DOI: 10.1002/cmdc.200600068

Synthesis and Biological Evaluation of Boronic Acid Containing *cis*-Stilbenes as Apoptotic Tubulin Polymerization Inhibitors

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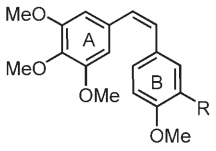
A series of boronic acid containing cis*-stilbenes as potent inhibitors of tubulin polymerization was synthesized by the introduction of boronic acid as an acceptor-type functional group into the aromatic ring B of the combretastatin framework. High cell-growth inhibition was observed with boron compounds* ***13c*** *and* ***13d****, in which a hydroxy group on the aromatic ring B of combretastatin A-4 was replaced with boronic acid; IC_{50} values toward B-16 and 1-87 cell lines are 0.48–2.1 μM. Compounds* ***13c*** *and* ***13d*** *exhibited significant inhibitory activity toward tubulin polymerization (IC_{50} = 21–22 μM). The carboxylic acid derivative* ***17****, which can be considered as a mimic of boronic acid* ***13c****, did not show significant inhibition of cell growth or tubulin polymerization. According to the FACScan analysis using Jurkat cells, apoptosis was induced after incubation for 8 h with* ***13c*** *at a concentration of $> 10^{-8}$ M. Growth inhibitory experiments against a panel of 39 human cancer cell lines revealed* ***13c*** *to inhibit growth differently than combretastatin A-4; the correlation coefficient (r) between the two compounds was 0.553 in the COMPARE analysis.*

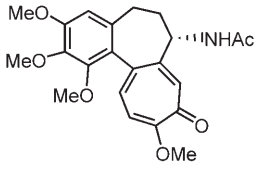
Introduction

Combretastatins isolated from the bark of the South African tree *Combretum caffrum* by Pettit and co-workers[1] are mitotic agents. In particular, combretastatin A-4 has been found to be a potent inhibitor of tubulin polymerization and a cytotoxic agent against a wide variety of human cancer cell lines, including multidrug-resistant cancer cell lines. Therefore, it is an attractive lead compound for the development of anticancer drugs.[2] Combretastatins consist of a *cis*-stilbene skeleton, and the three methoxy groups on the aromatic ring A are thought to interact with the colchicine binding site of β-tubulin.[3] Various combretastatin and colchicine derivatives have been developed. CA-4P[4] and ZD6126[5] are water-soluble sodium phosphate prodrugs of combretastatin A-4 and allocolchinol, respectively, and are currently in phase I/II clinical trials.[6] AC-7739[7] contains the HCl salt of the amine instead of the C3 hydroxy group in the aromatic ring B of combretastatin A-4, and AC-7700 (AVE-8062) is the L-serine amide HCl salt.[8] Not only have the *cis*-stilbene-type compounds[9] been synthesized as analogues of combretastatin A-4 but also the pseudo *cis*-oriented analogues such as dioxolanes,[10] heterocycles,[11] and benzophenone derivatives including phenstatins and 2-aminobenzophenones.[12] Combretastatins and these analogues have a donor-type functional group substituted on the aromatic ring B. We are interested in introducing boronic acid as an acceptor-type functional group into the aromatic ring B in the combretastatin framework.

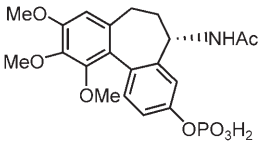
The use of boron atoms in pharmaceutical drug design possesses a high potential for discovery of new biological activity.[13,14] A boron atom has a vacant orbital and interconverts



1a: R = OH, combretastatin A-4
1b: R = OPO_3Na_2, CA-4P
1c: R = $NH_3^+Cl^-$, AC-7739
1d: R = NH-Ser HCl salt, AC-7700



colchicine



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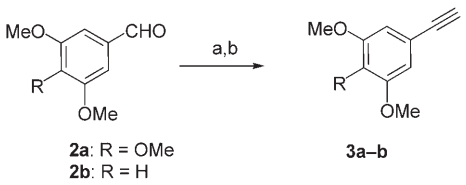
with ease between the neutral sp^2 and the anionic sp^3 hybridization states which generates a new stable interaction between a boron atom and a donor molecule through a covalent bond.[15] Therefore, it is expected that the boron atoms introduced into biologically active molecular frameworks would interact with a target protein not only through hydrogen bonds but also through covalent bonds, and this interaction would produce potent biological activity.[16] Among various boron compounds synthesized, much attention has been paid to boronic acid containing peptides.[17] In these boropeptides, a carboxylic acid has been replaced by a boronic acid group.

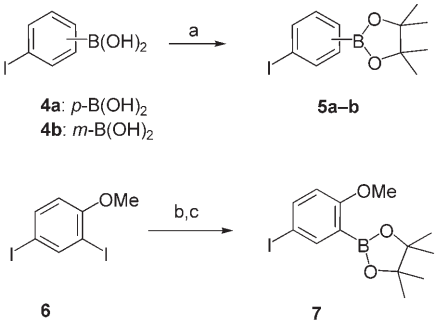
Our strategy for the design of boron compounds is based on the properties that make them different from conventional biologically active compounds.[18] Herein, we report the synthesis and biological evaluation of boronic acid containing combretastatin analogues.

Chemistry

Although there are a few examples of the synthesis of *cis*-stilbene compounds through the Horner–Wittig reaction[9a,c] and Suzuki–Miyaura coupling,[19] we designed the synthesis of boronic acid containing *cis*-stilbene analogues from phenylacetylenes and iodophenylboronic acids through a Sonogashira coupling reaction followed by *cis* reduction.[20] The phenylacetylenes **3a** and **3b** were synthesized from the corresponding aldehydes **2a** and **2b** through a Corey–Fuchs reaction as shown in Scheme 1.[21]



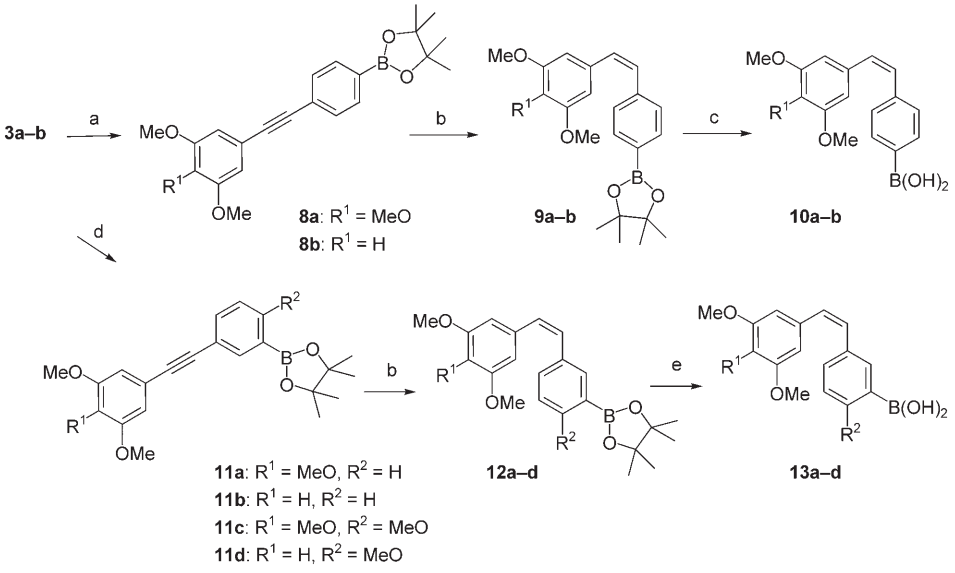
Scheme 1. Reagents and conditions: a) PPh_3, CH_2Br_2, 0 °C, 82–98%; b) *n*BuLi (2 equiv), −78 °C 1 h, then 20 °C, 83–88%.



Scheme 2. Reagents and conditions: a) pinacol, CH_2Cl_2, RT, quant; b) $B(OiPr)_3$, *n*BuLi, −78 °C, then HCl, 78%; c) pinacol, CH_2Cl_2, RT, quant.

Synthesis of iodophenylboronic acid pinacol esters **5a,** 5**b**, and **7** is shown in Scheme 2. The reactions of 4-iodophenylboronic acids **4a** and 3-iodophenylboronic acid **4b** with pinacol proceeded smoothly in ether to give the corresponding esters **5a** and **5b** quantitatively. 5-Iodo-2-methoxyphenylboronic acid pinacol ester **7** was synthesized from 2,4-diiodoanisole **6**. Treatment of **7** with *n*BuLi and isopropylborate at −78 °C, followed by protection with pinacol afforded 5-iodo-2-methoxyboronic acid pinacol ester **7** in 73% yield in two steps.[22]

