a variety of human cell lines from different organs. They also showed comparable or similar antitubulin activities (IC_{50} = 1.6, 2.1, 1.7, and 1.8 μM, respectively) to combretastatin A-4 and colchicine (IC_{50} = 1.4 and 3.2 μM, respectively). SAR data indicated that the NH_2 substituent at position 2 of ring A or ring B in combretastatin moieties apparently plays an important role in the activity of this series of compounds. The introduction of a polar amino group in the C2-position of combretastatin A-4 and **9** gave compound **20** and **21**, respectively, which resulted in a substantial activity. This information can be applied to other related combretastatin and colchicine analogue modifications for increasing their polarity without compromising activity.

Experimental Section

General Procedure for the Preparation of Substituted 2-Amino and 2′-Aminocombretastatins Derivatives (11−13, 15, 18, 20−21). 2′-Amino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (11). *n*-BuLi (1.6 M in hexane, 3.5 mL) was added dropwise to a well-stirred suspension of 3,4,5-trimethoxybenzyltriphenylphosphonium chloride (1.32 g, 2.76 mmol) in THF (30 mL) at −78 °C. The stirring was continued at −78 °C for 30 min and at room temperature for 1 h. The reaction mixture was recooled to −78 °C and then was added to a solution of 4-methoxy-2-nitrobenzaldehyde (**22**) in THF (16 mL) by an addition funnel. The stirring was continued at −78 °C for 1 h and at room temperature for 18 h. Ice water (30 mL) and ethyl acetate (10 mL) were added to the mixture. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (15 mL × 2) and methylene chloride (15 mL × 2). The combined organic layers were dried and evaporated to give a residue, which was further treated with Zn (9 g, 0.138 mol) in AcOH (50 mL) at room temperature for 1 h. The reaction mixture was filtrated and then evaporated to give a residue that was purified by

silica gel flash column chromatography (ethyl acetate/*n*-hexane = 1:2) to afford **11** as a pale yellow solid, yield 41%; mp 94.0−94.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 6H), 3.74 (s, 3H), 3.79 (s, 3H,), 6.25 (d, J = 2.4 Hz, 1H), 6.30 (dd, J = 8.4, 2.4 Hz, 1H), 6.42 (d, J = 12 Hz, 1H), 6.49 (d, J = 14.8 Hz, 3H), 7.03 (d, J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.1, 55.7, 60.7, 100.6, 104.2, 105.7, 115.985, 125.6, 130.5, 130.8, 132.2, 137.2, 144.8, 152.6, 160.0. MS (EI) *m*/*z* 315 (M^+, 90%), 300 (100%). HRMS (EI) calcd for $C_{18}H_{21}NO_4$ (M^+), 315.1471; found, 315.1471. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

2-Amino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (13). The title compound was obtained in 39% overall yield from (4-methoxybenzyl)-triphenylphosphonium bromide and 2-nitro-3,4,5-trimethoxybenzaldehyde (**24**). ^{1}H NMR (500 MHz, CD_3OD) δ 3.52 (s, 3H), 3.73 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.35 (d, J = 11.9 Hz, 1H), 6.45 (s, 1H), 6.55 (d, J = 12.0 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 55.6, 56.9, 60.8, 61.3, 110.3, 114.5, 120.0, 125.0, 130.8, 131.2, 131.4, 133.6, 142.8, 143.4, 146.6, 160.4. MS (EI) *m*/*z* 315 (M^+, 100%), 300 (58%). HRMS (EI) calcd for $C_{18}H_{21}NO_4$ (M^+), 315.1469; found, 315.1470. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

2-Amino-3,4′,5-trimethoxy-(*Z*)-stilbene (15). The title compound was obtained in 40% overall yield from (4-methoxybenzyl)-triphenylphosphonium bromide and 3,5-dimethoxy-2-nitrobenzaldehyde (**25**); mp 83.2−86.3 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.63 (s, 3H), 3.75 (s, 3H), 3.83 (s, 3H), 6.31 (d, J = 2.3 Hz, 1H), 6.39 (s, 1H), 6.41 (d, J = 12.9 Hz, 1H), 6.58 (d, J = 12.0 Hz, 1H), 6.73 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.0, 55.5, 98.4, 103.9, 113.4, 123.5, 124.2, 127.2, 129.2, 130.0, 130.8, 148.4, 152.1, 158.8. MS (EI) *m*/*z* 285 (M^+, 100%), 270 (29%). HRMS (EI) calcd for $C_{17}H_{19}NO_3$ (M^+), 285.1369; found, 285.1367. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

2,3′-Diamino-3,4,4′,5-tetramethoxy-(*Z*)-stilbene (21). The title compound was obtained in 34% overall yield from 2-nitro-3,4,5-(trimethoxybenzyl)triphenylphosphonium bromide (**31**) and 4-methoxy-3-nitrobenzaldehyde (**32**); mp 97.3−98.1 °C. ^{1}H NMR (500 MHz, acetone-d_6) δ 3.54 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 4.01 (s, 2H), 4.25 (s, 2H), 6.26 (d, J = 12.1 Hz), 6.39 (d, J = 12.1 Hz, 1H), 6.53 (s, 1H), 6.56 (dd, J = 8.2, 1.7 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 6.68 (d, J = 1.8 Hz, 1H). ^{13}C NMR (125 MHz, acetone-d_6) δ 56.1, 57.1, 61.0, 61.4, 110.5, 111.2, 117.0, 120.4, 120.9, 124.9, 131.3, 132.1, 133.6, 137.3, 143.0, 143.5, 146.8, 148.8. MS (EI) *m*/*z* 330 (M^+, 100%), 315 (27%). HRMS (EI) calcd for $C_{18}H_{22}N_2O_4$ (M^+), 330.1578; found, 330.1570. Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

2-Amino-3′-hydroxy-3,4,4′,5- tetramethoxy-(*Z*)-stilbene (20). The title compound was obtained in 30% overall yield from 2-nitro-(3,4,5-trimethoxybenzyl)triphenylphosphonium bromide (**31**) and 3-(*tert*-butyldimethylsilyloxy)-4-methoxybenzaldehyde (**33**) according to the above procedure and one extra procedure, which was 3 equiv of tetra-*n*-butylammonium fluoride/THF at room temperature, stirring for 1 h. ^{1}H NMR (500 MHz, CD_3OD) δ 3.55 (s, 3H), 3.79 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.33 (d, J = 12 Hz, 1H), 6.49 (s, 1H, H-6), 6.50 (d, J = 11.9 Hz, 1H), 6.71 (dd, J = 8.2, 1.9 Hz, 1H), 6.74 (d, J = 1.8 Hz, 1H), 6.77 (d, J = 8.2 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 56.3, 57.0, 60.9, 61.3, 110.4, 112.3, 116.5, 120.0, 122.0, 125.1, 131.4, 131.5, 133.5, 142.9, 143.4, 146.7, 147.1, 148.5. MS (EI) *m*/*z* 331 (M^+, 100%), 284 (25%). HRMS (EI) calcd for $C_{18}H_{21}NO_5$ (M^+), 331.1422; found, 331.1421. Anal. ($C_{18}H_{21}NO_5$) C, H, N.

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Supporting Information Available: Spectral data of compounds **12**, **14**, **16**−**19**, **22**−**25**, **30**, **31**, **33** and experimental procedures for biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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