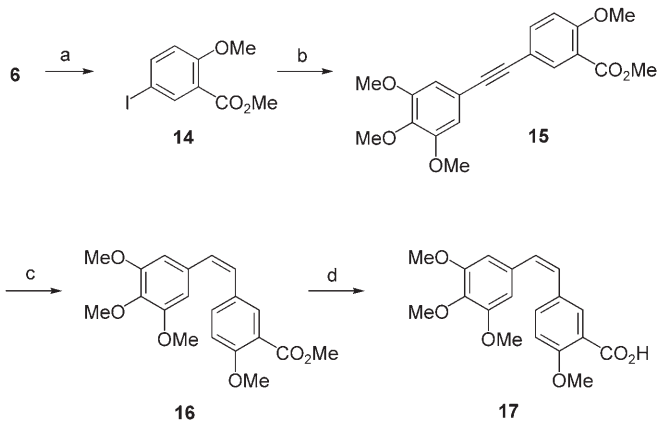
We next examined Sonogashira coupling reactions between the acetylenes **3a** and **3b**, and the iodide **5a** (Scheme 3). The reaction of the acetylene **3a** with the iodide **5a** proceeded in the presence of $[Pd(PPh_3)_4]$ and CuI catalysts in CH_3CN at 70 °C, to give the corresponding alkyne **8a** in 70% yield. *cis* Reduction of the alkyne **8a** was accomplished by using dicyclohexylborane in THF to give the *cis*-stilbene **9a** in 67% yield. KHF_2 was employed for the deprotection of the ester group on **9a** and the *para*-boronic acid **10a** was obtained in 46% yield. In a similar manner, the boronic acid **10b** was synthesized from the acetylene **3b** and the iodide **5a** in three steps. The *meta*-boronic acid derivatives **13a–d** were also synthesized from **3a** and **3b**. The acetylenes **3a** and **3b** underwent Sonogashira coupling with the iodides **5b** or **7** to afford the corresponding alkynes **11a–d**. *cis* Reduction of **11a** with dicyclohexylborane gave the *cis*-stilbene **12a** in 53% yield, and protection of **12a** with KHF_2 gave the desired product **13a** in very poor yield (8%). After several trials,



Scheme 3. Reagents and conditions: a) **5a**, $[Pd(PPh_3)_4]$, CuI, TEA, CH_3CN, 70 °C, 71–73%; b) dicyclohexylborane, THF, 0 °C, 40–68%; c) KHF_2, ether, then HCl, 25–46%; d) **5b** or **7**, $[Pd(PPh_3)_4]$, CuI, TEA, CH_3CN, 70 °C, 46–82%; e) diethanolamine, then HCl, 46–68%.

we found that transesterification with diethanolamine followed by hydrolysis under acidic conditions was efficient for the deprotection of the boronic acid pinacol ester **12a**, and the corresponding boronic acid **13a** was obtained in 38% yield in two steps. The boronic acid pinacol ester **12b**, which was synthesized from the acetylene **3b** and the iodide **5b** through Sonogashira coupling followed by *cis* reduction, underwent a deprotection reaction with diethanolamine to give the boronic acid **13b** in 68% yield. Synthesis of the 4-methoxy-substituted (R^2 = MeO) *cis*-stilbenes **13c,d** was also accomplished in the same manner. The reaction of the acetylenes **3a** and **3b** with 5-iodo-2-methoxyboronic acid pinacol ester **7** afforded the corresponding alkynes **11c** and **11d** in 55% and 82% yields, respectively. Use of 5-bromo-2-methoxyboronic acid pinacol ester was not effective for this coupling reaction. Reduction of **11c** and **11d** with dicyclohexylborane gave the *cis*-stilbenes **12c** and **12d** in 40% and 43% yields, respectively. Deprotection of **12c** and **12d** was performed using transesterification to give **13c** and **13d** in 46% and 68% yields, respectively.

The carboxylic acid derivative **17** was also synthesized for comparison with the boronic acid **13c**, as shown in Scheme 4.



Scheme 4. Reagents and conditions: a) 1. *n*BuLi, THF, −78 °C, then CO_2; 2. $SOCl_2$, CH_2Cl_2, 3. TEA, CH_3OH, 61%; b) **3a**, $[Pd(PPh_3)_4]$, CuI, TEA, CH_3CN, 70 °C, 95%; c) dicyclohexylborane, THF, 0 °C, 54%; d) LiOH, THF/H_2O, 79%.

The reaction of 2,4-diiodoanisole **6** with *n*BuLi afforded the *ortho*-lithiated anisole, which was treated with CO_2 to give the corresponding carboxylic acid. The carboxylic acid was then converted to **14** in 61% yield. The methyl ester **14** underwent Sonogashira coupling with **3a**, giving the corresponding alkyne **15** in 95% yield. Reduction of **15** afforded the *cis*-stilbene **16** in 54% yield, and finally hydrolysis of **16** with LiOH in aqueous THF gave the carboxylic acid **17** in 79% yield.

Biological Results and Discussion

In vitro cell growth inhibition assay

Inhibition of cell growth by boronic acid analogues was examined using B-16 and 1-87 cell lines. The results are summarized in Table 1. The alkynes **8a** and **8b** exhibited 50% inhibition of cell growth at a concentration range of 20–36 μM. The *cis*-stilbenes **10a** and **10b**, which have a boronic acid group at the *para* position on the aromatic ring B of the combretastatin framework, exhibited higher inhibition of cell growth (2.6–12 μM). The corresponding *meta*-substituted boronic acids **13a** and **13b** were more effective at inhibiting cell growth (0.48–2.1 μM). Finally, we found that compound **13c**, which has a methoxy group substituted at the *para* position on the aromatic ring B, significantly inhibits cell growth. The IC_{50} values of **13c** toward B-16 and 1-87 cells are 0.0063 μM and 0.013 μM, respectively. These inhibitory activities are similar to those of the amide derivative **1c** (B-16 cells: 0.0080 μM, 1-87 cells: 0.011 μM), although combretastatin A-4 (**1a**) is more efficient for the inhibition of cell growth (B-16 cells: 0.0046 μM, 1-87 cells: 0.0085 μM). Three methoxy groups substituted on the aromatic ring A were necessary to obtain higher inhibition of cell growth (**10a**, **13a**, and **13c** versus **10b**, **13b**, and **13d**), as reported by Cuchman, Hamel and co-workers.[23] Unprecedentedly, the carboxylic acid derivative **17**, which is considered a mimic of the boronic acid **13c**, showed 10000-fold less cell growth inhibition than **13c** toward B-16 and 1-87 cell lines (160 and 110 μM, respectively).

Table 1. Cell growth inhibition by boronic acid derivatives.

Compd	[IC_{50} μM][a] B-16[b]	1-87[c]
8a	20 ± 1.1	22 ± 1.4
8b	32 ± 1.7	36 ± 0.92
10a	2.6 ± 0.14	3.2 ± 0.12
10b	12 ± 0.72	9.1 ± 0.3
13a	0.49 ± 0.019	2.1 ± 0.13
13b	1.8 ± 0.13	2.0 ± 0.12
13c	0.0063 ± 0.0015	0.013 ± 0.0044
13d	0.019 ± 0.0032	0.028 ± 0.0052
17	160 ± 15	110 ± 13
combretastatin A-4 (**1a**)	0.0046 ± 0.0005	0.0085 ± 0.0011
1c	0.0080 ± 0.0012	0.011 ± 0.0032
colchicine	0.056 ± 0.008	0.012 ± 0.0035

[a] Compounds were assayed at least three times, and the IC_{50} values reported are mean ± SD of an average of three experiments. [b] Mouse B-16 melanoma cell line. [c] Human lung carcinoma 1-87 cell line.

Inhibition of in vitro tubulin polymerization

To investigate inhibitory activities of the synthesized boronic acids toward the microtubule system, their effects on in vitro polymerization of tubulin were examined. Tubulin was purified from porcine brains according to a modified Shelanski protocol.[24,25] The results are shown in Table 2. Inhibitory activity of compound **10a** was not observed at 100 μM, whereas compound **10b** exhibited enhanced inhibition with an IC_{50} value of 79 μM. Compounds **13a** and **13b** did not show significant inhibition at a concentration of 100 μM. The methoxy group substituted at the *para* position on the benzene ring B enhanced inhibition of tubulin polymerization, and significant inhibitory activity was observed with compounds **13c** and **13d**, with relatively low IC_{50} values of 22 and 21 μM, respectively. Two meth-

Table 2. Inhibition of tubulin polymerization by boronic acid derivatives.[a]

Compd	IC_{50} [μM][b]
10a	>100
10b	79±7.0
13a	>100
13b	>100
13c	22±2.1
13d	21±2.6
16	100±16
17	>100
combretastatin A-4 (**1a**)	1.8±0.10
1c	5.0±0.21

[a] Polymerization reactions of tubulin (1.0 mg mL^{-1}) in assembly buffer were performed at 37 °C in the presence of the compounds for 30 min. Tubulin polymerization was monitored by measuring the increase in absorbance values at 350 nm. [b] IC_{50} is the drug concentration needed for 50% inhibition of tubulin polymerization following incubation for 30 min. The compounds were assayed three times, and the values reported are mean ± SD of an average of three experiments.

oxy groups substituted at positions 3 and 5 on ring A resulted in potent inhibition of tubulin polymerization.[23] The carboxylic acid **17** and its methyl ester derivative **16**, a mimic of the boronic acid **13c**, were also tested in the inhibition assay. However, no significant inhibition was observed with a concentration of 100 μM. These results indicate that boronic acid is more effective than carboxylic acid, although electron donor groups such as hydroxy (in **1a**) and amine (in **1c**) are more suitable for the inhibition of tubulin polymerization. Figure 1 shows time-dependent tubulin polymerization (microtubule assembly) in the presence of various concentrations of the boronic acid **13c**.[25] This compound caused a decrease in the rate and extent of microtubule formation in a concentration-dependent manner. Microtubule formation, as assessed after 30 min incubation, was decreased by 30% and 70% in the presence of 10 and 30 μM **13c**, respectively, relative to control values obtained in the absence of **13c**. The IC_{50} was estimated to be 22 μM.

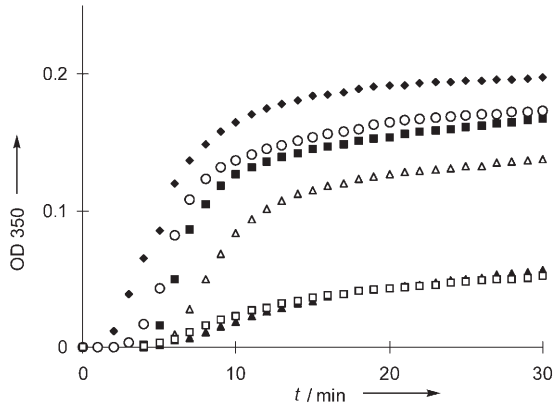


Figure 1. Effects of various concentrations of **13c** on time-dependent polymerization of tubulin. Polymerization reactions of tubulin (1.0 mg mL^{-1}) were performed in assembly buffer at 37 °C in the absence of (◆), or in the presence of **13c** at concentrations of 1(○), 3(■), 10(△), 30(▲), and 100 μM(□). The increase in the absorbance values at 350 nm represents the polymerization of tubulin into microtubules.

Cell-cycle effects

To characterize the significant inhibition of cell growth and tubulin polymerization induced by **13c**, FACScan flow cytometry analysis was used.[26] Treatment of Jurkat cells with **13c** and combretastatin A-4 at graded concentrations (10^{-6}–10^{-9} M) for 24 h led to profound changes in cell-cycle profiles as shown in Figure 2. The cell-cycle profile was not affected by **13c** at 10^{-9} M. At 10^{-8} M, the G1 cell population dramatically decreased, while the sub-G1 cell population gradually increased. With higher drug concentrations (10^{-7}–10^{-6} M), the G2 phase fraction disappeared, and a massive accumulation of cells was observed in the sub-G1 phase (Figure 2a). In contrast, combretastatin A-4 induced a massive accumulation of cells in the sub-G1 phase at $>10^{-9}$ M. The progressive generation of cells with a hypodiploid DNA content (sub-G1 material) is characteristic of apoptosis and reflects fragmentation of DNA.

Evaluation of apoptotic activity of 13c

To further characterize the cell-death mechanism induced by **13c**, a biparametric cytofluorimetric analysis was performed using FITC-labeled annexin V and propidium iodide (PI), which stain phosphatidylserine residues and DNA, respectively. Annexin V is a Ca^{+}-dependent phospholipid-binding protein with a high affinity for phosphatidylserine. Jurkat cells were treated with **13c** (10^{-7} M) for 16 h and then stained with PI and FITC-labeled annexin V to specifically detect phosphatidylserine residues exposed at the cell surface. The resulting green (FITC) and red (PI) fluorescence was monitored with flow cytometry. As indicated in Figure 3, the number of annexin V-positive and PI-negative cells increased following treatment with **13c**. These results indicate that apoptosis was induced by **13c**. In addition, Figure 4 shows dose-dependant apoptotic activity. Following incubation with **13c** and combretastatin A-4 for 8–16 h, Jurkat cells were labeled, then washed, and the resulting FITC and PI fluorescence was monitored by flow cytometry. Apoptosis was induced by incubation with **13c** for 8 h at concentrations $>10^{-8}$ M. The percentage of apoptotic cells increased with increasing incubation time (8–16 h), and apoptosis was induced in 24% of cells by concentrations $>10^{-7}$ M (Figure 4c). Combretastatin A-4 exhibited 10-fold higher apoptotic activity than **13c**. Apoptosis was induced in the cells following 8 h incubation with $>10^{-9}$ M combretastatin A-4, and the population of apoptotic cells increased to 24% following 16 h incubation at concentrations $>10^{-8}$ M. Similar dose-dependant effects on inhibition of tubulin polymerization were observed with **13c** and combretastatin A-4, as shown in Table 2.

Growth inhibition against a panel of 39 human cancer cell lines

Compound **13c** and combretastatin A-4 were tested for in vitro antiproliferative activity against a panel of 39 human cancer cell lines, which is similar to the panel developed by the National Cancer Institute.[27–29] More than 200 standard

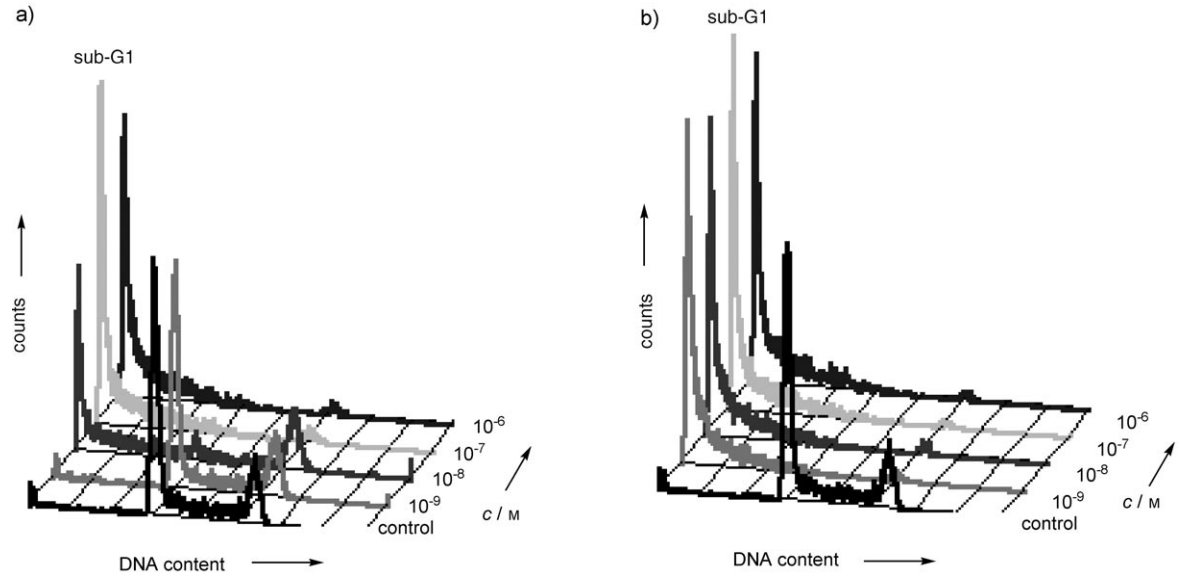


Figure 2. Cell-cycle distribution determined by FACScan flow cytometry of Jurkat cells treated for 24 h with a) compound **13c** or b) combretastatin A-4 at graded concentrations (10^{-6}–10^{-9} M). Results show a typical experiment which was repeated three times.

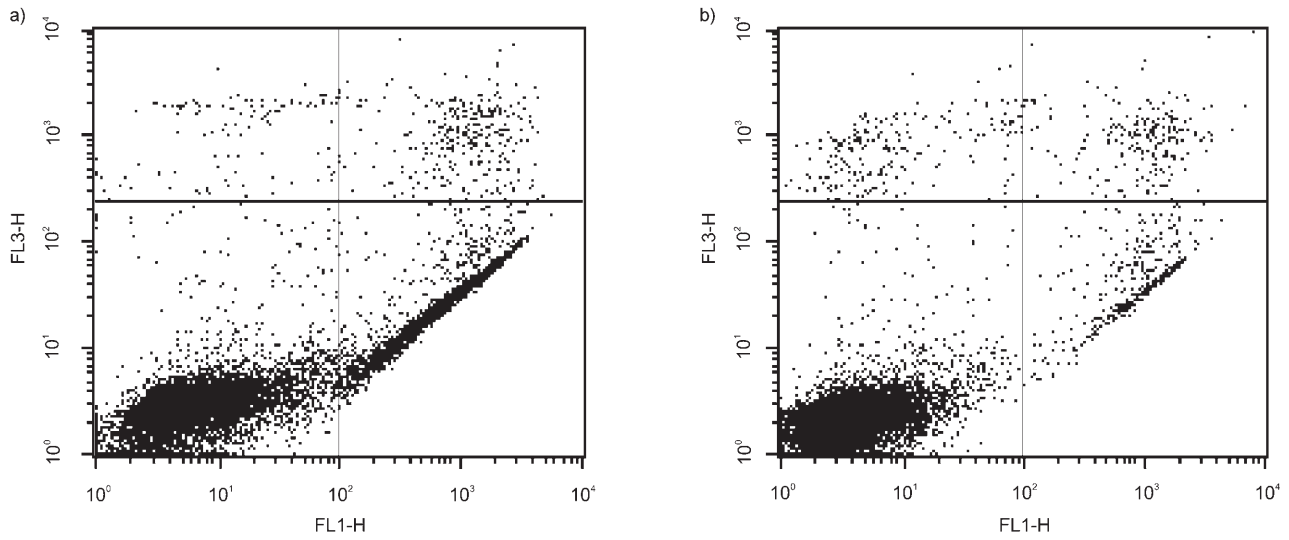


Figure 3. Externalization of phosphatidylserine residues. a) Jurkat cells were treated with **13c** at 10^{-7} M for 16 h and then stained with propidium iodide (PI) and FITC-labeled annexin V specifically detecting the exposure of phosphatidylserine residues at the cell surface. b) Control cells were not exposed to **13c** but were otherwise treated in an identical manner. The fluorescence intensity of FITC-labeled annexin V (green) is plotted on the horizontal axis and that of PI (red) is plotted on the ordinate. Both fluorescence intensities are shown as log scales.

compounds, including various anticancer drugs and various types of inhibitors, have been evaluated using this panel. We compared standard drugs with each other for the mean graph pattern using COMPARE analysis and confirmed that drugs sharing a certain mode of action clustered together, as described previously.[30,31] Figure 5 shows the mean graphs of compound **13c** and combretastatin A-4 based on the growth inhibition parameter, GI_{50}. Compound **13c** and combretastatin showed differential growth inhibition. The mean log GI_{50} values of **13c** and combretastatin A-4 were −7.86 and −8.08, respectively. Enhanced inhibitory activities of **13c** were observed in HT-29, AS49, OVCAR-4, OVCAR-5, and PC-3 cell lines, and the Delta value was 1.14. Lower inhibitory activities were observed with HBC-4 and MKN45, and the Range value was 2.74. In the mean graphs of a panel screening, a compound that has Delta and Range values of >0.5 and >1, respectively, is evaluated as positive for differential activity, therefore, **13c** and combretastatin A-4 possess differential activities toward certain cancer cells. The COMPARE analysis of the mean graphs revealed a correlation ranking of **13c** with combretastatin A-4 ($r=0.553$), bleomycin, which is a DNA strand-breaking agent, ($r=0.532$), taxol ($r=0.455$), and vincristine ($r=0.436$) (see Table 3. In COMPARE analysis, positive correlation between two agents that possess similar modes of action results in *r* values

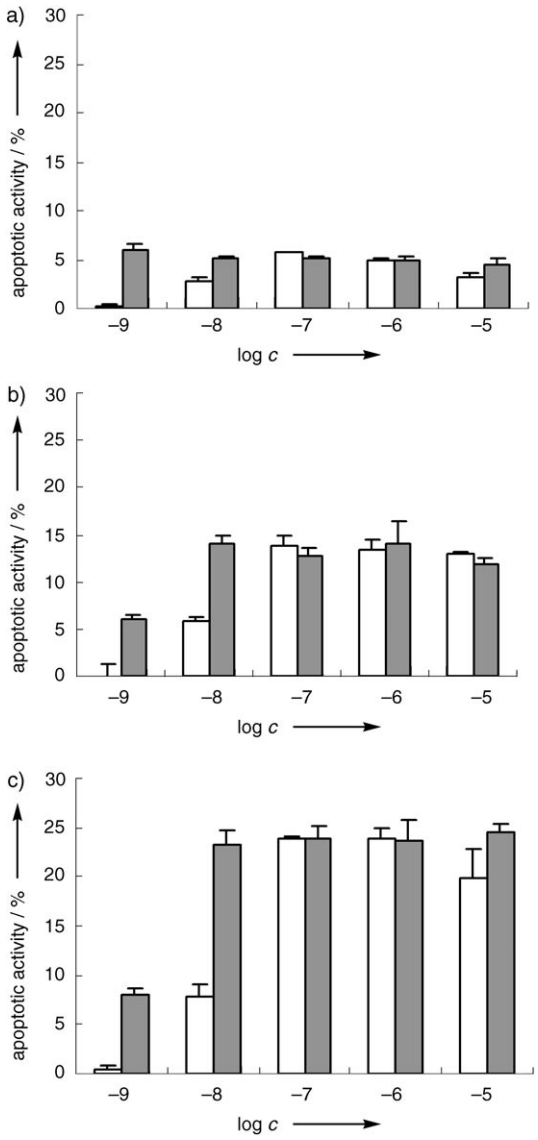


Figure 4. Apoptotic activity of **13c** (white bars) and combretastatin A-4 (grey bars) toward Jurkat cells after incubation for a) 8 h, b) 12 h, and c) 16 h. Apoptotic cells were detected by FITC-labeled annexin V using flow cytometric analysis; c = concentration in M.

greater than 0.75; a compound that is correlated to a certain agent with r values between 0.5 and 0.75 has a possibility of both different and similar modes of action to the agent. Therefore, the current analysis suggests that compound **13c** may inhibit tubulin polymerization by a different mode of action than that of combretastatin A-4.

Conclusions

We succeeded in the synthesis of a series of boron-containing *cis*-stilbenes. High inhibition of cell growth was observed with compounds **13c** and **13d**; their IC_{50} values are 100-fold lower than those of **13a** and **13b**, and 1000-fold lower than those of **10a** and **10b** (Table 1). The inhibitory activity of **13c** was essentially similar to the reported activity of combretastatin A-4 (**1a**) and **1c**. Compounds **13c** and **13d** were also found to be potent inhibitors of tubulin polymerization, although their IC_{50} values were a little higher than those of **1a** and **1c** (Table 2). The carboxylic acid derivative **17**, which can be considered a mimic of the boronic acid **13c**, showed no significant inhibition of either cell growth or tubulin polymerization. Furthermore, **13c** was characterized as an apoptotic agent by the FACScan-flow cytometry analysis, and it was clarified that significant inhibition of cell growth by **13c** was due to its highly cytotoxic property—induction of apoptosis. It should be noted that more potent effects on cytotoxicity and inhibition of tubulin polymerization by boronic acid **13c** in comparison with carboxylic acid **17** may be because it is a Lewis acid, rather than a proton donor. **13c** was basically equipotent with combretastatin A-4 in cell growth inhibition. However, the ability of **13c** to inhibit tubulin polymerization was 10-fold less than that of combretastatin A-4, and the correlation between **13c** and combretastatin A-4 in the COMPARE analysis was not very high. Therefore unlike combretastatin A-4, the boronic acid **13c** may affect cellular processes other than inhibition of tubulin polymerization.

Experimental Section

General: ^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL JNM-AL 300 (300 MHz) or a Varian Unity-Inova 400 (400 MHz) spectrometer. Chemical shifts of ^{1}H NMR were expressed in parts per million downfield from $CDCl_3$ as an internal standard (δ = 7.24 ppm) in $CDCl_3$ or from CD_3OD as an internal standard (δ = 3.35 ppm). Chemical shifts of ^{13}C NMR were expressed in parts per million downfield from $CDCl_3$ as an internal standard (δ = 77.0 ppm) in $CDCl_3$ or from CD_3OD as an internal standard (δ = 49.3 ppm). Analytical thin layer chromatography (TLC) was performed on glass plates (Merck Kieselgel 60 F_{254}, layer thickness 0.2 mm, or RP-18 F_{254s}, layer thickness 0.2 mm). Visualization was accomplished by UV light (254 nm), I_2 and $KMnO_4$. Column chromatography was performed on silica gel (Merck Kieselgel 70–230 mesh). Further purification was performed with GPC (JAIGEL-1H and 2H) using a recycling preparative HPLC system (LC-918: Japan Analytical Industry). All reactions were carried out under argon atmosphere using standard Schlenk techniques. Most chemicals and solvents were analytical grade and used without further purification.

5-Iodo-2-methoxyphenylboronic acid pinacol ester (**7**): Isopropyl borate (1.8 mL, 7.9 mmol) at −80 °C was added to a mixture of 2,4-diiodoanisole (**6**), (2.4 g, 6.5 mmol) in THF (6 mL) and toluene (24 mL) under Ar, and the mixture was stirred for 30 min. *n*BuLi (1.6 M in hexane; 4.1 mL, 6.5 mmol) was added to the reaction mixture dropwise, and the reaction mixture was allowed to warm to −20 °C for 1 h while stirring. The reaction was quenched by HCl (1 N), and the mixture was stirred for 10 min. After neutralization with a saturated solution of aqueous $NaHCO_3$, the mixture was extracted with ether, dried over anhydrous $MgSO_4$, filtered, and then concentrated. The white solid obtained was dissolved in dichloromethane (15 mL), and pinacol (0.61 g, 5.1 mmol) was added. The reaction mixture was stirred for 3 h at room temperature, dried over anhydrous $MgSO_4$, filtered, and then concentrated to give **7** (1.8 g, 5.1 mmol) in 78% yield: mp 99–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (s, 1H, Ar), 7.63 (d, ^3J = 8.8 Hz, 1H, Ar), 7.62 (d, ^3J =

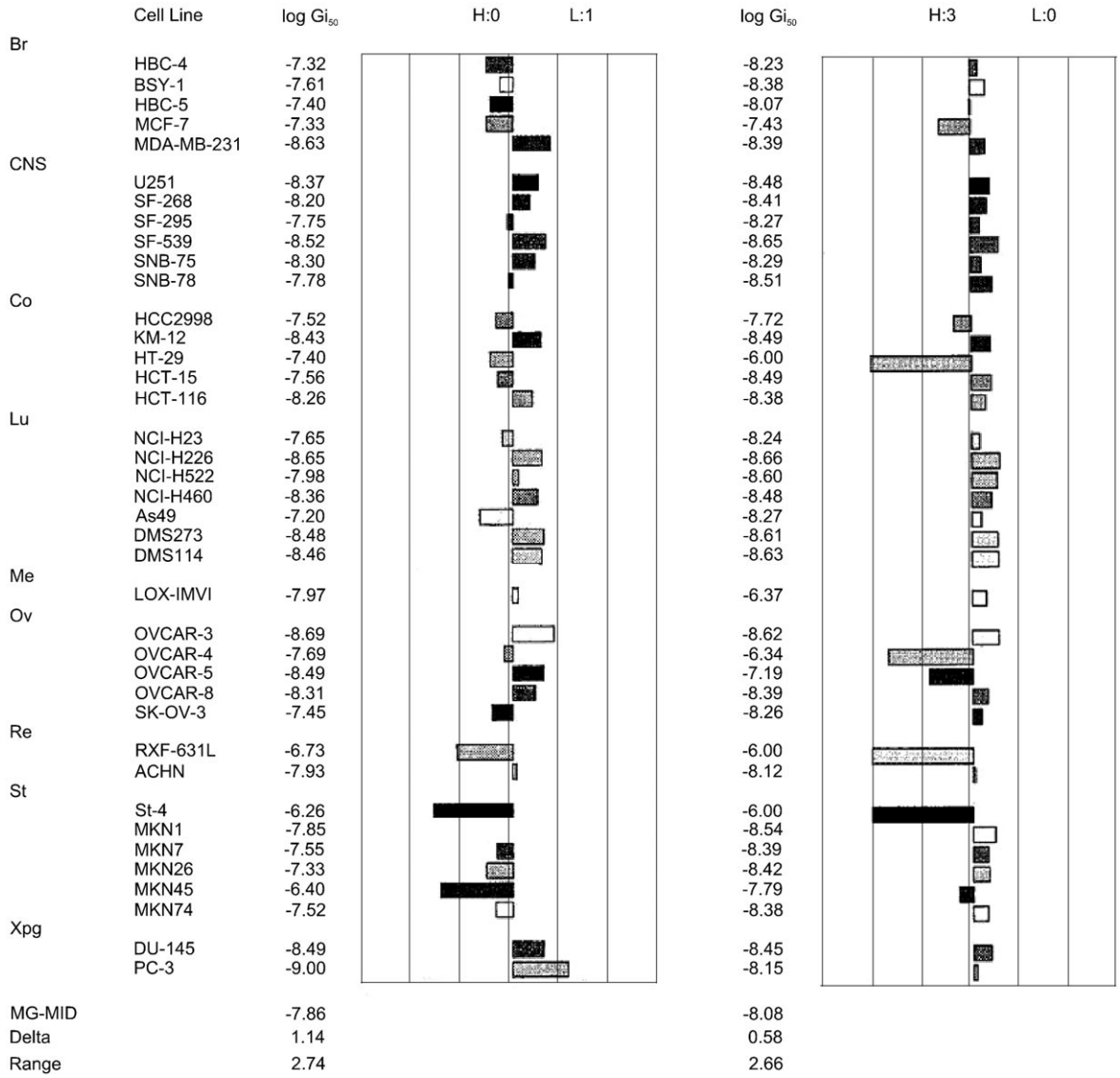


Figure 5. Growth inhibition of **13c** (left graph) and combretastatin A-4 (right graph) against a panel of 39 human cancer cell lines. The mean graph was produced by computer processing of the GI_{50} values as described in the Experimental Section. The log GI_{50} value for each cell line is indicated. Columns extending to the right represent sensitivity to compounds; columns extending the left represent resistance to compounds. Each gradation represents one logarithm difference. MG-MID is the mean of log GI_{50} values for 39 cell lines. Delta is the logarithm of difference between the MG-MID and the log GI_{50} of the most sensitive cell line. Range is the logarithm of difference between the log GI_{50} of the most resistant cell line and that of the most sensitive one.

Table 3. COMPARE analysis of **13c**.[a]

Ranking Order	Compd	r[b]
1	combretastatin A-4	0.553
2	bleomycin	0.532
3	taxol	0.455
4	vincristine	0.436

[a] The mean graph of **13c** was compared with those of 200 standard drugs and combretastatin A-4 in the DOS database using the COMPARE algorithm. The top four compounds with high r values are listed in ranking order. [b] Correlation coefficient.

8.8 Hz, 1H, Ar), 3.78 (s, 3H, OCH_3), 1.32 ppm (s, 12H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 163.8$ (Ar), 144.8 (Ar), 140.9 (Ar), 112.9 (Ar), 83.7 ($C(CH_3)_2$), 82.9 (Ar), 55.9 (OCH_3), 24.8 ppm (CH_3).

4-[(3,4,5-Trimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**8a**): **3a** (0.72 g, 3.8 mmol), **5a** (1.2 g, 3.8 mmol) and triethylamine (1.6 mL, 11 mmol) were added to a mixture of CuI (0.072 g, 0.38 mmol) and $[Pd(PPh_3)_4]$ (0.22 g, 0.19 mmol) in CH_3CN (30 mL), and the reaction mixture was stirred at 60 °C for 1.5 h under Ar. After the reaction was quenched with a saturated solution of aqueous NH_4Cl, the mixture was extracted with ether, dried over anhydrous $MgSO_4$, filtered, and then concentrated. Purification by column chromatography on silica gel with hexane/ethyl acetate (3:1) gave **8a** (1.1 g, 71%) as a white solid: mp 101–103 °C; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.77$ (d, $^3J = 8.4$ Hz, 2H, 3-H), 7.50 (d, $^3J = 8.4$ Hz, 2H, 2-H), 6.76 (s, 2H, 2′-H), 3.86 (s, 6H, 3′-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 1.33 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta =$ 153.1 (3′-C), 138.9 (4′-C), 134.6 (2-C), 130.7 (3-C), 125.8 (4-C), 118.1 (1′-C), 108.8 (2′-C), 90.7 (alkyne), 88.7 (alkyne), 84.0 ($C(CH_3)_2$), 61.0 (4′-OCH_3), 56.1 (3′-OCH_3), 24.9 ppm (CH_3); HRMS (ESI) *m/z*: calcd for $C_{23}H_{27}BO_5$: 395.2030 $[M+H]^+$, found: 395.2025.

4-[(3,5-Dimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**8b**) was synthesized from **3b** (0.88 g, 5.4 mmol) and **5b** (1.8 g, 5.4 mmol) using the procedure described for **8a**, to give **8b** (1.4 g, 73%) as a white solid: mp 111–113 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.77 (d, ^3J = 8.0 Hz, 2H, 3-H), 7.51 (d, ^3J = 8.0 Hz, 2H, 2-H), 6.68 (s, 2H, 2′-H), 6.45 (s, 1H, 4′-H), 3.78 (s, 6H, OCH_3), 1.33 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 160.5 (3′-C), 134.6 (3-C), 130.8 (2-C), 125.7 (1′-C), 124.4 (4-C), 109.3 (2′-C), 102.0 (4′-C), 90.7 (alkyne), 89.1 (alkyne), 83.9 ($C(CH_3)_2$), 55.4 (CH_3O), 24.9 ppm (CH_3); HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}BO_4$: 365.1924 $[M+H]^+$, found: 365.1920.

3-[(3,4,5-Trimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**11a**) was synthesized from **3a** (1.2 g, 6.0 mmol) and **5b** (2.0 g, 6.0 mmol) using the procedure described for **8a** to give **11a** (1.7 g, 72%) as a white solid: mp 88–90 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (s, 1H, 2-H), 7.74 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.58 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.34 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.74 (s, 2H, 2′-H), 3.86 (s, 6H, 3′-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 1.33 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 153.1 (3′-C), 138.1 (4′-C), 134.3 (2-C), 133.9 (6-C), 127.7 (4-C), 122.7 (5-C), 118.4 (1′-C), 108.9 (2′-C), 89.5 (alkyne), 88.5 (alkyne), 84.0 ($C(CH_3)_2$), 60.9 (4′-OCH_3), 56.2 (3′-OCH_3), 24.9 ppm (CH_3); HRMS (ESI) *m/z*: calcd for $C_{23}H_{27}BO_5$: 395.2030 $[M+H]^+$; found: 395.2027.

3-[(3,5-Dimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**11b**) was synthesized from **3b** (0.64 g, 4.0 mmol) and **5b** (1.3 g, 4.0 mmol) using the procedure described for **8a** to give **11b** (1.0 g, 70%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.99 (s, 1H, 2-H), 7.74 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.59 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.34 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.66 (d, ^4J = 2.4 Hz, 2H, 2′-H), 6.44 (t, ^4J = 2.4 Hz, 1H, 4′-H), 3.79 (s, 6H, 3′-OCH_3), 1.34 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 160.5 (3′-C), 138.2 (2-C), 134.4 (6-C), 134.1 (4-C), 127.7 (5-C), 124.6 (1′-C), 122.6 (3-C), 109.3 (2′-C), 101.8 (4′-C), 89.4 (alkyne), 88.9 (alkyne), 84.0 (($C(CH_3)_2$), 55.4 (3′-OCH_3), 24.9 ppm (CH_3); HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}BO_4$: 365.1924 $[M+H]^+$; found: 365.1921.

2-Methoxy-4-[(3,4,5-trimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**11c**) was synthesized from **3a** (0.29 g, 1.5 mmol) and **7** (0.51 g, 1.4 mmol) using the procedure described for **8a** to give **11c** (0.28 g, 46%) as a white solid: mp 103–105 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.85 (s, 1H, 6-H), 7.54 (d, ^3J = 8.8 Hz, 1H, 4-H), 6.82 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.73 (s, 2H, 2′-H), 3.86 (s, 6H, 3′-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 3.84 (s, 3H, 2-OCH_3), 1.34 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.0 (2-C), 153.0 (3′-C), 140.2 (6-C), 138.4 (4′-C), 135.6 (4-C), 118.7 (1′-C), 114.8 (5-C), 110.3 (3-C), 108.5 (2′-C), 88.5 (alkyne), 88.0 (alkyne), 83.6 ($C(CH_3)_2$), 60.9 (4′-OCH_3), 56.0 (3′-OCH_3), 55.8 (2-OCH_3), 24.8 ppm (CH_3); HRMS (ESI) *m/z*: calcd for $C_{24}H_{29}BO_6$: 425.2136 $[M+H]^+$; found: 425.2137.

2-Methoxy-4-[(3,5-dimethoxyphenyl)ethynyl]phenylboronic acid pinacol ester (**11d**) was synthesized from **3b** (0.77 g, 4.8 mmol) and **7** (1.7 g, 4.8 mmol) using the procedure described for **8a** to give **11d** (1.5 g, 82%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.86 (d, ^4J = 2.0 Hz, 1H, 6-H), 7.55 (dd, ^3J = 8.8, 2.0 Hz, 1H, 4-H), 6.81 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.65 (d, ^4J = 2.4 Hz, 2H, 2′-H), 6.42 (t, ^4J = 2.4 Hz, 1H, 4′-H), 3.83 (s, 3H, 2-OCH_3), 3.77 (s, 6H, 3′-H), 1.34 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.1 (2-C), 160.5 (3′-C), 140.4 (6-C), 135.8 (4-C), 125.0 (1′-C), 114.8 (5-C), 110.4 (3-C), 109.1 (2′-C), 101.5 (4′-C), 89.0 (alkyne), 88.11 (alkyne), 83.7 (C-(CH_3)$_2$), 55.8 (2-OCH_3), 55.4 (3′-OCH_3), 24.8 ppm (CH_3); ESIMS *m/z*: 417 $[M+H]^+$.

(*Z*)-4-[(3,4,5-Trimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**9a**): BH_3·SMe_2 (1.0 M in CH_2Cl_2, 1.1 mL, 1.1 mmol) was added dropwise at 0 °C to a solution of cyclohexene (0.22 mL, 2.2 mmol) in THF (1 mL). After stirring for 1 h, a solution of **8a** (0.39 g, 1.0 mmol) in THF (2 mL) was added to the reaction mixture at 0 °C and stirred for 1 h. The reaction was allowed to come to room temperature, and the mixture was stirred for 40 min. The reaction mixture was cooled to 0 °C and acetic acid (0.38 mL, 6.6 mmol) was added. After stirring for 2.5 h, the reaction was quenched with a saturated solution of aqueous $NaHCO_3$, and the mixture was extracted with ether, dried over $MgSO_4$ anhydride, filtered, and then concentrated. Purification by column chromatography on silica gel with hexane/ethyl acetate (10:1) gave **9a** (0.17 g, 45%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.67 (d, ^3J = 7.6 Hz, 2H, 2-H), 7.27 (d, ^3J = 7.6 Hz, 2H, 2-H), 6.56 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.50 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.44 (s, 2H, 2′-H), 3.81 (s, 3H, 4′-OCH_3), 3.62 (s, 6H, 3′-OCH_3), 1.31 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 152.9 (3′-C), 140.3 (4′-C), 137.4 (4-C), 134.6 (2-C), 132.3 (1′-C), 130.7 (*cis*-alkene), 129.9 (*cis*-alkene), 106.2 (2′-C), 83.7 ($C(CH_3)_2$), 60.8 (4′-OCH_3), 55.9 (3′-OCH_3), 24.8 (CH_3); ESIMS *m/z*: 419 $[M+Na]^+$.

(*Z*)-4-[(3,5-Dimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**9b**) was synthesized from **8b** (0.80 g, 2.2 mmol) using the procedure described for **9a** to give pure **9b** (0.55 g, 68%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.66 (d, ^3J = 7.6 Hz, 2H, 3-H), 7.26 (d, ^3J = 7.6 Hz, 2H, 2-H), 6.59 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.54 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.38 (d, ^4J = 2.4 Hz, 2H, 2′-H), 6.30 (t, ^4J = 2.4 Hz, 1H, 4′-H), 3.62 (s, 6H, 3′-OCH_3), 1.32 ppm (s, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.5 (2′-C), 140.0 (1′-C), 138.9 (4-C), 130.8 (alkene), 130.6 (alkene), 128.2 (3-C), 106.7 (2′-C), 99.8 (4′-C), 83.7 ($C(CH_3)_2$), 55.2 (3′-OCH_3), 24.8 (CH_3); ESIMS *m/z*: 367 $[M+H]^+$, 389 $[M+Na]^+$.

(*Z*)-3-[(3,4,5-Trimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**12a**) was synthesized from **11a** (1.2 g, 3.1 mmol) using the procedure described for **9a** to give pure **12a** (0.65 g, 53%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (s, 1H, 2-H), 7.61 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.38 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.21 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.56 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.46 (s, 2H, 2′-H), 6.45 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 3.80 (s, 3H, 4′-OCH_3), 3.61 (s, 6H, 3′-OCH_3), 1.30 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 152.8 (3′-C), 137.2 (4′-C), 136.7 (2-C), 135.3 (3-C), 133.5 (6-C), 132.4 (1′-C), 131.7 (5-C), 129.9 (alkene), 129.8 (alkene), 127.5 (4-C), 106.1 (2′-C), 83.8 ($C(CH_3)_2$), 60.9 (4′-OCH_3), 55.8 (3′-OCH_3), 24.8 ppm (CH_3); ESIMS *m/z*: 419 $[M+Na]^+$.

(*Z*)-3-[(3,5-Dimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**12b**) was synthesized from **11b** (0.88 g, 2.4 mmol) using the procedure described for **9a** to give pure **12b** (0.41 g, 47%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.73 (s, 1H, 2-H), 7.62 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.37 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.19 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.60 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.49 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.39 (s, 2H, 2′-H), 6.30 (s, 1H, 4′-H), 3.60 (s, 6H, 3′-OCH_3), 1.32 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 160.5 (3′-C), 139.0 (2-C), 136.6 (1′-C), 135.5 (3-C), 133.6 (6-C), 131.7 (5-C), 130.5 (alkene), 130.1 (alkene), 127.4 (4-C), 106.7 (2′-C), 100.1 (4′-C), 83.8 ($C(CH_3)_2$), 55.2 (3′-OCH_3), 24.8 ppm (CH_3); ESIMS *m/z*: 389 $[M+Na]^+$.

(*Z*)-2-Methoxy-5-[(3,4,5-trimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**12c**) was synthesized from **11c** (1.1 g, 2.5 mmol) using the procedure described for **9a** to give pure **12c** (0.44 g, 40%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (s, 6H, 2-H), 7.31 (d, ^3J = 8.8 Hz, 1H, 4-H), 6.70 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.51 (s, 2H, 2′-H), 6.48 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.38 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 3.81 (s, 3H, 4′-OCH_3), 3.79 (s,

3H, 2-OCH_3), 3.66 (s, 6H, 3′-OCH_3), 1.31 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.1 (2-C), 152.7 (3′-C), 137.2 (4′-C), 133.0 (6-C), 132.7 (4-C), 129.2 (alkene), 128.9 (5-C), 128.3 (alkene), 109.9 (3-C), 105.9 (2′-C), 83.3 ($C(CH_3)_2$), 60.6 (4′-OCH_3), 55.7 (3′-OCH_3), 55.6 (2-OCH_3), 24.7 ppm (CH_3); ESIMS *m/z*: 449 $[M+Na]^+$.

(*Z*)-2-Methoxy-5-[(3,5-dimethoxyphenyl)ethenyl]phenylboronic acid pinacol ester (**12d**) was synthesized from **11d** (1.5 g, 3.9 mmol) using the procedure described for **9a** to give pure **12d** (0.66 g, 43%) as a white solid: mp 75–79 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.61 (d, ^3J = 2.4 Hz, 1H, 6-H), 7.30 (dd, ^3J = 8.8, 2.4 Hz, 1H, 4-H), 6.67 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.51 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.43 (d, ^4J = 1.6 Hz, 2H, 2′-H), 6.41 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.29 (t, ^4J = 1.6 Hz, 1H, 4′-H), 3.78 (s, 3H, 2-OCH_3), 3.63 (s, 6H, 3′-OCH_3), 1.31 ppm (s, 12H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.3 (3′-C), 160.5 (2-C), 139.4 (6-C), 137.7 (1′-C), 131.1 (alkene), 130.1 (alkene), 128.9 (5-C), 128.5 (4-C), 109.9 (3-C), 106.4 (2′-C), 99.8 (4′-C), 83.5 ($C(CH_3)_2$), 55.8 (3′-OCH_3), 55.2 (2-OCH_3), 24.7 ppm (CH_3); ESIMS *m/z*: 419 $[M+Na]^+$.

(*Z*)-4-[(3,4,5-Trimethoxyphenyl)ethenyl]phenylboronic acid (**10a**): KHF_2 (4 N in methanol, 1 mL) was added to a solution of **9a** (0.15 g, 0.37 mmol) in methanol (5 mL), and the mixture was stirred for 30 min at room temperature. The white precipitate was filtered, washed with cold water, and then washed with cold ether. The filtrate was dissolved in a stirred solution of ethyl acetate (5 mL) and aqueous HCl (1 N, 5 mL) and the stirring was continued for 30 min. The organic layer was separated, washed with a saturated solution of aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and then concentrated. The solid obtained was purified by recrystallization from hexane and CH_2Cl_2 to give **10a** (0.054 g, 46%) as a white solid: mp 114–116 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) monomer: δ = 7.64 (d, ^3J = 7.6 Hz, 2H, 3-H), 7.31 (d, ^3J = 7.6 Hz, 2H, 2-H), 6.58 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.53 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.44 (s, 2H, 2′-H), 4.91 (bs, 2H, $B(OH)_2$), 3.81 (s, 3H, 4′-OCH_3), 3.63 ppm (s, 6H, 3′-OCH_3); trimer: δ = 8.09 (d, ^3J = 7.6 Hz, 2H, 3-H), 7.40 (d, ^3J = 7.6 Hz, 2H, 2-H), 6.63 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.58 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.48 (s, 2H, 2′-H), 3.83 (s, 3H, 4′-OCH_3), 3.64 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) monomer: δ = 152.8 (3′-C), 140.2 (4′-C), 133.6 (4-C), 132.4 (2-C), 130.8 (alkene), 129.8 (alkene), 128.4 (1′-C), 125.9 (3-C), 106.1 (2′-C), 60.9 (4′-OCH_3), 55.9 ppm (3′-OCH_3); trimer: δ = 152.9 (3′-C), 141.9 (4′-C), 135.5 (4-C), 132.3 (2-C), 131.3 (alkene), 129.8 (alkene), 128.5 (1′-C), 126.0 (3-C), 106.1 (2′-C), 60.9 (4′-OCH_3), 55.9 ppm (3′-OCH_3). It is well known that there is an equilibrium between the monomer and cyclic trimer in boronic acids. We assigned chemical shifts as the monomer, the intensity of which increased when D_2O was added to the NMR sample. ESIMS *m/z*: 365 $[C_{17}H_{17}O_3B(OMe)_2+Na]^+$; elemental analysis calcd (%) for $C_{17}H_{19}BO_5$: C 65.00, H 6.10; found: C 64.85, H 6.16.

(*Z*)-4-[(3,5-Dimethoxyphenyl)ethenyl]phenylboronic acid (**10b**): A mixture of **9b** (0.50 g, 1.4 mmol) and diethanolamine (0.13 mL, 1.4 mmol) in isopropanol (3 mL) was stirred at room temperature for 5 min. Then ether (15 mL) was added and the solution was stirred for 3 h. The precipitate was filtered and washed with cold ether. The filtrate was dissolved in THF (4 mL), and HCl (1 N in water, 4 mL) was added. After the mixture was stirred for 30 min, the organic layer was separated, washed with a saturated solution of aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography on silica gel with hexane/ethyl acetate (2:1) gave pure **10b** (0.021 g, 25%) as a white solid: mp 138–140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.06 (d, ^3J = 7.6 Hz, 2H, 3-H), 7.38 (d, ^3J = 7.6 Hz, 2H, 2-H), 6.66 (d, ^3J = 12.4 Hz, 1H, alkene), 6.61 (d, ^3J = 12.4 Hz, 1H, alkene), 6.41 (d, ^4J = 2.0 Hz, 2H, 2′-H), 6.33 (t, ^4J = 2.0 Hz, 1H, 4′-H), 3.64 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 160.6 (3′-C), 141.6 (1′-C), 138.9 (4-C), 135.5 (2-C), 131.5 (alkene), 130.5 (alkene), 128.5 (3-C), 106.7 (2′-C), 100.0 (4′-C), 55.2 ppm (3′-OCH_3); ESIMS *m/z*: 335 $[C_{16}H_{15}O_2B(OMe)_2+Na]^+$; elemental analysis calcd (%) for $C_{16}H_{17}BO_4$: C 67.64, H 6.03; found: C 67.93, H 5.74.

(*Z*)-3-[(3,4,5-Trimethoxyphenyl)ethenyl]phenylboronic acid (**13a**) was synthesized from **12a** (0.5 g, 1.3 mmol) using the procedure described for **10b** to give **13a** (0.20 g, 50%) as a white solid: mp 81–83 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.09 (s, 1H, 2-H), 7.97 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.52 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.37 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.69 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.57 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.49 (s, 2H, 2′-H), 3.78 (s, 3H, 4′-OCH_3), 3.64 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 152.9 (3′-C), 137.3 (2-C), 137.0 (4′-C), 136.1 (3-C), 134.5 (6-C), 133.3 (1′-C), 132.4 (5-C), 130.4 (alkene), 129.8 (alkene), 127.7 (4-C), 106.1 (2′-C), 60.9 (4′-OCH_3), 55.9 ppm (3′-OCH_3); ESIMS *m/z*: 365 $[C_{17}H_{17}O_3B(OMe)_2+Na]^+$; elemental analysis calcd (%) for $C_{17}H_{19}BO_5$: C 65.00, H 6.10; found: C 64.73, H 6.11.

(*Z*)-3-[(3,5-Dimethoxyphenyl)ethenyl]phenylboronic acid (**13b**) was synthesized from **12b** (0.33 g, 0.89 mmol) using the procedure described for **10b** to give **13b** (0.17 g, 68%) as a white solid: mp 42–44 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.09 (s, 1H, 2-H), 7.92 (d, ^3J = 7.6 Hz, 1H, 4-H), 7.49 (d, ^3J = 7.6 Hz, 1H, 6-H), 7.35 (t, ^3J = 7.6 Hz, 1H, 5-H), 6.70 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.60 (d, ^3J = 12.4 Hz, 1H, *cis*-alkene), 6.44 (d, ^4J = 2.0 Hz, 2H, 2′-H), 6.31 (d, ^4J = 2.0 Hz, 1H, 4′-H), 3.61 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 160.6 (3′-C), 139.0 (2-C), 136.7 (1′-C), 136.1 (3-C), 134.6 (6-C), 133.4 (alkene), 130.5 (alkene), 127.8 (4-C), 106.7 (2′-C), 100.1 (4′-C), 55.2 ppm (3′-OCH_3); ESIMS *m/z* 335: $[C_{16}H_{15}O_2B(OMe)_2+Na]^+$; elemental analysis calcd (%) for $C_{16}H_{17}BO_4$: C 67.64, H 6.03; found: C 67.53, H 6.11.

(*Z*)-2-Methoxy-5-[(3,4,5-trimethoxyphenyl)ethenyl]phenylboronic acid (**13c**) was synthesized from **12c** (0.44 g, 1.0 mmol) using the procedure described for **10b** to give **13c** (0.16 g, 46%) as a white solid: mp 113–115 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.81 (d, ^4J = 2.0 Hz, 1H, 6-H), 7.35 (dd, ^3J = 8.4, 2.0 Hz, 1H, 4-H), 6.75 (d, ^3J = 8.4 Hz, 1H, 3-H), 6.51 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.49 (s, 2H, 2′-H), 6.43 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.16 (bs, 2H, $B(OH)_2$), 3.87 (s, 3H, 2-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 3.67 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.6 (2-C), 152.9 (3′-C), 137.7 (6-C), 137.1 (4′-C), 133.4 (1′-C), 132.8 (5-C), 130.1 (4-C), 129.2 (alkene), 129.1 (alkene), 109.7 (3-C), 106.0 (2′-C), 60.9 (4′-OCH_3), 55.9 (2-OCH_3), 55.6 ppm (3′-OCH_3); ESIMS *m/z*: 395 $[C_{18}H_{19}O_4B(OMe)_2+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{21}BO_6$: C 62.82, H 6.15; found C 62.52, H 6.28.

(*Z*)-2-Methoxy-5-[(3,5-dimethoxyphenyl)ethenyl]phenylboronic acid (**13d**) was synthesized from **12d** (0.12 g, 0.3 mmol) using the procedure described for **10b** to give **13d** (0.064 g, 68%) as a white solid: mp 99–101 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.77 (d, ^4J = 2.4 Hz, 1H, 6-H), 7.34 (dd, ^4J = 8.8, 2.4 Hz, 1H, 4-H), 6.73 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.53 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.46 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.41 (d, ^4J = 2.0 Hz, 2H, 2′-C), 6.30 (t, ^4J = 2.4 Hz, 1H, 4′-C), 5.93 (bs, 2H, $B(OH)_2$), 3.86 (s, 3H, 2-OCH_3), 3.65 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 163.7 (3′-C), 160.7 (2-C), 139.4 (6-C), 137.8 (1′-C), 133.3 (alkene), 130.2 (alkene), 129.9 (5-C), 129.3 (4-C), 109.8 (3-C), 106.8 (2′-C), 99.9 (4′-C), 55.6 (2-OCH_3), 55.2 ppm (3′-OCH_3); elemental analysis calcd (%) for $C_{17}H_{19}BO_5$: C 65.00, H 6.10; found: C 64.88, H 6.30.

Methyl 5-iodo-2-methoxybenzoate (**14**): *n*BuLi (1.6 M in hexane, 1.8 mL) at −80 °C was added to a solution of **6** (0.99 g, 2.7 mmol) in THF (2.5 mL) and toluene (10 mL), and the mixture was stirred

for 30 min. Carbon dioxide was bubbled for 30 min while stirring at −80 °C, and the reaction mixture was then allowed to warm to room temperature. The reaction was quenched by aqueous NaOH and washed with ether. The aqueous layer was neutralized with HCl and extracted CH_2Cl_2. The organic layer was separated and concentrated to give crude 5-iodo-2-methoxybenzoic acid, which was treated with thionyl chloride (3 mL) for 30 min, and the extra thionyl chloride was evaporated. The acid chloride obtained was dissolved in MeOH (3 mL), and triethylamine (0.29 mL) was added at room temperature. The mixture was stirred for 10 min and then the solvent was removed. The residue was dissolved in water and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography on silica gel eluted with hexane/ethyl acetate (10:1) gave **14** (0.49 g, 61%) as a white solid. Compound **14** was confirmed by comparison with the reported spectral data.[25]

Methyl 2-methoxy-5-[(3,4,5-dimethoxyphenyl)ethynyl]benzoate (**15**) was synthesized from **3a** (0.28 g, 1.4 mmol) and **14** (0.42 g, 1.4 mmol) using the procedure described for **8a** to give **15** (0.49 g, 95%) as a white solid: mp 129–131 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.96 (d, ^4J = 2.0 Hz, 1H, 6-H), 7.60 (dd, ^3J = 8.8, 2.0 Hz, 1H, 4-H), 6.94 (d, ^3J = 8.8 Hz, 1H, 3-H), 6.73 (s, 2H, 2′-H), 3.91 (s, 3H, CO_2CH_3), 3.88 (s, 3H, 4′-OCH_3), 3.86 (s, 6H, 3′-OCH_3), 3.85 ppm (s, 3H, 2-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 165.8 ($ArCO_2$-), 158.9 (2-C), 153.1 (3′C), 138.8 (4′-C), 136.4 (4-C), 135.0 (6-C), 120.2 (1′-C), 118.2 (5-C), 115.1 (1-C), 112.1 (3-C), 108.7 (2′-C), 88.8 (alkyne), 87.3 (alkyne), 61.0 (4′-OCH_3), 56.1 (3′ and 2-OCH_3), 52.1 ppm (CO_2CH_3).

Methyl (*Z*)-2-methoxy-5-[(3,4,5-dimethoxyphenyl)ethenyl]benzoate (**16**) was synthesized from **15** (0.51 g, 1.4 mmol) using the procedure described for **9a**. Further purification was accomplished by GPC eluted with $CHCl_3$ to give **16** (0.28 g, 54%) as a white solid: mp 77–79 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.74 (d, ^4J = 2.4 Hz, 1H, 6-H), 7.35 (dd, ^3J = 8.4, 2.4 Hz, 1H, 4-H), 6.81 (d, ^3J = 8.4 Hz, 1H, 3-H), 6.46–6.44 (4H, *cis*-alkene and 2′-H), 3.84 (s, 3H, CO_2CH_3), 3.81 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, 2-OCH_3), 3.65 ppm (s, 6H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 166.4 ($ArCO_2$-), 158.0 (2-C), 152.9 (3′-C), 137.2 (4′-C), 134.0 (4-C), 132.3 (alkene), 132.2 (alkene), 129.7 (1′-C), 129.1 (6-C), 128.2 (5-C), 119.7 (3-C), 111.6 (1-C), 105.8 (2′-C), 60.9 (3′-OCH_3), 56.0 (4′-OCH_3), 55.8 (2-OCH_3), 52.0 ppm (CO_2CH_3); elemental analysis calcd (%) for $C_{20}H_{22}O_6$: C 67.03, H 6.19; found: C 67.04, H 6.31.

(*Z*)-2-Methoxy-5-[(3,4,5-dimethoxyphenyl)ethenyl]benzoic acid (**17**): LiOH (1 N in water, 1 mL) was added to a solution of **16** (0.10 g, 0.29 mmol) in THF (1 mL), and the mixture was stirred for 18 h at room temperature. The mixture was washed with CH_2Cl_2 to remove the organic layer, and the aqueous layer obtained was neutralized with 1 N HCl. The white precipitate was extracted with CH_2Cl_2, and the organic layer was dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography on silica gel eluted with ethyl acetate gave **17** (0.078 g, 79%) as a white solid: mp 91–93 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.06 (d, ^4J = 2.4 Hz, 1H, 6-H), 7.42 (dd, ^3J = 8.4, 2.4 Hz, 1H, 4-H), 6.86 (d, ^3J = 8.4 Hz, 1H, 3-H), 6.50 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.44 (d, ^3J = 12.0 Hz, 1H, *cis*-alkene), 6.42 (s, 2H, 2′-H), 3.99 (s, 3H, 4′-OCH_3), 3.79 (s, 3H, 2-OCH_3), 3.64 ppm (s, 6H, 4′-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 165.6 ($ArCO_2$-), 157.0 (2-C), 153.0 (3′-C), 137.4 (4′-C), 135.3 (4-C), 134.2 (6-C), 132.1 (alkene), 131.1 (alkene), 130.6 (1′-C), 127.7 (5-C), 117.4 (3-C), 111.3 (1-C), 105.8 (2′-C), 60.8 (3′-OCH_3), 56.7 (4′-OCH_3), 55.9 ppm (2-OCH_3); elemental analysis calcd (%) for $C_{19}H_{20}O_6$: C 66.27, H 5.85; found: C 65.99, H 6.01.

Cell growth inhibitory assay: Mouse B-16 melanoma cells and human lung carcinoma 1-87 cells were maintained in RPMI-1640 medium containing 10% fetal bovine serum at 37 °C in a 5% CO_2 humidified incubator. The cells (0.5×10^4 cells/well) were exposed to various concentrations of the test compounds in 96-well culture plates for 72 h. The medium was removed, and the cells were washed three times with PBS(−) and stained with crystal violet (0.4% in MeOH) to enable cell counting with a microplate reader. The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically relative to control. Compounds were examined in a minimum of three independent experiments.

Tubulin polymerization in vitro assay: Tubulin was purified according to a modified method of Shelanski et al.[23,24] Porcine brain tissue was obtained within 2 h of slaughter and chilled to 0 °C. All subsequent procedures were carried out at 0–4 °C unless otherwise indicated. Cerebral tissue was freed of meninges and clotted blood, and 1.5 mL of GTP-RB buffer (1 mM) was added per gram of tissue. The RB buffer contained MES (100 mM, pH 6.8), EDTA (1 mM), and $MgCl_2$ (0.5 mM). Homogenization was performed in a Waring blender operated at 7000 rpm^{-1} for three periods of 15 s with intervals of 45 s. The homogenate was centrifuged at 5000 *g* for 10 min, and the supernatant was centrifuged at 70000 *g* for 90 min. The supernatant was mixed with 0.5 volume of 12 M glycerol, kept at 37 °C for 30–45 min, and then centrifuged at 70000 *g* for 90 min at room temperature. The pellet was suspended in an appropriate volume of the mixture of the 1 mM GTP-RB buffer and 12 M glycerol (1:2, *v/v*) and stored at −20 °C. Before use, the tubulin was mixed with an equal volume of the 1 mM GTP-RB buffer supplemented with 0.2 M potassium acetate (pH 6.5), warmed to 37 °C for 30–45 min, and centrifuged at 30000 *g* for 60 min at room temperature. The pellet was suspended in the 1 mM GTP-RB buffer and centrifuged at 30000 *g* for 60 min at 4 °C. The supernatant was taken as a tubulin preparation (final concentration 1.0 mg mL^{-1}). The tubulin solution (95 μL $well^{-1}$) and a dimethyl sulfoxide solution (5 μL) containing the test compounds at various concentrations were placed in a 96-well microplate. The increase in absorbance was measured at 350 nm in a microplate reader at 37 °C and recorded at one-minute intervals for 30 min. The IC_{50} was calculated by nonlinear regression based on the absorbance of untreated control at 0 min and 30 min (0 and 100% polymerization of tubulin, respectively).

Cell-cycle analysis: Jurkat cells were suspended at 1×10^6 cells mL^{-1} in 24-well plates. Test compounds were prepared with the medium and added to the cell plate at final concentrations of 10^{-6}–10^{-9} M. The cells were incubated for 24 h at 37 °C under an atmosphere of 5% CO_2 and then washed with 1 mL PBS(−). After centrifugation, the cell pellet was resuspended in 1 mL cold ethanol for 1 h at 4 °C. The ethanol was removed by centrifugation, and the cell pellet was washed with 1 mL PBS(−). The cells were incubated for 30 min in a solution of propidium iodide (50 μg mL^{-1}) containing 100 μg mL^{-1} RNase. Cell samples were analyzed by a flow cytometer (FACSCalibur, Becton-Dickinson).

Apoptotic activity: Jurkat cells were suspended at 5×10^5 cells mL^{-1} in 24-well plates. Test compounds were prepared with the medium and added to the cell plate at final concentrations of 10^{-5}–10^{-9} M. The cells were incubated for 8–16 h at 37 °C under an atmosphere of 5% CO_2. The cells were then centrifuged, washed with PBS(−), and suspended in a conjugation buffer (HEPES/NaOH, 10 mM, pH 7.4; NaCl, 140 mM; $CaCl_2$, 2.5 mM; 195 μL). FITC-labeled annexin V solution (Wako Pure Chemicals; 5 μL) was added to the cell suspension and incubated for 10 min

(FITC = fluorescein isothiocyanate). The cells were washed with 500 μL conjugation buffer and resuspended in 190 μL conjugation buffer on ice. Propidium iodide 10 μL, 20 $\mu g\,mL^{-1}$ was added to the cell suspension and incubated for 5 min. After dilution with the conjugation buffer (final volume 500 μL), the analysis of apoptotic cells was performed by flow cytometry.

Human cancer cell line panel screening: To evaluate drugs for the cell growth inhibition profile, we established a human cancer cell line panel combined with a database. The system as a whole was developed according to a modified method of the National Cancer Institute.[27–29] The panel consisted of 39 human cancer cell lines. With this system, we examined the antiproliferative effect of more than 200 standard compounds, including various anticancer drugs, and established a new database, as described below.

Measurements of cell growth inhibition and data analysis: The details of measuring cell growth inhibition are described elsewhere.[30,31] Briefly, the cells were plated at proper density in 96-well plates in RPMI-1640 with 5% fetal bovine serum and allowed to attach overnight. The cells were exposed to the test compounds for 48 h. Then, the cell growth was determined according to the sulforhodamine B assay described by Skehan et al.[32] Data calculations were made according to a previously described method.[31] Absorbance for the control well (C) and the test well (T) were measured at 525 nm. Moreover, at time 0 (addition of compounds), absorbance for the test well (T_0) was also measured. Using these measurements, cell growth inhibition (percentage of growth) by each concentration of compound was calculated as: $\%\ \text{growth} = 100[(T-T_0)/(C-T_0)]$, when $T > T_0$ and $\%\ \text{growth} = 100[(T-T_0)/T]$, when $T < T_0$. By using the computer to process % growth values, the 50% growth inhibition parameter (GI_{50}) was determined. GI_{50} values were calculated as $100[(T-T_0)/(C-T_0)] = 50$. The mean graph, which shows the differential growth inhibition of the drug in the cell line panel, was drawn based on a calculation using a set of GI_{50} values.[29,30] To analyze the correlation between the mean graphs of compounds A and B, a COMPARE computer algorithm was developed according to the method described by Paull et al.[29] Correlation coefficients were calculated using the following formula: $r = (\Sigma(x_i-x_m)(y_i-y_m))/(\Sigma(x_i-x_m)^2\Sigma(y_i-y_m)^2)^{1/2}$, for which x_i and y_i are log GI_{50} of compounds A and B, respectively, against each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively.

Acknowledgements

Part of this work was supported by the Uehara Memorial Foundation, Japan. We thank Professor M. Kohno (Nagasaki University) for discussions on the tubulin polymerization inhibition assay.

Keywords: antitumor agents · biological activity · boron · *cis*-stilbenes · tubulin polymerization

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Received: March 15, 2006
Published online on May 23, 2006

